Original Investigation

Animal Research in Otorhinolaryngology: Shifts in Publication and Practice Over Two Time Periods

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Abstract

Objective: The aim of this bibliometric analysis is to assess the evolving trends in animal research in otorhinolaryngology over two different time periods.

Methods: Articles published in the Science Citation Index Expanded general otorhinolaryngology journals in 2005-2007 and 2018-2020 were retrieved. The relationship of these studies to the Replacement, Reduction, and Refinement (3Rs) principles, emphasizing evidence of Reduction, and Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines were examined, and the predominant subspecialties and the contributions of journals and countries were evaluated. Keyword, research focus, and citation analyses were performed using VOSviewer.

Results: Despite a 51.8% increase in the total number of publications between the two study periods, the number of animal studies decreased by 41.5%, along with a 49.1% reduction in the number of animals used. *In vivo* studies lacking reported animal numbers constituted 10.9% in the first period and 10.3% in the second. "Cochlea" and "ototoxicity" were the most frequent keywords, with seven of the top ten appearing in both periods. Although 58.6% of the animal studies received ten or fewer citations, The Laryngoscope published the most animal studies and was the most-cited journal.

Conclusion: This study underscores the importance of adopting the 3Rs and enhancing adherence to guidelines such as ARRIVE. Evaluating the outcomes of animal studies will be essential for responsible and impactful research in otorhinolaryngology. By revealing current research focuses, leading journals, and countries, this study also presents clues for future animal research in the field.

Keywords: ARRIVE, animal research, otorhinolaryngology, quality of reporting, 3Rs

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Introduction

Animal use in scientific research has long been debated, raising concerns despite the contributions it has made to science (1). In response, the Replacement, Reduction, and Refinement (3Rs) were established to support responsible animal research (2). Likewise, the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines were introduced and updated to promote standardization in reporting animal studies (3,4). With the growing use of animals in research, the volume of publications related to these studies has also increased (5-7).

The aim of this bibliometric analysis is to assess the evolving trends in animal research in otorhinolaryngology by revealing subspecialties, keywords, research focuses, citations, and contributions over two different time periods.

Methods

Two different three-year periods (2005-2007 and 2018-2020), separated by a 10-year interval, were selected to evaluate the 3Rs principles, with an emphasis on evidence of Reduction; to compare the periods before and after the publication of the ARRIVE guidelines; and to provide an up-to-date assessment of the literature and its trends.

Articles published in otorhinolaryngology journals listed in the Science Citation Index Expanded during these years were retrieved. On the website "https://jcr.clarivate.com/jcr/browse-journals" the category "Otorhinolaryngology" was chosen in the "Category" area, and "Science Citation Index Expanded" was selected in the "Citation Index" part for the specified years. Only English-language journals with accessible archives for both periods were included, while subspecialty-specific journals were excluded. The lists of included and excluded journals for each study period are presented in Supplementary Tables S1-S4.

For the total number of publications, the journal title was entered into the search field of the Web of Science (WoS) Core Collection database, and "Article" was selected under the "Document Types" category in the "Refine Results" section. Review articles, editorials, and letters were excluded by not selecting these document types.

For the animal study publications, "Custom Range" was selected in the "Publication Date" column in the PubMed database. For each journal and year, the start and end dates for the specified years were entered separately. Then "Other Animals" was selected in the "SPECIES" section of the "Additional Filters" field. Further, each journal archive was manually searched and compared to publications obtained from the PubMed database.

The Methods section of each publication was reviewed to determine the study type and the source of the animal subjects. *In vivo* animal studies and *ex vivo/in vitro* studies using animals euthanized specifically for research purposes were included. Studies using animals obtained from slaughterhouses or euthanized for unrelated purposes were excluded. Additionally, three animal studies that were not indexed in the WoS Core Collection database were excluded from the VOSviewer-based analysis.

Categorization decisions were made by evaluating the full text of the article and keywords. Each animal study was assigned to one subspecialty—otology, rhinology, or head and neck/laryngology—based on the anatomical site studied and the research focus. Research topics were determined through keyword analysis and article review, with attention to anatomy, pathophysiology, and the interventions studied.

Data was extracted from the WoS Core Collection and PubMed databases on March 1, 2023, and journal archives were manually verified. The relationship of these studies to the 3Rs principles, with an emphasis on evidence of Reduction, and to the ARRIVE guidelines was examined. ARRIVE guideline adherence was assessed solely based on whether the number of animals used was reported in the publication. Other guideline items were not analyzed.

Ethics committee approval was not required because the study involved a retrospective bibliometric analysis and did not include any human participation or personal data.

