vaccines

MDPI

Review

Skin-Based Vaccination: A Systematic Mapping Review of the Types of Vaccines and Methods Used and Immunity and Protection Elicited in Pigs

Inés Có-Rives *, Ann Ying-An Chen and Anne C. Moore

School of Biochemistry and Cell Biology, University College Cork, T12 XF62 Cork, Ireland
* Correspondence: icorives@ucc.ie

Abstract: The advantages of skin-based vaccination include induction of strong immunity, dose-sparing, and ease of administration. Several technologies for skin-based immunisation in humans are being developed to maximise these key advantages. This route is more conventionally used in veterinary medicine. Skin-based vaccination of pigs is of high relevance due to their anatomical, physiological, and immunological similarities to humans, as well as being a source of zoonotic diseases and their livestock value. We conducted a systematic mapping review, focusing on vaccine-induced immunity and safety after the skin immunisation of pigs. Veterinary vaccines, specifically anti-viral vaccines, predominated in the literature. The safe and potent skin administration to pigs of adjuvanted vaccines, particularly emulsions, are frequently documented. Multiple methods of skin immunisation exist; however, there is a lack of consistent terminology and accurate descriptions of the route and device. Antibody responses, compared to other immune correlates, are most frequently reported. There is a lack of research on the underlying mechanisms of action and breadth of responses. Nevertheless, encouraging results, both in safety and immunogenicity, were observed after skin vaccination that were often comparable to or superior the intramuscular route. Further research in this area will underlie the development of enhanced skin vaccine strategies for pigs, other animals and humans.

Keywords: vaccine; pig; skin; intradermal; transdermal; transcutaneous; epidermal; epicutaneous; percutaneous; needle-free

check for updates

Citation: Có-Rives, I.; Chen, A.Y.-A.; Moore, A.C. Skin-Based Vaccination: A Systematic Mapping Review of the Types of Vaccines and Methods Used and Immunity and Protection Elicited in Pigs. *Vaccines* **2023**, *11*, 450. https://doi.org/10.3390/vaccines11020450

Academic Editors: Jun Li, Jianli Shi and Chen Li

Received: 12 January 2023
Revised: 10 February 2023
Accepted: 13 February 2023
Published: 16 February 2023

Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Skin-based immunisation is defined as the administration of a vaccine into, onto or through the skin. This route of administration is not new; the inoculation into the skin of variola virus, termed variolation, popularly dates back at least to the time of the Ottoman Empire in the seventeenth century, with other reports from China in the eleventh century. Variolation consisted of rubbing contents of virus-laden pustules onto scratched skin in an attempt to protect against fatal smallpox [1]. This ancient technique was further developed by Edward Jenner's use of cowpox instead of smallpox pustules, which was also administered to superficial incisions on the skin. Scarification, consisting of scraping the skin to break the outer layers with a lancet or, in modern times, a bifurcated needle, was subsequently adopted as the vaccination delivery technique [2]. The smallpox vaccine, given by the skin route, led to the first and only, eradication of a human disease in 1980 [3].

The skin harbours cells from both the innate and the adaptive immune system [4]. This has been suggested as a reason why stronger systemic immune responses can be elicited by skin-based vaccination. Potent responses may permit vaccine dose-sparing, already proven for influenza, rabies and hepatitis B vaccines [5,6]. This dose-sparing advantage of skin-based immunization could be key to overcome vaccine shortages. In addition, new delivery technologies, such as microneedle patches, are being developed, primarily for human use. Some of these should ease the administration process, reducing the need for

highly trained personnel [7–10]. It is, therefore, timely to review the state of the art with respect to skin-based vaccination. Within this field, the pig is a suitable large animal model due to their anatomical and immunological similarity to humans [11–14]. Their central role in the One Health approach as food industry animals [15] and source of zoonotic diseases [16] also situates them as a target species for some vaccines [17,18]. The aim of this review is to evaluate the field of skin-based vaccination in the pig, focusing on vaccine-induced immune responses and safety.

1.1. Skin-Based Vaccination

The skin is an attractive vaccine administration site due to its physiological and immunological characteristics. It is the biggest and outermost organ in the human body and consists of four distinct layers: the outermost barrier; the stratum corneum, then the epidermis, dermis, and hypodermis (Figure 1a). The stratum corneum is a lipid barrier to the surrounding environment that must be traversed to gain access to deeper skin layers containing immune cells [4]. The abundant presence of components of the immune system in the skin has led to the concept that targeting vaccines to this immune-rich organ could have benefits with respect to vaccine potency and the magnitude and quality of the response. Safety and strong immunogenicity of vaccines delivered by the skin route has been reported in pre-clinical [19–23] and clinical trials of human-targeting vaccines [5,24–26]. The presence of different subsets of antigen presenting cells (APCs) in the different layers of the skin is of special interest, suggesting a potentially distinct response depending on the cells targeted. Langerhans cells and dermal dendritic cells are mainly found in the epidermis and dermis, respectively [4,27–30] (Figure 1b), and they have key roles in driving humoral and cellular immune responses [31–35]. Langerhans cells have dichotomous roles, balancing between immunity and tolerance [36]. In addition, they have been shown to be able to prime $CD8^+$ T cells and $CD4^+$ Th2 cells [33,35,37]. In contrast, dermal dendritic cells have been associated with inducing $CD4^+$ Th1 cells [37] and having a faster migration to differential locations in the lymph nodes [38]. Lymphocytes can also be found in the skin. Resident T lymphocytes, mainly $CD4^+$ T cells, are found in high quantities in human skin, under steady state conditions [39]. Most lymphocytes localise to the dermis, with the exception of a low percentage of $CD8^+$ T lymphocytes found in mouse and human epidermis [39–41] (Figure 1b). In humans, T cells express diverse TCR repertoire and belong preferentially to the alpha/beta population, with very few belonging to the gamma/delta subset [39,42,43]. In contrast, in mice, gamma/delta T cells constitute the majority of T cells in epidermis and dermis [42,44,45]. T cells in human skin predominantly show a memory effector phenotype ($CD45RO^+$, CD62L−, CCR7−), hence they are ready for an immediate protective effector response when re-encountering antigens [39,46]. The presence of a memory [40] and plasma [47] resident B cells phenotype in the skin has also been recently demonstrated. Thus, rapid action by these cells is expected upon antigen stimulation. Furthermore, skin immunisation has been proposed to provide a greater magnitude of response by inducing mucosal immune responses in mice and pigs capable of fighting pathogens at their entry site [19,23,48–51].

Potentially stronger immune responses at the skin level could permit the use of reduced antigen doses in a vaccine without compromising efficacy and/or for a broader and stronger response to be induced by a full dose of the vaccine, which could potentially lead to single dose vaccines. The potential of skin-based vaccination to elicit broader immune responses, which has been demonstrated in mice [52,53], would contribute to making currently licensed vaccines [52] and developing vaccine platforms [53] more universal. Dose-sparing has been proven in clinical trials, with immunogenicity being at least non-inferior to that of intramuscular vaccination for influenza, rabies, and hepatitis B vaccines [5,6]. Thus, the smaller dose required for the skin-based route of immunisation could facilitate wider distribution of vaccines. This is crucial for emergency response during epidemics and pandemics in addition to enhancing routine immunisation programmes [54]. Furthermore, this strategy would address ever-rising vaccine shortages [55]. However, the

dose-sparing effect is not observed for all skin-delivered vaccines. For example, the use of fractional dose inactivated polio vaccine (IPV) administered intradermally demonstrated contradictory results in clinical trials [5]. While some trials have observed comparable seroconversion rates between fractional and full doses [56,57], others have highlighted the inferiority of the reduced dose in eliciting immune response, especially in very young infants [58–60]. In addition, scarce availability of studies for some skin-delivered vaccines make it difficult to extract precise conclusions, despite the promising results observed [5]. Thus, the dose-sparing effect is a possibility worth further evaluation in clinical trials, considering potential differences between vaccines, modes of injection and vaccination subjects, which will need to be adjusted.

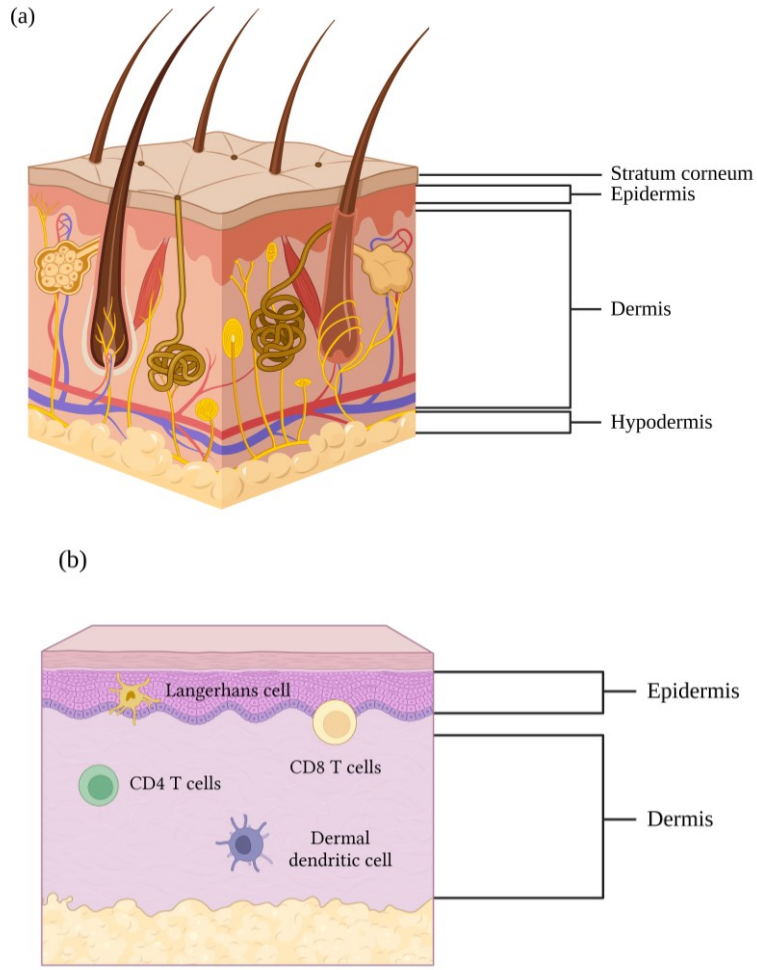


Figure 1. (**a**) Schematic representation of the structure of the skin. (**b**) Schematic representation of immune cells location in the skin. Created with Biorender.com.

