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Chapter

Perspective Chapter: The Most Natural Possible Vaccine Administered in the Most Natural Possible Way - Noninvasive over Injectable Vaccine Delivery Routes

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Abstract

This chapter provides perspective on the routes of vaccine administration, comparing invasive and noninvasive delivery methods. We begin with an analysis of the most frequently used routes of administration: invasive, such as traditional needle-based injections (intramuscular and subcutaneous) and noninvasive, including oral and intranasal routes. We discuss recent advancements, for example, aerosols and jet injectors, as well as other novel administration methods for immunization such as improved mucosally-administered vaccines. Finally, we provide an update on how different delivery methods can impact consumer (vaccine recipients) compliance rates and vaccine availability (e.g., cold chain logistics in areas of the world with infrastructure limitations) from the perspectives of both the vaccine provider and the vaccine recipient.

Keywords: vaccine administration, vaccine delivery routes, mucosal vaccines, oral vaccines, noninvasive vaccine delivery

1. Introduction

Over the past couple of decades our understanding of the microbial factors and host immune responses that contribute to effective control of infection and long-lasting immunity have enabled exciting new advances in next-generation vaccine

technologies against multiple globally-problematic diseases (e.g., noninvasive vaccine delivery methods such as mucosally active oral vaccines against mucosally acquired infections, shelf-stable vaccines to avoid cold chain requirements, and mRNA-based vaccine technologies) that are changing the landscape of infection prevention [1–5]. Vaccines remain the most cost-effective infection intervention, and today's vaccines are much safer and more effective than ever before. The greatest advances have been in the areas of developing safe nontoxic or greatly attenuated antigens that are more efficiently presented to the immune system, either through targeted uptake by antigen-presenting cells or by tailored stimulation *via* adjuvants of appropriate immune cell responses that can more rapidly clear the pathogen from the body. Adjuvants are ingredients incorporated into vaccines to stimulate, amplify and prolong the immune response induced, thereby enhancing the effectiveness of vaccines and potentiating a robust and defensive immune response in vaccine recipients [6, 7]. Many of these improvements have been incorporated into the most recent vaccines licensed for use [8]. Despite these enormous strides, a remaining challenge in the field is the design of vaccines to take into account the entry route of the infectious pathogens, that is, where the immune system first encounters the pathogen, which in most cases is at the mucosal interface, and thereby prevent pathogen entry in the first place.

An ideal vaccine against mucosal pathogens must elicit a mucosal immune response, which is most effectively stimulated by the delivery of the antigen to mucosal surfaces [3, 9–13]. This entails the development of alternative vaccine delivery approaches to target oral, intranasal, and other mucosal delivery routes. Prophylactic vaccines effective in preventing infections are still inaccessible to a vast majority of the global population due to their high cost and challenges regarding the need for multiple administrations (usually *via* needle injection) that must be performed in a medical setting by healthcare professionals. The added value of the new oral and intranasal delivery approaches toward vaccine development is that they also provide improved distribution and administration advantages.

In this chapter, we will compare the advantages and disadvantages of the emerging noninvasive vaccines with traditional needle-based invasive vaccines. Our perspective is informed by a comprehensive survey of vaccine providers and industry experts, granting us insights into their priorities and perceptions, their target populations, and how alternative vaccination routes can improve multiple facets (e.g. accessibility, acceptance, cost-effectiveness, and consumer compliance rates, etc.) of the healthcare system [2]. Though the survey was conducted in the United States (US), we believe that the perspectives we present in this chapter are applicable on a global scale. Oral vaccination presents the most desirable of approaches for mass vaccination campaigns, compared with injectable vaccines for the ease and convenience of administration, the option to self-administer, the high levels of acceptance by target populations compared to parenteral routes, the absence of pain compared to invasive needle-based methods, enhanced effectiveness against mucosal infections, and the stability and relative ease of production, storage, and distribution.

2. Current vaccination methods and practices

Vaccines in the US are administered by four routes: intramuscular, subcutaneous, oral, and intranasal [14]. Here, we present an overview of each of these approaches and their current applications in practice.

2.1 Needle-based injection routes: intramuscular, subcutaneous, or intradermal

Needle injection-based vaccines can be administered *via* three routes: intramuscularly by direct injection into the muscle, subcutaneously by injection into the fat layer under the skin, or intradermally into the dermis layer just underneath the epidermis (upper skin layer). Antigens are more easily and rapidly absorbed by intramuscular injection. Since the blood supply to the subcutaneous tissue is less compared to muscle, the rate of adsorption is slower, and local dendritic cells are better able to facilitate the capture of the vaccine antigens to stimulate local inflammation that induces maturation of the dendritic cells and migration to lymph nodes [13, 15]. Intradermal injections have the advantage that lower doses can be used to achieve similar immune protection as subcutaneous injections because the dermis has more immune cells [16].

