An Estimation of the Extent of Animal Use in Research in Brazil, as Determined by Bibliographic Sampling from Journals Published in the State of Paraná

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Summary — Animal use in research is an issue of increasing ethical concern. The objective of this work was to investigate animal use in research described in the papers appearing in 18 journals published in the State of Paraná in 2006. The fields used in the bibliographic sampling were: agrarian science, biological science, biological and health science, environmental science, food technology, and health science. Of the 865 papers analysed, 41% involved the use of animals — a total of 3,497,653 animals, of which 216,223 were vertebrates. Procedures which were classified as A or B for degree of invasiveness were involved in 67% of the papers; 571 fish were employed in procedures classified as E. Only 11% of the journals required certificates from Animal Use Ethics Committees. These results suggest that Brazil is important in the context of worldwide animal use for research, in terms of both the total numbers of animals and the numbers of vertebrates used. Bibliographical sampling is a useful method for estimating the extent of animal use in experiments in Brazil. However, there are limitations to this approach, resulting from the geographical distribution of the authors, the existence of papers presenting insufficient information, and the exclusive inclusion of animal experimentation that actually reaches publication. Thus, the introduction of a formal system to record and control laboratory animal use in Brazil, is urgently required.

Key words: *animal use statistics, animal welfare, experimentation, invasiveness, sentience.*

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Introduction

Animal use in research is of increasing ethical concern worldwide. The gathering of data related to animal use in experiments is essential, because it forms a basis for consistent and informed discussion (1). In some countries, the use of animals in research is officially registered (2). In 2005, the number of animals used in research in Europe increased, as compared with 2002 and 1996 (3). In the United Kingdom, a reduction in the annual number of procedures which involved the use of animals had been observed since 1976; this downward trend then stabilised in 1990, but recently began to increase. Figures from the latest statistics show that almost 3.7 million scientific procedures were started in 2008, a rise of 14% on 2007 figures. This increase is mainly due to the increased use of turkeys, pigs and fish (4). In the USA, animal use data have been kept since 1973 (5), but the information kept relates exclusively to birds and mammals — with the exception of birds bred for research, and rats and mice (6). Thus, the numbers reported must be interpreted very carefully, because the most widely used species (mice and rats) are not considered in the US data.

Animal use in research is linked to the generation of scientific knowledge by a given country.

According to the Coordination for the Improvement of Superior Education Human Resources (7), Brazil is ranked 15th in the world for scientific knowledge generation. In 2007, 19,428 papers were published in Brazil, this representing 2.0% of the total world scientific paper publication, above Switzerland (1.9%) and Sweden (1.8%), and close to The Netherlands (2.5%) and Russia (2.7%). Brazil's main area of knowledge contribution at a global level is in the field of agriculture, with 4,139 papers published between 2003 and 2007, representing 4% of the total world production (7). The southern Brazilian State, Paraná, has an area of 199,314.850km^2, with 10,284,503 inhabitants (2002 data), representing 5.6% of the Brazilian population (8). In terms of research output, 339 PhD diplomas were granted in Paraná in 2007. This number represents 3.4% of the number granted throughout the whole of Brazil (9), which could be taken as indicative of the relative level of research undertaken in the region.

Up to 2008, there were no official data available on the numbers and taxonomic groups of animals used in research in Brazil, the procedures employed, the institutions involved, and the existence of Animal Use Ethics Committees. After the publication of a resolution from the Federal Veterinary Council in February 2008 (10), and the

approval of the *Federal Act No. 11.794* (11) in October 2008, there are now provisions for regulations, such as mandatory Animal Use Ethics Committees. However, these regulations need further detailed guidance in order to become fully effective.

Bibliographic sampling can be employed to contextualise animal use in Brazil. Our objective was to investigate the extent of animal use in research described in scientific papers appearing in journals published in the State of Paraná in 2006, according to the geographical origin of the authors, the taxonomic groups of animals and the degrees of invasiveness of the procedures used, and the requirement for Animal Use Ethics Committee certificates by journal editors.

