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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

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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

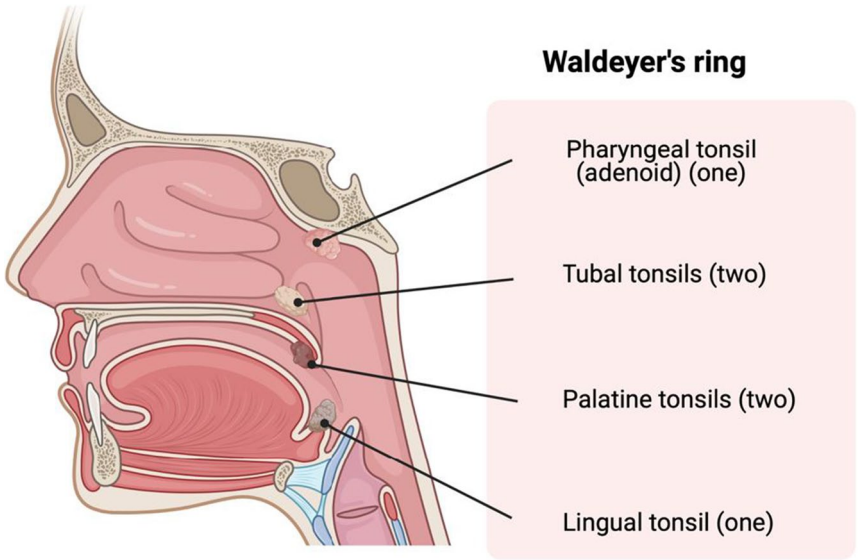
Anatomical and Physiological Differences

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

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

Diagrams and Median Sections	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).

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 (continued)

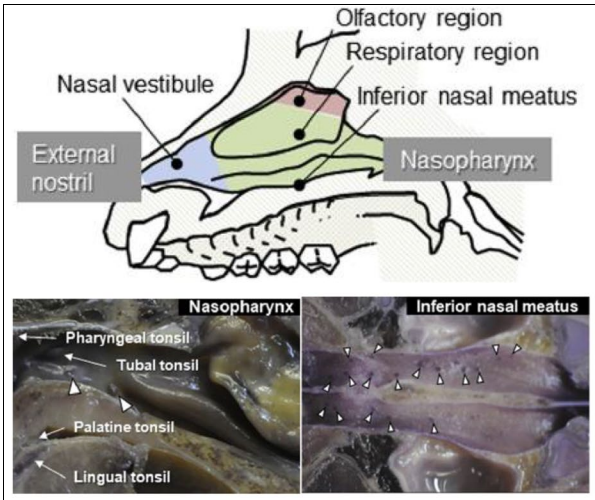
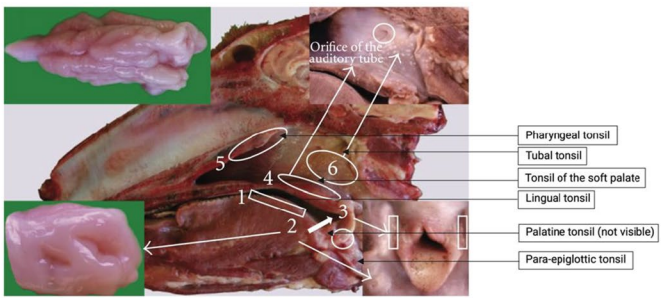
	Cynomolgus monkey: Waldeyer's ring similar to that of humans and isolated NALT through the nasal cavity. (Upper) Nasal cavity of monkeys (Lower) Harris hematoxylin staining of posterior region and inferior nasal meatus of the monkey nasal cavity (white arrowheads, NALT) (201). With copyright permission from Elsevier.
	Sheep have a Waldeyer's ring consisting of a lingual tonsil, palatine tonsil, para-epiglottic 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)

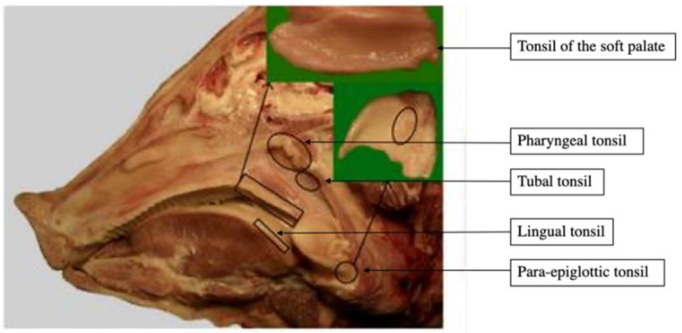
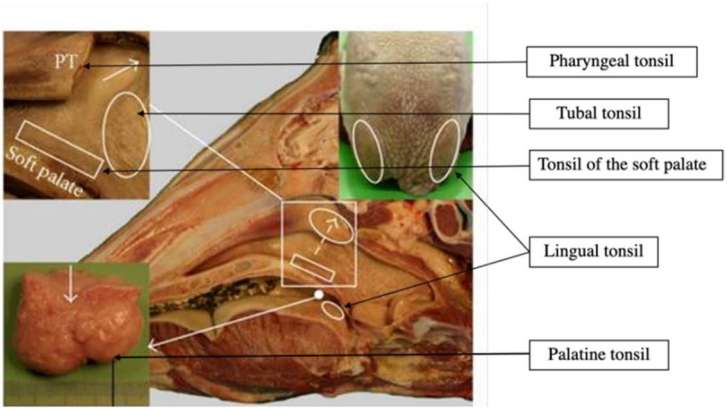
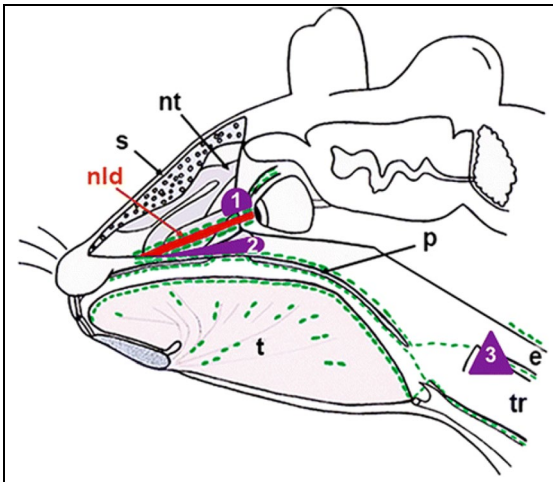
	Pigs have a Waldeyer's ring consisting of a tonsil of the soft palate, pharyngeal tonsil, tubal tonsil, lingual tonsil, and para-epiglottic tonsil (167). Copyright 2011 Christophe Casteleyen et al. This is an open access article distributed under the Creative Commons Attribution License.
	Cattle have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

Table 1 (continued)

	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.