Ease of administration is another key factor needed for successful immunisation campaigns. Skin vaccination using scarifiers, needles and syringes using the intradermal Mantoux technique was conventional practice for the smallpox vaccine, but presents limitations regarding the reproducibility of administered quantities and precision at the site of injection [61–63]. New technologies aim to allow precise and easy-to-perform administration, thereby reducing or eliminating the need for highly trained personnel. This will contribute to increased compliance and vaccine uptake. They consist of needle-free or minimally invasive administration techniques at the epidermis and dermis levels, including small needles, jet injectors, gene guns, and microneedle array patches (MAPs) [7,9,64,65]. These techniques have also proven to be immunogenic in animal models [23,52,53,66–68], humans [24,69–75], and veterinary medicine [76,77]. The use of jet injectors in veteri-

nary medicine has increased in recent decades, with some being available for the routine immunisation of pigs [78,79].

The strong immunogenic potential, the potential for dose-sparing, and development of easy-to-use delivery technologies have led to an increased focus on the use of the skin route for a wide range of vaccines. However, skin immunisation still has challenges to overcome, including inferiority in immunogenicity observed for some vaccines when compared to the intramuscular route and adaptation of vaccine formulation to new delivery methods.

1.2. Pigs as a Model for Skin-Based Vaccination

The most used animal model in biomedical research is the mouse. However, they present limitations, particularly with respect to lack of physiological and immunological similarity to humans [45]. Focusing on skin-based immunisation, swine are a highly relevant model for research on the immune system and the skin [80–85]. Pigs are used in a wide range of research fields and they share physiological, anatomical and immunological similarities with humans [86]. The genes of pigs' immune system show preservation of orthology with human genes, 13 times higher than between human and mice, being especially representative in members of the chemokine and cytokine families [13,14]. There is a higher percentage of similarity between protein sequences and structures, with predicted responses for some chemokines, pattern recognition receptors and T lymphocytes being better preserved between pigs and humans compared with mice and humans [13]. Moreover, organs from the immune system, such as the thymus, present similarities in structure and development although some differences, for example, the presence of inverted structure of mucosa-associated lymph nodes in the pig, are observed [87].

The skin of pigs is anatomically very similar to that of humans, with similar thickness and composition of epidermis and dermis and both presenting age-dependant differences [11,12,88]. Chemical and structural closeness has also been observed [89], with similar permeability [11] and mechanical properties [90], which are very useful characteristics for vaccine research. There is also a resemblance between the two species at the cellular level. Studies have identified pig dendritic cell subsets that are phenotypically and functionally similar to their human counterparts, fitting the classification of Langerhans cells and dermal DCs that are found in similar locations in both species [91–93]. Regarding the adaptive immune system, markers for both B and T cells and migration patterns are similar to those used in humans [94,95]. Porcine B cells have been observed to respond to TLR ligands in a comparable way to humans, leading to their differentiation [95]. Pigs, like humans, also possess alpha/beta T cells, which are classified according to their CD4 and CD8 markers. However, some differences do exist. Anatomically, pigs have a decreased number and size of elastic fibres and a higher number of blood vessels compared with humans. Pigs, unlike humans, have some granulose cells near the blood vessels and present apocrine sweat glands instead of eccrine glands [11]. Immunologically, the proportion of alpha/beta and gamma/delta T cells in blood is reversed, especially in young pigs, with a gradual increase in the proportion of alpha/beta T cells observed with age [94]. Aside from their role in wound healing and homeostasis [44], gamma/delta T cells have been suggested to have an active role in the early phases of immune response to infection and/or vaccination [96]. Circulating $CD4^+$ $CD8^+$ double positive T cells can be found in a higher percentage in healthy pigs compared with humans, and have been associated with memory and effector functions [97,98]. Understanding the anatomical and immunological similarities and differences to humans renders pigs as relevant models for skin-based vaccination studies.

1.3. One Health Perspectives

One Health is defined by the One Health High Level Expert Panel as "an integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals, and ecosystems. It recognizes the health of humans, domestic and wild animals, plants, and the wider environment (including ecosystems) are closely linked and inter-

dependent" [99]. Pigs are a source of zoonotic diseases [16], which makes them a crucial part of the One Health approach. As an example, the 2009 pandemic influenza virus derived from pigs when human, avian and pig influenza viruses reassorted, leading to this novel, pandemic H1N1 virus strain [100,101]. The presence of human and avian influenza receptors in pigs leads to the perfect environment for genetic reassortment of viruses that can then potentially infect humans and lead to epidemics and pandemics [102]. This highlights the centrality of pigs in relation to infectious diseases, such as influenza and Nipah viruses, that are of high concern to human spill over events of pandemic potential. This further validates their use as models for human diseases [82,103] and vaccine development [17,18], particularly for pathogens shared with humans [82].

Pigs are also important livestock animals, with pork being the most consumed animal worldwide (36%) according to the UN-FAO. The European Union is one of the biggest exporters and the second biggest producer of pork after China [15]. High quality pig health care, including preventive and therapeutic treatments such as vaccination, are required to ensure food security. Vaccine development in pigs is a growing field, with respect to vaccines specific for pigs and also their use as a large animal model for human vaccines. Multiple vaccines for pigs are licensed (Table 1). Six intradermally delivered vaccines have been licensed at least in one member state of the European Union. The devices used for intradermal administration of these vaccines are jet injectors.

As the interest in skin-based vaccination in human medicine is growing, it is timely to review developments in relation to skin-based vaccination in pigs. The aim of this review is to perform a comprehensive evaluation and to map the literature relating to skin-based vaccination in pigs. We focus on reviewing vaccine-induced humoral and cellular immune responses assessed at a systemic and mucosal level, as well as safety assessments. We quantitatively analyse the relative focus on different pathogens, the type of vaccine platforms and adjuvants used as well as the vaccination route and device. The current state of knowledge, gaps and future directions in this area are discussed.

Table 1. Pig vaccines against infectious pathogens centrally licensed by EMA [104] or by at least one EU-member state [105].

Target Pathogen	Vaccine Name	Type of Vaccine	Route of Administration	Marketing Authorisation Holder
Lawsonia intracellularis	Porcilis Lawsonia ID [a]	Inactivated	ID	Intervet Ireland Limited
Mycoplasma hyopneumoniae	M Hyo ID ONCE [a]	Inactivated	ID	Intervet Ireland Limited
Mycoplasma hyopneumoniae + PCV2 [b]	Mhyosphere PCV ID	Inactivated (Mhyo)/recombinant subunit (PCV2)	ID	HIPRA
PCV2	Porcilis PCV ID	Subunit	ID	Intervet International BV
PRRSV [b]	Porcilis© PRRS	Live attenuated	ID	Intervet International BV
PRRSV	UNISTRAIN® PRRS [a]	Live attenuated	ID	HIPRA
Actinobacillus pleuropneumoniae	Coglapix	Inactivated	IM	Ceva-Phylaxia Veterinary Biologicals Co. Ltd.
Bordetella bronchiseptica + *Pasteurella multocida*	Rhiniseng	Inactivated (Bb)/subunit (Pm)	IM	HIPRA
Bordetella bronchiseptica + *Pasteurella multocida*	Porcilis AR-T DF	Inactivated (Bb)/subunit (Pm)	IM	Intervet International BV

Table 1. *Cont.*

Target Pathogen	Vaccine Name	Type of Vaccine	Route of Administration	Marketing Authorisation Holder
Clostridium difficile and perfringens	Suiseng Diff/A	Subunit (toxoid)	IM	HIPRA
Clostridium perfringens + *Escherichia coli*	Enteroporc COLI AC	Subunit (toxoids + fimbriae)	IM	Ceva Santé Animal
Clostridium perfringens + *Escherichia coli*	Porcilis ColiClos	Subunit (toxoids + fimbriae)	IM	Intervet International BV
CSFV [b]	Suvaxyn CSF Marker	Viral vector	IM	Zoetis
Escherichia coli	VEPURED	Subunit	IM	HIPRA
Escherichia coli	Enteroporc COLI	Subunit (fimbriae)	IM	Ceva Santé Animal
Escherichia coli	Ecoporc SHIGA	Subunit	IM	Ceva Santé Animal
Escherichia coli	Porcilis Porcoli Diluvac Forte	Subunit	IM	Intervet International BV
Escherichia coli	Neocolipor	Inactivated	IM	Boehringer Ingelheim Vetmedica GmbH
Escherichia coli	Coliprotec F4/F18	live	Oral	Elanco GmbH
Erysipelothrix rhusiopathiae	Eryseng	Inactivated	IM	HIPRA
FMDV [b]	AFTOVAXPUR DOE	Inactivated	IM	Boehringer Ingelheim Vetmedica GmbH
Influenza virus	Respiporc FLUpan H1N1	Inactivated	IM	Ceva Santé Animale
Influenza virus	Respiporc Flu3	Inactivated	IM	Ceva Santé Animal
Lawsonia intracellularis	Porcilis Lawsonia [a]	Inactivated	IM	Intervet Ireland Limited
Mycoplasma hyopneumoniae	Suvaxyn M Hyo [a]	Inactivated	IM	Zoetis
Mycoplasma hyopneumoniae	Suvaxyn MH-One [a]	Inactivated	IM	Zoetis
Mycoplasma hyopneumoniae + PCV2	CircoMax Myco	Inactivated (Mhyo)/inactivated recombinant chimeric (PCV2)	IM	Zoetis
Mycoplasma hyopneumoniae + PCV2	Suvaxyn Circo+MH RTU	Inactivated (Mhyo)/inactivated recombinant chimeric (PCV2)	IM	Zoetis
Mycoplasma hyopneumoniae + PCV2	Porcilis PCV M Hyo	Inactivated (Mhyo)/subunit (PCV2)	IM	Intervet International BV
Porcine parvovirus	Porcilis© Parvo [a]	Inactivates	IM	Intervet Ireland Limited
PCV2	Circovac	Inactivated	IM	CEVA-PHYLAXIA
PCV2	Ingelvac CircoFLEX	Subunit	IM	Boehringer Ingelheim Vetmedica GmbH
PCV2	Porcilis PCV	Subunit	IM	Intervet International BV
PCV2	Suvaxyn Circo	inactivated recombinant chimeric	IM	Zoetis
PCV2	CircoMax	Inactivated recombinant chimeric	IM	Zoetis

Table 1. *Cont.*

Target Pathogen	Vaccine Name	Type of Vaccine	Route of Administration	Marketing Authorisation Holder
Porcine parvovirus	ReproCyc ParvoFLEX	Subunit	IM	Boehringer Ingelheim Vetmedica GmbH
Porcine Parvovirus + *Erysipelothrix rhusiopathiae*	Eryseng Parvo	Inactivated	IM	HIPRA
Porcine Parvovirus + *Erysipelothrix rhusiopathiae*	BIOSUIS ParvoEry [a]	Inactivated	IM	Bioveta
PRRSV	Suvaxyn PRRS MLV	Live attenuated	IM	Zoetis
PRRSV	UNISTRAIN® PRRS [a]	Live attenuated	IM	HIPRA
PRRSV	Ingelvac PRRSFLEX EU [a]	Live attenuated	IM	Boehringer Ingelheim
PRRSV	Porcilis© PRRS	Live attenuated	IM	Intervet International BV
PRRSV	ReproCyc PRRS EU [a]	Live attenuated	IM	Boehringer Ingelheim
PRV [b]	Suvaxyn Aujeszky 783 + O/W	Live attenuated	IM	Zoetis
Salmonella enterica	BIOSUIS Salm [a]	Inactivated	IM	Bioveta

[a]: Not central Marketing Authorisation. [b]: Abbreviations PCV2; Porcine Circovirus type 2. CSFV; Classical Swine Fever Virus. FMDV; Food-and-Mouth Disease Virus. PRRSV; Porcine Reproductive and Respiratory Syndrome virus. PRV; Pseudorabies Virus.