Materials and Methods

Journals from relevant scientific areas, published in the State of Paraná in 2006, were analysed. Journal names were sourced by using websites such as the National Catalogue of Scientific Journals (www.ibict.br), Ulrich's Periodicals Directory (http://www.ulrichsweb.com/ulrichsweb/), SciELO (Scientific Electronic Library Online; www.scielo.org), the Virtual Library on Health (www.bireme.br/php/index.php), Google (www.google.com.br), and the Brazilian Education Ministry (portal.mec.gov.br). From the list of 43 journals, 18 journals were randomly sampled for analysis, in view of the time and human resources available. The papers published in the selected journals in 2006 were analysed to determine their geographical origin and the taxonomic groups and numbers of animals used. The numbers of vertebrates used were given special attention, since this group represents a high probability of consciousness (12) or sentience (13). The procedures reported were classified into five degrees of invasiveness, according to Canadian Council on Animal Care definitions (14; see Table 1). The requirement of a certificate from an Animal Use Ethics

Committee, as a form of licence document, was searched for in the *Instructions to Authors* from each journal. Also, the journal's quality, or '*Qualis*', was verified. As defined by the Brazilian federal agency, CAPES (9), '*Qualis*' is the classification (reviewed annually) of channels of communication used by post-graduate programmes to publish their research output, which is based on a journal's median impact factor in the specific area of knowledge.

Results and Discussion

Of the 43 scientific journals initially identified, 18 were from the field of health science, 12 from agrarian science, nine from biological science, two from environmental science, one from biological and health science, and one from food technology. Eighteen journals of different quality were randomly chosen from this initial set of 43 journals, and papers describing experiments involving animal use were identified in this subset. These papers represented 42% of the total number of papers published in 2006 in the 18 journals (Table 2). The higher quality of a scientific journal is represented by A, followed by B and C. Since the majority are classified between B and C, it would be possible to improve the quality of several journals that publish research papers featuring animal experiments. A recording of publication quality could be incorporated in the regulatory mechanisms for animal use, so the '*Qualis*' of journals could be easily accessed and controlled. Of the journals studied, four (22%) did not publish papers featuring animal experiments; for this reason, the results refer only to the 14 journals which published research papers based on animal use.

The journals studied published papers from different geographical locations (Figure 1). The origin of the majority of the papers was the State of Paraná (128 papers), followed by São Paulo (55 papers), Rio Grande do Sul (32 papers), Minas Gerais (33 papers), Rio de Janeiro (28 papers), Santa Catarina

Table 1: Descriptions of the degrees of invasiveness of animal experiments

Degree of invasiveness	Description
A	Experiments on most invertebrates or on live isolates
B	Experiments which cause little or no discomfort or stress
C	Experiments which cause minor stress or pain of short duration
D	Experiments which cause moderate to severe distress or discomfort
E	Procedures which cause severe pain near, at, or above the pain tolerance threshold of unanaesthetised conscious animals

From Reference 14.

Table 2: Details of eighteen scientific journals published in 2006, in the State of Paraná

Knowledge area	City of origin	Journal name	*Qualis*	Number of papers published (2006)		
				Total	All animals	Vertebrate animals
Agrarian science	Curitiba	Archives of Veterinary Science	B	43	37	37
	Curitiba	Revista Acadêmica Ciências Agrárias e Ambientais	–	39	11	9
	Maringá	Agronomy — UEM	B	80	9	0
	Maringá	Animal Sciences — UEM	A	62	55	50
	Umuarama	Arquivos de ciências Veterinárias e Zoologia da UNIPAR	B	21	12	12
Biological science	Curitiba	Acta Biológica Paranaense	–	11	7	2
	Curitiba	Brazilian Archives of Biology and Technology	A	137	45	28
	Curitiba	Revista Brasileira de Zoologia	A	153	123	63
	Maringá	Biological Sciences — UEM	C	55	33	28
Biological and health science	Londrina	Semina Revista Cultural e Científica da UEL	C	61	7	5
	Curitiba	Cogitare Enfermagem	B	37	0	0
	Curitiba	Família, Saúde e Desenvolvimento	C	24	0	0
	Curitiba	Interação em Psicologia	C	40	2	2
Health science	Curitiba	Revista Médica do Paraná	B	17	4	4
	Maringá	Health Sciences — UEM	B	25	7	7
	Umuarama	Arquivos de Ciências da Saúde da UNIPAR	–	18	0	0
Food technology	Curitiba	Boletim do Centro de Pesquisa e Processamento de Alimentos	C	29	3	3
Environmental science	Curitiba	Pesticidas: Revista de Ecotoxicologia e Meio Ambiente	C	13	0	0
Overall Totals				**865**	**355**	**250**