The majority of all routine prophylactic (preventive) vaccinations and therapeutic (postinfection) vaccine administrations in the US, and globally, use invasive, needle-based methods—including those with the option to be administered either intramuscularly or subcutaneously—despite the needle-associated discomfort and potential blood-contamination issues, adverse side-effects, and cold chain-associated high storage/transportation costs [2, 14, 17–21]. The term "cold chain" refers to a supply chain that maintains low-temperature control of a product from its manufacture to consumption. An uninterrupted cold chain involves a continuous sequence of refrigerated or freezing activities, including production, storage, and distribution, and is supported by appropriate equipment and logistics. This systematic approach is crucial in maintaining the safety and efficacy of temperature-sensitive vaccines, which can significantly increase distribution costs and limit the availability of vaccines in areas that are either remote or have insufficient infrastructure [22]. Other challenges and limitations of using needle-based vaccines include (i) the discomfort of administering multiple vaccines during either a single visit or over a multiple-dose series, (ii) a higher incidence of needle fear among children [20], which reduces vaccine availability in certain settings (e.g., supermarket pharmacies) where providers seek to avoid noisy disruptions, (iii) the need for a dedicated (often private) space to administer vaccines, (iv) specialized training requirements and the associated regulatory limitations on the healthcare professionals who are eligible to be trained on vaccine administration, and (v) high cost, minimum-order requirements that preclude some locations in low-demand, rural areas from ordering or offering certain vaccines [2].

Of the 94 vaccines currently licensed for use in the US, nearly all are administered by either intramuscular or subcutaneous routes [8], with only five vaccines administered orally and two intranasally. Most subcutaneous vaccines may also be administered intramuscularly. The only exception to this is the MMR vaccine PRIORIX, which must be administered subcutaneously, as it allows slow release of the vaccine at a constant rate compared with intramuscular injection [14]. Other less common vaccines, such as smallpox and monkeypox (JYNNEOS) vaccine and the dengue tetravalent (DENGVAXIA) vaccine, must also be administered by the subcutaneous route [23, 24].

In the US, there are currently no routine vaccines licensed for administration by the intradermal route [25]. Fluzone Intradermal (Influenza Virus Vaccine) received FDA approval in 2011, but Sanofi Pasteur discontinued the product at the end of the 2017–2018 influenza season [26]. Erythema, induration, swelling, and pruritus were the most common side effects associated with Fluzone Intradermal and occurred more frequently when compared with the Fluzone-intramuscular route [27].

During a Public Health Emergency in 2022, the JYNNEOS vaccine was allowed to be administered intradermally to individuals at high risk of monkeypox infection [28]. This route was given Emergency Use Authorization (EUA) [29] as an alternative to the standard regimen of subcutaneous injection that uses an injection volume of 0.5 mL compared with 0.1 mL for intradermal route [30]. This was done to increase the number of available vaccine doses as the lower intradermal dose was shown to be immunologically as effective as the standard subcutaneous dose [31].

As exemplified by JYNNEOS, a major advantage of intradermal vaccines is their potential to generate a similar immune response by using only one-fifth—or even one-tenth—the volume of a subcutaneous or intramuscular dose, and this less-invasive method also removes the risk of injury to nerves, blood vessels, or joint spaces [32]. In addition, the intradermal influenza vaccine has been shown to have similar safety and immunogenicity compared to the intramuscular influenza vaccine in immunocompromised populations and may be a way to increase compliance [33]. The major factors hampering the advancement of this vaccine delivery strategy are that it needs a larger multifunctional T-cell population for efficacy, and there is more pain associated with its administration, compared with intramuscular injection [13, 34].

2.2 Intranasal route

The comfort and convenience of the intranasal route have the potential to increase vaccine compliance as the pain and discomfort associated with needles are avoided. However, this route could introduce unique uncertainties for healthcare workers and vaccine recipients. For instance, one might question whether a dose of nasal spray should be repeated if the recipient sneezes immediately after administration (the answer is "no"). Also, a recipient may not accurately assess how their nasal congestion might interfere with the reception of the dose [25, 35].

The only vaccine currently administered by the intranasal route is the live, attenuated trivalent, or tetravalent influenza (LAIV [FluMist]) vaccine [14]. It is approved for nonpregnant individuals ages 2 through 49 years old [36]. Introduced in 2003, MedImmune FluMist nasal spray was marketed as a convenient, comfortable alternative to the intramuscular influenza vaccine—especially for children. However, an aggressive pricing strategy (over four times that of the intramuscular vaccine) and public skepticism over the new delivery method led first-year sales to fall more than 75% short of projections [37]. These issues dampened the adoption of FluMist in its early years, and efficacy issues after the 2009 influenza pandemic led the Advisory Committee on Immunization Practices (ACIP) to recommend against the product during the 2016–2017 and 2017–2018 seasons [38]. However, FluMist was once again recommended in the 2018–2019 season after MedImmune began using new ingredients in production. Due to its limited use in the US, there are no recent efficacy estimates for the new, revised FluMist; but data from other countries have shown that it offers comparable protection to the standard-dose, egg-based inactivated flu vaccine among children [36].

2.3 Oral route

The human body's extensive network of mucosal surfaces, including the oral, oropharyngeal, urogenital, and gastrointestinal (GI) tracts, provides an interface that must be breached by a mucosally invading pathogen [39]. Robust immune responses to invading pathogens at these sites have the potential to inhibit or limit the establishment of infection and subsequent disease. However, the majority of licensed

vaccines do not act at these mucosal surfaces and instead are administered by intramuscular or subcutaneous injection, generally inducing systemic humoral and cellular immune responses against invading pathogens, with limited or absent mucosal activity [40]. In contrast, immunization with vaccines active at mucosal surfaces has the potential to induce effective surface sIgA responses in addition to systemic IgG responses directed against such pathogens [41]. Despite this clear benefit, there are relatively few oral vaccines currently licensed for use (**Table 1**), and oral vaccines are a minority among the vaccines in developmental stages. As mentioned above, 94 vaccines are licensed in the United States for clinical use to prevent 36 diseases, among which only five vaccines are administered orally to provide protection against four diseases: Adenovirus, Cholera, Rotavirus, and Typhoid [8].

