Numbers of Publications Related to Laboratory Animals

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Summary

Laboratory animals are widely utilized in biomedical research, so a search of scientific publications can give us useful information on the use of animals. We retrieved the PubMed biomedicine database and searched for publications related to laboratory animals from 1966 to 2005. We found that rats and mice constitute the vast majority of species used in biomedical research; C57BL and BABL/c inbred mice, and Sprague Dawley and Wistar outbred rats are the most common strains. Recently, the numbers of publications relating to traditionally used animals such as rats, guinea pigs, dogs, cats, and sheep decreased slightly, whereas the numbers relating to mice, fish, Drosophila and Caenorhabditis elegans increased from 1995 to 2005, with annual mean growth rates of 4.5%, 8.22%, 1.95%, and 10.3%, respectively. Publications involving transgenic mice increased dramatically from the mid-1980s. This survey provides significant clues for predicting the future direction of biomedical research.

Introduction

Laboratory animals are widely used in biomedical research on how the body works, the identification and development of new treatments, and the understanding of diseases and their progression. Every year, about 8 million animals are used in Japan (*Ninomiya et al., 1998*), 2 million in Canada (*Rowsell, 1988*), 2.8 million in the UK (*Home Office, 2005*), and 18-22 million in the USA (*Mukerjee, 1997*). However, it is unknown exactly how many animals are used in biomedical research worldwide. Scientific research publications published by biomedical journals reflect the work of biomedical scientists and can give useful information on the animals used in biomedical research.

Materials and Methods

Selection of database

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Medline is the largest biomedical database in the world and is the most commonly used search engine (*Ebbert et al., 2003*). PubMed is the internet version of Medline (www.ncbi.nlm.nih.gov/entrez/query. fcgi?db=PubMed), containing bibliographic citations and author abstracts from more than 4,800 biomedical journals published in the USA and 70 other countries. The Medical Subject Headings (MeSH) system determined by the National Library of Medicine is used for indexing papers, cataloguing materials, and searching MeSH-indexed databases. A PubMed search was conducted for publications related to laboratory animals.

Selection of publication years

We used the MeSH system to search PubMed for publications related to laboratory animals such as mice, rats, and rabbits, and received numerous items that matched the retrieval requirements. After analysis of the search results from 1966 to 2005, a preliminary assessment of the importance of laboratory animals in biomedical research was made.

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Selection of laboratory animal species

In this study, the term "laboratory animal" is applied to vertebrates and invertebrates (*Liu, 2004; Zutphen et al., 2001*) but excludes animal materials such as mouse cell lines and monoclonal antibodies. Based on our experience and data from the literature (*Home Office, 2005; Liu, 2004; Mukerjee, 1997; Ninomiya et al., 1998; Rowsell, 1988; Zutphen et al., 2001*) the common vertebrates mice, rats, rabbits, guinea pigs, hamsters, gerbils, pigs, dogs, sheep, goats, ferrets, cats, non-human primates, cattle, horses, donkeys, chickens, fish, toads and frogs, and the invertebrates *Drosophila* and *Caenorhabditis (C.) elegans* were selected as MeSH. Universal inbred and outbred strains such as C57BL, BABL/c, C3H, DBA/2, ICR, CBA, A, and NOD mice, and Wistar, Sprague Dawley (SD), F344, Lew, WKY, and SHR rats were also selected. PubMed was searched for all publications related to these animals.

Selection of retrieval method and sample survey

PubMed uses MeSH indexing terms, and within PubMed MeSH can be identified by looking in the Thesaurus section. The Thesaurus contains carefully constructed sets of terms. An extensive literature search of the PubMed database was conducted using MeSH relevant to laboratory animals, such as "rats", "mice", in the search strategy. For example, we input "mice [MH] not review [PT] not editorial [PT] not comment [PT] and 1995/01/01[DP]: 2005/12/31 [DP]" into the Query box and selected the Go button. This search was limited to papers relevant to mice published from 1 January 1995 to 31 December 2005, excluding reviews, editorials, and comments. Subsequently, a manual review of relevant papers in 2003 was performed to determine whether the animals were used or only mentioned. One hundred mouse- or rat-relevant abstracts or full-text manuscripts identified during the literature search were chosen by systematic sampling (Antony, 2002; Lwanga et al., 1991) and reviewed independently by two investigators.