2. Methodology

A PubMed search was conducted to identify published peer-reviewed literature that used skin routes of administration. The specific search was as follows: (((pigs OR porcine OR swine) AND (vaccin*)) AND (intradermal OR transdermal OR transcutaneous OR epidermal OR epicutaneous OR percutaneous OR needle-free OR skin OR scarification)) NOT (guinea). The inclusion criteria were (i) peer-reviewed papers; (ii) must be in the English language; (iii) contain primary data; (iv) feature pigs as animal model; (v) contain in vivo work; (vi) include a vaccine; (vii) inoculation to be performed through the skin route and (viii) evaluation of the safety and/or efficacy of vaccine. Efficacy includes protection or immune response that is a correlate of protection, elicited by the vaccine.

Each paper was analysed and classified according to the following parameters: immunisation regimens and vaccines used, including the administration route and device, target pathogen, type of vaccine platform, type of adjuvant and breed of pig, that could modulate the immunological endpoints. Where papers fit into more than one category in any variable, the paper was classified in all appropriate categories.

The search returned with 389 articles published through 8 September 2022. Screening of the papers led to the exclusion of 272 papers which did not comply with at least one of the inclusion criteria set (Figure 2). A total of 117 papers were included in this review. The first author reviewed, screened and characterised all articles. The two other authors reviewed the categorisation and reviewed articles that required re-examination. The authors examined articles independently. No automation tools were used in the process. Frequencies of multiple variables within the literature selected were analysed using the ggplot2 package from RStudio.

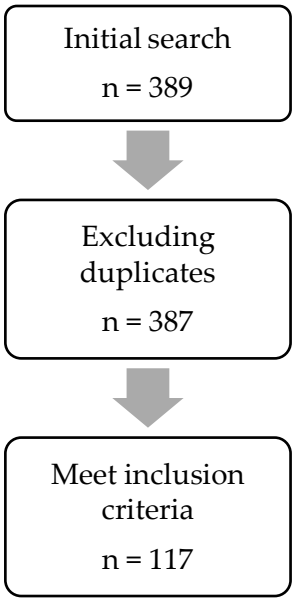


Figure 2. Number of papers identified, and numbers excluded during analysis.

3. Results and Discussion

3.1. Type of Pigs

There are 10 species of living pigs within the Sus genus including domestic pigs and their ancestors, wild boars [106]. Miniature pigs, micro-pigs and domestic pigs are all classified as the same species [107]. We analysed the frequency of use of each species and breed in the skin vaccination field and determined their proportionate use. Articles tend to use the term "commercial domesticated pigs"; this contains many breeds. Among the included literature, 35 papers use Yorkshire, also known as Large White, and Landrace, or crossbreeds of the same. In a few studies, minipigs were used as they are regarded as a suitable model when it comes to vaccination through the skin [83]. It is noted that almost half of the identified papers (n = 57), do not name the pig breed, often referring to it only as commercial breed [49,51,76,108–161]. All mentioned breeds are from domestic pigs.

3.2. Vaccine Targets for Skin-Based Immunisation in Pigs

We next determined the range and frequencies of the pathogens targeted by vaccines using the skin route of administration. The pathogens were first defined as viruses, bacteria, and parasites and a fourth category, labelled "other", which includes studies using vaccines consisting of model antigens (namely ovalbumin, OVA and keyhole limpet haemocyanin, KLH). Almost 80% of skin-delivered vaccines target viruses (Figure 3). The remainder focused on bacteria (16.5%), parasites (2.5%) and other targets (5%).

Within viruses, it is evident that vaccines targeted to PRRSV and influenza viruses predominate (Figure 3). This reflects the magnitude of the PRRSV problem and the importance of pigs as a zoonotic source in influenza vaccine development efforts in addition to vaccines for swine influenza viruses.

Porcine Reproductive and Respiratory Syndrome virus (PRRSV) is an arterivirus, which are enveloped, positive strand RNA virus. It is one of the leading diseases affecting farm pigs and a cause of major economic losses, with the EU estimating an annual cost of €1.5 billion and the US reporting an estimate of over US$600 million [162,163]. PRRSV can affect pigs of all ages but the clinical manifestations vary, being reproductive failure in sows and respiratory difficulties and pneumonia in piglets. Mild symptoms and asymptomatic infections are common. A number of commercial vaccines are routinely used for PRRSV, some of which are administered intradermally (Table 1) [164,165]. PRRSV vaccines are mostly modified live vaccines, with some inactivated vaccines also licensed. However, most live attenuated and inactivated vaccines induce weak immune responses and are

unable to cross-protect against different variants [166]. In addition, live attenuated vaccines are treated with caution due to concerns related to shedding and potential reversion to virulence [166,167]. There is a critical need to develop more effective PRRSV vaccines. The skin route of immunisation has become a focus in this effort, with promising results in eliciting stronger immune responses and enhanced protection [134,135,139] and its improved animal welfare when using needle-less technology [168]. New vaccine platforms and the use of new adjuvants and microneedle patches have been assessed for PRRSV vaccines (see below).

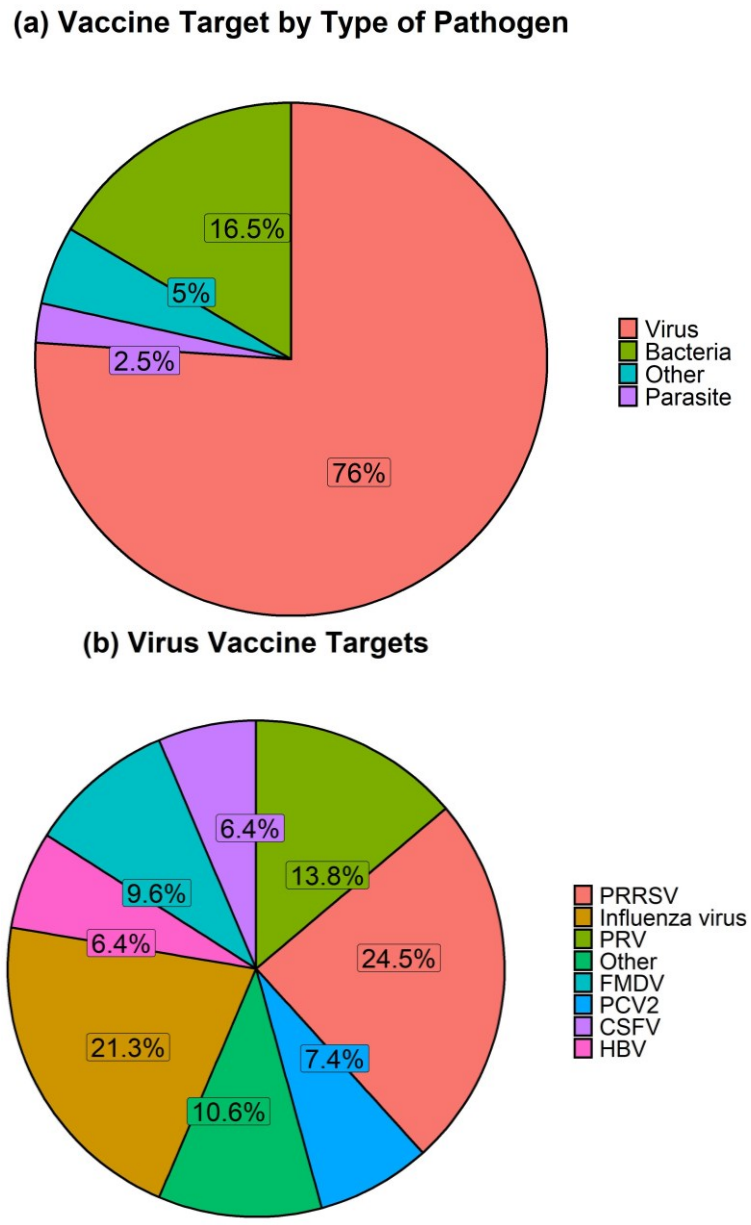


Figure 3. (**a**) Frequency of skin-based vaccination used in pigs according to whether the target of the vaccine was a virus, bacteria, parasite or other pathogen, as identified in 117 published papers. (**b**). Within viruses, frequency of skin-based vaccination used in pigs according to disease indication in 92 published papers. PRRSV: Porcine Reproductive and Respiratory Syndrome Virus, PRV: Pseudorabies virus, FMDV: Food and Mouth Disease Virus, HBV: Hepatitis B virus, CSFV: Classical Swine Fever Virus, PCV2: Porcine Circovirus 2.