UEM = Universidade Estadual de Maringá; UEL = Universidade Estadual de Londrina; UNIPAR = Universidade Paranaense.

Qualis *is determined by the official federal agency, CAPES (9).* A *indicates the highest quality rating, and* C *the lowest. '–' indicates that the journal is not classified by CAPES (i.e. no rating).*

Fourteen out of the eighteen journals were used in the subsequent analyses, as four did not include any research papers featuring experiments involving the use of animals.

(12 papers), and Pernambuco (12 papers). The publication of less than 10 relevant papers each was ascribed to fourteen other Brazilian states. There were six papers from other countries: USA (2 papers), Antarctica (1 paper), Argentina (1 paper), Mexico (1 paper), and Tunisia (1 paper).

Although the analysis of the data published in the State of Paraná shows that this State represents the main geographical origin, it constituted only 36% of the papers. This means that there were limitations related to the bibliographic sampling technique regarding the determination of animal use in specific locations, since 64% of the papers came from outside of the State of Paraná. At the same time, several papers produced in this State were published in other states or countries. For these reasons, the best way to obtain this infor-

mation seems to be through a registration system, which considers individuals, their origin, as well as procedures of the processes related to the conduct of scientific experiments involving animal use, particularly during project planning. It would then be possible to have the total numbers of animals used, including those used in experiments which are featured in papers published abroad and those which are not published. *Resolution 879* (10), and the recently-approved *Federal Law No. 11.794* (11), constitute concrete steps toward the construction of a system for the recording and control of laboratory animal use in Brazil. The bibliographic sampling does not substitute such formal mechanisms in any way.

In the 14 journals studies, 355 out of 865 scientific papers described experiments involving the

Figure 1: The origins of the research papers involving animal use, which appeared in fourteen scientific journals published in the State of Paraná in 2006

The map shows the country of Brazil, with its constituting states outlined. Two percent of the papers were authored by researchers from other countries, and 14% were from other states (data not shown on map).

use of animals (41%). Within those papers, 105 (30%) employed invertebrate animals, 246 (69%) vertebrate animals, and four (1%) both vertebrates and invertebrates (Tables 3 and 4). In 133 papers (37%), the number of animals used was not mentioned, and of these, 61 (17%) reported the use of vertebrates. The animals used in these papers were not included in our analysis, and this is one significant limitation of the application of the bibliographic sampling method. Among other relevant

information regarding the implementation of the Three Rs (15) — whose concept embraces the *Reduction* of animals used in research, their *Replacement* by alternative methods, and the *Refinement* of experimental procedures where their use is currently unavoidable — it is important that researchers and editors from journals in the State of Paraná look for an improvement with regard to the recording of numbers and species of animals used in the published experiments. All

Table 3: Taxonomic classification of Phylum Chordate (vertebrates [23, 24]) which were used in experiments described in the studied papers

