REVIEW ARTICLE

Factors Limiting the Translatability of Rodent Model–Based Intranasal Vaccine Research to Humans

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Abstract
The intranasal route of vaccination presents an attractive alternative to parenteral routes and offers numerous advantages, such as the induction of both mucosal and systemic immunity, needle-free delivery, and increased patient compliance. Despite demonstrating promising results in preclinical studies, however, few intranasal vaccine candidates progress beyond early clinical trials. This discrepancy likely stems in part from the limited predictive value of rodent models, which are used frequently in intranasal vaccine research. In this review, we explored the factors that limit the translatability of rodent-based intranasal vaccine research to humans, focusing on the differences in anatomy, immunology, and disease pathology between rodents and humans. We also discussed approaches that minimize these differences and examined alternative animal models that would produce more clinically relevant research.

Keywords Lymphoid tissues · Microbiome · Disease history · Comparative medicine

Introduction
In light of the coronavirus disease (COVID-19) pandemic, intranasal vaccines have drawn increased interest, with intranasal vaccine candidates such as DelNS1-2019-nCoV-RBD-OPT1 and COVI-VAC currently in clinical trials. Nasally administered vaccines offer numerous advantages over parenteral ones. While parenteral vaccines prevent systemic infection, they often cannot stop the pathogen from entering the host [1]. In contrast, mucosal vaccines such as intranasal vaccines can prevent pathogen entry [2], likely because they induce greater mucosal immune responses than parenteral vaccines do [3], in addition to systemic immune responses [2]. They promote patient compliance, avoid needlestick injuries, have the potential for self-administration, and may result in increased vaccination in children [4–6]. Finally, compared to other mucosal routes such as oral vaccination, vaccines administered intranasally avoid the enzymatic degradation and low pH of the gastrointestinal tract [7].

However, only one intranasal vaccine, FluMist, has been approved by the Food and Drug Administration (FDA) for use in the USA to date, and many intranasal vaccines do not progress beyond phase 1 or 2 clinical trials despite producing promising results in animal models. For example, a live-attenuated intranasal influenza vaccine was able to elicit neutralizing antibodies as well as show sufficient replication in both mice and ferrets, but these results were not predictive of the vaccine’s immunogenicity or infectivity in humans in clinical trials [8]. Cotton rats also frequently fail to predict the efficacy of vaccines against respiratory syncytial virus (RSV) in humans [9]. For instance, SynGEM, an intranasal RSV vaccine, elicited high titers of neutralizing antibodies in cotton rats and mice [10], but failed to do the same in human subjects and did not progress beyond phase 1 trials [11]. A live-attenuated intranasal vaccine against metapneumovirus was also found to be highly immunogenic in hamsters [12], but was found to be over-attenuated in seronegative children [13]. An H2N3 influenza virus also elicited a robust serum antibody response in mice and ferrets [14], but a
live-attenuated vaccine based on the same virus failed to do so in human subjects [15] [personal communication with J Treanor, January 26, 2021].

The discrepancy between results from intranasal vaccination studies in humans and rodent models can stem from a variety of reasons, one being the insufficient predictive value of experimental animal models [16]. In this review, we explored the limitations of rodent models for evaluating intranasal vaccine candidates, particularly for the respiratory diseases that have been the focus of most intranasal vaccine research and development. We also systemically analyzed and compared the differences in anatomy, immunology, and disease pathology among rodents, humans, and alternative animal model candidates and discussed the implications of these differences on the translatability of preclinical research. To reach an evidence-based conclusion, we conducted a meticulous and critical review of all relevant published studies using PubMed and Google Scholar. We selected peer-reviewed papers with rigorous study designs and considered both older established papers as well as current ones that supported or refuted previous ideas to avoid bias. Recent papers (published within 10 years) were highlighted/discussed in this review, although we included papers from the year 1960 to 2022 in our search criteria.

It is important to note that 80–90% of NIH extramural funding for animal research uses mouse models [17]. Considering that much of that research is not translated to humans, utilizing potentially more representative large animal models for intranasal vaccine research could not only improve the predictive value of preclinical studies but also significantly reduce costs and minimize unnecessary animal use in the long run. Consequently, animal models that may more reliably indicate the outcomes of intranasal vaccination in human subjects as well as measures that could increase the predictive value of rodent models were also explored.

### Selecting a Model

In order to produce the most translatable results, selected animal models should be as similar as possible to humans in terms of relevant anatomy, physiology, and immunology, as well as the clinical presentation, pathogenesis, and progression of the disease being studied. Animal models can also be natural or surrogate ones. As a natural model, the animal will develop disease in response to the pathogen, and the pathogen is usually similar to the one that infects humans (e.g., cattle infected with bovine RSV to model human RSV) but does not infect humans. Surrogate models are infected by the human pathogen, often under experimental conditions. The host is permissive to the pathogen to some extent, although disease presentation can vary.