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 also develops in a manner distinct from most secondary lymphoid organs. Its development begins after birth [47]. On the other hand, both the primary follicles [48] and crypts [49] of human tonsils arise starting 16 weeks into gestation, with germinal centers developing after birth [50], although their development does not require the infection or danger signal needed by rodent

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]. IgA_1 is far more abundant in serum than IgA_2. IgA_1 and IgA_2 are found within mucosal secretions in an approximate 3:2 ratio [52], although the nasal mucosa contains mostly IgA_1 [53]. Rodents, on the other hand, only produce one type of IgA. This is significant because IgA_1 and IgA_2 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]	Natural model (murine PIV3, or Sendai virus) and surrogate model for hPIV3 [103, 104]
Mouse	Surrogate model for hRSV [80]	Surrogate model [59, 60]	Surrogate model [94]	Natural model (murine PIV3, or Sendai virus) [99, 100]
Sheep	Natural model (oRSV and bRSV) 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	Natural model (bPIV3) [189]
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]	N/A
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 phyla 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 Bacteriodetes, with relatively low abundances of Actinobacteria and Tenericutes. Common genera included *Prevotella*, *Weeksella*, *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 crypts [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 *Promicromonospora. 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.

References

1. Otczyk DC, Cripps AW. Mucosal immunization: a realistic alternative. Human Vacc. 2010;6(12):978–1006.
2. Ramvikas M, Arumugam M, Chakrabarti S, Jaganathan K. Nasal vaccine delivery. Micro Nanotech Vacc Dev: Elsevier. 2017:279–301.
3. Wang T, Wei F, Liu J. Emerging role of mucosal vaccine in preventing infection with avian influenza a VIRUSES. Viruses. 2020;12(8):862.
4. Lemoine C, Thakur A, Krajišnik D, Guyon R, Longet S, Razim A, et al. Technological approaches for improving vaccination compliance and coverage. Vaccines. 2020;8(2):304.
5. Burgess TH, Murray CK, Bavaro MF, Landrum ML, O'Bryan TA, Rosas JG, et al. Self-administration of intranasal influenza vaccine: immunogenicity and volunteer acceptance. Vaccine. 2015;33(32):3894–9.
6. Fogel B, Hicks S. Influenza vaccination rates in children decline when the live attenuated influenza vaccine is not recommended. Vaccine. 2017;35(39):5278–82.
7. Jabbal-Gill I. Nasal vaccine innovation. J Drug Target. 2010;18(10):771–86.
8. Karron RA, Talaat K, Luke C, Callahan K, Thumar B, DiLorenzo S, et al. Evaluation of two live attenuated cold-adapted H5N1 influenza virus vaccines in healthy adults. Vaccine. 2009;27(36):4953–60.
9. Besteman SB, Bont LJ. Fail-fast in respiratory syncytial virus vaccine development. AmThoracic Soc. 2019.
10. Rigter A, Widjaja I, Versantvoort H, Coenjaerts FE, Van Roosmalen M, Leenhouts K, et al. A protective and safe intranasal RSV vaccine based on a recombinant prefusion-like form of the F protein bound to bacterium-like particles. PLoS One. 2013;8(8):e71072.
11. Ascough S, Vlachantoni I, Kalyan M, Haijema B-J, Wallin-Weber S, Dijkstra-Tiekstra M, et al. Local and systemic immunity against RSV induced by a novel intranasal vaccine: a randomised, double-blind, placebo-controlled trial. Am J Respir Crit Care Med. 2019.
12. Pham QN, Biacchesi S, Skiadopoulos MH, Murphy BR, Collins PL, Buchholz UJ. Chimeric recombinant human metapneumoviruses with the nucleoprotein or phosphoprotein open reading frame replaced by that of avian metapneumovirus exhibit improved growth in vitro and attenuation in vivo. J Virol. 2005;79(24):15114–22.
13. Karron RA, San Mateo J, Wanionek K, Collins PL, Buchholz UJ. Evaluation of a live attenuated human metapneumovirus vaccine in adults and children. J Pediatric Infect Dis Soc. 2018;7(1):86–9.
14. Chen GL, Lamirande EW, Yang C-F, Jin H, Kemble G, Subbarao K. Evaluation of replication and cross-reactive antibody responses of H2 subtype influenza viruses in mice and ferrets. J Virol. 2010;84(15):7695–702.
15. Evaluation of the Safety and Immunogenicity of a Live Attenuated Virus Vaccine for the Prevention of H2N3 Influenza.
16. Denayer T, Stöhr T, Van Roy M. Animal models in translational medicine: validation and prediction. New Horizons Trans Med. 2014;2(1):5–11.
17. Knoblaugh SE, Hohl TM, La Perle KM. Pathology principles and practices for analysis of animal models. ILAR J. 2018;59(1):40–50.
18. Casadei E, Salinas I. Comparative models for human nasal infections and immunity. Dev Compar Immunol. 2019;92:212–22.
19. Asanuma H, Thompson AH, Iwasaki T, Sato Y, Inaba Y, Aizawa C, et al. Isolation and characterization of mouse nasal-associated lymphoid tissue. J Immunol Methods. 1997;202(2):123–31.
20. Hellings P, Jorissen M, Ceuppens J. The Waldeyer's ring. Acta Otorhinolaryngol Belg. 2000;54(3):237–41.
21. Mutoh M, Kimura S, Takahashi-Iwanaga H, Hisamoto M, Iwanaga T, Iida J. RANKL regulates differentiation of microfold cells in mouse nasopharynx-associated lymphoid tissue (NALT). Cell Tissue Res. 2016;364(1):175–84.