Statistical Analysis

Microsoft Excel (Microsoft 365, Microsoft Corp., Redmond, WA, USA) was used for data organization, preliminary statistical aggregation (e.g., annual publication counts, percentage changes), and cross-tabulation of publication trends across journals, years, countries, and subspecialties. Keyword, research focus, and citation analyses were performed using VOSviewer (version 1.6.19, Leiden University Center for Science and Technology Studies, The Netherlands).

Results

A total of 12 journals were studied in 2005, 13 in 2006 and 2007, 18 in 2018 and 2019, and 19 in 2020. The initial period (2005-2007) included 7,193 publications across 13 journals, while the subsequent period (2018-2020) included 10,921 publications across 19 journals, representing a 51.8% increase.

During both periods, The Laryngoscope exhibited the highest article publication numbers, rising by 69.6% from 1,225 to 2,077 articles. Certain journals, such as the American Journal of Otolaryngology (112.9%) and the European Archives of Oto-Rhino-Laryngology (118.6%), experienced notable growth, while JAMA Otolaryngology-Head & Neck Surgery declined by 42.9% (Table 1).

A total of 518 animal study publications were identified during the first period (174, 168, and 176 annually, respectively) and 303 during the second period (122, 116, and 65 annually, respectively). These numbers corresponded to 63.1% of animal studies in the first period and 36.9% in the second, indicating a 41.5% decrease. The Laryngoscope published the most animal studies, accounting for 27.2% in the first period and 32.3% in the subsequent period. The four journals with the highest number of animal studies accounted for 71% of these publications in the first period and 61% in the second. In the first period, Clinical Otolaryngology and B-ENT, and in the second period, European Annals of Otorhinolaryngology-Head and Neck Diseases, published no animal studies (Table 1).

Table 1. Total publications, animal study publications, and animal study citations by journals

Journal	First period		Second period		Animal study citations
	Publications	Animal study publications	Publications	Animal study publications	
American Journal of Otolaryngology	286	9	609	5	126
Clinical Otolaryngology	190	0	386	1	5
JAMA Otolaryngology-Head & Neck Surgery	517	37	295	1	723
Otolaryngology-Head and Neck Surgery	1,175	73	873	19	1,140
The Annals of Otology, Rhinology & Laryngology	465	63	517	12	1,491
Auris Nasus Larynx	243	15	471	25	272
B-ENT	115	0	162	2	0
European Archives of Oto-Rhino-Laryngology	630	28	1,377	28	520
International Journal of Pediatric Otorhinolaryngology	757	27	1,399	35	675
The Journal of Laryngology & Otology	676	9	512	4	94
The Laryngoscope	1,225	141	2,077	98	5,019
ORL-Journal for Oto-Rhino-Laryngology, Head and Neck Surgery	174	21	110	5	299
Acta Oto-Laryngologica	740	95	597	22	1,270
Ear, Nose & Throat Journal			586	8	15
Acta Otorhinolaryngologica Italica			174	2	4
Brazilian Journal of Otorhinolaryngology			247	15	94
European Annals of Otorhinolaryngology-Head and Neck Diseases			229	0	0
Clinical and Experimental Otorhinolaryngology			131	18	109
Laryngoscope Investigative Otolaryngology			169	3	4
Total	7,193	518	10,921	303	

Animal study citations represent the total number of citations received by animal studies in both periods

Overall, 34 countries contributed to animal study publications in the first period and 23 in the second. Countries contributing more than 5 animal study publications are shown in Table 2. The top four contributors in both periods were the United States, Japan, Türkiye, and South Korea. The United States led with 320 publications (39.1% of the total 818 animal study publications), followed by Japan (127 publications) and Türkiye (113 publications).

In the first period, the United States (220 publications), Japan (84), Türkiye (48), and South Korea (42) were the four leading contributing countries. In the second period, the United States, Japan, Türkiye, and South Korea contributed 100, 43, 65, and 43 publications, respectively. Contributions from the United States and Japan decreased by 54% and 48%, respectively, whereas publications from Türkiye increased by 35% between the two periods.

The animal species most commonly utilized in the study periods were rats, mice, rabbits, guinea pigs, and dogs. The number of *in vivo* animal studies was 449 in the first period and 290 in the second. In the first period, 49 studies (10.9%) did not specify the exact number of animals utilized, while in the second period, this was 30 studies (10.3%). In total,

13,173 animals were used in the first period, whereas 6,695 were used in the second period, accounting for a 49.1% decrease (Table 3).