Super class/ class	Order	Family/genus/species	Animals featured in the papers	Number of animals	
Mammalian	Artiodactyla	*Sus domesticus*	Pigs	25,489	
		Sus scrofa	Wild boar	5	
			Bovids (cattle & buffalos)	15,037	partial[a]
		Capra aegagrus	Caprines (goats)	44	
		Ovis sp.	Ovines (sheep)	129	partial
	Chiroptera		Bats	1,924	
	Primates		Non-human primates	1,351	
	Lagomorpha	Leporidae	Rabbits	1,267	partial
	Perissodactyla	*Equus caballus*	Equines (horses)	361	
	Carnivora	Canidae	Canids (dogs)	153	partial
		Otaria flavescens	Sea lions	not informed	
		Felis catus	Felids (cats)	70	
	Cetacea		Cetaceans	130	partial
	Didelphimorphia	Didelphidae	Opossums	57	
	Rodentia	*Hydrochaeris hydrochaeris*	Capybaras	50	
		Agouti paca	Pacas (cuniculus rodents)	6	
		Dasyprocta aguti	Agouties (dasyproct rodents)	3	
		Ratus	Rats	743	partial
Aves	Galliformes	*Gallus gallus*	Broiler hens	3,228	
			Laying hens	408	
			Other birds	915	partial
Reptilia			Reptiles	307	
Amphibia	Anura		Anuran amphibians	34	
Pisces			Fish	164,512	partial

[a]*Numbers followed by the word "partial" refer to an incomplete total number of animals, due to the existence of studied papers that did not actually state the numbers of animals used; see text for more explanation.*

papers should contain information pertaining to the taxonomic groups and the numbers of animals used, as well as detailed explanation of the methods and procedures conducted in every phase of the research.

The numbers of animals used in each taxonomic group showed that the majority belonged to the crustacean group, followed by (in numerical order) fish, arthropods (excepting crustaceans), pigs, bovids (cattle and buffalos), broiler hens, bats, non-human primates, rabbits, medusae (jellyfish), birds (excepting broiler and laying hens), rats, molluscs, laying hens, horses, reptiles, canids (dogs), cetaceans (marine mammals), ovines (sheep), felids (cats), opossums, capybaras, caprines (goats), anuran amphibians, pacas (cuniculus rodents), wild boars and agouties (dasyproct rodents). In total, 3,497,653 animals were used in

the experiments featured in the papers studied. This data organisation permits the calculation of the percentages of different types of animals that were used in the experiments described (Figure 2a). A total of 216,223 vertebrate animals were used (Figure 2a and b), these mainly being fish (Figure 2a).

The estimated worldwide use of laboratory animals in 179 countries in 2005 was given as 58,339,972 animals, including vertebrates and invertebrate species such as cephalopods (16). If animals which are killed for tissue supply, used to maintain genetically-modified colonies and surplus bred are included, the number increases to 115,279,785. Since 3,497,653 animals were used in experiments featured in 42% of the papers appearing in 18 journals published in one Brazilian State in 2006, it can be extrapolated that, in the whole of

Table 4: Taxonomic classification of invertebrates (23, 24) which were used in experiments described in the studied papers

Phylum	Animals cited on the papers	Number of animals	
Arthropoda	Crustaceans	3,226,922	partial[a]
	Other arthropods	52,722	partial
Cnidaria	Medusae	1,092	partial
Mollusca	Molluscs	694	partial
Nematoda	Nematodes	not informed	
Platyhelminthes	Cestodes	not informed	
Porifera	Poriferans	not informed	

[a]*Numbers followed by the word "partial" refer to an incomplete total number of animals, due to the existence of studied papers that did not actually state the numbers of animals used; see text for more explanation.*

Brazil, the total number of animals used every year is extremely high in terms of the worldwide use of animals in experimentation. Additionally, the majority of other Brazilian states also publish scientific journals, particularly in the south and south-eastern regions. It should also be noted that these numbers include only those animals used in published experiments, and not in those that did not reach the publication stage. Furthermore, animals used in experiments featured in research papers that were sent to journals published in other Brazilian states or abroad, are also excluded, although there this may be counter-balanced by the inclusion of papers submitted from other geographical regions.