22. Fujimura Y. Evidence of M cells as portals of entry for antigens in the nasopharyngeal lymphoid tissue of humans. Virchows Arch. 2000;436(6):560–6.
23. Park S-K, Kim G-Y, Lim J-Y, Kwak J-Y, Bae Y-S, Lee J-D, et al. Acidic polysaccharides isolated from Phellinus linteus induce phenotypic and functional maturation of murine dendritic cells. Biochem Biophys Res Commun. 2003;312(2):449–58.
24. Csencsits KL, Walters N, Pascual DW. Cutting edge: dichotomy of homing receptor dependence by mucosal effector B cells: αE versus L-selectin. J Immunol. 2001;167(5):2441–5.
25. Fukuyama Y, Tokuhara D, Kataoka K, Gilbert RS, McGhee JR, Yuki Y, et al. Novel vaccine development strategies for inducing mucosal immunity. Expert Rev Vacc. 2012;11(3):367–79.
26. Meeusen EN. Exploiting mucosal surfaces for the development of mucosal vaccines. Vaccine. 2011;29(47):8506–11.
27. He Q, Martinez-Sobrido L, Eko FO, Palese P, Garcia-Sastre A, Lyn D, et al. Live-attenuated influenza viruses as delivery vectors for chlamydia vaccines. Immunol. 2007;122(1):28–37.
28. Russell MW, Moldoveanu Z, White PL, Sibert GJ, Mestecky J. Salivary, nasal, genital, and systemic antibody responses in monkeys immunized intranasally with a bacterial protein antigen and the cholera toxin B subunit. Infect Immun. 1996;64(4):1272–83.
29. Gherardi MM, Pérez-Jiménez E, Nájera JL, Esteban M. Induction of HIV immunity in the genital tract after intranasal delivery of a MVA vector: enhanced immunogenicity after DNA prime-modified vaccinia virus Ankara boost immunization schedule. J Immunol. 2004;172(10):6209–20.
30. Shim S, Soh SH, Im YB, Ahn C, Park H-T, Park H-E, et al. Induction of systemic immunity through nasal-associated lymphoid tissue (NALT) of mice intranasally immunized with Brucella abortus malate dehydrogenase-loaded chitosan nanoparticles. PLoS One. 2020;15(2):e0228463.
31. Kammona O, Bourganis V, Karamanidou T, Kiparissides C. Recent developments in nanocarrier-aided mucosal vaccination. Nanomedicine. 2017;12(9):1057–74.
32. Giri PK, Sable SB, Verma I, Khuller GK. Comparative evaluation of intranasal and subcutaneous route of immunization for development of mucosal vaccine against experimental tuberculosis. FEMS Immunol Med Microbiol. 2005;45(1):87–93.
33. Jegaskanda S, Mason RD, Andrews SF, Wheatley AK, Zhang R, Reynoso GV, et al. Intranasal live influenza vaccine priming elicits localized B cell responses in mediastinal lymph nodes. J Virol. 2018;92(9).
34. Lee H, Ruane D, Law K, Ho Y, Garg A, Rahman A, et al. Phenotype and function of nasal dendritic cells. Mucosal Immunol. 2015;8(5):1083–98.
35. Koornstra P, De Jong F, Vlek L, Marres E, van Breda VP. The Waldeyer ring equivalent in the rat: a model for analysis of oronasopharyngeal immune responses. Acta Otolaryngol. 1991;111(3):591–9.
36. Csencsits KL, Jutila MA, Pascual DW. Nasal-associated lymphoid tissue: phenotypic and functional evidence for the primary role of peripheral node addressin in naive lymphocyte adhesion to high endothelial venules in a mucosal site. J Immunol. 1999;163(3):1382–9.
37. Bourges D, Wang C, Chevaleyre C, Salmon H. T and IgA B lymphocytes of the pharyngeal and palatine tonsils: differential expression of adhesion molecules and chemokines. Scand J Immunol. 2004;60(4):338–50.
38. Miniggio H. Biological significance of palatine tonsillar epithelium: microstructure and disease. S Afr Dental J. 2016;71(10):496–9.
39. Perry M, Whyte A. Immunology of the tonsils. Immunol Today. 1998;19(9):414–21.
40. Brandtzaeg P. Potential of nasopharynx-associated lymphoid tissue for vaccine responses in the airways. Am J Respir Crit Care Med. 2011;183(12):1595–604.
41. Brandtzaeg P. Secretory immunity with special reference to the oral cavity. J Oral Microbiol. 2013;5(1):20401.
42. Park H-S, Francis KP, Yu J, Cleary PP. Membranous cells in nasal-associated lymphoid tissue: a portal of entry for the respiratory mucosal pathogen group a streptococcus. J Immunol. 2003;171(5):2532–7.
43. Perry ME. The specialised structure of crypt epithelium in the human palatine tonsil and its functional significance. J Anat. 1994;185(Pt 1):111.
44. Roberts S, Evans D, Mehanna H, Parish JL. Modelling human papillomavirus biology in oropharyngeal keratinocytes. Philos Trans R Soc B. 2019;374(1773):20180289.
45. Scadding GK. Immunology of the tonsil: a review. J R Soc Med. 1990;83(2):104–7.
46. Cesta MF. Normal structure, function, and histology of mucosa-associated lymphoid tissue. Toxicol Pathol. 2006;34(5):599–608.
47. Fukuyama S, Hiroi T, Yokota Y, Rennert PD, Yanagita M, Kinoshita N, et al. Initiation of NALT organogenesis is independent of the IL-7R, LTβR, and NIK signaling pathways but requires the Id2 gene and CD3− CD4+ CD45+ cells. Immunity. 2002;17(1):31–40.
48. Brugman S, Perdijk O, van Neerven RJ, Savelkoul HF. Mucosal immune development in early life: setting the stage. Arch Immunol Ther Exp. 2015;63(4):251–68.
49. von Gaudecker B, Müller-Hermelink HK. The development of the human tonsilla palatina. Cell Tissue Res. 1982;224(3):579–600.
50. Zuercher AW, Coffin SE, Thurnheer MC, Fundova P, Cebra JJ. Nasal-associated lymphoid tissue is a mucosal inductive site for virus-specific humoral and cellular immune responses. J Immunol. 2002;168(4):1796–803.
51. Li Y, Jin L, Chen T. The effects of secretory IgA in the mucosal immune system. Biomed Res Int. 2020;2020.
52. Papista C, Berthelot L, Monteiro RC. Dysfunctions of the Iga system: a common link between intestinal and renal diseases. Cell Mole Immunol. 2011;8(2):126–34.
53. Woof J, Russell M. Structure and function relationships in IgA. Mucosal Immunol. 2011;4(6):590–7.
54. Steffen U, Koeleman CA, Sokolova MV, Bang H, Kleyer A, Rech J, et al. IgA subclasses have different effector functions associated with distinct glycosylation profiles. Nat Commun. 2020;11(1):1–12.
55. Bakema J, Van Egmond M. The human immunoglobulin a fc receptor FcαRI: a multifaceted regulator of mucosal immunity. Mucosal Immunol. 2011;4(6):612–24.
56. Majde JA, Kapás L, Bohnet SG, De A, Krueger JM. Attenuation of the influenza virus sickness behavior in mice deficient in toll-like receptor 3. Brain Behav Immun. 2010;24(2):306–15.
57. Wong JP, Saravolac EG, Clement JG, Nagata LP. Development of a murine hypothermia model for study of respiratory tract influenza virus infection. Lab Anim Sci. 1997;47(2):143–7.
58. Edenborough KM, Gilbertson BP, Brown LE. A mouse model for the study of contact-dependent transmission of influenza a virus and the factors that govern transmissibility. J Virol. 2012;86(23):12544–51.
59. Fukushi M, Ito T, Oka T, Kitazawa T, Miyoshi-Akiyama T, Kirikae T, et al. Serial histopathological examination of the lungs of mice infected with influenza a virus PR8 strain. PLoS One. 2011;6(6):e21207.
60. Thangavel RR, Bouvier NM. Animal models for influenza virus pathogenesis, transmission, and immunology. J Immunol Methods. 2014;410:60–79.
61. Hirst GK. Studies on the mechanism of adaptation of influenza virus to mice. J Exp Med. 1947;86(5):357–66.