Influenza virus is an orthomyxovirus. It is an enveloped, negative strand RNA virus, containing a genome segmented into eight RNA strands. Influenza viruses constantly drift

and escape from pre-existing immunity and sometimes shift to create new strains to which the population is immunologically naïve. Influenza virus infection causes disease and death in humans (human influenza virus) and pigs (swine influenza virus; SIV or SIAV), among other species. It can be highly contagious and causes respiratory illness in both species. While swine usually experience mild infection and low mortality [169,170], in humans it can vary between mild to severe symptoms and mortality can be high depending on strain and susceptibility [171], with an estimated average of 650,000 deaths worldwide every year [172]. Morbidity can be high in both species, with a high disease burden observed in humans, estimated in an annual economic cost of US $11.2 billion [173]. The financial consequences of influenza in the pig industry are difficult to determine. However, the general loss of weight [174] coupled with an estimated global seroprevalence of 49.9% [175] could be an indicator of highly associated but unquantified health and financial costs of swine influenza virus infections. Despite the availability of vaccines, their effectiveness varies due to the requirement for appropriately matched vaccines to seasonally circulating influenza viruses [176]. Viral reassortment in pigs is also of major concern with respect to the evolution of viruses with pandemic potential [100,177]. Efforts are being directed towards the development of new influenza vaccines capable of eliciting a broader, more universal protective response in pigs. A skin-based approach can be beneficial and there is an increasing focus on this route for influenza vaccines in pigs. We identified 20 studies including both human and swine vaccines using the skin route [109–111,115,118,129,136,146,156,178–188] compared to approximately 60 studies evaluating either the intramuscular or subcutaneous routes for influenza vaccines in pigs, the latter also identified in a PubMed search. Some studies focus on inducing mucosal immunity by intradermal vaccine administration [115,118,182].

Pseudorabies virus, the causative agent of Aujeszky's Disease, is the third most targeted virus, with 13 studies examining skin-based pseudorabies vaccines. This virus is an alpha herpesvirus that can affect pigs of all ages, fatal in young piglets, and presenting a wide variety of signs from respiratory disease, to reproductive failure and nervous system signs [189]. Vaccination campaigns have worked to eliminate this pathogen from domestic pigs, resulting in the majority of the literature being >15 years old. However, this pathogen still circulates in wild boars and new variants have emerged in China which escape current vaccines [190] and lead to economic losses [191]. In addition, although controversial, this pathogen can, on rare occasions, infect humans and cause encephalitis [190,192]. If dose-sparing was possible using the skin route and/or impacts on the quality of the immune response, then skin-based immunisation could potentially help tackle these escaped variants and/or a hypothetical zoonotic outbreak.

Within the "other" category we classified viruses representing less than 4% and included: porcine epidemic diarrhoea virus (PEDV), Hepatitis C virus (HCV), African swine fever virus (ASFV), Respiratory syncytial virus (RSV), Human immunodeficiency virus (HIV), rotavirus and poxvirus.

Within targeted bacteria, the most commonly reported is *Mycoplasma hyopneumoniae* (38%). This is an important agent associated with respiratory health problems in pigs. Currently available vaccines have sub-optimal efficacy against infection, disease, and transmission. Experimental vaccines are under development [193]. Studies targeting enterotoxigenic E. coli (ETEC) represented 23.8% of all publications targeting bacterial diseases. This bacteria is still a major cause for concern for humans in low- and middle-income countries where access to preventive and therapeutic treatment is limited [194], and for farm pigs where a universal vaccine is lacking [195]. The remaining mentioned bacteria consisted of *Mycobacterium avium, Lawsonia intracellularis, Mycobacterium tuberculosis, Group E Streptococci, Chlamydia trachomatis and Actinobacillus pleuropneumoniae* (Table 2).

Only two parasites were the target of a vaccine, *Toxoplasma gondii* [196] and *Schistosoma japonicum* [197,198] both of which are zoonotic pathogens.

From this analysis, it is clear that the most reported pathogens in both the virus and bacteria category correspond to causal agents of pig diseases; PRRSV, PRV and *M. hyopneu-*

moniae, and to a lesser extent, pathogens that are both shared between pigs and humans, namely influenza virus and ETEC. Overall, the majority of skin-based vaccine research in pigs focuses on veterinary-specific routine immunisation in pigs, with only a minor focus on the pig as an animal model for human diseases.

Table 2. Reference and year of publication of papers using skin-based vaccines in pigs according to the target pathogen.

Pathogen	References	Publication Year
Model antigens	[117,144,199–202]	2016–2021
A. pleuropneumoniae	[203]	2008
ASFV *	[132]	2021
C. trachomatis	[204]	2012
CSFV *	[49,120,128,137,145,205]	1948, 2002–2011
ETEC *	[147,150,206–208]	2004–2008
FMDV *	[114,130,131,140,154,209–212]	1971, 1999–2009, 2018–2020
Group E streptococci	[213]	1973
HBV *	[83,142,178,214–216]	2002–2003, 2009–2017
HCV *	[217–219]	2006, 2016, 2019
HIV *	[149]	2006
Influenza virus	[109–111,115,118,129,136,146, 156,178–188]	1998–2002, 2013–2022
L. intracellularis	[119,220]	2020, 2021
M. avium	[127,221]	1983, 1978
M. hyopneumoniae	[51,113,133,153,160,161,220, 222]	2012–2022
M. tuberculosis	[223]	2015
PCV2 *	[108,116,153,159–161,220]	2008, 2020–2022
PEDV *	[121,122]	2017, 2021
Poxvirus	[224]	1989
PRRSV *	[66,76,88,112,123,134,135,139, 141,151,155,157,168,220,225–233]	2003–2009, 2013v2022
PRV *	[50,124–126,138,148,152,158,234–238]	1991–2000, 2005, 2011, 2016
Rotavirus	[239]	2016
RSV *	[143]	2016
S. japonicum	[197,198]	2000, 2010
T. gondii	[196]	2008

* Abbreviations: ASFV; African Swine Fever Virus, CSFV; Classical Swine Fever Virus, ETEC; Enterotoxigenic E. coli, FMDV; Food-and-Mouth Disease Virus, HBV; Hepatitis B virus, HCV; Hepatitis C virus, HIV; Human Immunodeficiency Virus, PCV2; Porcine circovirus 2, PEDV; Porcine Endemic Diarrhoea virus, PRRSV; Porcine Reproductive and Respiratory Virus, PRV; Pseudorabies virus, RSV; Respiratory Syncytial Virus.

3.3. Vaccine Platforms and Adjuvant Systems

We next determined the frequency of different vaccine platforms that are administered via the skin to pigs. We classified vaccine platforms as live attenuated (weakened organisms), inactivated (killed organisms), nucleic acid (DNA or RNA), subunit (including subunit, virus like particles (VLP) and toxoid vaccines) and virus vector vaccines.

The frequency of four of the five main platforms in skin-based immunisation in pigs is practically equivalent within the reviewed published papers (Figure 4).

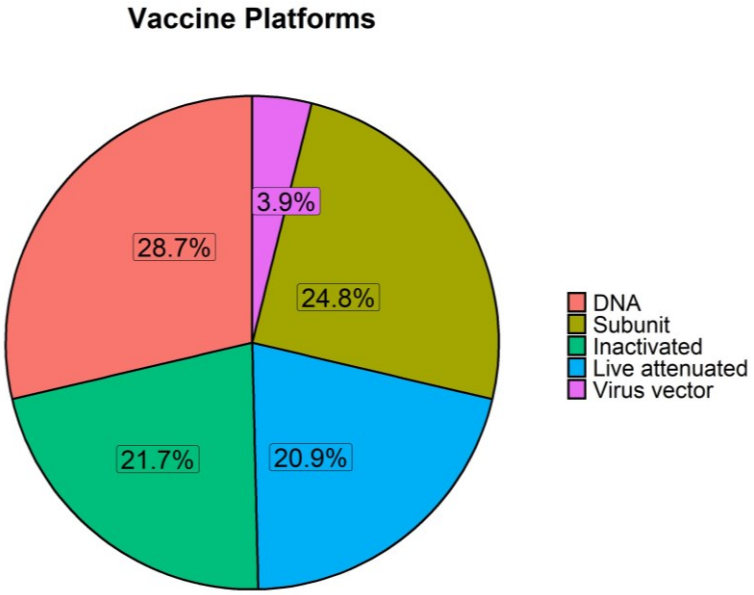


Figure 4. Frequency of use in 117 published papers of skin-based vaccine in pigs according to the type of vaccine platform.

Live attenuated and inactivated platforms have been the traditional vaccines in veterinary medicine. However, advances in the field have led to the development of new platforms that are increasingly being used for pig viral vaccines [240]. DNA vaccines, which consist of plasmids encoding the antigen of interest, present advantages such as faster re-derivation of the vaccine to new strains and the inability to revert to virulence. The capacity to use specific antigens instead of the whole organism also permits the differentiation of infected from vaccinated animals (DIVA) [241,242]. Moreover, DNA vaccines can overcome maternal antibodies in the neonate pigs via the skin-based route [115,182]. Some approaches identified in the literature utilise DNA-based vaccines with the aim of improving heterologous protection to influenza [109,115,182]. However, only one DNA vaccine has been licensed for equine use, and none for pigs. This is largely due to a limited immune response observed in large animal models [241,243]. Nevertheless, advances in the field, including better vectors, new delivery methods, and use of adjuvants, have led to improved humoral and cellular immune responses to vaccination observed in pigs and in clinical trials [244,245]. The first DNA vaccine, for SARS-CoV-2, received approval for human use in 2021 in India [246,247]. This vaccine is administered into human skin.

The European Medicines Agency (EMA) defines adjuvants as ingredients in a medicine that increase or modify the activity of the other ingredients. Adjuvants are used to enhance and modulate vaccine-induced immunity. Over 60% of identified papers using skin-based vaccination in pigs included adjuvants; the majority mixed or co-inoculated with the vaccine [51,76,88,108,110,112,113,115,117–125,129,131–134,137–144,146,151,153,154,156,158–161,168,179,180,182,186–188,196,198,199,201–203,206,208,210–215,218,220–222,226–229,232,233,236] and on a few occasions administered separately [147,178,206,207], to modulate the immune response. Numerous studies formulate multiple adjuvants together [51,108,113,117,119,133,139,146,156,182,202,220,222,226,227] while others compare safety and/or efficacy of different adjuvants [88,120,121,132,144,146,156,180,186,198,199,201,206,211,213,220,227,228]. We examined the proportional use of different adjuvants by the skin route in pigs. We classified them based on the EMA definitions [248] (Figure 5). Emulsions include surfactants, oils, oil-in-water, water-in-oil, water-in-oil-in-water and oil-based emulsions. Endogenous immuno-modulators include cytokines, CTLA4, perforin and cGAMP; and TLR agonists constitute a separate category. Microbial derivatives consist of cholera toxin, enterotoxin

and cdAMP; particulate adjuvants include liposomes and nanoparticles. A final category includes alum-based adjuvants. The adjuvants not fitting any of these categories were classified as "other", including vitamin C, polymers and phosphazene.