In Europe, the total number of vertebrate animals used in 25 countries in 2005 was 12.1 million (3); in this present study, 216,222 vertebrates were used, considering data from 2006. The results show that, in Paraná, rodents and rabbits are not the most commonly-used species, corresponding to only 0.05% of the total of animals used, while in Europe these animals correspond to 78%. This fact might be related to the inclusion

Figure 2: Taxonomic groups of animals used in the procedures described in studied papers
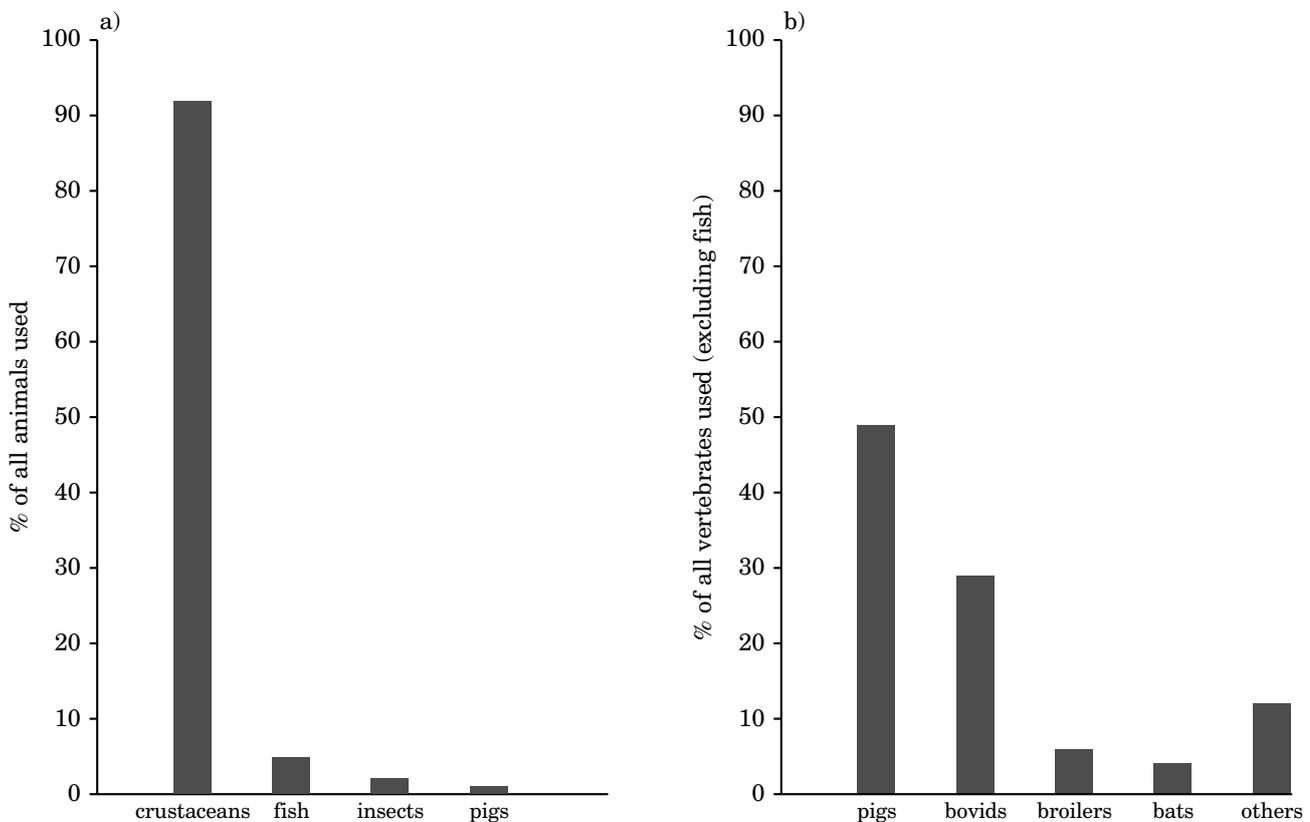
a) All animals (bovids, broilers and others each totalled < 1%, so were not included in this graph); b) vertebrate animals, except fish (which corresponded to 75% of the vertebrate total, and would impair visualisation of the other proportions if included in the plotted data).

in the European figures of animals used in toxicology and product safety testing, the production and control of products used by humans, dentistry and veterinary medicine, teaching, and the diagnosis of disease.