The oral route is used for the rotavirus oral-drop vaccines (RV1 [Rotarix], RV5 [RotaTeq]), which are routinely given to infants between 2 and 6 months of age [42]. The oral cholera (Vaxchora) and typhoid (Vivotif) vaccines are recommended for those traveling to regions where these diseases are endemic [43, 44]. Vaxchora is ingested after mixing with water [45], and Vivotif is distributed to consumers in capsule form [46]. The oral Adenovirus Type 4 and Type 7 tablets are administered as a single dose for US military personnel—it is not available to the general public [47, 48]. The oral-drop poliovirus vaccine (OPV) may be available in other parts of the world but is no longer licensed or administered in the US [49]. Since poliovirus spreads *via* fecal-oral routes, the live-attenuated oral polio vaccine (Sabin vaccine), which triggers intestinal/mucosal immunity, is more effective than the inactivated injectable vaccine (Salk vaccine) against polio virus. However, vaccine-derived virulent poliovirus reemergence and several outbreaks have caused serious adverse events with the Sabin polio vaccine, especially in areas with low vaccination rates [50–52].

The utilization of novel technological advancements has led to the development of oral vaccines with stabilized viral genomes that prevent reversion to virulence, and plant-created, safe, and cost-effective oral vaccines are being developed with improved levels of stabilized antigen production [51]. Triggering the mucosal immunity by vaccination *via* mucosal routes (e.g., oral or nasal) is very important and desirable for mucosally acquired infections such as respiratory diseases to inactivate the virus at the host entry stage. However, minimizing the viral transmission among humans by restricting vaccine delivery to injection routes that do not induce mucosal immunity may confine the virus to animals, potentially leading to unexpected outbreaks as happened with certain coronaviruses [53, 54]. The dearth of licensed oral vaccines (**Table 1**) and investigational oral vaccines (**Table 2**) calls for a redoubling of efforts directed toward their development.

Disease	Vaccine trade name	Vaccine type	Vaccine strain	Manufacturer	Dosing and indication
Adenovirus	Adenovirus Type 4 and Type 7 vaccine, Live, oral	Live virus (lyophilized; tablets)	Type 4 and Type 7	Barr Labs, Inc.	Single dose (2 tablets). For use in military populations aged 17 through 50 years of age
Cholera	Dukoral	Whole cell and subunit (combined)	Cholera toxin B subunit and inactivated *Vibrio cholerae* 01 whole cells	Valneva	Two doses given 14 days apart. Indicated fr/or travelers to cholera-affected areas

Disease	Vaccine trade name	Vaccine type	Vaccine strain	Manufacturer	Dosing and indication
Cholera	Vaxchora	Live attenuated (CVD-10-HgR)	Genetically manipulated *Vibrio cholerae* 01 Inaba strain	Emergent Travel Health Inc.[*]	Single dose. Indicated for adults traveling to cholera-affected areas
Poliomyelitis	N/A[#]	Live attenuated	Whole cell monovalent, bivalent, and trivalent vaccines	N/A	N/A - Replaced by inactivated polio vaccine (IPV)
Rotavirus	Rotarix	Live attenuated (liquid)	Human Rotavirus strain G1P	GSK[##]	Two doses given at least 4 weeks apart in infants 6–24 weeks of age
Rotavirus	Rotavac	Live attenuated (liquid)	Human Rotavirus strain 116E (G9P)	Bharat Biotech, Int, Ltd.	Three doses given 4 weeks apart in infants at least 6 weeks of age
Rotavirus	Rotavin-M1	Live attenuated (liquid)	Human Rotavirus strain G1P	CRPVB, Vietnam	Two doses given 60 days apart in infants at least 6 weeks of age
Rotavirus	Rotateq	Pentavalent live vaccine (liquid)	Human rotavirus strains G1, G2, G3, G4, and G9	Merck and Co., Inc.	Three doses given 4–10 weeks apart in infants 6–32 weeks of age
Rotavirus	Rotasiil	Pentavalent live vaccine (lyophilized)	Human rotavirus strains G1, G2, G3, G4, and G9	Serum Institute (India)	Three doses given 4 weeks apart in infants at least 6 weeks of age
Typhoid	Vivotif	Live attenuated vaccine	Ty21a	Emergent Travel Health Inc.[*]	Four doses given every other day in children at least 6 years of age, and in adults

N/A—not applicable.
[*]*Manufacturing and distribution of both Vaxchora and Vivotif were temporarily discontinued in May 2023 by Emergent Travel Health Inc. due to a reduction in global travel during the COVID-19 pandemic.[#]Most oral polio vaccines have been discontinued.[##]GSK—GlaxoSmithKline.*

Table 1.
Oral vaccines licensed for clinical use.