### Limitations of Rodent Models For Intranasal Vaccination Studies

#### Anatomical and Physiological Differences

Rodents, especially mice, are commonly employed for preclinical vaccine research, as they are inexpensive, easy to handle, and are well-characterized genetically and immunologically. Rodents also have well-characterized, organized nasopharynx-associated lymphoid tissue (NALT) that can be excised for further analysis [18]. Rodent NALT consists of bilateral aggregations of lymphoid tissue at the base of the nasal cavity [19], while humans possess a Waldeyer’s ring (Table 1), which is comprised of the lingual, pharyngeal, palatine, and tubal tonsils [20]. Nasopharyngeal lymphoid tissues play a critical role in immunity following intranasal vaccination. While the mechanism is not fully elucidated, intranasal vaccination likely results in antigen uptake by Microfold or M cells in the epithelium of NALT [21] and tonsils [22]. The antigen is presented to dendritic cells (DCs), which activate submucosal T cells that allow B cells to become IgA-secreting plasma cells [23]. B and T cells in NALT also express specific molecules that guide their migration to specific regional and distal mucosal sites following activation [24– 26], which explains how intranasal vaccination can lead to cellular or humoral responses at the reproductive tract [27–29], and belies the existence of a common mucosal immune system [24]. Intranasal vaccination can induce the production of systemic IgG and IgA antibodies due to the activation of leukocytes within NALT [30]. Following the activation in NALT, lymphocytes can enter systemic circulation [31]. Regional lymph nodes may also be involved in generating a systemic response [32, 33].

NALT in rodents is considered functionally analogous to Waldeyer’s ring in humans, and the two share several characteristics. Both NALT and tonsils have a predominance of B over T cells at a steady state [34, 35]. Both structures also express high levels of antiperipheral node addressin (PNAd) adhesion molecules and fewer mucosal addressin cell adhesion molecule-1 (MAdCAM) in high endothelial venules (HEVs), which impact leukocyte trafficking and distinguish nasal lymphoid tissue from other mucosal lymphoid tissues, such as Peyer’s patches in the gastrointestinal tract. For instance, leukocytes that can enter Peyer’s patches may not be able to enter NALT [36, 37].

However, few rigorous functional comparisons between NALT and Waldeyer’s ring have been conducted, and the two have significant structural differences. First, tonsils contain crypts [38] that increase antigen retention times and exposure of underlying lymphoid tissue to antigen [38, 39]. They also play a role in increasing B cell diversity and
memory in humans following antigen stimulation [40]. NALT, on the other hand, lacks crypts [41], which may also explain why rodents can develop a lesser memory response following intranasal vaccination [40]. Secondly, rodent NALT is covered by ciliated simple columnar epithelium interspersed with M cells, goblet cells, and intra-epithelial

Table 1  Diagrams and Median Sections Indicating Anatomical Locations of NALT and/or Waldeyer’s Ring in Humans and Various Animal Models

| Components of NALT and/or Waldeyer’s Rings |
|-------------------------------------------|
| Humans:                                   |
| Adults: Waldeyer ring consisting of one pharyngeal (adenoid) tonsil, two palatine tonsils, two tubal tonsils, and one lingual tonsil. |
| Infants: Waldeyer’s ring in addition to more diffuse lymphoid follicles throughout the nasal cavity (in order of most to least in terms of percentage of total NALT: upper nasal cavity, middle concha, palate, inferior nasal concha, superior nasal concha) (133). |

Diagrams and Median Sections

![Diagrams and Median Sections of NALT and Waldeyer's Ring](image)
Table 1 (continued)

Cynomolgus monkey:

Waldeyer’s ring similar to that of humans and isolated NALT through the nasal cavity.

(Upper) Nasal cavity of monkeys (Lower) Harris hematoxylin staining of posterior region and inferior nasal meatus of the monkey nasal cavity (white arrowheads, NALT) (201). With copyright permission from Elsevier.

Sheep have a Waldeyer’s ring consisting of a lingual tonsil, palatine tonsil, paraepiglottic tonsil, tonsil of the soft palate, pharyngeal tonsil, and tubal tonsil. Note that the tonsil of the soft palate is on the posterior side of the palate, while the palatine tonsil is on the anterior side (typical for sheep and other ungulates (167). Copyright 2011 Christophe Casteleyen et al. This is an open access article distributed under the Creative Commons Attribution License.
Table 1 (continued)

Pigs have a Waldeyer’s ring consisting of a tonsil of the soft palate, pharyngeal tonsil, tubal tonsil, lingual tonsil, and para-epiglottic tonsil (167). Copyright 2011 Christophe Casteleyen et al. This is an open access article distributed under the Creative Commons Attribution License.

Cattle have a Waldeyer’s ring consisting of a pharyngeal tonsil, tubal tonsil, palatine tonsil, lingual tonsil, and a tonsil of the soft palate. Arrow in image indicates part of the pharyngeal tonsil that has been cut away to show the tubal tonsil. There is no para-epiglottic tonsil (167). Copyright 2011 Christophe Casteleyen et al. This is an open access article distributed under the Creative Commons Attribution License.
lymphocytes [34, 42]. While the tubal and pharyngeal tonsils are covered by ciliated columnar epithelium, the lingual and palatine tonsils are covered by non-keratinized or para-keratinized stratified squamous epithelium [38]. Crypt epithelium is also distinct from both the epithelium over NALT and the epithelium lining the tonsillar surface, as it becomes highly reticulated and desquamated and lacks a basal lamina [43, 44]. These reticulations permit leukocytic infiltration of the crypts, which are filled with lymphocytes, macrophages, and DCs [39]. Tonsils are also rich in germinal centers, while NALT is not [45, 46].