A comparison with the numbers from the USA requires careful interpretation. Before the *Farm Security and Rural Investment Act (2002)*, the *Animal Welfare Act (1985)* excluded rats, mice and birds from the figures, and it still excludes birds bred for research, and rats and mice (6). With these considerations in mind, in the USA, 1.1 million birds and mammals were used in 2004; in the state of Paraná 46,076 individuals from the same taxonomic groups were used in 2006, or 21% of the total number of vertebrates. This low percentage is in agreement with statements by other authors with regard to the serious deficiency of recording in the USA (1) and its incoherence (17).

Concerning the degree of invasiveness of the procedures employed, 110 papers corresponded to degree A, 137 to B, 57 to C, 55 to D, and 7 to E (Figure 3). Thus, while most of the papers involved degree B of invasiveness, several involved a higher degree of invasiveness, causing considerable animal suffering. In some papers, the limited description of the methods applied made it difficult to understand and classify the procedures. There is clearly a need for more explicit information regarding the employment of the Three Rs (18) in the *Materials and Methods* sections of published papers (15). Seven pairs of papers (14 papers) had notably similar information on the number of animals, types of procedures and authors, so it is possible that these publications were based on the use of the same animals.

The majority of the animal procedures applied were classified as being of degree A of invasive-

Figure 3:   Degrees of invasiveness of procedures involving animal use, which were described in the studied papers
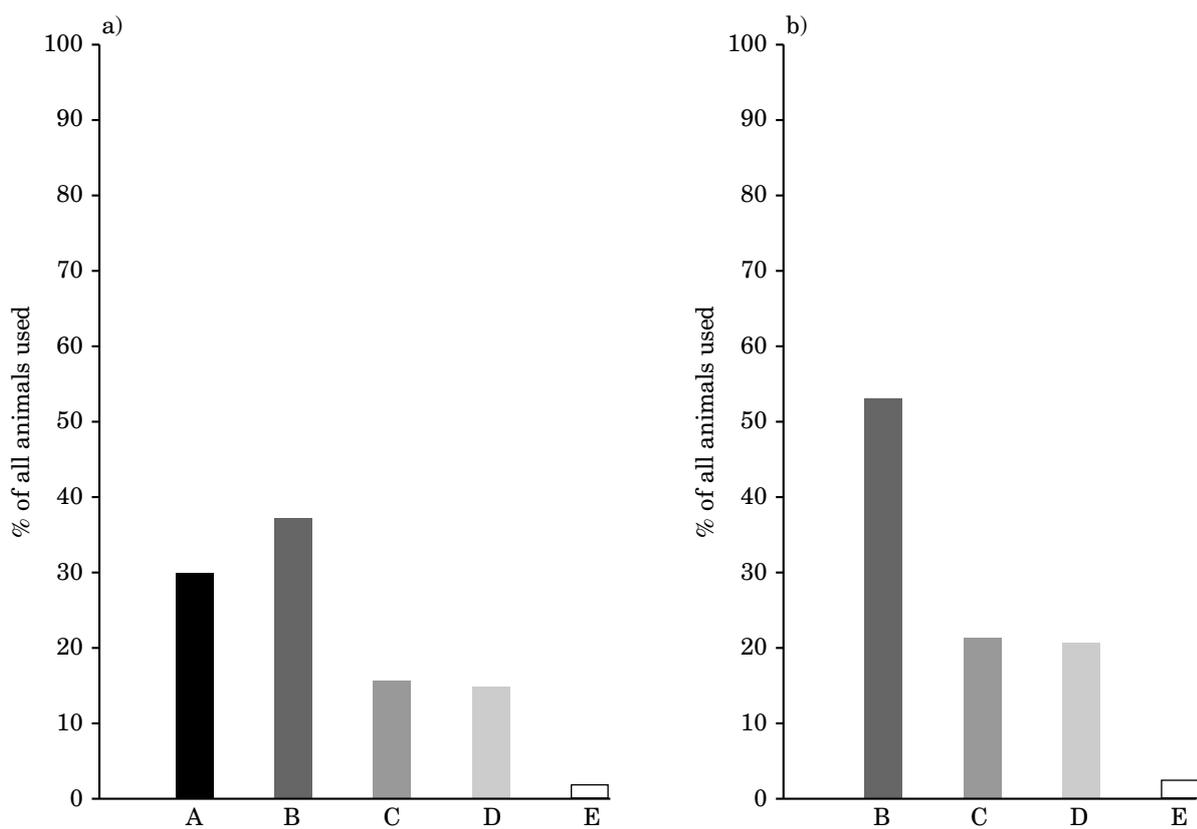
= experiments on most invertebrates or on live isolates; = experiments which cause little or no discomfort or stress; = experiments which cause minor stress or pain of short duration; = experiments which cause moderate to severe distress or discomfort; = procedures which cause severe pain near, at, or above the pain tolerance threshold of unanaesthetised conscious animals