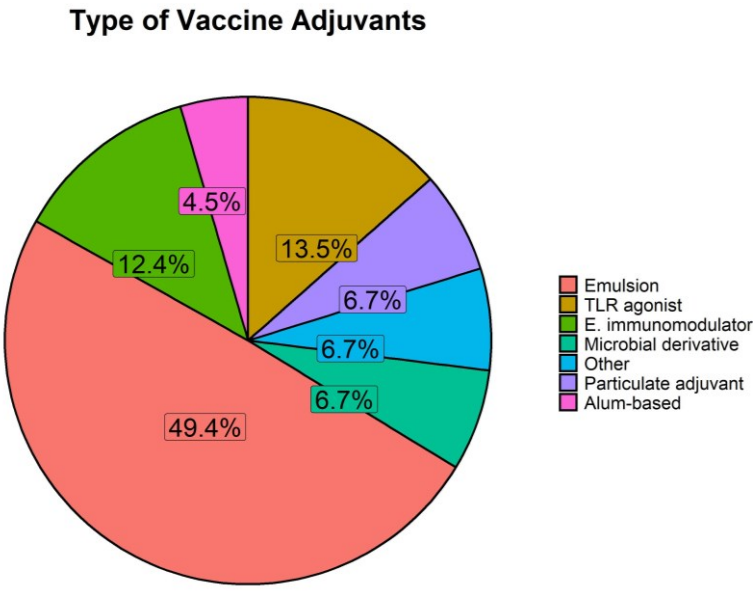


Figure 5. Frequency of use of adjuvant types in skin-based vaccines in pigs in 117 reviewed papers. E. immunomodulators; endogenous immunomodulators.

The most commonly reported adjuvants are emulsions (49.4%, Figure 5). Emulsions are formed by two immiscible liquids brought together and are a common choice of adjuvant in veterinary vaccines [249,250]. They are particularly used for livestock vaccines given their low cost, ease of preparation and use, and efficacy [250–253]. Special attention needs to be taken when choosing the type of emulsion, as the benefit of protection against virus challenge must be greater than skin-based reactogenicity, as seen for a PEDV vaccine [121] but not for other vaccines [132,253]. Some emulsion-based adjuvants have also shown to be safe for skin administration, inducing a strong response mediated by skin DCs [125,199,210]. Emulsions, like the licensed Emulsigen, have shown a more balanced local reaction and immune system activation when delivered to skin in pigs [199,254]. Various emulsion adjuvants are licensed for use in pigs, including various under the band Montanide [250,255]. There are fewer licensed human vaccines using emulsion adjuvants, likely due to unwanted reactogenicity profiles of early emulsion adjuvants used in clinical trials. Such issues have been resolved through improved manufacturing processes and better understanding of how emulsion properties are linked to reactogenicity and immunogenicity [256,257]. TLR agonists represent 12.9% and endogenous immunomodulators 11.8% of the reported adjuvants in skin-based vaccines in pigs. Substantial research has focused on developing adjuvants based on innate sensing of pathogen patterns, such as the TLR, STING and NOD systems [88,187,228,258]. Alum was the first adjuvant to be approved for human use, and it is widely used in humans [259]. However, it only represents a 4.3% of adjuvants reported in skin-based vaccines in pigs within the identified studies. A possible explanation could be the potential for causing granuloma if incorrectly administered into the intradermal space and/or its strong local reactogenicity. Contrary to what we observe in human vaccines, where adjuvants are generally reserved for subunit vaccines, many of the pig skin-based whole vaccines (live attenuated and inactivated) incorporate adjuvants. An example of this is the commercial vaccine Porcilis PRRSV consisting of a modified live virus (MLV) accompanied by dl-α-tocopherol acetate adjuvant mixed to form an emulsion [76,141,151,168,220,232,233].

Therefore, similar to human vaccines, new adjuvants suitable for skin use are being developed, however, a number of adjuvanted vaccines, particularly emulsions are routinely and safely used in pigs.

3.4. Routes and Devices

There are various skin administration routes that have been defined by regulatory bodies such as the FDA. The epidermal and intradermal route delivers vaccines into the epidermis and dermis, respectively. Transdermal routes administer material through the dermal layer of the skin to systemic circulation by diffusion. Percutaneous is defined as delivery through the skin, whereas epicutaneous is delivery onto the surface of the skin [260].

Intradermal immunisation accounts for 82.6% of the routes used in the included studies (Figure 6). While there is no specific platform that stands out for the intradermal route, 83% of epidermal vaccinations use DNA platforms. Some of the studies evaluated the combination of the skin-based route of administration with intramuscular, oral, subcutaneous, or intranasal, simultaneously [112,132] or through heterologous prime-boost regimens using the same or different vaccines [111,122,142,147,150,202,206,208,217,223,229,234,238,261], with the objective to elicit a stronger immune response.

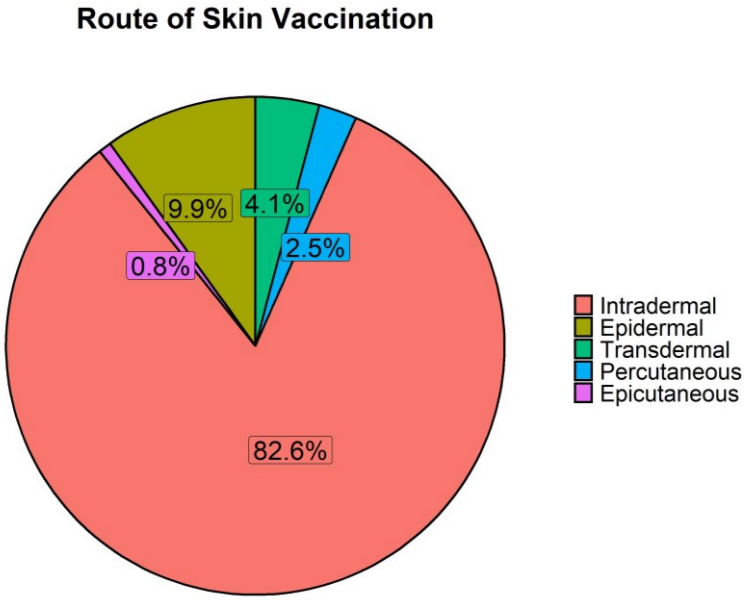


Figure 6. The proportion of different reported skin-based routes of administration, or based on the device specifications (pressure, needle length) in the 117 published papers reviewed.

A range of delivery technologies are used for skin-based administration in addition to conventional needle-and-syringe (Figure 7). Jet injectors were the most frequently used device (35%). These are needle-free devices used to deliver liquid vaccines or drugs into intradermal, subcutaneous, or intramuscular tissues by using high pressure to create a narrow stream that can penetrate the skin. They have been used since the 1950s [262,263], although the use of the first generation of injectors was stopped due to rising concerns of iatrogenic transmission of pathogens between individuals. These issues were overcome in the newer generation of jet injectors [264] which present various advantages, especially in the pork industry. These advantages are mainly shared with all needle-free devices, compared with the classical needle and syringe, including ease of administration, no generation of hazardous sharps waste, elimination of needle-related accidents, reduced pain and stress, and better reproducibility [108,265,266]. The MSD IntraDermal Application of Liquids (IDAL) device was used in 26 of the "skin immunisation" studies reviewed; accounting for 56.5% of the jet injectors mentioned. IDAL is an example of a needle-free jet injector that has been in use since the 2000s [78,79,267]. It is approved for administration of

vaccines against PCV2, PRRSV and *Mycoplasma hyopneumoniae*, some of the most common infections in pigs [78,267] (Table 1), and has proven to be beneficial in animal welfare [108]. Jet injectors have been demonstrated to result in a less painful and less aversive experience compared to intramuscular injection, proven to further enhance animal welfare [108,225]. By controlling several factors, including pressure applied, size of orifice, angle of injection and knowing the thickness of the skin, jet injectors can target the dermis [268] and, thus, are used for intradermal vaccination. Intradermal administration was used with jet injectors in the majority of studies reviewed.

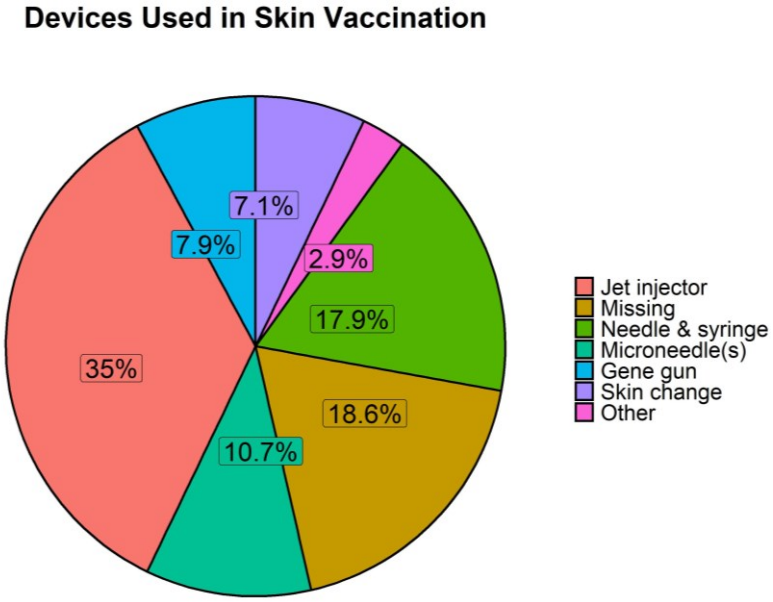


Figure 7. The proportion of different reported devices used in skin-based route of administration in the 117 published papers reviewed.

Needle and syringe, including short intradermal needles, represent 17.9% of the devices mentioned. Microneedle(s), both in array and single format, constitute 10.7% of the devices. Skin structural changes, by means of skin ablation or electroporation, is reported with a frequency of 7.1% and usually accompanies other devices, such as jet injectors, needles, or microneedles (Figure 7).

Gene guns, including ballistic injectors, were assessed at a frequency of 7.9% (Figure 7). Most immunisations use the epidermal route use a gene gun, with the exception of one study using a combined laser and array patch [201] and one comparing gene gun to electroporation [109]. Gene guns propel DNA-coated gold particles into the skin using gas pressure at 400–600 psi (154). They are limited to targeting the epidermis [66,269]. Finally, the category "other" includes all devices/techniques used in less than 5% of cases. Condensation chambers, scarification and cover slip methods conform this category, and correspond to the epicutaneous (the former) and percutaneous (the other two) routes of administration [126,143,197,224].