Murine NALT consists of paired aggregations of lymphoid tissue at the nasopharyngeal duct. Shown in a diagram of a mouse head. “2” indicates the location of NALT, which is inferior to the nasal turbinates (“nt”) but superior to the palate (“p”). Antigens from NALT are drained along the green lines, which go to “3,” lymph nodes of the neck (202). Copyright 2018 Melanie Lohrberg et al. This is an open access article distributed under the terms of the Creative Commons CC BY License.
NALT. Tonsillar crypts are also rich in commensal bacteria, which may play a role in postnatal germinal center formation [40]. However, the absence of such crypts in mice means that the microbiome within the nasal cavity is organized differently in rodents versus humans, which could affect how microbiota interact with nasal lymphoid tissue and the subsequent response to vaccination.

Furthermore, intranasal vaccination induces mucosal immunity, which results in the production of IgA. Humans produce two subclasses of IgA, both of which can form the dimeric secretory IgA that is important for mucosal immunity [51]. IgA1 is far more abundant in serum than IgA2. IgA1 and IgA2 are found within mucosal secretions in an approximate 3:2 ratio [52], although the nasal mucosa contains mostly IgA1 [53]. Rodents, on the other hand, only produce one type of IgA. This is significant because IgA1 and IgA2 are processed differently, elicit different levels of pro-inflammatory responses, and carry out distinct effector functions [54], but the differential impact of vaccines on the production of each subclass of IgA cannot be understood or translated from rodent studies. Mice in particular also lack a myeloid receptor for serum IgA, or FcαRI, which is present in humans and plays a significant role in immune defense [55]. The vastly different IgA systems of rodents and humans are one of the reasons that the effects of different IgA subclasses have not been fully elucidated and could also limit an accurate or complete understanding of intranasal vaccine responses in humans.

### Natural Disease History

In addition to anatomical and physiological differences, rodent models sometimes fail to accurately reproduce human disease pathology. For instance, rodents may not accurately represent respiratory diseases such as influenza, pertussis, or RSV for which intranasal vaccines are frequently developed (Table 2). Although mice are frequently used for influenza research, there are significant drawbacks to their use. For instance, mice do not present with the same clinical picture as humans, responding with hypothermia instead of fever [56, 57] and failing to sneeze or produce nasal discharge [58]. In addition, influenza in mice results in lethal pneumonia without progressing first through the upper respiratory infection seen in humans [59, 60]. Transmission of influenza viruses between mice is also inefficient compared to transmission between humans [60], and mice to human transmission of influenza virus has not been reported. Consequently, influenza viruses must be adapted if they are to infect mice, but undergoing serial passages can lead to mutations that alter the virulence and growth kinetics of influenza viruses [61–63]. These changes can lead to non-predictive results from viral challenge studies and live-attenuated influenza vaccination studies, which use weakened strains of influenza viruses. In contrast to mice, however, most human influenza viruses do not have to be adapted to cause disease in cotton rats [64, 65]. However, cotton rats also show hypothermia rather than fever and predominantly

### Table 2 Animal Models for Respiratory Diseases

| Animal model | Disease                      | Respiratory syncytial virus (RSV) | Influenza | Bordetella pertussis | Parainfluenza virus 3 |
|--------------|------------------------------|-----------------------------------|-----------|---------------------|----------------------|
| Rat          | Surrogate model for hRSV [80] | Surrogate model [64–66], especially the cotton rat | Surrogate model [98] |                     |                      |
| Mouse        | Surrogate model for hRSV [80] | Surrogate model [59, 60]          | Surrogate model [94] |                     |                      |
| Sheep        | Natural model (oRSV and hRSV) and surrogate model for hRSV [163, 169, 170] | N/A | Surrogate model for B. pertussis, but infected naturally by B. parapertussis [180] | Natural model (bPIV3 and ovine PIV3 viruses) and surrogate model for hPIV3 [173, 174] |
| Cattle       | Natural model (bRSV) [190]    | N/A                               | N/A       |                     |                      |
| Pig          | N/A                          | Natural model (swine influenza) and surrogate model [184–186] | Surrogate model for B. pertussis, but naturally infected by B. bronchiseptica [94, 180, 182] | Natural model (bPIV3) [189] |
| Non-Human Primates (NHP) | Surrogate model for hRSV, especially chimpanzees and African green monkeys [143, 163] | Surrogate model [148–150] | Surrogate model [147] | Surrogate model, especially African green monkeys and chimpanzees [147] |

N/A not available
pulmonary lesions despite viral replication occurring in both the upper and lower respiratory tract [66, 67], and there are relatively few studies on using cotton rats as models for influenza vaccine efficacy. Currently, ferrets are the preferred small-animal model for influenza virus studies, as they are highly susceptible to human influenza viruses [68–70]. Infection occurs predominantly in their upper respiratory tract [71, 72], with age-dependent severity of human-like symptoms that include sneezing, fever, and nasal discharge [73–77]. However, ferret nasal cavities also differ anatomically from human ones. Ferrets have double-scrolled turbinates [78] and no Waldeyer’s ring [18], although they do have a pharyngeal tonsil [79]. Like mice, inoculum doses via the intranasal route in ferrets are also much higher than typical infectious doses in humans, and ferrets are likely to swallow the inoculum. Both factors make intranasal vaccination studies in ferrets more challenging [76].