a) All degrees of invasiveness (see Table 1 for definitions); b) degrees of invasiveness in vertebrates, after the exclusion of degree A.

ness, given the invertebrate use that corresponded to 93% of the total. Taking into account the taxonomic groups of the vertebrates which were more frequently used, the majority of the procedures performed were classified as follows: in fish, as degrees B (140,055) and D (21,739); in pigs, as B (25,147) and C (300); in bovids, as B (14,906) and D (99); in broiler hens, as B (3,228); in bats, as B (1,226) and C (670); and in non-human primates, as B (1,351; see Table 5). These results suggest that fish and bovids are submitted to more-invasive procedures, followed by bats and pigs.

Procedures classified as E were performed on 571 fish. Experiments that involve degree E should be discouraged, as the conduct of animal procedures that involve severe pain and suffering in research are generally not approved (19). This scenario is currently commonplace in North America, but a similar trend may occur in Brazil, since there is increasing concern over experimental animal use — suggesting that society is becoming increasingly sensitive about animal welfare issues.

The analysis of projects by Animal Use Ethics Committees has been mandatory in Paraná since 2003 (20). However, proof of adoption of this practice does not appear to be a systematic requirement by scientific journals, before a research paper will be considered for publication. Of the 18 journals studied, only two journals required a certificate from an Animal Use Ethics Committee as a pre-requisite to manuscript submission, according to their *Instructions to Authors*. The Animal Use Ethics Committee has a basic objective of evaluating projects that involve the use of animals in teaching, training and scientific procedures (21). The main action of such committees is encourage the minimisation of pain and suffering and to promote animal welfare (22). In this sense, reinforcing the law requiring the analysis of projects by Animal Use Ethics Committees should be a matter of high priority, to improve the welfare of the animals used, and to control the overall use of animals in experimentation.

Conclusions

Brazil is an important contributor to the vast numbers of animals used in research worldwide. The results reported here show that most of the animals used were subjected to only minor suffering. However, thousands of vertebrate animals were subjected to procedures associated with higher degrees of suffering. The bibliographic sampling method permits the collation of important information related to animal use in research. However, the construction of an official register of such data in Brazil is essential. Such a system should include data on the numbers of animals used in research, teaching, product testing and diagnosis, as well as some classification of the procedures employed. Also important are the assessment of projects by Animal Use Ethics Committees, and the need for Brazilian scientific journals to require authors to declare that studies involving the use of animals are properly licensed. Such information should be readily available, creating a solid basis for a well-grounded discussion between animal protection groups, laboratory animal users, and society in general, with the ultimate goals of ethical improvement, increased satisfaction for all involved, and a reduction of animal numbers and animal suffering.

Acknowledgements

Table 5:   The most frequently-used taxonomic groups of animals, further classified by degree of invasiveness of the procedures, which were described in the 355 studied papers

Taxonomic group	Degree of invasiveness[a] ascribed to the procedures (as a percentage of the total procedures described for the taxonomic group)				
	A	**B**	**C**	**D**	**E**
Fish	0	85	1	13	1
Pigs	0	99	1	0	0
Bovids	0	99	1	0	0
Broiler hens	0	100	0	0	0
Bats	0	64	35	1	0
Non-human primates	0	100	0	0	0

[a]*See Table 1 for definitions.*

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