62. Choi E-J, Lee YJ, Lee J-M, Kim Y-J, Choi J-H, Ahn B, et al. The effect of mutations derived from mouse-adapted H3N2 seasonal influenza a virus to pathogenicity and host adaptation. PLoS One. 2020;15(1):e0227516.
63. Grimm D, Staeheli P, Hufbauer M, Koerner I, Martínez-Sobrido L, Solórzano A, et al. Replication fitness determines high virulence of influenza a virus in mice carrying functional Mx1 resistance gene. Proc Natl Acad Sci. 2007;104(16):6806–11.
64. Blanco JC, Pletneva LM, Wan H, Araya Y, Angel M, Oue RO, et al. Receptor characterization and susceptibility of cotton rats to avian and 2009 pandemic influenza virus strains. J Virol. 2013;87(4):2036–45.
65. Ottolini M, Blanco J, Porter D, Peterson L, Curtis S, Prince G. Combination anti-inflammatory and antiviral therapy of influenza in a cotton rat model. Pediatr Pulmonol. 2003;36(4):290–4.
66. Ottolini MG, Blanco JC, Eichelberger MC, Porter DD, Pletneva L, Richardson JY, et al. The cotton rat provides a useful small-animal model for the study of influenza virus pathogenesis. J Gen Virol. 2005;86(10):2823–30.
67. Eichelberger MC, Prince GA, Ottolini MG. Influenza-induced tachypnea is prevented in immune cotton rats, but cannot be treated with an anti-inflammatory steroid or a neuraminidase inhibitor. Virology. 2004;322(2):300–7.
68. Jayaraman A, Chandrasekaran A, Viswanathan K, Raman R, Fox JG, Sasisekharan R. Decoding the distribution of glycan receptors for human-adapted influenza a viruses in ferret respiratory tract. PLoS One. 2012;7(2):e27517.
69. Imai M, Watanabe T, Hatta M, Das SC, Ozawa M, Shinya K, et al. Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets. Nature. 2012;486(7403):420–8.
70. Houser KV, Pearce MB, Katz JM, Tumpey TM. Impact of prior seasonal H3N2 influenza vaccination or infection on protection and transmission of emerging variants of influenza a (H3N2) v virus in ferrets. J Virol. 2013;87(24):13480–9.
71. Bodewes R, Kreijtz JH, van Amerongen G, Hillaire ML, Vogelzang-van Trierum SE, Nieuwkoop NJ, et al. Infection of the upper respiratory tract with seasonal influenza a (H3N2) virus induces protective immunity in ferrets against infection with a (H1N1) pdm09 virus after intranasal, but not intratracheal, inoculation. J Virol. 2013;87(8):4293–301.
72. Haff R, Schriver P, Stewart R. Pathogenesis of influenza in ferrets: nasal manifestations of disease. Br J Exp Pathol. 1966;47(5):435.
73. Paquette SG, Huang SS, Banner D, Xu L, León A, Kelvin AA, et al. Impaired heterologous immunity in aged ferrets during sequential influenza a H1N1 infection. Virology. 2014;464:177–83.
74. Maher JA, DeStefano J. The ferret: an animal model to study influenza virus. Lab animal. 2004;33(9):50–3.
75. Herlocher ML, Elias S, Truscon R, Harrison S, Mindell D, Simon C, et al. Ferrets as a transmission model for influenza: sequence changes in HA1 of type a (H3N2) virus. J Infect Dis. 2001;184(5):542–6.
76. Belser JA, Eckert AM, Tumpey TM, Maines TR. Complexities in ferret influenza virus pathogenesis and transmission models. Microbiol Mol Biol Rev. 2016;80(3):733–44.
77. Moore IN, Lamirande EW, Paskel M, Donahue D, Qin J, Subbarao K. Severity of clinical disease and pathology in ferrets experimentally infected with influenza viruses is influenced by inoculum volume. J Virol. 2014;88(23):13879–91.
78. Funk KA, Frantz C, Dyke AS, Ryan PC, Jin H, Kemble G, et al. Nasal turbinate enlargement due to cartilage and bone proliferation: a normal developmental finding in young ferrets. Toxicol Pathol. 2010;38(7):1064–9.
79. Perrott MR, Sigurdson CJ, Mason GL, Hoover EA. Mucosal transmission and pathogenesis of chronic wasting disease in ferrets. J Gen Virol. 2013;94(Pt 2):432.
80. Taylor G. Animal models of respiratory syncytial virus infection. Vaccine. 2017;35(3):469–80.
81. Prince GA, Jenson AB, Hemming V, Murphy B, Walsh E, Horswood R, et al. Enhancement of respiratory syncytial virus pulmonary pathology in cotton rats by prior intramuscular inoculation of formalin-inactiva ted virus. J Virol. 1986;57(3):721–8.
82. Kim HW, , et al. Respiratory syncytial virus disease in infants despite prior administration of antigenic inactivated vaccine. Am J Epidemiol. 1969;89(4):422–434.
83. Moore EC, Barber J, Tripp RA. Respiratory syncytial virus (RSV) attachment and nonstructural proteins modify the type I interferon response associated with suppressor of cytokine signaling (SOCS) proteins and IFN-stimulated gene-15 (ISG15). Virol J. 2008;5(1):1–11.
84. Johnson JE, Gonzales RA, Olson SJ, Wright PF, Graham BS. The histopathology of fatal untreated human respiratory syncytial virus infection. Mod Pathol. 2007;20(1):108–19.
85. Graham BS, Perkins MD, Wright PF, Karzon DT. Primary respiratory syncytial virus infection in mice. J Med Virol. 1988;26(2):153–62.
86. Openshaw PJ. The mouse model of respiratory syncytial virus disease. Challenges Opport Resp Syncytial Virus Vacc. 2013:359–69.
87. Cormier SA, You D, Honnegowda S. The use of a neonatal mouse model to study respiratory syncytial virus infections. Expert Rev Anti-Infect Ther. 2010;8(12):1371–80.
88. Bilawchuk LM, Griffiths CD, Jensen LD, Elawar F, Marchant DJ. The susceptibilities of respiratory syncytial virus to nucleolin receptor blocking and antibody neutralization are dependent upon the method of virus purification. Viruses. 2017;9(8):207.
89. Organization WH. State of the art of new vaccine and development: World Health Organization; 2006.
90. Wright PF, Karron RA, Madhi SA, Treanor JJ, King JC, O'Shea A, et al. The interferon antagonist NS2 protein of respiratory syncytial virus is an important virulence determinant for humans. J Infect Dis. 2006;193(4):573–81.
91. Wright PF, Karron RA, Belshe RB, Thompson J, Crowe JE Jr, Boyce TG, et al. Evaluation of a live, cold-passaged, temperature-sensitive, respiratory syncytial virus vaccine candidate in infancy. J Infect Dis. 2000;182(5):1331–42.
92. Groppo R, DiNapoli J, Il Jeong K, Kishko M, Jackson N, Kleanthous H, et al. Effect of genetic background and delivery route on the preclinical properties of a live attenuated RSV vaccine. PLoS One. 2018;13(6):e0199452.
93. Cunningham CK, Karron R, Muresan P, McFarland EJ, Perlowski C, Libous J, et al., editors. Live-attenuated respiratory syncytial virus vaccine with deletion of RNA synthesis regulatory protein M2-2 and cold passage mutations is overattenuated. Open forum infectious diseases; 2019: Oxford University Press US.
94. Mills KH, Gerdts V. Mouse and pig models for studies of natural and vaccine-induced immunity to Bordetella pertussis. J Infect Dis. 2014;209(suppl_1):S16–S9.
95. Elahi S, Holmstrom J, Gerdts V. The benefits of using diverse animal models for studying pertussis. Trends Microbiol. 2007;15(10):462–8.
96. Pittman M, Furman BL, Wardlaw AC. Bordetella pertussis respiratory tract infection in the mouse: pathophysiological responses. J Infect Dis. 1980;142(1):56–66.
97. Scanlon KM, Snyder YG, Skerry C, Carbonetti NH. Fatal pertussis in the neonatal mouse model is associated with pertussis toxin-mediated pathology beyond the airways. Infect Immun. 2017;85(11).