A large proportion (18.6%) of publications did not state the device that was used or the method of skin administration and are categorised as "missing". The only detail mentioned is the use of an intradermal route. This lack of detail and the use of a potentially generic term of "intradermal" could bias the higher frequency of studies using the intradermal route as opposed to the other skin-based administration routes (Figure 6). The lack of specific details prevents the re-use and reproducibility of these findings.

3.5. Induction of Immunity

3.5.1. Adaptive Immune Responses

Out of the 117 papers, 10 focus on safety aspects with no mention of the immune response [108,130,149,154,160,168,185,200,201,216] and 3 focus on the innate immune re-

sponse [88,199,207]. The remaining 104 papers assess vaccine-induced adaptive immunity and/or efficacy.

3.5.2. Humoral and Cellular Immune Responses Elicited by Skin-Based Immunisation

Systemic immune responses

Antigen-specific antibody responses were the most frequently evaluated adaptive immune response; only 6 out of 104 publications did not analyse humoral immunity. Systemic antibody responses are predominantly assessed, including antigen and/or pathogen-specific antibodies, neutralizing antibodies, and hemagglutinin inhibition (HI) titers for influenza virus. T cell responses were evaluated in less than half of the studies while only eight publications investigated B cell responses [50,122,133,204,206,208,219,227].

Most publications evaluated PRRSV-specific antibodies (20 out of 23 papers) and T cells (15 out of 23 papers), whereas only one paper evaluated B cell responses. Out of the 20 studies that examined PRRSV-specific antibodies subsequent to skin-based immunisations, 12 reported achieving seroconversion after vaccination [76,134,135,139,141,151,155,157,220,225,226,233]. Seroconversion is defined as sample to positive (S/P) ratios above a 0.4 threshold. From those 12 studies, 11 studies assessed a live attenuated vaccine and 1 tested a DNA vaccine. Strong antibody responses, especially after challenge, were induced in a number of studies, for example, after three DNA immunisations [66] or subsequent to two adenoviral vector-based vaccine immunisations [231]. Compared with placebo, strong humoral responses induced by skin-based immunisation led to better protection after homologous, heterologous virus strain challenge or natural infection, shown as a decrease in clinical signs, lung lesions, viral shedding or viremia [76,112,134,135,139,157,220]. However, increased antibodies do not always correlate with protection, as observed by partial, limited or no efficacy of a subunit [123] or an inactivated vaccine [227] after challenge despite an increase in specific-antibodies. Furthermore, some PRRSV vaccines, such as an adjuvanted inactivated vaccine delivered into the skin, did not induce seroconversion even after challenge, and no protection was observed [228]. Protection, however, is not necessarily only linked to antibodies, as cellular immune responses to PRRSV play an important role as well [76,112,135,139,141,157]. Seroconversion and efficacy with no significant differences in protection against challenge have been observed between the single vaccines and multivalent vaccines, the latter of which can contain PCV2, *M. Hyopneumoniae* and *L. intracellularis* [220].

The second most frequently assessed vaccine indication in the literature is influenza virus. Studies that examined skin-based delivery of influenza vaccines (n = 20) focused mainly on evaluating the antibody response after vaccination (n = 19); seven studies evaluated T cell responses, and none evaluated B cell responses. A virus challenge was performed in 11 of these studies. For this pathogen, antigen-specific antibodies; mostly IgG, neutralizing antibodies, and hemagglutinin inhibition (HI) titers were commonly evaluated. A little over half of the studies (n = 11) on influenza used DNA vaccines for immunisation [109–111,115,118,136,146,181,182,184,188], with the remaining studies testing inactivated [129,178,179,183,186] and subunit vaccines [156,180,187]. Independently of the platform used, large increases in virus-specific antibodies after skin-based vaccination were reported in 15 studies, with 12 of them also reporting HI antibodies' greater than the seroprotective threshold and/or neutralizing titters [109,110,115,118,129,136,156,178,180–184,186,188]. Significant increases in IFNg-, IL17- or IL13-secreting cells or proliferation recall responses in blood or lymph nodes in vaccinated compared to controls were observed in 3 studies [110,129,186], while no T cell responses were observed in response to a subunit vaccine [156]. A study with an inactivated AS03-adjuvanted vaccine showed increase in antibodies but considerable variability observed between individual animals [179]. Two studies evaluating a DNA [146] or a subunit [187] vaccine adjuvanted by microbial derivatives or endogenous immunomodulators, point towards increased antibody titters compared to unadjuvanted vaccine or HI titters higher than the threshold, respectively. Antibody responses are mostly associated with enhanced protection to both homologous and heterologous

virus challenges, described as reduction or elimination in viral shedding, load or clinical signs [109,115,136,182,184,186,188]. Nevertheless, a subunit vaccine, using conserved influenza antigens fused to an anti-CD11c antibody adjuvanted with CpG resulted in exacerbation of disease after challenge despite a significant increase in antibodies after intradermal administration of the vaccine compared to controls [180]. Failure to protect pigs either against homologous or heterologous virus challenges, despite higher specific-antibody titters induced after immunisation with inactivated and DNA vaccines [118,129], points towards other type of responses needed to induce protection, namely cellular responses.

Similar to PRRSV and influenza, all 13 studies targeting pseudorabies virus (PRV) focused on antibody responses, whereas only 5 evaluated T cells [50,124,125,152,235] and one assessed B cell responses [50]. Neutralizing antibodies were the main response evaluated after PRV vaccination. Seroconversion according to neutralizing titters after skin vaccination with DNA, subunit, live attenuated or viral vector vaccines was observed in 9 trials, with differences being observed between pre- and post-vaccination stage and/or between vaccinees and controls [50,124–126,138,148,152,235,236]. Antibodies increased after challenge. Significant increase in systemic antigen-specific IFNg-secreting T cells or lymphoproliferation responses compared to controls was observed in 2 out 5 studies evaluating T cell responses [125,235], with the other three showing increases but high variability or insufficient statistical power [50,124,152]. Positive correlation between neutralizing antibody titters and T cell responses was demonstrated in one study [125]. At least partial protection, and/or reduced virus shedding was often seen after vaccination and challenge [50,124,126,138,148,152,235]. However, lack of correlation of protection between neutralizing titters and/or cellular responses and protection has also been observed [124,236], underlining the importance of other responses. Additionally, a significant increase in IgG1- and IgG2-secreting B cells in skin-draining lymph nodes, after immunisation with an unadjuvanted subunit vaccine, has been demonstrated by ELISPOT [50].

Vaccine-induced B cell responses at a systemic level tend to differ between studies. Two studies demonstrated a significant increase in IgG-and IgA-secreting B cells in the blood and draining lymph nodes of animals intradermally vaccinated with subunit and inactivated vaccines when compared to controls. This B cell response additionally, correlated with protection [50,133]. Memory B cells have also been detected in the blood of pigs vaccinated with a subunit HCV vaccine [219]. However, lack of significant increase in percentage of B cell [227] or low correlation with protection [204] has also been reported.

Mucosal immune responses

It has become increasingly evident that mucosal immunity can be required for sterile protection against infection. Intradermal vaccination has shown potential for inducing this kind of immunity in various species, including humans [270]. Within the papers analysed in this review, 14 studies have documented vaccine-induced mucosal immunity. These mucosal samples include saliva [49], bronchoalveolar lavage (BAL) [51,115,182], nasal mucosa [50,118,202,204,226,229], colostrum [121], gut [122,206,208] and vaginal mucosa [202,204].

Very few studies have looked at mucosal antibodies after PRRSV vaccination. Although a certain tendency to higher nasal IgG was hinted after skin-based immunisation with a DNA vaccine alone, or in combination with a live attenuated vaccine by the intramuscular route, levels remain low and not significant, with no IgA detected [226,229]. A slight increase was also reported for nasal and BAL antibodies (IgG and IgA) after influenza vaccination with DNA vaccines and challenge [115,118,182]. In the case of vaccination against PRV, a significant increase in nasal IgA after vaccination and intranasal challenge compared to controls was demonstrated using a subunit vaccine platform. Specifically, a positive correlation between protection and amount of nasal IgA was observed, although to a lesser extend than that observed for serum IgG [50]. Furthermore, inactivated and viral vector vaccines targeting *M. hyopneumoniae*, Classical Swine Fever virus and Porcine Epidemic Diarrhea Virus have been able to elicit mucosal antibodies in pigs after intradermal immunisation by needles or jet injector [49,51,121]. Mucosal antibodies were proposed to

improve survival of vaccinated sows [49] and partial protection of their piglets through colostrum [121].

Even less frequent is the analysis of B cell responses in mucosal tissues. Generally low levels of antibody-secreting B cells in the gut have been described [122,206,208]. A heterologous prime-boost regimen consisting of DNA intradermal priming with subunit oral booster reported the induction of antibody secreting cells (ASC) in various mucosal lymphoid tissues [206,208]. Therefore, between the 8 studies evaluating B cell responses, the most documented significant effects on these cells are observed in the blood or administration site draining lymph nodes, while only weaker or no responses are identified in mucosal tissues.

Further studies addressing mucosal immunity and B cell responses induced by skin vaccination are needed to better understand the magnitude and mechanisms of action of this immunisation route.

3.6. Comparison of Routes of Immunisation

Many of the published papers administering vaccines through the skin route in pigs compare this route to the more predominant intramuscular route, with fewer articles comparing to intranasal and subcutaneous administrations.

Similar humoral and/or cellular responses and/or protection are achieved either by intradermal or intramuscular vaccination against PRRSV using live attenuated platform with or without adjuvants, with intradermal being superior in some cases [76,134,135,139,141,151,155,225]. These studies all used live attenuated PRRSV vaccine. However, on two occasions, the intramuscular route was shown to be more immunogenic compared with the skin route with inactivated vaccines [227,228]. Similarly, in PRV skin-based immunisation with DNA, live attenuated, viral vector and/or subunit vaccines, comparable or even stronger and faster immune responses were observed with skin-based immunisation [50,125,126,138,152,158,235,236,236]. An adjuvant sparing effect was observed in one trial with a subunit vaccine administered by the skin route [50]. Influenza subunit [180], inactivated [179] or DNA vaccines [136], however, induced higher responses when the intramuscular route was used, including the prevention of disease exacerbation. On occasion, skin-based immunisation showed encouraging results [186].