Target indication	Vaccine type	Mode of oral administration	Development phase	developer	Source
Human respiratory syncytial virus (hRSV)	Subunit	Chewable pills, vaccine puree, oral drops, and nasal spray	Preclinical	FruitVaccine	fruitvaccine.org
SARS-CoV-2	Subunit (RBD[*])	Tablet	Preclinical	MigVax	migvax.com

Target indication	Vaccine type	Mode of oral administration	Development phase	developer	Source
Norovirus	Adenovirus type 5-vectored	Tablet	Phase II	Vaxart	clinicaltrials.gov vaxart.com
Shigella and ETEC[**]	Live Attenuated	Tablet	Phase II	Eveliqure Biotechnologies GmbH	clinicaltrials.gov

[*]*Receptor Binding Domain of SARS-CoV-2 Spike protein.*[**]*Enterotoxigenic* Escherichia coli.

Table 2.
Investigational oral vaccines.

We provide here a summary of the primary advantages and disadvantages associated with using oral vaccines, as well as challenges that have hampered progress in their development.

2.3.1 Advantages of oral administration of vaccines

1. Ease of administration. Oral formulations can be self-administered and minimize the need for trained healthcare personnel. This has a potential beneficial effect on cost reduction for vaccine programs [55].

2. Greater acceptance (due to safety and comfort) and compliance. Enhanced vaccine acceptance and adherence in routine prophylactic and reactive outbreak vaccination programs results in more widespread vaccine distribution and access, particularly in resource-limited settings [2, 56, 57].

3. Decreased risk of injury. Needle-free administration that eliminates occupational needlestick injuries and associated intentional/unintentional hazards, such as HIV and hepatitis infections [58].

4. Enhanced stimulation of mucosal immunity. Stimulation of local immune responses at the point-of-entry mucosal interface between the host and pathogen contributes to a potential disease transmission-blocking effect [59].

5. Enhanced stability and shelf life. Oral-pill vaccines offer greater resilience to environmental conditions, have zero or minimal cold chain requirements, minimize greenhouse gas emissions, present a huge reduction in healthcare costs, and are associated with longer storage shelf life [60–62].

2.3.2 Potential disadvantages and challenges of oral vaccines

1. Survival of the acidic properties encountered in the stomach. For those vaccines that must be delivered to the GI tract, there may be a need to develop formulations that withstand the highly acidic environment of the stomach [63].

2. Survival of the digestive properties of the GI tract. There is a potential for the degradation of protein-based vaccines by proteolytic enzymes within the GI tract [63].

3. Absorption at undesirable sites in the GI tract. Some oral vaccines delivered to the gut undergo absorption into the systemic circulation *via* the small intestine, where there is a limited absorption time of 3–4 hours as these formulations are slow to dissolve in intestinal fluids [64]. This can be avoided by formulations designed to be absorbed systemically through the oral and oropharyngeal mucosa rather than more distally within the GI tract.

4. Requirement for higher doses of antigen. Compared to parenterally-administered vaccines, orally-administered vaccines generally require a higher dose of antigen to induce an effective immune response, thereby limiting the immunogen payload included in certain formulations—particularly those that include a vector or carrier molecule [65]. Such higher doses may increase the risk of induction of tolerance instead of protective immunity, and thereby necessitate the need for the inclusion of potent adjuvants to override the vaccine-induced tolerance [66–68]. However, immune tolerance is strictly dose-dependent [69], and the doses used for oral vaccinations are unlikely to induce immune tolerance, as the amount of immunogen administered as an oral vaccine is too low to induce tolerance, especially with novel technologies such as the use of virus-like particles (VLPs), immunostimulants, optimal dosages and boosters [69–74].

Despite these reported challenges, some of which have already been addressed, the many strengths outlined above render oral vaccination among the most desirable approaches for mass vaccination campaigns.

3. Emerging methods for the delivery of vaccines

3.1 Aerosols

Aerosolized vaccines are considered a valuable option to provide rapid mucosal immunization for large populations, especially in regions with high population densities and crisis areas. It is recommended that these be introduced by nasal spray inhalation, which happens to be more beneficial for children and the elderly [75, 76]. Aerosol vaccines have also been shown to offer stronger immunity than intranasal vaccines [77]. While a nasal spray may only reach the nose and throat, an aerosolized vaccine penetrates deeper into the lungs, providing a stronger immune response to infections of the lower respiratory tract. This is especially beneficial for infections caused by influenza, hRSV, and SARS-CoV-2 that tend to be more severe in the lower —rather than upper—respiratory system [78]. A study evaluating two trivalent adenoviral-vectored COVID-19 vaccines confirmed that respiratory mucosal delivery protected against challenges with a number of SARS-CoV-2 variants by stimulating optimal B- and T-cell immunity [79].

There are several examples of large-scale immunizations using aerosolized vaccines. In Russia, thousands of individuals have received such vaccinations against anthrax, plague, tularemia, and smallpox [80]. In Mexico, more than 4 million children received aerosolized measles immunizations with high compliance rates, lower costs, and fewer recorded side effects in comparison to the subcutaneous vaccination route [75]. The potential for widespread, rapid distribution of aerosolized vaccines was highlighted in one comparative study, which showed that two medical professionals could administer the aerosolized plague vaccine to 1248 individuals by using a

dynamic exposure chamber (i.e., room or tent), in 2 hours and 40 minutes. In contrast, six medical professionals could immunize only 150 persons by using parenteral methods in the same time period [75, 81]. More recently, a 2022 study compared aerosol and intramuscular delivery of an adenovirus-vectored tuberculosis vaccine, and concluded that aerosol delivery achieved comparable safety with improved performance eliciting respiratory mucosal immunity [77].