Results

Number of publications related to animals

From 1966 to 2005, the total number of publications in PubMed increased by 3.28% per year. Publications related to the top 10 animals in 1995 to 2005 are shown in Table 1. The vast majority of species were rats, mice, rabbits, pigs, or dogs (Fig. 1). From 1995 to 2005, the total number of publica-

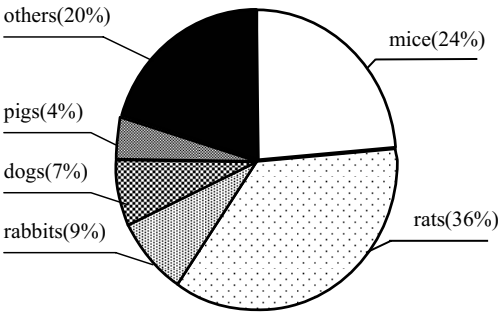

Figure 1. Proportions of publications by animal species from 1995 to 2005. Other animals include guinea pigs, hamsters, gerbils, sheep, goats, ferrets, cats, non-human primates, cattle, horses, donkeys, chickens, fish, toads, frogs, Drosophila, and C. elegans.

Table 1. Top 10 animals in publications related to laboratory animals from 1 January 1995 to 31 December 2005

	Rats	Mice	Rabbits	Pigs	Dogs	Non-human primates	Hamsters	Fish	Chickens	Sheep
Number	365,208	314,136	55,422	46,651	44,618	40,432	32,793	31,680	20,945	20,313
AMGR	−1.2%	4.5%	−5.64%	0.69%	−1.76%	4.37%	−1.18%	8.22%	−0.30%	−2.27%

AMGR: annual mean growth rate = $(N_{2005}/ N_{1995})^{1/10} - 1$, where N_{2005} is the number of publications in 2005 and N_{1995} is the number of publications in 1995.

tions in PubMed increased by 4.57% per year, while publications related to pigs, non-human primates, *Drosophila*, or mice increased slightly, with annual mean growth rates (AMGRs) of 0.69%, 4.37%, 1.95%, and 4.5%, respectively. Publications related to fish and *C. elegans* increased steeply, at 8.22% and 10.3%, respectively. Publications related to rats, rabbits, dogs, cats, guinea pigs, sheep, chickens, hamsters showed declines in AMGR of 1.2%, 5.64%, 1.76%, 3.35%, 8.07%, 2.27%, 0.30%, and 1.18%, respectively, from 1995 to 2005 (Table 1).

Mice have become more popular than rats

Rats and mice are the most widely used animals in biomedical research. Publications related to them from 1966 to 2005 are shown in Fig. 2A. The numbers of publications related to rats were 1,059,047 in 1966 to 2005 and 365,208 in 1995 to 2005; the proportions of all publications were 8.18% and 7.36%, respectively. The proportions of all publications related to mice were 5.37% from 1966 to 2005 and 6.34% from 1995 to 2005. Interestingly, publications related to rats decreased from 1996 (Fig. 2A), while those related to mice exceeded rats during this period, became the majority in 2003, and have continued to increase (Fig. 2A). Publications related to mice have increased almost continuously since 1966, while publications related to rats increased from 1966 to 1995 then began to decline progressively from 1996 (Fig. 2A). The proportion of publications related to rats among all publications increased from 1966 to 1972, showed two plateaus (from 1972 to 1979 and from 1980 to1997), then began to decrease dramatically (Fig. 2B). The proportion of publications related to mice

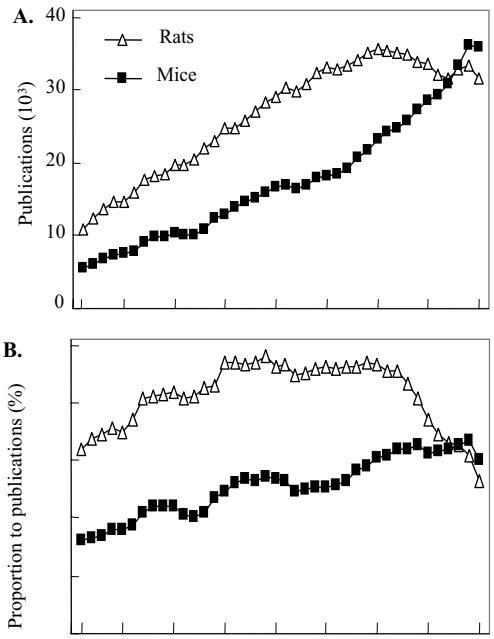

Figure 2. Publications related to mice and rats (A), proportions of all publications from 1966 to 2005 (B). Publications related to rats increased by 4.18% per year from 1966 to 1995, then decreased by 1.2%, and were exceeded by mice in 2003 (A). The proportion of publications related to mice to all publications showed an increasing tendency. The proportion of publications related to rats to all publications increased slowly from the early 1970s, and after two stable periods decreased steeply from 1998 (B). 66: 1966, and so on.