98. Woods DE, Franklin R, Cryz S, Ganss M, Peppler M, Ewanowich C. Development of a rat model for respiratory infection with Bordetella pertussis. Infect Immun. 1989;57(4):1018–24.
99. Walter MJ, Morton JD, Kajiwara N, Agapov E, Holtzman MJ. Viral induction of a chronic asthma phenotype and genetic segregation from the acute response. J Clin Invest. 2002;110(2):165–75.
100. Tyner JW, Uchida O, Kajiwara N, Kim EY, Patel AC, O'Sullivan MP, et al. CCL5-CCR5 interaction provides antiapoptotic signals for macrophage survival during viral infection. Nat Med. 2005;11(11):1180–7.
101. Walter M, Kajiwara N, Karanja P, Castro M, Holtzman MJ. IL-12 p40 production by barrier epithelial cells during airway inflammation. J Exp Med. 2001;193:339–52.
102. Chang P, Hsiung G. Experimental infection of parainfluenza virus type 5 in mice, hamsters and monkeys. J Immunol. 1965;95(4):591–601.
103. Mostafa HH, Vogel P, Srinivasan A, Russell CJ. Non-invasive imaging of Sendai virus infection in pharmacologically immunocompromised mice: NK and T cells, but not neutrophils, promote viral clearance after therapy with cyclophosphamide and dexamethasone. PLoS Pathog. 2016;12(9):e1005875.
104. Ottolini MG, Porter DD, Hemming VG, Hensen SA, Sami IR, Prince GA. Semi-permissive replication and functional aspects of the immune response in a cotton rat model of human parainfluenza virus type 3 infection. J Gen Virol. 1996;77(8):1739–43.
105. Stearns JC, Davidson CJ, McKeon S, Whelan FJ, Fontes ME, Schryvers AB, et al. Culture and molecular-based profiles show shifts in bacterial communities of the upper respiratory tract that occur with age. ISME J. 2015;9(5):1246–59.
106. Peterson SW, Knox NC, Golding GR, Tyler SD, Tyler AD, Mabon P, et al. A study of the infant nasal microbiome development over the first year of life and in relation to their primary adult caregivers using cpn60 universal target (UT) as a phylogenetic marker. PLoS One. 2016;11(3):e0152493.
107. Khamash DF, Mongodin EF, White JR, Voskertchian A, Hittle L, Colantuoni E, et al., editors. The association between the developing nasal microbiota of hospitalized neonates and Staphylococcus aureus colonization. Open forum infectious diseases; 2019: Oxford University Press US
108. Vintini E, Villena J, Alvarez S, Medina M. Administration of a probiotic associated with nasal vaccination with inactivated Lactococcus lactis-PppA induces effective protection against pneumoccocal infection in young mice. Clin Exp Immun. 2010;159(3):351–62.
109. Harata G, He F, Hiruta N, Kawase M, Kubota A, Hiramatsu M, et al. Intranasal administration of lactobacillus rhamnosus GG protects mice from H1N1 influenza virus infection by regulating respiratory immune responses. Lett Appl Microbiol. 2010;50(6):597–602.
110. Davidson LE, Fiorino A-M, Snydman DR, Hibberd PL. Lactobacillus GG as an immune adjuvant for live-attenuated influenza vaccine in healthy adults: a randomized double-blind placebo-controlled trial. Eur J Clin Nutr. 2011;65(4):501–7.
111. Ding T, Song T, Zhou B, Geber A, Ma Y, Zhang L, et al. Microbial composition of the human nasopharynx varies according to influenza virus type and vaccination status. MBio. 2019;10(4).
112. Bassis CM, Tang AL, Young VB, Pynnonen MA. The nasal cavity microbiota of healthy adults. Microbiome. 2014;2(1):1–5.
113. Kumpitsch C, Koskinen K, Schöpf V, Moissl-Eichinger C. The microbiome of the upper respiratory tract in health and disease. BMC Biol. 2019;17(1):1–20.
114. Schenck LP, McGrath JJ, Lamarche D, Stämpfli MR, Bowdish DM, Surette MG. Nasal tissue extraction is essential for characterization of the murine upper respiratory tract microbiota. Msphere. 2020;5(6).
115. Ritchie ND, Ijaz UZ, Evans TJ. IL-17 signalling restructures the nasal microbiome and drives dynamic changes following Streptococcus pneumoniae colonization. BMC Genomics. 2017;18(1):1–20.
116. Bosch AA. Piters WAdS, van Houten MA, Chu MLJ, Biesbroek G, Kool J, et al. maturation of the infant respiratory microbiota, environmental drivers, and health consequences. A prospective cohort study. Am J Respir Crit Care Med. 2017;196(12):1582–90.
117. Teo SM, Mok D, Pham K, Kusel M, Serralha M, Troy N, et al. The infant nasopharyngeal microbiome impacts severity of lower respiratory infection and risk of asthma development. Cell Host Microbe. 2015;17(5):704–15.
118. Salk HM, Simon WL, Lambert ND, Kennedy RB, Grill DE, Kabat BF, et al. Taxa of the nasal microbiome are associated with influenza-specific IgA response to live attenuated influenza vaccine. PLoS One. 2016;11(9):e0162803.
119. Biesbroek G, Tsivtsivadze E, Sanders EA, Montijn R, Veenhoven RH, Keijser BJ, et al. Early respiratory microbiota composition determines bacterial succession patterns and respiratory health in children. Am J Respir Crit Care Med. 2014;190(11):1283–92.
120. Man WH, van Houten MA, Mérelle ME, Vlieger AM, Chu MLJ, Jansen NJ, et al. Bacterial and viral respiratory tract microbiota and host characteristics in children with lower respiratory tract infections: a matched case-control study. Lancet Respir Med. 2019;7(5):417–26.
121. Ichinohe T, Pang IK, Kumamoto Y, Peaper DR, Ho JH, Murray TS, et al. Microbiota regulates immune defense against respiratory tract influenza a virus infection. Proc Natl Acad Sci. 2011;108(13):5354–9.
122. Chaves-Moreno D, Plumeier I, Kahl S, Krismer B, Peschel A, Oxley AP, et al. The microbial community structure of the cotton rat nose. Environ Microbiol Rep. 2015;7(6):929–35.
123. Beura LK, Hamilton SE, Bi K, Schenkel JM, Odumade OA, Casey KA, et al. Normalizing the environment recapitulates adult human immune traits in laboratory mice. Nature. 2016;532(7600):512–6.
124. Pizzolla A, Wang Z, Groom JR, Kedzierska K, Brooks AG, Reading PC, et al. Nasal-associated lymphoid tissues (NALTs) support the recall but not priming of influenza virus-specific cytotoxic T cells. Proc Natl Acad Sci. 2017;114(20):5225–30.
125. Sada-Ovalle I, Talayero A, Chavéz-Galán L, Barrera L, Castorena-Maldonado A, Soda-Merhy A, et al. Functionality of CD4+ and CD8+ T cells from tonsillar tissue. Clin Exp Immun. 2012;168(2):200–6.
126. Thome JJ, Bickham KL, Ohmura Y, Kubota M, Matsuoka N, Gordon C, et al. Early-life compartmentalization of human T cell differentiation and regulatory function in mucosal and lymphoid tissues. Nat Med. 2016;22(1):72.
127. Schreider JP, Raabe OG. Anatomy of the nasal-pharyngeal airway of experimental animals. Anat Rec. 1981;200(2):195–205.
128. Scheerlinck J-PY, Snibson KJ, Bowles VM, Sutton P. Biomedical applications of sheep models: from asthma to vaccines. Trends Biotechnol. 2008;26(5):259–66.
129. Citron MP, Patel M, Purcell M, Lin S-A, Rubins DJ, McQuade P, et al. A novel method for strict intranasal delivery of non-replicating RSV vaccines in cotton rats and non-human primates. Vaccine. 2018;36(20):2876–85.
130. McCluskie MJ, Weeratna RD, Davis HL. Intranasal immunization of mice with CpG DNA induces strong systemic and mucosal responses that are influenced by other mucosal adjuvants and antigen distribution. Mol Med. 2000;6(10):867–77.
131. Southam D, Dolovich M, O'byrne P, Inman M. Distribution of intranasal instillations in mice: effects of volume, time, body position, and anesthesia. American journal of physiology-lung cellular and molecular. Physiology. 2002;282(4):L833–L9.