3.2 Jet injectors and transcutaneous/transdermal vaccines

Jet injectors use compressed gas or springs to penetrate the skin with a narrow, high-pressure stream of fluid. This route of administration was used extensively in the US during mass vaccination campaigns beginning in the 1950s [82] and became the standard method of immunization by the Department of the Army in 1961 [83]. However, these multiuse nozzle jet injectors (MUNJIs) often went unsterilized between consecutive vaccine recipients, which increased the risk of transmitting bloodborne illness [84]. In 1984–1985, jet injectors were associated with vehicle transmission of the hepatitis B virus during an outbreak of hepatitis B at a weight clinic [85]. After additional studies determined that MUNJIs could transmit pathogens between vaccine recipients, multiuse jet injectors were withdrawn from the market in the 1990s, and the US Department of Defense discontinued their use in 1997 [86].

Since those earlier applications, a new generation of disposable-syringe jet injectors (DSJIs) was developed, where the injector is refilled with a single-use, needle-free cartridge between each vaccine recipient [84]. These DSJIs mitigate the risk of pathogen transmission between vaccine recipients and remove the risk of needlestick injuries and potential sterilization problems that could occur with traditional injection methods. In 2020, a review of fourteen randomized controlled trials compared the efficacy of vaccines administered by jet injection and needle syringe routes, concluding that both routes produced similar immunogenicity [87, 88]. For individuals who experience needle apprehension, jet injectors are preferred over needle-based injections [87]. In addition, it was noted that jet injectors yielded a higher number of reactions at the injection site, but fewer systemic adverse events [87]. In the US, one jet injector (AFLURIA Quadrivalent) was approved for use during the 2022–2023 influenza season [89].

Another relatively noninvasive alternative to injectable vaccines is transcutaneous immunization, which uses the skin as a vaccination site to induce T-cell or B-cell response by accessing antigen-presenting cell (APC) populations in the skin. Transcutaneous/transdermal delivery employs a variety of techniques such as microneedles, electroporation, laser ablation, sono- or iontophoresis, jet/powder injectors, and particle-based systems to deliver adjuvanted vaccines to APCs. However, potential skin damage can be a drawback with this administration method [90–92].

3.3 Natural plant-derived & mucosally-administered vaccines

Plant-based vaccines were first pioneered in the 1990s using easily grown and genetically manipulatable tobacco and potato plants [93–97]. This was aided by a wealth of existing literature not only on how to genetically introduce foreign genes but also the gene-expression technologies to control protein production in these model laboratory plants [98]. At the time, the concept was to produce the antigen in the plants in large quantities, which could then be extracted and used for large-scale vaccine production. An advantage of this approach was the ability to harvest the

plants and store them under moderate conditions until the time of production. In contrast to traditional vaccine production that requires complex and costly techniques using mammalian (e.g., eggs or mammalian tissue culture) or microbial cell culture for large-scale manufacturing, plant-based vaccines are easier to produce. Significant advancements in the use of natural plants as the factories to create improved plant-based vaccines have been reported recently: (1) the incorporation of stabilized immunogen constructs into plants *via* recombinant immune complex (RIC) vaccines; and (2) virus-like particles (VLPs) eliciting efficient vaccine delivery mechanisms with the potential to be effective without adjuvants [99–111].

With these improvements to early efforts, much of the development impetus has shifted more toward plants that can generate vaccines for mucosal administration. These vaccines are produced in and delivered through natural plants, which have been genetically engineered through the introduction of antigen genes. These transgenic plants are then used to manufacture the desired protein immunogens and can be administered mucosally (e.g. orally or nasally). This stimulates a mucosal immune response that confers protection against the targeted mucosal disease by triggering the immune system at effector sites (i.e., lymphoid tissue) associated with the oral, oropharyngeal, respiratory, and gastrointestinal tract mucosa. Mucosally delivered vaccines offer the advantage of inducing both mucosal and systemic immunity. Major plant species explored in the development of plant-based, mucosally-administered vaccines include potato, rice, tobacco, banana, tomato, maize, spinach, lettuce, alfalfa, and carrots [95–97, 112–118]. If effective dosages can be formulated, orally- and nasally-administered vaccines are also a preferable option due to their safety, lower production cost, and rapid scalability. For instance, it is estimated that only 200 acres of land would be needed to produce enough orally-administered hepatitis B vaccine for all infants worldwide, annually [117]. Ease of administration, lack of cold chain issues, and vegan- and environmental-friendliness are other major benefits of mucosally-administered plant-based vaccines. It is expected that, at the global scale, the local costs of manufacturing, labor, and sourcing of raw materials for plant-created mucosal vaccines will be significantly less than cost estimates in the US (**Table 3**).