Heterologous prime-boost administration strategies featuring skin immunisation with other routes or the use of different vaccines in the prime and boost have been tested in the pig model. An increase in antibody or cellular responses coupled with clinical protection was observed in a heterologous intradermal-intramuscular strategy in PRRSV [229,230], influenza [111] and PRV [234] vaccination, when compared with each route on their own or in combination with intranasal vaccination. The simultaneous use of these routes with adjuvanted inactivated or DNA vaccines seems to also benefit the induction of antibody responses [112,238]. Epidermal and intradermal skin administration for DNA vaccines has also shown superiority in terms of antibody production and cellular responses, compared to the combination of intradermal and intramuscular routes in PRRSV [66] and PRV [235] trials.

Overall, there is some evidence of skin-based immunisation being similar or superior to the classical intramuscular route. A clear advantage in reduced intradermal doses has been observed, reaching comparable responses to those observed in intramuscular immunisation and even surpassing those induced by higher intradermal doses [203].

However, despite the encouraging evidence favouring skin-based immunisation, it will most likely take time for new routes to replace intramuscular administration, which continues to be the most frequently licensed route in the pig industry (Table 1). Practically, immunization of pigs should be efficient, easy to perform on each animal and across a herd, and it should not decrease the animal's welfare or health. Ideally, it would induce higher quality, durable responses, potentially with lower vaccine doses. Conventional intradermal vaccination by needle-and-syringe is time consuming and prone to variability. The new needle-free technologies can be more expensive than use of a needle-and-syringe by the

intramuscular route. This underscores the need for further development and optimisation of vaccine delivery technologies to the skin route to enhance vaccine-induced protection against pathogen challenge.

3.7. Safety and Adverse Events

Safety is a crucial parameter to consider both for the pig industry and possible translation into human medicine. Skin-based immunisation has been associated with local and sometimes general adverse events [271]. From the 117 papers included in this review, 63 evaluated safeness associated either to the vaccine platform, adjuvant or device used in skin-based immunisation.

The general systemic side effects of vaccination, including fever, weight loss and fatigue, were only mild or absent in the pigs vaccinated using the skin route [110,113,123,131,132,134,135,138,140,151,153,157,158,160,179,188,203,221,222,227,228,233,239]. In addition, co-administration of multiple vaccines into the skin has shown the same safety profile as individual vaccines for PCV2, PRRSV, *M. hyopneumonia* and *L. intracellularis* [153,220], which is not only convenient for the farmer but can also reduce the stress of handling and potential pain to the animals. Special attention is given to the safety of newly developed vaccines for skin administration. Live attenuated viral strains of PRV [234,238], PRRSV [76,139,155] and FMDV [130] were shown to be safe, as observed by the absence or mild presence of clinical signs, lack of transmission, no recovery from tissues and limited dissemination of vaccine virus, when administered intradermally. Moreover, studies with poxviruses in the 1980s and 1990s [126,224] evaluated their possible use as viral vector in the pig, proving their safety. A virus replicon particle for CSFV [145] has proven to be a safe candidate for vaccine development after showing mild or no clinical signs and inefficient replication after skin administration. In addition, DNA vaccines for FMDV [131], HBV [216] and PRRSV [157] are also well tolerated by the skin route, with acceptable biodistribution and no toxicity.

Local reactogenicity after intradermal vaccination generally includes swelling, redness, erythema, induration and skin lesion. Skin-based vaccine administration is more frequently associated with local reactions than the intramuscular or subcutaneous routes, as reactions in skin can be observed compared to reactions that occur deeper in the body such as the muscle [132,140,179,180,203,214,215,222,224,227,228]. Unwanted reactogenicity could be decreased by the use of smaller volumes of vaccine [210]. However, local side effects are particularly detected when needle and syringe or scarification techniques are used. Thus, the development of alternative devices for skin immunisation is important to guarantee decreased reactogenicity. For instance, needle-free jet injectors have proven to reduce local side effects and avoided pathogen transmission between animals [155], leading to better tolerability of the skin route. Other devices like microneedles, microneedle patches, gene guns or electroporators can also prevent severe skin lesions or any prolonged or permanent damage [142,183,216,228].

An appropriate balance is needed between immunopotentiation and safety when choosing adjuvants for skin immunisation. Acceptable transient skin reactions or oedema are commonly observed after skin vaccination with diverse type of adjuvants, including liposomes, emulsions, polymers, endogenous immunomodulators and microbial derivatives [117,121,132,187,210,214]. Strong reactogenicity has been associated with alum-based adjuvants and certain emulsions such as highly reactogenic Freund's adjuvants [132,199]. TLR7/8 agonists, such as resiquimod, induce macroscopic and microscopic lesions that are milder or absent for TLR1/2 or TLR9 agonists (Pam3Cys or CpG oligodeoxynucleotides) [88,228]. Unwanted local reactions can be mitigated by adjusting the amount of adjuvant used [186], or adapting to different skin administration methods such as topical application or epidermal powder delivery [178,201]. However, this must be balanced with maintaining or improving potency and efficacy.

Overall, the skin route of vaccination in pigs is generally safe, with mild or no systemic adverse events recorded. However, balance needs to be achieved to improve animal's welfare, industry benefits and translatability into human medicine, when relevant.

4. Future and Directions for Pigs in Skin Immunisation

The skin represents an attractive target for immunisation. Given the similarity between pigs and humans coupled with the central role of pigs in the One Health approach, the pig represents a very relevant animal to examine vaccine-induced immunity by skin immunisation. Within our PubMed search, a predominance of veterinary vaccine research, specifically focusing on viral targets, has been noted as being opposed to pigs being used as a model for human vaccines. This could be explained due to an increased interest in this route for its potential to facilitate immunisation and increase the welfare of farm pigs. These veterinary vaccine findings emphasise the advantages of skin immunisation for humans. In particular, the safe use of adjuvants, particularly emulsion-based adjuvants in pigs, could set a precedence for skin-based vaccines for humans. Further emphasis should be put on the pig as a model for skin immunisation, as given its common characteristics with humans and its position as a source of zoonotic diseases, the potential for translatability increases.

Multiple routes of administration into the skin exist, but a lack of precision in reporting and clarity in devices used makes it difficult to evaluate potential differences in elicited immune responses. Better adherence to the official definitions of routes and further details when describing immunisation could help further characterise immune response after skin immunisation and improve the reproducibility and re-use of published results.

Despite the promising immune responses observed, future research should focus on the underlying mechanisms of action and anatomical breadth of responses to further understand how vaccines administered to the skin are working in systemic and mucosal compartments. In addition, a better understanding of how immune responses correlate with protection is needed. The development of a wider range of reagents specific for pig assays could help overcome challenges in this field.

Despite the predominance of more classical routes of immunisation, increased interest in the skin within the pig industry coupled with the further development of administration technologies should lead to the increased use of skin-based immunisation in the future. Lessons learned from these farm animals can also benefit human medicine.

Author Contributions: Conceptualization, A.C.M. and I.C.-R.; methodology, A.C.M. and I.C.-R.; formal analysis, I.C.-R., A.Y.-A.C. and A.C.M.; investigation, I.C.-R., A.Y.-A.C. and A.C.M.; data curation, I.C.-R.; writing—original draft preparation A.C.M. and I.C.-R.; writing—review and editing, I.C.-R., A.Y.-A.C. and A.C.M.; visualization, I.C.-R.; supervision, A.C.M.; project administration, A.C.M.; funding acquisition, A.C.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Science Foundation Ireland, grant number FFP-A-8882.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Boylston, A. The origins of inoculation. *J. R. Soc. Med.* **2012**, *105*, 309–313. [CrossRef] [PubMed]
2. Belongia, E.A.; Naleway, A.L. Smallpox Vaccine: The Good, the Bad, and the Ugly. *Clin. Med. Res.* **2003**, *1*, 87–92. [CrossRef] [PubMed]
3. World Health Organization. Smallpox [Internet]. Available online: https://www.who.int/health-topics/smallpox (accessed on 19 September 2022).
4. Yazdi, A.S.; Röcken, M.; Ghoreschi, K. Cutaneous immunology: Basics and new concepts. *Semin. Immunopathol.* **2016**, *38*, 3–10. [CrossRef] [PubMed]
5. Schnyder, J.L.; De Pijper, C.A.; Garcia Garrido, H.M.; Daams, J.G.; Goorhuis, A.; Stijnis, C.; Schaumburg, F.; Grobusch, M.P. Fractional dose of intradermal compared to intramuscular and subcutaneous vaccination—A systematic review and meta-analysis. *Travel Med. Infect. Dis.* **2020**, *37*, 101868. [CrossRef] [PubMed]