Table 2. Top five mouse and rat strains in publications related to mouse and rat strains from 1 January 1995 to 31 December 2005

	Mouse					Rat				
	C57BL	BALB/c	C3H	ICR	CBA	SD	Wistar	F344	Lew	SHR
Number	57,587	44,983	9,300	7,734	5,714	120,984	99,904	11,976	8,672	5,479
AMGR	8.95%	1.26%	–4.11%	2.49%	–5.35%	–0.92%	–1.02%	–3.08%	–2.98%	–5.72%

AMGR: annual mean growth rate = $(N_{2005}/ N_{1995})^{1/10} - 1$, where N_{2005} is the number of publications in 2005 and N_{1995} is the number of publications in 1995.

increased continuously, exceeded rats in 2003 (Fig. 2B), and then became the majority; these findings are consistent with the numbers of publications related to mice (Fig. 2A).

Standard laboratory animals play an important role in biomedical research

Several hundreds of laboratory animal strains have been generated since the 1900s. We searched for publications related to common strains from 1995 to 2005. As shown in Table 2, the most common inbred strain is the C57BL mouse, while the most common outbred strain is the SD rat. The proportions of the retrieved publications related to these strains are 28% and 34%, respectively. Publications related to rat strains decreased, though some mouse strains increased. Publications related to BALB/c were exceeded by C57BL in 1997, and the AMGR of C57BL was 8.95% from 1995 to 2005 (Table 2).

Publications related to transgenic animals increased dramatically

Transgenic animals are important models in biomedical research. "Transgenic" was introduced as a MeSH in PubMed in 1988 and "knock-out" in 1994; transgenic mice include knock-out mice in MeSH. Thus, we searched for publications related to transgenic mice from 1988 and knock-out mice from 1994. As shown in Fig. 3, the number of publications increased steeply; the AMGR of transgenic mice was 24.76% from 1988 and that of knock-out mice was 41.88% from 1994.

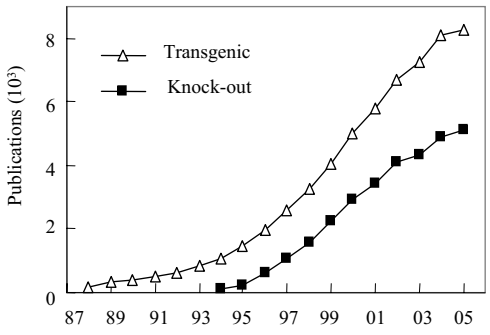

Figure 3. Publications related to transgenic mice from 1988 to 2005.

Sample results supported the above conclusions

According to method of systematic sampling (Antony, 2002; Lwanga et al., 1991), we sampled 100 publications related to mice from 2003. Among these publications, mice only were used in 66 publications, mice and other species were used in eight, and no animals were used in 26. C57BL and BALB/c were the most common strains, and transgenic mice were used in 22 publications (Table 3). Rats were used in 83 relevant publications, while in 17 publications no animals were used or mentioned (Table 3). SD and Wistar were the most common rat strains, in accordance with Table 2.

Discussion

We searched for publications related to laboratory animals in PubMed, and calculated numbers, proportions, and AMGRs to obtain important information on current use and trends in the use of laboratory animal in biomedical research. PubMed cannot be considered a complete bibliographic database until around 1980 to 1985. Thus, we paid more attention to the results retrieved from 1995 to 2005. Although we excluded reviews and editorials, some publications could not be included in our search, and some overlapped or were not matched. For example, publications of mouse gene sequences might not be retrieved, and some publications related to farm animals (e.g. pigs, chickens) may be retrieved in the field of agricultural research but not in biomedical research. Nevertheless, when we evaluated the reliability of our retrieval results, we found that 74% of publications related to mice were matched real fact; for rats, this rate was higher, at 83%. We think that the search results are acceptable.