Among the subspecialties, otology accounted for the highest number of animal studies (358, 43.6%), followed by head and neck/laryngology (355, 43.2%) and rhinology (96, 11.7%). Frequently investigated topics included physiology, treatment methods, ototoxicity, and pathophysiology in otology; oncology, treatment methods, reconstruction, and wound healing in head and neck/laryngology; and allergy, sinusitis, nasal/paranasal pathophysiology, and treatment methods in rhinology (Table 4).

The keywords "cochlea" and "ototoxicity" were the most frequently used keywords in animal study publications, each appearing 38 times. The keyword "larynx" followed with 34 occurrences, "wound healing" with 27, and "hearing loss" with 26. Other commonly used keywords included "vocal fold" with 25 occurrences, "tissue engineering" with 24, "inner ear" with 19, "recurrent laryngeal nerve" with 18, and "otitis media" with 15. At least half of the top ten keywords were directly related to otology, and seven of these were present in both periods.

Table 2. Animal study publications and citations by country (both periods combined)

Country	Number of animal study publications	Percentage	Citations
USA	320	39.1%	6,490
Japan	127	15.5%	1,572
Türkiye	113	13.8%	1,131
South Korea	85	10.4%	883
China	65	7.9%	465
Sweden	32	3.9%	556
Germany	28	3.4%	531
Australia	16	2.0%	178
Brazil	16	2.0%	164
Canada	14	1.7%	132
England	12	1.5%	172
Spain	12	1.5%	114
Egypt	7	0.9%	67
France	7	0.9%	100
Denmark	6	0.7%	79
Taiwan	6	0.7%	47

Table 3. Number of animals used by species and study period

Animal species	First period	Second period
Rat	5,719	3,122
Mouse	2,633	1,594
Guinea pig	1,878	419
Rabbit	1,738	958
Gerbil	289	0
Dog	279	146
Chinchilla	215	159
Pig	158	71
Hamster	96	0
Monkey	50	0
Cat	46	35
Ferret	20	34
Bullfrog	18	0
Asian house shrew	15	0
Pigeon	10	0
Sheep	7	22
Cow	1	0
Camel	1	0
Zebrafish	0	125
Lamb	0	10
Total	13,173	6,695

Table 4. Research topics of animal studies by subspecialty

Topic in otology	Number of studies
Physiology	68
Treatment technique/method	66
Ototoxicity	57
Pathophysiology	48
Otitis media	30
Noise-induced hearing loss	22
Facial nerve	19
Wound healing	17
Otitis media with effusion	11
Endolymphatic hydrops/Meniere's disease	8
Cochlear implantation	6
Cholesteatoma	6

Topic in head and neck/laryngology	Number of studies
Oncology	63
Treatment technique/method	46
Reconstruction	45
Wound healing	40
Physiology	33
Laryngotracheal stenosis	32
Pathophysiology	28
Recurrent laryngeal nerve and laryngeal innervation	24
Injection laryngoplasty	14
Reflux	10
Thyroid and parathyroid	8
Swallowing and swallowing disorders	8
Laryngeal transplantation	4

Topic in rhinology	Number of studies
Allergy	24
Sinusitis	14
Nasal and paranasal sinus pathophysiology	12
Treatment technique/method	11
Smell	9
Septorhinoplasty	8
Wound healing	6
Immunity	5
Snoring and obstructive sleep apnea syndrome	4
Nasal polyposis	2
Anatomy	1

Number of studies indicates the number of animal studies addressing each topic across both periods

The co-occurrence overlay visualization (Figure 1) encompasses both study periods; nevertheless, unique keyword networks specifically evolved during the second period. In otology, keywords such as "DPOAE," "cisplatin," and "hydrogel" appeared alongside "ototoxicity" and "electromyography" was associated with the "facial nerve." In head and neck/laryngology, "platelet-rich plasma," "vocal fold scar," and "subglottic stenosis" clustered around "wound healing," while "thyroidectomy," "electromyography," and "vocal fold paralysis" were linked to the "recurrent laryngeal nerve." In rhinology, "inflammation" was linked to "allergic rhinitis" (Figure 1). Collectively, these keyword networks highlight the principal research focus studied in the second period.

A total of 818 animal study publications received 11,860 citations (range 0-478, mean 14.4). Among these, 480 publications (58.6%) received 10 or fewer citations, while 36 earned no citations at all (Table 5).

The Laryngoscope was the most cited journal, with 5,019 citations (Table 1). The United States had the highest number of citations (6,490), followed by Japan (1,572) and Türkiye (1,131) (Table 2).