132. Shimizu S. Routes Adminis Lab Mouse. 2004:527–41.
133. Debertin A, Tschernig T, Tönjes H, Kleemann W, Tröger H, Pabst R. Nasal-associated lymphoid tissue (NALT): frequency and localization in young children. Clin Exp Immunol. 2003;134(3):503–7.
134. Sepahi A, Salinas I. The evolution of nasal immune systems in vertebrates. Mol Immunol. 2016;69:131–8.
135. Sabirov A, Metzger DW. Intranasal vaccination of infant mice induces protective immunity in the absence of nasal-associated lymphoid tissue. Vaccine. 2008;26(12):1566–76.
136. Gross EA, Swenberg JA, Fields S, Popp J. Comparative morphometry of the nasal cavity in rats and mice. J Anat. 1982;135(Pt 1):83.
137. Xi J, Si X, Zhou Y, Kim J, Berlinski A. Growth of nasal and laryngeal airways in children: implications in breathing and inhaled aerosol dynamics. Respir Care. 2014;59(2):263–73.
138. Contencin P, Gumpert L, Sleiman J, Possel L, De Gaudemar I, Adamsbaum C. Nasal fossae dimensions in the neonate and young infant: a computed tomographic scan study. Arch Otolaryngol–Head Neck Surg. 1999;125(7):777–81.
139. Harkema JR, Plopper CG, Hyde DM, Wilson DW, St. George JA, Wong VJ. Nonolfactory surface epithelium of the nasal cavity of the bonnet monkey: a morphologic and morphometric study of the transitional and respiratory epithelium. Am J Anatomy. 1987;180(3):266–79.
140. Mizuno D, Kimoto T, Sakai S, Takahashi E, Kim H, Kido H. Induction of systemic and mucosal immunity and maintenance of its memory against influenza a virus by nasal vaccination using a new mucosal adjuvant SF-10 derived from pulmonary surfactant in young cynomolgus monkeys. Vaccine. 2016;34(16):1881–8.
141. Ichinohe T, Ainai A, Ami Y, Nagata N, Iwata N, Kawaguchi A, et al. Intranasal administration of adjuvant-combined vaccine protects monkeys from challenge with the highly pathogenic influenza a H5N1 virus. J Med Virol. 2010;82(10):1754–61.
142. Hewitt JA, Lutz C, Florence WC, Pitt MLM, Rao S, Rappaport J, et al. Activating resources for the COVID-19 pandemic: in vivo models for vaccines and therapeutics. Cell Host Microbe. 2020.
143. Altamirano-Lagos MJ, Díaz FE, Mansilla MA, Rivera-Pérez D, Soto D, McGill JL, et al. Current animal models for understanding the pathology caused by the respiratory syncytial virus. Front Microbiol. 2019;10:873.
144. Crowe JE Jr, Bui PT, Siber GR, Elkins WR, Chanock RM, Murphy BR. Cold-passaged, temperature-sensitive mutants of human respiratory syncytial virus (RSV) are highly attenuated, immunogenic, and protective in seronegative chimpanzees, even when RSV antibodies are infused shortly before immunization. Vaccine. 1995;13(9):847–55.
145. Inaba I, Inamori S. Blood picture in experimental whooping cough. Am J Dis Childr. 1934;48(6):1193–200.
146. Jiang W, Wei C, Mou D, Zuo W, Liang J, Ma X, et al. Infant rhesus macaques as a non-human primate model of Bordetella pertussis infection. BMC Infect Dis. 2021;21(1):1–12.
147. Warfel JM, Beren J, Kelly VK, Lee G, Merkel TJ. Nonhuman primate model of pertussis. Infect Immun. 2012;80(4):1530–6.
148. Rimmelzwaan G, Baars M, Van Beek R, Van Amerongen G, Claas E, Osterhaus A. Induction of protective immunity against influenza virus in a macaque model: comparison of conventional and iscom vaccines. J Gen Virol. 1997;78(4):757–65.
149. Villinger F, Miller R, Mori K, Mayne A, Bostik P, Sundstrom J, et al. IL-15 is superior to IL-2 in the generation of long-lived antigen specific memory CD4 and CD8 T cells in rhesus macaques. Vaccine. 2004;22(25-26):3510–21.