Both mice and cotton rats are often used to study RSV. Cotton rats, however, do not reliably predict results in humans [9]. While more permissible to RSV infection than mice [80], cotton rats do not manifest clinical signs of the disease and show different activation patterns of inflammatory cells compared to human infants [81, 82]. While mice do develop signs of RSV, the pathogenesis between humans and mice differs, as RSV primarily infects alveolar epithelium in mice but bronchioles in humans [83, 84]. Older mice are also more susceptible to RSV than younger mice [80, 85], while the opposite is true for humans, where the highest risk of bronchiolitis is in children under 6 months old. In addition, human strains of RSV are impossible to adapt to mice [86], so high inoculums are required to infect mice [87]. The virus is also delivered in relatively crude suspensions that introduce considerable cellular debris into murine lungs [86, 88], which, in addition to confounding results, also does not mimic natural infection in humans. Such differences between rodent and human models may also explain why live-attenuated intranasal RSV vaccines found effective in rodents are often found under-attenuated or over-attenuated in humans during clinical trials [89–93].

Likewise, mice can be infected with Bordetella pertussis, but their presentation does not always mimic that of humans [94]. Mice cannot cough and consequently do not develop the characteristic paroxysmal cough associated with pertussis. Adult mice also usually do not show symptoms [95]. Conversely, neonatal mice inoculated with a lethal dose of B. pertussis can show weight loss, spleen atrophy, hypoglycemia, and leukocytosis, which is also seen in humans. In contrast to humans, however, neonatal mice show hypothermia when given a lethal dose and little change in temperature at a sublethal dose, rather than fever [96]. Adult mice also do not transmit pertussis to each other [97]. On the other hand, Sprague-Dawley rats infected with B. pertussis develop a presentation consistent with a disease in humans, including paroxysmal cough, leukocytosis, weight loss, and hypoglycemia [98].

Mice are also natural models for parainfluenza viruses, as they can be infected by mouse parainfluenza virus type 1 Sendai virus and produce symptoms and patterns of inflammation similar to that of humans [99–101]. However, they are poorly infected by human parainfluenza virus 3 (hPIV3) [102]. While cotton rats can be infected by hPIV3 and will show significant pulmonary histopathology, they are generally asymptomatic [103, 104].

**Nasal Microbiome**

Growing research suggests that the nasal microbiome modulates disease and immunological responses. Commensal bacteria in the nasal mucosa carry out important functions, ranging from facilitating immune system development, as mentioned earlier in “Anatomical and Physiological Differences,” to resisting colonization by pathogens [105]. Namely, nasal microbiota generally matures from large in total number, low in microbial diversity, and less stable to low in total number, high in diversity, and more stable during the first few years of life [105, 106]. As the maturation of the nasal microbiome occurs in the first few years of life when children are most susceptible to respiratory infection, this change reinforces the notion that nasal microbiome composition impacts disease. Indeed, lower nasal microbial diversity was linked to greater Staphylococcus aureus colonization in infants [107], and this link between microbiome composition and disease susceptibility underscores the importance of developing an animal model that accurately represents the human nasal microbiome. More specifically, nasal microbiota impact disease susceptibility by shaping the immune response. For example, administration of probiotics via both nasal and oral routes prior to intranasal vaccination against Streptococcus pneumoniae induced higher titers of IgA and IgG than the vaccines alone did [108]. Prior intranasal exposure to Lactobacillus rhamnosus GG also reduced symptoms in mice following influenza challenge and led to greater cytokine mRNA expression [109]. Likewise, co-administration of Lactobacillus and a live-attenuated influenza vaccine (LAIV) resulted in higher rates of seroconversion against certain strains of influenza viruses in healthy adult subjects [110]. Certain nasal microbiome compositions can also predispose to infections such as influenza, while others can be protective [111]. At the same time, vaccination may also exert some of its protective effects through modification of the nasal microbiome [69]. For example, lack of influenza vaccination was linked to higher overall nasal microbial diversity, with greater representation of pathogenic species.
associated with hospital-acquired infections. Vaccination may help limit the growth of opportunistic pathogens [152].