Discussion

Overall, this bibliometric analysis shows that, although total otorhinolaryngology publications increased by 51.8% between the two periods, animal studies, the number of animals used, and the number of contributing countries all decreased. This shift shows a changing practice in animal research within the field. The increase in total publications is consistent with the literature showing the rise in publications within otorhinolaryngology (8). In addition to the emergence of new journals, financial support, collaboration, and academic promotion requirements may be the reasons for this increase (9).

A review of the journals revealed that The Laryngoscope was the leading journal in both periods, with the majority of animal study publications appearing in only four journals. Moreover, some journals published few or no animal studies. The choice of an appropriate journal influences a study's visibility, impact, and publication timing (10). In this regard, our study may help researchers in choosing an appropriate journal for their study.

It has been reported that the contribution to animal research can reflect the economic and scientific development of countries (11). In terms of country contributions, the United States was the leader in animal study publications, consistent

Table 5. Number of animal study publications by citation count

Citation/s	Publications
0	36
1	54
2	39
3	54
4	42
5	64
6	51
7	43
8	29
9	39
10	29

Only animal study publications with 0-10 citations are presented; data represent both study periods
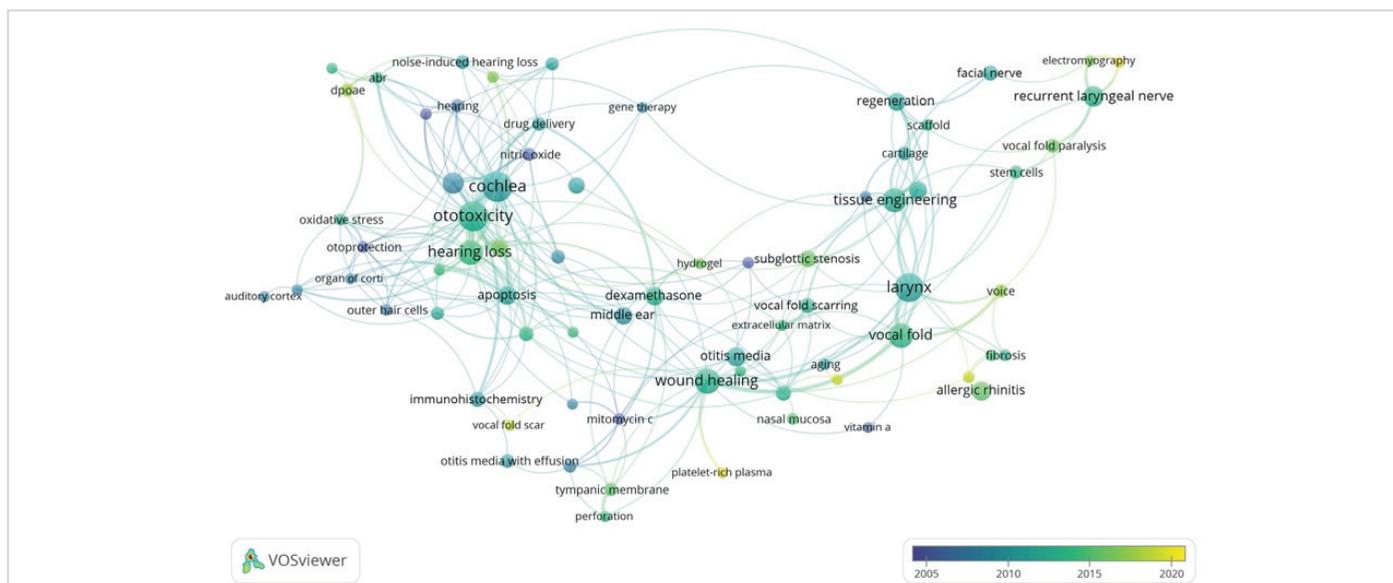

Figure 1. Keyword co-ocurrence overlay visualisation map

with prior reports of its prominent role in animal research (12). However, while contributions from the United States and Japan, the top two contributors, decreased between the two periods, Türkiye, the third leading country, showed an increase in animal study publications. Identifying countries with significant engagement in animal research may facilitate collaborations, enhance experience, and ultimately improve research outcomes.

Animal studies are widely conducted worldwide, while underlying motives may differ. Mayir et al. (13) reported that in Türkiye's general surgery clinics, such research was frequently undertaken for thesis preparation or academic advancement. The authors emphasized that animal studies should be conducted for scientific purposes and that goals must be clearly defined. Considering Türkiye's significant contributions to animal studies in otorhinolaryngology, it is seen as an important issue whether similar motivations lead to this result.