150. Baskin CR, García-Sastre A, Tumpey TM, Bielefeldt-Ohmann H, Carter VS, Nistal-Villán E, et al. Integration of clinical data, pathology, and cDNA microarrays in influenza virus-infected pigtailed macaques (Macaca nemestrina). J Virol. 2004;78(19):10420–32.
151. Moncla LH, Ross TM, Dinis JM, Weinfurter JT, Mortimer TD, Schultz-Darken N, et al. A novel nonhuman primate model for influenza transmission. PLoS One. 2013;8(11):e78750.
152. Kuiken T, Rimmelzwaan G, Van Amerongen G, Osterhaus A. Pathology of human influenza a (H5N1) virus infection in cynomolgus macaques (Macaca fascicularis). Vet Pathol. 2003;40(3):304–10.
153. Plopper C, Harkema JR. The respiratory system and its use in research. The laboratory primate: Elsevier Ltd.; 2005. p. 503–26.
154. Yeh H, Brinker R, Harkema J, Muggenburg B. A comparative analysis of primate nasal airways using magnetic resonance imaging and nasal casts. J Aerosol Med. 1997;10(4):319–29.
155. Mills RP, Christmas HE. Applied comparative anatomy of the nasal turbinates. Clin Otolaryngol Allied Sci. 1990;15(6):553–8.
156. Harkema JR, Carey SA, Wagner JG. The nose revisited: a brief review of the comparative structure, function, and toxicologic pathology of the nasal epithelium. Toxicol Pathol. 2006;34(3):252–69.
157. Li X, Trivedi U, Brejnrod AD, Vestergaard G, Mortensen MS, Bertelsen MF, et al. The microbiome of captive hamadryas baboons. Animal Microbiome. 2020;2(1):1–10.
158. Cole AL, Sweeney YC, Lasseter AG, Gray JM, Beavis AC, Chong CF, et al. Evaluation of the pig-tailed macaque (Macaca nemestrina) as a model of human Staphylococcus aureus nasal carriage. Infect Immun. 2018;86(6).
159. Nagpal R, Wang S, Solberg Woods LC, Seshie O, Chung ST, Shively CA, et al. Comparative microbiome signatures and short-chain fatty acids in mouse, rat, non-human primate, and human feces. Front Microbiol. 2018;9:2897.
160. Vinson A, Raboin MJ. A practical approach for designing breeding groups to maximize genetic diversity in a large colony of captive rhesus macaques (Macaca mulatta). J Am Assoc Lab Anim Sci. 2015;54(6):700–7.
161. Sasseville VG, Mansfield KG. Overview of known non-human primate pathogens with potential to affect colonies used for toxicity testing. J Immunotoxicol. 2010;7(2):79–92.
162. Hein WR, Griebel PJ. A road less travelled: large animal models in immunological research. Nat Rev Immunol. 2003;3(1):79–84.
163. Gerdts V, Wilson HL, van Meurens F, et al. Large animal models for vaccine development and testing. ILAR J. 2015;56(1):53–62.
164. Gizurarson S. Animal models for intranasal drug delivery. Acta Pharm Nord. 1990;2(2):105–22.
165. Cho H-J, Joo NS, Wine JJ. Defective fluid secretion from submucosal glands of nasal turbinates from CFTR-/-and CFTR ΔF508/ΔF508 pigs. PLoS One. 2011;6(8):e24424.
166. Engle T, Klingborg DJ, Rollin BE. The welfare of cattle: CRC Press; 2018.
167. Casteleyn C, Breugelmans S, Simoens P, Van den Broeck W. The tonsils revisited: review of the anatomical localization and histological characteristics of the tonsils of domestic and laboratory animals. Clin Dev Immunol. 2011;2011.
168. Gizurarson S. Anatomical and histological factors affecting intranasal drug and vaccine delivery. Curr Drug Deliv. 2012;9(6):566–82.
169. Derscheid RJ, Ackermann MR. Perinatal lamb model of respiratory syncytial virus (RSV) infection. Viruses. 2012;4(10):2359–78.
170. Derscheid RJ, van Geelen A, Gallup JM, Kienzle T, Shelly DA, Cihlar T, et al. Human respiratory syncytial virus Memphis 37 causes acute respiratory disease in perinatal lamb lung. BioResearch open access. 2014;3(2):60–69.
171. Kawashima K, Meyerholz DK, Gallup JM, Grubor B, Lazic T, Lehmkuhl HD, et al. Differential expression of ovine innate immune genes by preterm and neonatal lung