Because specific species of commensal bacteria can modulate the immune response to intranasal vaccines, the average microbiome composition of any animal model would ideally resemble that of humans. However, while microbiome composition can vary within species, there are also significant differences between humans and rodents. While few studies have directly compared the microbiota of rodent and human nares, a study on healthy adults found that human nares were dominated by Actinobacteria and Firmicutes, with lower percentages of Proteobacteria [112]. The predominant genera are Bifidobacterium, Staphylococcus, Streptococcus, Moraxella, Corynebacterium, and Dolosigranulum [113]. In contrast, murine nares were dominated by Proteobacteria such as Pasteurella, Shigella, and E. coli, as well as Firmicutes, including Staphylococcus and Streptococcus species, with fewer Bacteroidetes and Actinobacteria. Mice also have small Moraxella, Corynebacterium, and Dolosigranulum populations [114, 115]. These differences in microbiome composition may lead to differential immune responses between humans and rodents and impact the ability of rodents to model natural infection. For example, Moraxella is common in human nares and has also been found to be associated with respiratory infections including influenza and RSV in humans [111, 116, 117], but it is relatively scarce in the nares of mice. Another study showed that L. helveticus and B. ovatus were associated with increased IgA titers after LAIV administration in humans [118], suggesting that specific species of commensal bacteria can impact vaccine response. However, while the genera Lactobacillus and Bacteroides are present in murine nasal cavities, whether or not the two specific species are present or predict an increased IgA response in mice is unclear. Corynebacterium and Dolosigranulum are also far less abundant in murine nasal cavities than in human ones, but both are negatively associated with respiratory infection in humans [116, 119, 120]. Furthermore, rodents do not always show the same changes in the nasal microbiome in response to infection that humans do. Humans showed reduced Corynebacterium in association with influenza, while administration of neomycin to mice led to both a decreased immune response against influenza virus challenge and an increase in the relative abundance of Corynebacterium spp. within the nasal cavity [121].

The cotton rat nasal microbiome also does not closely resemble the human nasal microbiome. While there have been relatively few studies on the nasal microbiome of the cotton rat, one study found Campylobacter, Acholeplasma, Streptobacillus, and Catonella, with many of the specific species unknown. The authors concluded that the cotton rat’s nasal microbiome, dominated by many new and unknown species, is highly distinct from the human one [122].

**Immunological History**

Laboratory mice have less representative immune systems due to their unique living environment. Raised in pathogen-free environments, they often have a general scarcity of memory CD8+ T-cells, particularly effector-differentiated CD8+ T cells [123]. This is important because NALT also appears to be the site of memory CD8+ T cell expansion following upper respiratory infection in mice [124]. While there is little research comparing the abundance of T-cell subsets in the NALT of inbred laboratory mice to outbred mice, there is a general lack of memory CD8+ T cells in murine NALT at rest, although it increases following infection [124]. In contrast to rodent NALT, a study showed that by the ages of 4–9 years old, human tonsils have substantial memory T cell populations, including effector-differentiated memory CD4+ and CD8+ T cells, even at rest [125]. As a result, the relative deficiency of memory CD8+ T cells in mice compared to humans may also influence translatability of vaccine studies on laboratory mouse NALT to tonsils. It is interesting, however, to note that neonates are more similar to mice and generally lack effector-differentiated memory T cells in both their lymphoid and non-lymphoid tissues, although these memory T cells are found by young adulthood [126].

In contrast to laboratory mice, feral and pet store mice have significantly more memory CD8+ T-cells, including the effector-differentiated phenotype, which were also induced in C57BL/6 mice following exposure to pet store mice, indicating that environmental exposure significantly affects the immune composition. Consequently, mice raised in sterile environments such as laboratories lack the immunological “experience” that makes their immune systems resemble that of adult humans [123]. One possible remedy to this problem is to expose laboratory mice to a more complete microbial profile by placing them in natural environments.

**Practical Limitations**

There are tremendous differences in the lengths, volumes, and surface areas between rodent and adult human nasal cavities [127]. In addition to their impact on the distribution of vaccines in the nasal cavity upon administration, these differences also confer practical limitations. Because rodents are relatively small, intranasal vaccine doses for humans (usually around 150 μL) must be concentrated into significantly smaller volumes for delivery into rodents’ nasal cavity (approximately 3 μL). These small volumes are difficult to administer, so practically, relatively large volumes (around 12–50 μL) are used. However, this results in rodents’ nasal
tissues being bathed in a vaccine, which does not occur in humans [128]. Inoculation of volumes greater than 10 μL can also result in some distribution beyond the nasopharynx [129]. The relatively large volumes administered to rodents means that immune responses originating solely from NALT would be difficult to separate from responses originating from bronchus-, lung-, or gut-associated lymphoid tissue [130]. Delivery protocols and positioning of the rodent can also vary, resulting in inhalation or swallowing of the vaccine. The likelihood of inhalation or swallowing is also greater if the mouse is lightly anesthetized [131], which occurs frequently in intranasal vaccination studies [132].

**Rodent Models, Young Children and Infants, and Adult Humans**

Children under 2 years old possess both a Waldeyer’s ring and disseminated follicles and aggregates, particularly in their upper nasal cavity and middle concha [133]. While these aggregates eventually regress, they appear to resemble rodent NALT at the base of the nasal cavity [134, 135], perhaps more so than the tonsils of Waldeyer’s ring. Furthermore, the nasal cavity sizes of infants and rodents are more comparable than that of adults and rodents [136–138].