The low publication rates of completed animal studies represent a significant issue. ter Riet et al. (14) reported that approximately 50% of animal studies submitted to journals could be published. While another study found that only 23% of animal studies conducted for thesis preparation were published (15). These findings indicate that the number of animal studies conducted far exceeds the number published in literature. This shows how important a meticulous approach is in the design and execution of animal studies.

Analysis of the number and species of the animals used in otorhinolaryngology revealed a decrease. Species selection is critical in study design (16). Researchers should review the literature to select species based on study focus. Choosing the appropriate species may improve clinical translatability and support more effective research (17).

Studies in medicine or other fields reported variable levels of animal use over time (12,18). Here, we noted that the number of animal studies, along with the total number of animals used, decreased. This result may suggest adoption of 3Rs over time. This shift may also result from ethical considerations, financing limitations, stricter regulations, and editorial policies. The fact that only general otorhinolaryngology journals were included in the analysis, and subspecialty-specific journals were not, may have influenced the findings.

Insufficient reporting of animal numbers is an ongoing issue in scientific papers. Previous studies have identified deficiencies in research reporting, and one of the common deficiencies is to report the number of animals used (19,20). Smith et al. (19) reported that 30% of the studies failed to report animal numbers, while Frommlet and Heinze (21) noted that poor reporting of animal numbers could result in biased outcomes and compromise reproducibility.

Research by Bezdjian et al. (22) and Leung et al. (23) suggest that adherence to ARRIVE guidelines is insufficient in otorhinolaryngology. This bibliometric analysis revealed that 10.9% of the articles in the first period and 10.3% in the second did not report the numbers of animals. This shows that the underreporting of animal numbers in publications still seems to be a significant problem. Strict control by ethical committees and further studies into reporting quality may improve the standard of reporting in animal research.

This study is the first to reveal the topics that animal studies focus on and trends through keyword analysis in otorhinolaryngology. Such information can direct future research strategies and focal points.

Citation analysis is a method frequently used to evaluate the scientific impact of a publication and the status of a research field (24,25). Although open to discussion, it is considered one of the important elements that provide information on this issue (25,26). Kinikoğlu et al. (26) stated that animal studies did not receive a significant number of citations; consequently, their scientific contribution was questionable. Öztürk and Ersan (27) reported that 38% of the 142 animal studies in orthopedics were cited only once or not at all. Hackam and Redelmeier (28) reported that even highly cited animal studies were adapted to randomized clinical trials by about one-third. In this study, 58.6% of animal studies received ten or fewer citations. This is notable given this study examined the leading, high-impact journals in otorhinolaryngology. Considering this, the necessity of using animals and the quality of research should be assessed carefully.

Study Limitations

One limitation of this study is that the assessment of adherence to the ARRIVE guideline was limited to whether or not the numbers of animals used were reported. Further studies evaluating all items of the guideline would be valuable to provide an overview of their adoption. Second is the lack of comparisons with citation counts from clinical studies conducted concurrently. Further, the number of publications or citations are not a definitive indicator of the quality or impact of research. A significant measure of scientific contribution is the development of patented treatments. While there are studies evaluating patenting outcomes in medicine, there is presently no data available for animal research in otorhinolaryngology (29,30). There is a need for studies in our field that will more definitively demonstrate the impact of animal studies on science.

Conclusion

This bibliometric analysis shows persistent reporting gaps in animal numbers and highlights a deficiency in adherence

to guidelines. This emphasizes the necessity of improving reporting standards. By revealing current research focuses, leading journals, and countries, this study also gives clues for future animal research in the field. The adoption of the 3Rs, enhanced adherence to guidelines such as ARRIVE, and evaluation of the outcomes of animal studies are essential for responsible and impactful otorhinolaryngology research.

Ethics

Supplementary Tables Link: https://d2v96fxpocvxx. cloudfront.net/df15911b-c92e-4fb1-a3c6-b4a159ab0d2e/ content-images/342399f4-9b5e-457b-8c1a-524c8868da0a. pdf

Main Points

- Despite the overall growth in otorhinolaryngology publications (51.8%), the number of animal studies and animals used significantly declined (41.5% and 49.1% respectively), reflecting a trend toward the Reduction principle of the 3Rs.
- A lack of animal number reporting was observed in 10.9% of in vivo studies during the first period and in 10.3% during the second period.
- Seven of the ten most frequently used keywords were the same in both study periods.
- Citation analysis revealed that 58.6% of the animal studies received ten or fewer citations.
- Strengthening the impact of animal research in otorhinolaryngology requires improved study design, responsible animal use, and consistent reporting, as underscored by this study.

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