epithelia infected with respiratory syncytial virus. Viral Immunol. 2006;19(2):316–23.
172. Meyerholz DK, Grubor B, Fach SJ, Sacco RE, Lehmkuhl HD, Gallup JM, et al. Reduced clearance of respiratory syncytial virus infection in a preterm lamb model. Microbes Infect. 2004;6(14):1312–9.
173. Ackermann MR. Lamb model of respiratory syncytial virus–associated lung disease: insights to pathogenesis and novel treatments. ILAR J. 2014;55(1):4–15.
174. Baghezza S, Mamache B, Bennoune O, Ghougal K. Pathological study and detection of bovine parainfluenza 3 virus in pneumonic sheep lungs using direct immunofluorescence antibody technique. Comp Clin Pathol. 2021;1-10.
175. Queen C, Ward AC, Hunter DL. Bacteria isolated from nasal and tonsillar samples of clinically healthy Rocky Mountain bighorn and domestic sheep. J Wildl Dis. 1994;30(1):1–7.
176. Barbour E, Nabbut N, Hamadeh S, Al-Nakhli H. Bacterial identity and characteristics in healthy and unhealthy respiratory tracts of sheep and calves. Vet Res Commun. 1997;21(6):421–30.
177. Alley M. The bacterial flora of the respiratory tract of normal and pneumonic sheep. N Z Vet J. 1975;23(6):113–8.
178. Belz GT, Heath TJ. Tonsils of the soft palate of young pigs: crypt structure and lymphoepithelium. Anatomical Record: Off Publ Am Assoc Anatomists. 1996;245(1):102–13.
179. Suzuki S, Fuchimoto D. Fetal and early postnatal development of the porcine tonsils of the soft palate. Exp Anim. 2019:18–0142.
180. Elahi S, Brownlie R, Korzeniowski J, Buchanan R, O'Connor B, Peppler M, et al. Infection of newborn piglets with Bordetella pertussis: a new model for pertussis. Infect Immun. 2005;73(6):3636–45.
181. Braucher DR, Henningson JN, Loving CL, Vincent AL, Kim E, Steitz J, et al. Intranasal vaccination with replication-defective adenovirus type 5 encoding influenza virus hemagglutinin elicits protective immunity to homologous challenge and partial protection to heterologous challenge in pigs. Clin Vaccine Immunol. 2012;19(11):1722–9.
182. Elahi S, Thompson DR, Strom S, O'Connor B, Babiuk LA, Gerdts V. Infection with Bordetella parapertussis but not Bordetella pertussis causes pertussis-like disease in older pigs. J Infect Dis. 2008;198(3):384–92.
183. Meurens F, Summerfield A, Nauwynck H, Saif L, Gerdts V. The pig: a model for human infectious diseases. Trends Microbiol. 2012;20(1):50–7.
184. Rajao DS, Vincent AL. Swine as a model for influenza a virus infection and immunity. ILAR J. 2015;56(1):44–52.
185. Khatri M, Dwivedi V, Krakowka S, Manickam C, Ali A, Wang L, et al. Swine influenza H1N1 virus induces acute inflammatory immune responses in pig lungs: a potential animal model for human H1N1 influenza virus. J Virol. 2010;84(21):11210–8.
186. Everett HE, Nash B, Londt BZ, Kelly MD, Coward V, Nunez A, et al. Interspecies transmission of reassortant swine influenza a virus containing genes from swine influenza a (H1N1) pdm09 and a (H1N2) viruses. Emerg Infect Dis. 2020;26(2):273.
187. Correa-Fiz F, Fraile L, Aragon V. Piglet nasal microbiota at weaning may influence the development of Glässer's disease during the rearing period. BMC Genomics. 2016;17(1):1–14.
188. Weese JS, Slifierz M, Jalali M, Friendship R. Evaluation of the nasal microbiota in slaughter-age pigs and the impact on nasal methicillin-resistant Staphylococcus aureus (MRSA) carriage. BMC Vet Res. 2014;10(1):1–10.
189. Probert M, Stott E, Thomas L, Collins A, Jebbett J. An inactivated parainfluenza virus type 3 vaccine: the influence of vaccination regime on the response of calves and their subsequent resistance to challenge. Res Vet Sci. 1978;24(2):222–7.
190. Taylor G. Bovine model of respiratory syncytial virus infection. Challenges Opportunities Respir Syncyt Virus Vacc. 2013;372:327–45.
191. Mansoor F, Earley B, Cassidy JP, Markey B, Doherty S, Welsh MD. Comparing the immune response to a novel intranasal nanoparticle PLGA vaccine and a commercial BPI3V vaccine in dairy calves. BMC Vet Res 2015;11(1):1-11.
192. Cortese VS, Woolums A, Hurley DJ, Berghaus R, Bernard JK, Short TH. Comparison of interferon and bovine herpesvirus-1-specific IgA levels in nasal secretions of dairy cattle administered an intranasal modified live viral vaccine prior to calving or on the day of calving. Vet Immunol Immunopathol. 2017;187:35–41.
193. Metcalfe L, Chevalier M, Tiberghien MP, Jolivet E, Huňady M, Timothy S, et al. Efficacy of a live intranasal vaccine against parainfluenza type 3 and bovine respiratory syncytial virus in young calves with maternally derived antibodies. Veterinary Record Open. 2020;7(1):e000429.
194. McDaneld TG, Kuehn LA, Keele JW. Microbiome of the upper nasal cavity of beef calves prior to weaning. J Anim Sci. 2019;97(6):2368–75.
195. Seker E, Kuyucuoglu Y, Konak S. Bacterial examinations in the nasal cavity of apparently healthy and unhealthy Holstein cattle. J Ani Vet Adv. 2009;8(11):2355–9.
196. Yu CI, Gallegos M, Marches F, Zurawski G, Ramilo O, García-Sastre A, et al. Broad influenza-specific CD8+ T-cell responses in humanized mice vaccinated with influenza virus vaccines. Blood, J Am Soc Hematol. 2008;112(9):3671–8.
197. Abreu NA, Nagalingam NA, Song Y, Roediger FC, Pletcher SD, Goldberg AN, et al. Sinus microbiome diversity depletion and Corynebacterium tuberculostearicum enrichment mediates rhinosinusitis. Sci Transl Med. 2012;4(151):151–24.
198. Krishnamurthy A, McGrath J, Cripps AW, Kyd JM. The incidence of Streptococcus pneumoniae otitis media is affected by the polymicrobial environment particularly Moraxella catarrhalis in a mouse nasal colonisation model. Microbes Infect. 2009;11(5):545–53.
199. Miller MA, Stabenow JM, Parvathareddy J, Wodowski AJ, Fabrizio TP, Bina XR, et al. Visualization of murine intranasal dosing efficiency using luminescent Francisella tularensis: effect of instillation volume and form of anesthesia. PLoS One. 2012;7(2):e31359.
200. Bracken MB. Why animal studies are often poor predictors of human reactions to exposure. J R Soc Med. 2009;102(3):120–2.
201. Torikai Y, Sasaki Y, Sasaki K, Kyuno A, Haruta S, Tanimoto A. Evaluation of systemic and mucosal immune responses induced by a nasal powder delivery system in conjunction with an OVA antigen in cynomolgus monkeys. J Pharm Sci. 2020.
202. Lohrberg M, Pabst R, Wilting J. Co-localization of lymphoid aggregates and lymphatic networks in nose-(NALT) and lacrimal duct-associated lymphoid tissue (LDALT) of mice. BMC Immunol. 2018;19(1):1–8.