Secondly, the nasal microbiomes of children and adults differ in terms of composition and stability. Like adults, the nasal microbiota of young children is dominated by Actinobacteria and Firmicutes, with more limited Proteobacteria [106]. However, a comparative study found that young children have a larger proportion of Proteobacteria such as *Moraxella, Enterobacteriaceae,* and *Haemophilus* than adults. Adults, on the other hand, have a greater proportion of Firmicutes such as *Staphylococcus* and *Streptococcus,* Bacteroidetes, and certain Actinobacteria. As described in “Nasal Microbiome,” young children have distinct and less stable nasal microbiomes compared to adults, which can make the identification of a representative animal model more challenging. However, because children have a much broader and dynamic range of nasal microbiome compositions [105] and generally have more Proteobacteria and less *Corynebacterium* than adults, it is possible that the similarity between the nasal microbiomes of rodents and some children could exceed that of adults and rodents. As noted earlier, laboratory mice also have few memory CD8+ T-cells, particularly effector-differentiated memory CD8+ T cells, a characteristic shared by the lymphoid tissue of neonates.

As a result, the translatability of results from vaccine studies using rodents could depend on age, with the results of some rodent studies being more applicable to children than adults. This hypothesis would be difficult to test, however, because clinical trials for a nasal vaccine in young children or infants generally cannot commence before the safety and efficacy of the vaccine have been established in adult human subjects due to ethical reasons.

**Alternative Animal Models**

**Non-Human Primates**

Considering the significant array of differences between humans and rodents, other mammalian models should be considered when evaluating intranasal vaccine candidates. Originally, we hypothesized that because anatomical and physiological differences between rodents and humans are responsible for the limited predictive value of rodents, non-human primates (NHP) such as rhesus monkeys, cynomolgus monkeys, and marmosets would serve as more accurate animal models due to their greater anatomical and physiological resemblance to humans. A literature search found important similarities between humans and other primates. Namely, non-human primates (NHP) may more closely resemble humans immunologically, anatomically, and physiologically. Like humans, NHP have tonsils arranged in a Waldeyer’s ring (Table 1) [46], although they also have some discrete lymphoid aggregates along the nasopharynx [139] that could be analogous to the non-tonsillar nasal lymphoid tissues of infants. In addition, NHP produce an IgA response to intranasal vaccines and are susceptible to most human pathogens [140–142]. Like humans, NHP can produce two subclasses of IgA [54], which would allow vaccination studies to explore the immune response to vaccine candidates in a manner impossible in rodents. NHP can also accurately reproduce symptoms of many respiratory diseases in humans. For example, chimpanzees are completely permissive to human RSV [143], with seronegative chimpanzees considered the most applicable model for older seronegative infants for RSV vaccines [144]. African green monkeys are also often used to model RSV [144]. However, African green monkeys show few clinical symptoms or histopathological changes following RSV infection [80]. In addition, NHP, like humans but unlike mice, develop a paroxysmal cough and produce increased mucus when infected with *B. pertussis*; they also develop leukocytosis [145] and transmit the disease to each other [146]. Baboons in particular are highly permissive to *B. pertussis* and may more accurately reproduce the disease compared to rats and pigs [147]. However, while rhesus, cynomolgus, and pig-tailed monkeys can be infected with the influenza virus [148–150], primates are not natural hosts for influenza. Transmission of influenza between marmosets, for instance, has also not been documented, although marmosets can be inoculated with influenza viruses and present with human-like symptoms such as nasal discharge and sneezing. In addition, influenza viruses may require significantly less modification prior to infection.
of marmosets than rodents [151]. Cynomolgus monkeys also show a wide range of symptoms and can present with significant histological findings following infection, depending on the strain of influenza virus [148, 152].

Like humans, NHP have nasal cavities with the primary function of breathing rather than olfaction [153]. This functional similarity may account for the greater structural likenesses between NHP and human nasal cavities [154]. For example, both humans and Old World monkeys have simple, single-scroll turbinates [155]. As the structure of turbinates affects absorption, deposition, and filtration of air particles [156], using such NHP may produce more accurate results when studying the administration of intranasal vaccines. In addition, the larger nasal cavities of NHP compared to rodents [127] mean that larger doses can be given, reducing the need to concentrate vaccines.

In addition, NHP possess nasal microbiomes that resemble that of humans. Although there are few studies on the NHP nasal microbiome, a study found that Firmicutes and Actinobacteria were the most prevalent phyla in the nares of hamadryas baboons. In addition, the two most abundant amplicon sequence variants in baboon nares belonged to *Corynebacterium* and *Dolosigranulum*. *Streptococcus* and *Lactobacillus* were also common, although *Bifidobacterium* was not [157]. While *Staphylococcus* was not noted at significant numbers in the baboon nares, pig-tailed monkeys displayed a high rate of *S. aureus* colonization in their nares and were considered a suitable physiological model for human *S. aureus* nasal carriage [158]. Taken together, the NHP nasal microbiome shares more similarities with the human nasal microbiome than the rodent nasal microbiome, a conclusion paralleled by results from a comparative study indicating that human gut microbiota more closely resembled that of NHP than of rodents [159]. In terms of immunological history, non-human primates (NHP), such as rhesus monkeys, used for research are genetically outbred and live in colonies [160]. While isolated, these colonies still offer the opportunity for NHP to develop a rich immunological history [161].