Several challenges have been reported in the past during the early development of plant-based vaccines. Primary among them include the dosage between fruits, plants,

Process	Cost/dose	Cost estimate guide	Assumptions on estimates
Seed costs	$0.01	$2/30 seeds	133 doses/plant
Vertical farm labor	$0.12	$1.15 labor cost/lb fruit	10 doses/lb fruit
Other growing costs	$0.04	$0.42 other cost/lb fruit	10 doses/lb fruit
Puree	$0.05	$0.50/lb to puree	10 doses/lb fruit
Lyophilizing & dosing	$0.04	$0.44/lb to freeze dry & dose	10 doses/lb fruit
Quality control Procedures	$5.00		$5 cost per dose
Packaging	$0.07		
Cost of goods sold[*]	$5.33		

[*]*Overhead not included.*

Table 3.
Manufacturing cost estimates in the US per dose of our FruitVax™ pills (USD) [119–121].

and generations may not stay consistent with each crop, and there are difficulties in the purification of the immunogens due to high levels of terpenes and other undesirable compounds in certain plants [122]. Also, foreign proteins tend to have lower expression levels in plants and face potential degradation in the gastrointestinal tract compared to proteins delivered *via* intranasal or parenteral routes. As a result, oral vaccine doses typically need to be significantly higher than those administered through other routes. For instance, the oral dose of the hepatitis B vaccine is typically 10–100 times higher than the parenteral dose. To achieve immunogenicity, 100 g of transgenic fresh potato in three separate doses were required [123]. Other challenges include the selection of transgenic plants that are not typically eaten raw—where cooking the food might neutralize the immunizing protein (e.g., potato)—and administration problems due to infants spitting up part of the dose.

Promising advances in expression vector design have now enabled significant improvements, particularly in the area of high-yield expression of various antigens in plants [106–111]. To illustrate, FruitVaccine, Inc. (http://www.fruitvaccine.org/) employs high-yield antigen expression technologies using the cherry tomato plant due to its pleasant tasting fruits, common use worldwide, easy genetic manipulability, and rapid crop growth. FruitVaccine uses cherry tomato fruits (containing the immunogen) as the vehicle for its mucosally-administered vaccine platforms (**Figure 1**).

The generally pleasant taste of cherry tomatoes and their suitability for raw consumption give them an advantage over other plants. In addition, tomatoes can be grown in greenhouses at a global scale, and engineered tomatoes can be pureed, dosed, and freeze-dried into pill form or processed into a dosed-paste to facilitate their delivery to regions, where mucosally-administered vaccines are most urgently needed. For instance, to address the need for an inexpensive and effective vaccine against hRSV, we have shown the potential for the use of the tomato fruit as an expression and oral delivery vehicle for FruitVaccine's plant-optimized and stabilized (by removing undesirable cleavages within desired epitopes) hRSV-F immunogen (hRSV-fusion-protein). Tomato, as a plant grown worldwide, has added benefits due
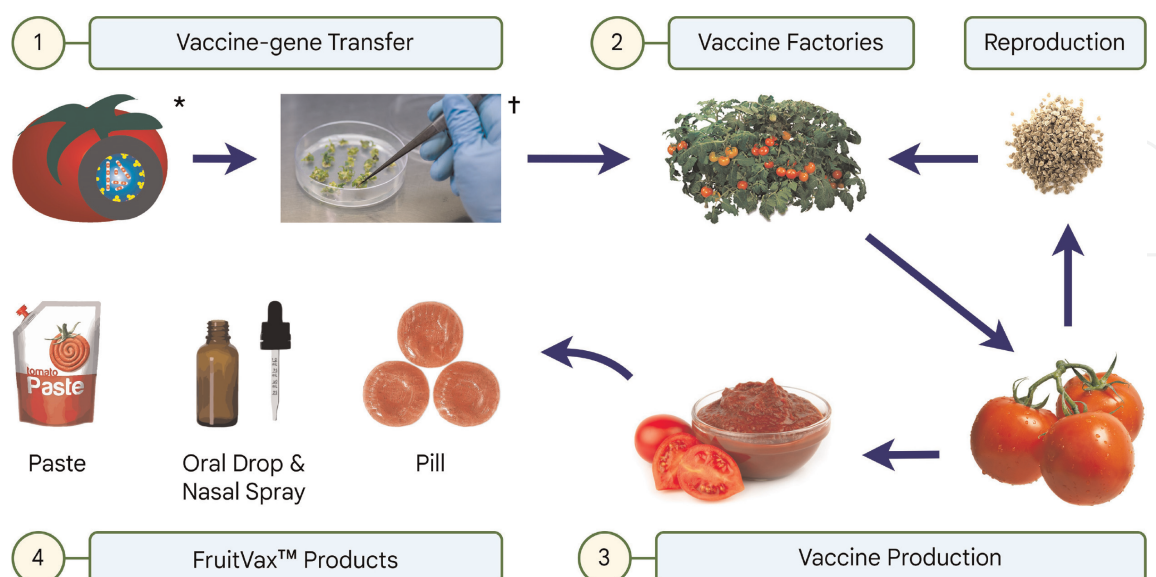

Figure 1.
*Using transgenic fruits to formulate FruitVaccine's mucosal vaccine platforms. Shown is a schematic flowchart of the production process using immunogen-containing plant parts (e.g., fruits in step 3) involved in formulating the vaccine platforms: chewable vaccine pills (FruitVax™ pills), puree/paste, and purified oral-drop or nasal-spray formulations [124]. Image sources: *[125], †[126].*

to its inherent adjuvant, tomatine, and other beneficial bioactive/immunogenic compounds [127]. Tomato has also shown expression and oral delivery capabilities against other infections, such as the Norwalk virus [118]. Expression vector enhancements *via* RIC vaccines and VLPs can greatly increase the expression of recombinant proteins in plants [106, 110, 128, 129], and as stated above, plant-based VLP and immune complex vaccine platforms are synergized to optimize immunogenicity [107].