Previous statistical data indicate that mice constitute the overwhelming majority of animals used in laboratories worldwide (*Home Office, 2005; Liu, 2004; Mukerjee, 1997; Ninomiya et al., 1998;*

Table 3. Results of sampling from 2003

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	None	One	Several	Animal strains				
				Transgenic	C 57BL	BALB/c	SD	Wistar
Mice	26	66	8	22	28	14	-	-
Rats	17	77	6	1	-	-	34	21

None: no animals used; one: only one species used; several: two or more species used.

Rowsell 1988; Zutphen et al., 2001); in which case, why was the number of publications related to mice less than of rats until 2003? We suggest that many mice are used to test the safety of a wide range of products including drugs, medical appliances, pesticides, household and industrial chemicals, food additives, cosmetics, and toiletries, and the results of such tests are not published; consequently, we could not search for these in PubMed. Such cases also exist for other animals. In the field of biomedical research, mice and rats constituted the vast majority of species, accounting for 80-85% of all animals used in the UK (*Home Office, 2005*) and 90% in Japan (*Ninomiya et al., 1998*). Publications related to rats declined from 1996, while mice have been dominant since 2003 (Fig. 2). Mice are used because of their small size, short reproductive cycle, and well-known genetic background, which facilitate many molecular biologic technologies. For example, a draft genome sequence of the inbred strain C57BL/6 was completed in 2002 (*Waterston et al., 2002*), and increasing numbers of scientists are therefore likely to select this strain for their research.

Gordon et al. created the first transgenic mouse in 1980 (*Gordon et al., 1980*), and new strategies to improve transgenesis have led to significant advances in biomedical research. Over 80% of mouse gene functions are the same as those in humans, and they are therefore an ideal human surrogate in the study of most diseases. Currently, over 90% of transgenic animals used in biomedical research are mice (*Home Office, 2005*). In our survey sample, nearly one-third (22/74) of publications related to mice used transgenic mouse models; only 1.2% (1/83) used transgenic rat models (Table 3). The transgenic animals used included rats (*Tesson et al., 2005*), rabbits (*Fan et al., 2003*), fish (*Rocha et al., 2004*), and large animals (goats, sheep, and pigs) (*Wheeler et al., 2003*). It is hoped that refinements of transgenesis techniques in mice will enable a reduction in the use of higher animals, such as dogs and non-human primates, in biomedical research.

The principle of the 3Rs (replacement, refinement, and reduction) was originally developed by Russell and Burch (*Russell et al., 1959*), and the 3Rs are now widely accepted internationally as criteria for humane use of animals in research and testing (Martin, 1986). Hagelin et al. suggested that efficiency increased in use of animals and due to refinement in animal research conditions (*Hagelin et al., 1999*). Statistics show that the use of animals in Europe and Japan has been declining since the end of the 1970s (*Home Office, 2005; Mukerjee, 1997; Ninomiya et al., 1998*). We found that publications related to lower animals such as fish and C. elegans increased from 1995 to 2005, with AMGRs of 8.22% and 10.3%. This finding is supported by the fact that animal numbers have remained constant in the UK and Canada, but fish have replaced mammals in many areas, especially toxicology (*Home Office, 2005; Mukerjee, 1997; Rowsell 1988*). The numbers of cats and dogs are declining; dogs are being replaced by pigs, calves, and other farm animals in the USA (*Mukerjee, 1997*).

This study has several limitations. The first relates to the difficulty in defining what constitutes publications related to laboratory animals. Secondly, searches using other databases such as EMBASE were not performed, as we focused on Medline, the most comprehensive and widely used database. Thirdly, there may potentially be more efficient and complex search strategies that were not identified in this study. Despite these limitations, our results allow several comments to be made. Firstly, to our knowledge, this is the first report of a comprehensive search identifying all publications related to laboratory animals published from 1966 to 2005. Secondly, to improve reliability, a sampling search was performed independently by two researchers using a statistical method. Finally, the team comprised investigators in laboratory animal science with a specific interest in PubMed searching.

In conclusion, we should emphasize that the data were obtained by researchers in laboratory animal science who are not experts in scientometrics. Nevertheless, the numbers of publications related to laboratory animals derived from our analysis may reflect the current and future direction of use of laboratory animals in biomedical research.

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