**Sheep, Pigs, and Cattle**

Because the use of NHP is limited by cost and ethical considerations, other animal models with tonsils may also be considered. While smaller mammals such as dogs, rabbits, chickens, and cats may also serve as representative models, herein we focus on large animals that are physiologically and immunologically more closely related to humans, such as sheep, cows, and pigs. For example, the nasal cavities of sheep and cattle display similar patterns of development to that of humans [162]. In addition, these ungulates are outbred, which allows for a more accurate representation of both the diversity and nature of immune responses encountered in human populations. Compared to rodents, they have neonatal periods more similar to that of human infants [163] and nasal cavities more comparative to human ones in terms of size and anatomy. In addition, a number of intranasal vaccines have already been approved for large animals, so their immune responses to intranasal vaccination are well understood. They can also model many respiratory infectious diseases (Table 2), and their nasal microbiomes bear some similarity to that of humans. Furthermore, unlike laboratory mice, large animals are sometimes housed outdoors or in natural settings, allowing for the development of greater immunological history. However, these animals lack the two IgA subclasses seen in certain NHP species and humans [54], and there are also some structural differences in their nasal cavities. For example, sheep, pigs, and cows have double-scrolled turbinates [164–166] instead of the single-scrolled turbinates seen in humans.

Sheep have tonsils highly analogous to human ones, although instead of a second pharyngeal or palatine tonsil, sheep have a para-epiglottic tonsil and tonsil of the soft palate (Table 1). In addition, like their human counterpart, the palatine tonsil of sheep contains crypts [167]. On the other hand, sheep have a longer nasal cavity than humans, which may lead to longer antigen retention times and prevent nasal delivery devices for humans from working effectively in sheep [128, 164, 168]. Sheep can also be infected by human RSV as well as ovine and bovine RSV [163, 169, 170] (Table 2). In addition, during pulmonary infection with bovine RSV, sheep exhibit similar age-dependent vulnerability, gross and histological pathology, and expression of innate immune molecules to humans [171, 172]. The clinical progression, histopathology, and inflammatory cytokine profile of the disease of lambs infected with human RSV also mirror that in infants, suggesting that the lamb can serve as a model for RSV vaccines in infants [170]. Sheep can also be infected by ovine, bovine, and human strains of parainfluenza virus 3 [173, 174], making them both a natural and surrogate model for parainfluenza virus 3. Based on our review of literature, we identified Proteobacteria, Firmicutes, and Actinobacteria as common genera in ovine nares [175–177]. A study conducted on healthy domestic and wild bighorn sheep identified *Staphylococcus*, *Streptococcus*, and *Pasteurella* as common genera in the nares. *Corynebacterium* and *Moraxella* were also present in the nasal cavity; however, *Corynebacterium* was found only in the nares of wild sheep, while *Moraxella* was present in both domestic and wild sheep [175]. The presence of *Corynebacterium* in the nares of wild but not domestic sheep also suggests that sheep can be colonized with this genus for relevant influenza vaccination studies if exposed to it. Both ovine and human nares can be colonized with *Corynebacterium*, *Staphylococcus*, *Streptococcus*, and *Moraxella*. In contrast to humans, however, *Dolosigranulum* and *Bifidobacterium* were not significantly present in sheep nares.
Pigs also have five tonsils arranged similarly to human tonsils (Table 1) [37], although they lack a palatine tonsil and most of their lymphoid tissues are concentrated in their tonsil of the soft palate rather than the pharyngeal and palatine tonsils, which is the case in humans [178]. Their tonsils contain deep crypts similar to those in humans [38, 167, 179]. Intranasal vaccination of pigs also results in mucosal secretory IgA and serum IgG production [180, 181]. Intranasal vaccines currently approved for pigs include vaccines for B. bronchiseptica and influenza A. Pigs are also excellent natural models of respiratory diseases such as pertussis [94, 180, 182] and influenza [183, 184] (Table 2). Pigs are naturally infected by swine strains of influenza [185, 186] and are often susceptible to human influenza viruses [184], leading to significantly lower viral adaptation requirements for a pig model than a mouse model. In the case of pertussis, pigs appear to be better models than mice [183], as the clinical symptoms and pathologic lung lesions of infected pigs appear to more closely resemble those of humans. Although both infected mice and piglets fail to develop a paroxysmal cough, piglets present with fever, breathing difficulties, and nasal discharge [94, 180], symptoms that are generally absent in mice but present in humans. One study found that pig nares were dominated by Proteobacteria, Firmicutes, and Bacteroidetes, with relatively low abundances of Actinobacteria and Tenericutes. Common genera included Prevotella, Weisella, Haemophilus, Streptococcus, Lactobacillus, and Moraxella [187]. Unhealthy piglets also showed a greater prevalence of Moraxella in their nasal cavities compared to healthy piglets, suggesting that Moraxella is linked to disease in pigs [187] like it is in humans. Lactobacillus, which has been used as an effective probiotic for influenza vaccination as described in “Nasal Microbiome,” is also associated with better health in pigs [187, 188]. In contrast to humans, however, Corynebacterium was not significantly present in pig nares. On the other hand, both Moraxella and Streptococcus are prevalent in porcine and human nares. While the porcine nasal microbiome is distinct from the human nasal microbiome, certain microbiota in pig and human nares may exert similarly protective or detrimental effects in terms of disease susceptibility, indicating that the pig model may have utility in predicting human responses to vaccination.