4. Comparative discussion: impact on vaccination rates: injectable vs. natural plant-created mucosal-vaccines

4.1 Cost: logistics

The costs of oral vs. injectable vaccines can be differentiated across three broad categories: (1) the manufacturing/formulation process, (2) storage requirements, and (3) administration. The manufacturing process for injectable vaccines is typically complex, requiring the cultivation of large quantities of the target pathogen, inactivation or attenuation, purification, and formulation into a final product. This production process often involves specialized equipment that could add to the already stringent quality control measures and contribute to higher costs. In contrast, the nature of plant-based vaccine production and the relative simplicity of formulating a mucosally active vaccine, such as an oral pill, will result in lower manufacturing costs (**Table 3**).

Many injectable vaccines require low or freezing temperatures to maintain their stability and efficacy, necessitating cold chain logistics in the form of both storage facilities and transport from the producer to the end-user/consumer [61, 130]. This need for continuous refrigeration/freezing significantly increases the costs of distribution. In contrast, an oral vaccine pill retains its efficacy at higher temperatures (stable storage at room temperature, or even higher), and homogenizing (pureeing) and lyophilization (freeze-drying) of vaccine fruits improves stability, mitigating the costs associated with cold chain requirements. Storage and transportation costs could be reduced further by the relative simplicity of decentralized, local plant-based manufacturing that does not require complex processing facilities, as mentioned above.

Administration of injectable vaccines is usually performed by healthcare professionals. The training of personnel and the workflow disruption of administering an injectable vaccine can be a financial net loss for providers in certain healthcare settings such as pharmacies and hospitals, where the speedy dispensing of an oral tablet would save both time and money [2]. In addition, the disposal of massive amounts of sharps and other medical waste into the environment is avoided with oral vaccines, which are typically self-administered by the vaccine recipient.

4.2 Accessibility

Based on 135 interviews with professionals in the vaccine business ecosystem, we found that economic buyers of vaccines (e.g., healthcare providers) in the US are using metaphorical "band-aids" to meet consumer needs in rural areas [2]. That is, many vaccines are shipped in multidose vials or multiunit packages. However, in areas with low demand, unused products from these multidose shipments would likely expire and be an unsustainable financial burden for these providers. These healthcare providers simply cannot afford the cost of wasted vaccines, and therefore forego

purchasing low-demand products that are shipped in bulk. To work around this issue, many of these healthcare providers transfer individual doses of certain vaccines among their local peers, as needed (e.g., regional health systems to local health departments or among independent pharmacies). These transfers circumvent the need for each provider to purchase a large quantity of a specific vaccine. However, they cause additional, cumbersome administrative work due to the current regulations surrounding vaccine transfer [2]. An oral vaccine pill that avoids the cold chain and the need for bulk packaging could ship for a lower cost and be individually packed, further eliminating the need for such workarounds in rural areas.

Globally, oral vaccine pills with high-temperature stability could be a boon for areas with little to no cold chain infrastructure. This includes low-income, remote, or isolated regions (e.g., islands or rural areas) with limited transportation, as well as conflict-affected regions that face additional obstacles to vaccine access due to disrupted healthcare systems, infrastructure damage, or limited resources. Middle-income regions with large urban populations or logistical challenges could likewise benefit as local vaccine stores can expand beyond cold chain limitations. Plant-based oral vaccines could be grown, formulated, and distributed locally, creating employment opportunities as an additional economic benefit to these areas.

4.3 Acceptability

The administration route plays an important factor in whether a target population finds a vaccine acceptable. Because injectable vaccines are so ubiquitous, needle fear and the associated anxiety of pain due to injection have traditionally been one of the most common reasons for vaccine avoidance [131, 132]. A systematic review and meta-analysis determined that over half of children exhibit needle fear, and this fear is also high in adolescents (range 20–50%) and adults (20–30%) [20]. These findings were mirrored in FruitVaccine's recent survey of healthcare providers, which revealed that about 24% of vaccine recipients (age-independent) expressed needle fear [2]. We believe an oral vaccine would remove the fear and anxiety that so many recipients, especially children, feel during the administration process, making the experience simple and uneventful, and undoubtedly leading to higher rates of compliance.

High vaccine hesitancy rates surrounding the COVID-19 vaccines [57, 133–136] could indicate how the public will react to future, novel vaccination methods [137, 138]. An encouraging recent study found that among adults, approximately 10% of COVID-19 hesitancy cases could be attributed to a fear of blood, needles, or injury during injection [139]. However, the SARS-CoV-2 pandemic has revealed new challenges in terms of the public acceptability of vaccines. While much of the hesitancy may stem from the vaccines' rapid development and EUA, side effects were cited as the top concern [140]. Therefore, the removal of needle fear may not be enough for target populations to immediately accept a novel, alternative oral vaccine.

4.4 Industry trends: challenges and future prospects

The vaccine providers we previously interviewed described serious challenges with the current state of needle-based vaccines. When questioned about the vaccination process, their most common concern was the need to improve workflow efficiency. This was especially true among independent pharmacists who generally expressed the need to streamline consumer services wherever possible. Many pharmacists explained that this need is driven by ever-shrinking reimbursement rates, as contracted with

their respective pharmacy benefit managers [2]. This has reduced the margin on services that pharmacies provide, requiring independent pharmacists to serve a higher volume of consumers to remain profitable. The administration of routine injectable vaccines is one of the most time-consuming services a pharmacist can provide, taking an average of 23 minutes per vaccine recipient. This may become especially troublesome during multiple vaccinations in one visit (e.g., during childhood vaccinations). In contrast, several prescriptions could be filled during this same time period, and most pharmacists said they make little to nothing—or even lose money—on each vaccine [2].