Like pigs, cattle have five tonsils (Table 1), although they have a palatine tonsil and no para-epiglottic tonsil. Their tonsils also contain cysts [167]. Cows are also natural models of parainfluenza and RSV, as they are susceptible to both bovine parainfluenza 3 and bovine RSV [189, 190]. Bovine RSV in calves also replicate many of the features of human RSV in infants, including fever, nasal discharge, coughing, and rapid breathing [190]. However, cattle are not susceptible to human RSV [163]. Cattle can also produce mucosal IgA and serum IgG in response to intranasal vaccination [191–193]. Several intranasal vaccines for cattle have already been approved, including vaccines for bovine herpesvirus type 1, parainfluenza 3, and bovine respiratory syncytial virus. The most common phyla overall in the nares of calves prior to weaning and consequently not exposed to other calves included Proteobacteria, Bacteroidetes, and Firmicutes, while the most common genera were Moraxella, Mannheimia, and Promicromonaspora. Streptococcus, Bacteroides, and Clostridium were present as well [194]. Increased Moraxella and Streptococcus were also noted in unhealthy cattle compared to healthy cattle. Corynebacterium was found in the nasal cavities of both healthy and unhealthy cattle, although there was no significant difference in abundance of Corynebacterium between the two groups [195]. In general, unlike in humans Bifidobacterium and Dolosigranulum were not generally reported to be present in cattle nares, but like in humans Moraxella and Corynebacterium were present in cattle nares.

In summary, NHPs appear to be the most predictive model for intranasal vaccination studies due to the similarity of their anatomy, immunology, and nasal microbiome to that of humans. However, sheep, cattle, and pigs are also promising and potentially more cost-effective models for intranasal vaccination studies. Regardless of the particular animal model chosen, factors such as the animal’s susceptibility to the disease being studied (Table 2), tonsillar arrangement, nasal anatomy, immunological history, and nasal microbiome should be considered during the selection process.

Improving The Predictive Value of Rodent Models and Future Directions

Besides using NHPs and other large animal models such as sheep, pigs, and cattle, measures can also be taken to increase the similarity of rodent models to humans. Researchers can modify laboratory rodents’ immunological history through exposure to non-laboratory rodents [123], employ outbred strains of mice, design humanized mice with immune systems more closely resembling that of humans [196], and inoculate mice and rats with commensal bacteria more common to the human nares. Mice, for instance, can be experimentally colonized with Corynebacterium and Moraxella [197, 198]. However, different bacterial populations can have distinct influences on mice versus humans, so additional comparative studies on the microbiota of human and murine nares are needed. Strategies such as delivery devices specifically designed to target rodent NALT and precise positioning devices for intranasal vaccination may also be developed. Efforts to understand the distribution of vaccines or challenge pathogens delivered to murine nasal cavities, such as through the use of bioluminescent bacteria [199] or fluorescently labeled antigens, should also be undertaken.
Our review did encounter limitations. We focused primarily on biological differences between animal models and human subjects, but other factors, such as differences in experimental design and quality of research, may also contribute to the limited translatability of animal studies to clinical trials [200]. In addition, many clinical trials lack published preclinical studies on animal models or formally published results, which made identifying the factors underlying the disparity in results from particular preclinical and clinical studies more challenging. Lastly, there were relatively few direct comparative studies on potential animal models and human counterparts in terms of their anatomy, physiology, and disease pathology, and these studies would have been valuable in clarifying the specific similarities and differences between the two.

Conclusions

Our literature review identified multiple factors that limit the translational value of intranasal vaccine research based on murine models, with physiological and anatomical differences being the most prominent. Some factors, such as microbiome composition and immunological history, can be modified in order to increase the predictive value of murine models. Animal models that minimize more non-modifiable differences, such as differences in nasal anatomy, immunology, and disease pathology, should also be considered in future vaccine research. Based on our review of these modifiable and non-modifiable factors, we expect the non-human primate to be the most predictive model for the evaluation of intranasal vaccine candidates. However, given the significant costs and challenges associated with using non-human primates, sheep, cattle, and pigs can also serve as excellent animal models.

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Declarations

Conflict of Interest The authors declare no competing interests.

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