FruitVaccine's previous survey of vaccine providers allowed us to learn about buyers' purchasing decisions. For instance, over 50% of FruitVaccine's interviewees expressed a willingness to experiment and try new products/ideas. Several providers pointed to their past adoption of the FluMist intranasal spray as an attempt to improve vaccine recipients' experience and speed up their workflow. Even during seasons when the efficacy of FluMist was questioned, these providers still purchased a limited number of doses for those consumers who would otherwise avoid vaccination over needle fear [2]. This indicates that novel administration methods to improve the vaccination process will likely be adopted by a good number of healthcare providers.

4.5 Ecosystem building

The FruitVaccine business ecosystem will consist of current and newly created entities such as certified vaccine-plant growers and services for pureeing, freeze-drying, purifying, and packaging vaccine-containing fruit products (**Figure 2**). FruitVaccine's customers will include regional healthcare systems, pharmacies, and the CDC's Vaccines for Children Program (VFC). We will use a direct sales revenue model and sell to regional/national distributors, who will supply their wholesalers and healthcare customers with FruitVax™ products to be administered to consumers/ vaccine recipients.

Because the FruitVaccine production method is different from traditional vaccine manufacturing, we will pursue contract services from large-scale medical plant growers and pill manufacturers to leverage their production experience. We anticipate FruitVaccine's product will receive FDA approval and reach the market in approximately 5–6 years with an attempt at a breakthrough drug classification as
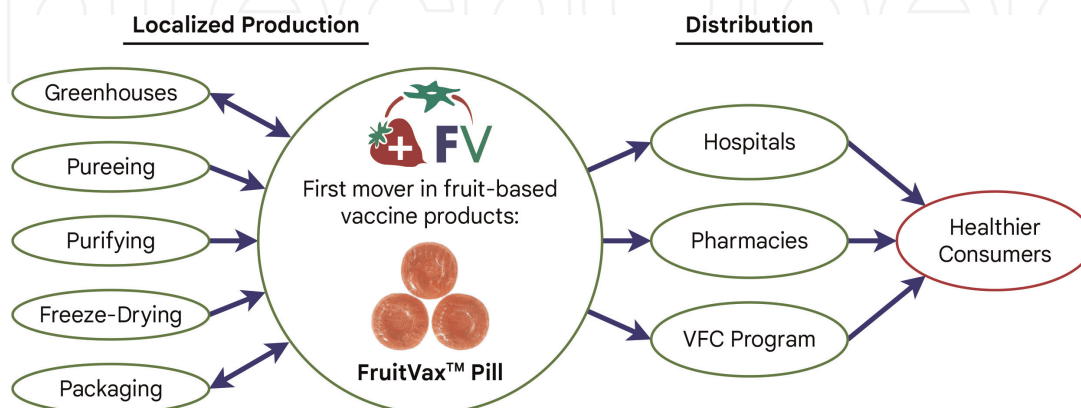

Figure 2.
An overview of the sustainable FruitVaccine business ecosystem. Shown is a diagram of the relationship among components of the FruitVaccine ecosystem, consisting of pre-existing (e.g., packaging services, hospitals, and pharmacies), and newly created (e.g., certified vaccine-plant growers, pureeing/freeze-drying agencies, and FruitVax™ pill makers) manufacturing and distribution systems.

vaccines are biologics. We believe that (1) the lower cost of FruitVaccine's disruptive innovation, (2) FruitVax™ products' drastic reduction in vaccine administration time, and (3) the vaccine recipient's preference for a noninvasive delivery method will give FruitVaccine a competitive advantage, driving rapid adoption of FruitVax™ product among healthcare providers.

5. Conclusion

Among the novel vaccination delivery methods in development, aerosols, jet injectors, and transcutaneous/transdermal administrations may provide advantages in terms of speed and comfort and require relatively less training to use than traditional needle injections. However, some aerosol devices require electric power [75], and DSJIs and transcutaneous or transdermal delivery methods can produce more undesirable reactions at their injection sites [87, 90–92]. Plant-based, orally-administered vaccines would pose neither of these issues and—because of their environmental resilience—could circumvent any disruptions to cold chain logistics networks that exist in either region with infrastructure limitations or conflict zones. Natural, plant-created vaccines that are formulated into chewable pills can be stored at room temperature—lowering transportation and storage costs—and their long shelf life should allow the fulfillment of multiunit orders at a price that will be affordable for rural providers.

On a societal level, we believe a long-term shift toward oral administration for the stimulation of mucosal immunity will make plant-based vaccines much more acceptable and accessible to all populations. The numerous advantages of oral vaccines over needle-based vaccines include their safety, comfort, convenience, lower production cost, efficient administration, and the generation of minimum disposables. Compared with injectable vaccines, oral vaccine pills can be safely self-administered, reduce the number of hospital visits and the need for specialized training of healthcare providers, activate mucosal—as well as systemic cell—mediated immunity, have greater acceptance and customer compliance rates, have increased shelf-life stability, minimize greenhouse gas emissions, and minimize overall vaccine-related healthcare costs [2, 55–62].

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