6. Egunsola, O.; Clement, F.; Taplin, J.; Mastikhina, L.; Li, J.W.; Lorenzetti, D.L.; Dowsett, L.E.; Noseworthy, T. Immunogenicity and Safety of Reduced-Dose Intradermal vs Intramuscular Influenza Vaccines: A Systematic Review and Meta-analysis. *JAMA Netw. Open.* **2021**, *4*, e2035693. [CrossRef]
7. Marshall, S.; Sahm, L.J.; Moore, A.C. The success of microneedle-mediated vaccine delivery into skin. *Hum. Vaccines Immunother.* **2016**, *12*, 2975–2983. [CrossRef]
8. Kim, Y.C.; Prausnitz, M.R. Enabling skin vaccination using new delivery technologies. *Drug Deliv. Transl. Res.* **2011**, *1*, 7–12. [CrossRef]
9. Hettinga, J.; Carlisle, R. Vaccination into the Dermal Compartment: Techniques, Challenges, and Prospects. *Vaccines* **2020**, *8*, E534. [CrossRef]
10. Kim, Y.C.; Jarrahian, C.; Zehrung, D.; Mitragotri, S.; Prausnitz, M.R. Delivery Systems for Intradermal Vaccination. *Intradermal. Immun.* **2011**, *351*, 77–112.
11. Khiao In, M.; Richardson, K.C.; Loewa, A.; Hedtrich, S.; Kaessmeyer, S.; Plendl, J. Histological and functional comparisons of four anatomical regions of porcine skin with human abdominal skin. *Anat. Histol. Embryol.* **2019**, *48*, 207–217. [CrossRef]
12. Liu, Y.; Chen J ying Shang H tao Liu C e Wang, Y.; Niu, R.; Wu, J.; Wei, H. Light Microscopic, Electron Microscopic, and Immunohistochemical Comparison of Bama Minipig (Sus scrofa domestica) and Human Skin. *Comp. Med.* **2010**, *60*, 142–148. [PubMed]
13. Dawson, H.D. A Comparative Assessment of the Pig, Mouse and Human Genomes: Structural and Functional Analysis of Genes Involved in Immunity and Inflammation. In *The Minipig in Biomedical Research*; McAnulty, P.A., Dayan, A.D., Ganderup, N.C., Hastings, K.L., Eds.; CRC Press: Boca Raton, FL, USA, 2012.
14. Dawson, H.D.; Loveland, J.E.; Pascal, G.; Gilbert, J.G.; Uenishi, H.; Mann, K.M.; Sang, Y.; Zhang, J.; Carvalho-Silva, D.; Hunt, T.; et al. Structural and functional annotation of the porcine immunome. *BMC Genom.* **2013**, *14*, 332. [CrossRef]
15. European Commission, Agriculture and Rural Development. Pork [Internet]. Available online: https://agriculture.ec.europa.eu/farming/animal-products/pork_en (accessed on 24 October 2022).
16. McLean, R.K.; Graham, S.P. The pig as an amplifying host for new and emerging zoonotic viruses. *One Health* **2022**, *14*, 100384. [CrossRef]
17. McLean, R.K.; Graham, S.P. Vaccine Development for Nipah Virus Infection in Pigs. *Front. Vet. Sci.* **2019**, *6*, 16. [CrossRef] [PubMed]
18. Keay, S.; Poljak, Z.; Klapwyk, M.; O'Connor, A.; Friendship, R.M.; O'Sullivan, T.L.; Sargeant, J.M. Influenza A virus vaccine research conducted in swine from 1990 to May 2018: A scoping review. *PLoS ONE* **2020**, *15*, e0236062. [CrossRef]
19. Belyakov, I.M.; Hammond, S.A.; Ahlers, J.D.; Glenn, G.M.; Berzofsky, J.A. Transcutaneous immunization induces mucosal CTLs and protective immunity by migration of primed skin dendritic cells. *J. Clin. Invest.* **2004**, *113*, 998–1007. [CrossRef] [PubMed]
20. Glenn, G.M.; Scharton-Kersten, T.; Vassell, R.; Mallett, C.P.; Hale, T.L.; Alving, C.R. Transcutaneous immunization with cholera toxin protects mice against lethal mucosal toxin challenge. *J. Immunol.* **1998**, *161*, 3211–3214. [CrossRef] [PubMed]
21. Godefroy, S.; Goestch, L.; Plotnicky-Gilquin, H.; Nguyen, T.N.; Schmitt, D.; Staquet, M.J.; Corvaïa, N. Immunization onto shaved skin with a bacterial enterotoxin adjuvant protects mice against respiratory syncytial virus (RSV). *Vaccine* **2003**, *21*, 1665–1671. [CrossRef]
22. Corbett, H.J.; Fernando, G.J.P.; Chen, X.; Frazer, I.H.; Kendall, M.A.F. Skin Vaccination against Cervical Cancer Associated Human Papillomavirus with a Novel Micro-Projection Array in a Mouse Model. *PLoS ONE* **2010**, *5*, e13460. [CrossRef] [PubMed]
23. Zaric, M.; Becker, P.D.; Hervouet, C.; Kalcheva, P.; Ibarzo Yus, B.; Cocita, C.; O'Neill, L.A.; Kwon, S.Y.; Klavinskis, L.S. Long-lived tissue resident HIV-1 specific memory CD8+ T cells are generated by skin immunization with live virus vectored microneedle arrays. *J. Control. Release* **2017**, *268*, 166–175. [CrossRef]
24. Rouphael, N.G.; Paine, M.; Mosley, R.; Henry, S.; McAllister, D.V.; Kalluri, H.; Pewin, W.; Frew, P.M.; Yu, T.; Thornburg, N.J.; et al. TIV-MNP 2015 Study Group. The safety, immunogenicity, and acceptability of inactivated influenza vaccine delivered by microneedle patch (TIV-MNP 2015): A randomised, partly blinded, placebo-controlled, phase 1 trial. *Lancet* **2017**, *390*, 649–658. [CrossRef] [PubMed]
25. Güereña-Burgueño, F.; Hall, E.R.; Taylor, D.N.; Cassels, F.J.; Scott, D.A.; Wolf, M.K.; Roberts, Z.J.; Nesterova, G.V.; Alving, C.R.; Glenn, G.M. Safety and immunogenicity of a prototype enterotoxigenic Escherichia coli vaccine administered transcutaneously. *Infect. Immun.* **2002**, *70*, 1874–1880. [CrossRef] [PubMed]
26. Meyer, J.; Harris, S.A.; Satti, I.; Poulton, I.D.; Poyntz, H.C.; Tanner, R.; Rowland, R.; Griffiths, K.L.; Fletcher, H.A.; McShane, H. Comparing the safety and immunogenicity of a candidate TB vaccine MVA85A administered by intramuscular and intradermal delivery. *Vaccine* **2013**, *31*, 1026–1033. [CrossRef]
27. Nicolas, J.F.; Guy, B. Intradermal, epidermal and transcutaneous vaccination: From immunology to clinical practice. *Expert Rev. Vaccines* **2008**, *7*, 1201–1214. [CrossRef] [PubMed]
28. Fehres, C.M.; Garcia-Vallejo, J.J.; Unger, W.W.J.; van Kooyk, Y. Skin-resident antigen-presenting cells: Instruction manual for vaccine development. *Front. Immunol.* **2013**, *4*, 157. [CrossRef] [PubMed]
29. Haniffa, M.; Gunawan, M.; Jardine, L. Human skin dendritic cells in health and disease. *J. Dermatol. Sci.* **2015**, *77*, 85–92. [CrossRef] [PubMed]
30. Klareskog, L.; Tjernlund, U.; Forsum, U.; Peterson, P.A. Epidermal Langerhans cells express Ia antigens. *Nature* **1977**, *268*, 248–250. [CrossRef]

31. Levin, C.; Bonduelle, O.; Nuttens, C.; Primard, C.; Verrier, B.; Boissonnas, A.; Combadière, B. Critical Role for Skin-Derived Migratory DCs and Langerhans Cells in TFH and GC Responses after Intradermal Immunization. *J. Invest. Dermatol.* **2017**, *137*, 1905–1913. [CrossRef]
32. Levin, C.; Perrin, H.; Combadiere, B. Tailored immunity by skin antigen-presenting cells. *Hum. Vaccines Immunother.* **2014**, *11*, 27–36. [CrossRef]
33. Liard, C.; Munier, S.; Joulin-Giet, A.; Bonduelle, O.; Hadam, S.; Duffy, D.; Vogt, A.; Verrier, B.; Combadière, B. Intradermal Immunization Triggers Epidermal Langerhans Cell Mobilization Required for CD8 T-Cell Immune Responses. *J. Invest. Dermatol.* **2012**, *132 Pt 1*, 615–625. [CrossRef]
34. Vogt, A.; Mahé, B.; Costagliola, D.; Bonduelle, O.; Hadam, S.; Schaefer, G.; Schaefer, H.; Katlama, C.; Sterry, W.; Autran, B.; et al. Transcutaneous anti-influenza vaccination promotes both CD4 and CD8 T cell immune responses in humans. *J. Immunol.* **2008**, *180*, 1482–1489. [CrossRef] [PubMed]
35. Combadière, B.; Vogt, A.; Mahé, B.; Costagliola, D.; Hadam, S.; Bonduelle, O.; Sterry, W.; Staszewski, S.; Schaefer, H.; van der Werf, S.; et al. Preferential Amplification of CD8 Effector-T Cells after Transcutaneous Application of an Inactivated Influenza Vaccine: A Randomized Phase I Trial. *PLoS ONE* **2010**, *5*, e10818. [CrossRef]
36. Mutyambizi, K.; Berger, C.L.; Edelson, R.L. The balance between immunity and tolerance: The role of Langerhans cells. *Cell. Mol. Life Sci. CMLS* **2009**, *66*, 831–840. [CrossRef] [PubMed]
37. Klechevsky, E.; Morita, R.; Liu, M.; Cao, Y.; Coquery, S.; Thompson-Snipes, L.; Briere, F.; Chaussabel, D.; Zurawski, G.; Palucka, A.K.; et al. Functional specializations of human epidermal Langerhans cells and CD14+ dermal dendritic cells. *Immunity* **2008**, *29*, 497–510. [CrossRef]
38. Kissenpfennig, A.; Henri, S.; Dubois, B.; Laplace-Builhé, C.; Perrin, P.; Romani, N.; Tripp, C.H.; Douillard, P.; Leserman, L.; Kaiserlian, D.; et al. Dynamics and Function of Langerhans Cells In Vivo: Dermal Dendritic Cells Colonize Lymph Node AreasDistinct from Slower Migrating Langerhans Cells. *Immunity* **2005**, *22*, 643–654. [CrossRef] [PubMed]
39. Clark, R.A.; Chong, B.; Mirchandani, N.; Brinster, N.K.; Yamanaka, K.I.; Dowgiert, R.K.; Kupper, T.S. The vast majority of CLA+ T cells are resident in normal skin. *J. Immunol.* **2006**, *176*, 4431–4439. [CrossRef] [PubMed]
40. Allie, S.R.; Randall, T.D. Resident Memory B Cells. *Viral Immunol.* **2020**, *33*, 282–293. [CrossRef] [PubMed]
41. Mueller, S.N.; Zaid, A.; Carbone, F.R. Tissue-Resident T Cells: Dynamic Players in Skin Immunity. *Front. Immunol.* **2014**, *5*, 332. [CrossRef]
42. Foster, C.A.; Yokozeki, H.; Rappersberger, K.; Koning, F.; Volc-Platzer, B.; Rieger, A.; Coligan, J.E.; Wolff, K.; Stingl, G. Human epidermal T cells predominantly belong to the lineage expressing alpha/beta T cell receptor. *J. Exp. Med.* **1990**, *171*, 997–1013. [CrossRef]
43. Quaresma, J.A.S. Organization of the Skin Immune System and Compartmentalized Immune Responses in Infectious Diseases. *Clin. Microbiol. Rev.* **2019**, *32*, e00034-18. [CrossRef]
44. Hu, W.; Shang, R.; Yang, J.; Chen, C.; Liu, Z.; Liang, G.; He, W.; Luo, G. Skin γδ T Cells and Their Function in Wound Healing. *Front. Immunol.* **2022**, *13*, 875076. [CrossRef] [PubMed]
45. Mestas, J.; Hughes, C.C.W. Of Mice and Not Men: Differences between Mouse and Human Immunology. *J. Immunol.* **2004**, *172*, 2731–2738. [CrossRef] [PubMed]
46. Sallusto, F.; Lenig, D.; Förster, R.; Lipp, M.; Lanzavecchia, A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature* **1999**, *401*, 708–712. [CrossRef] [PubMed]
47. Debes, G.F.; McGettigan, S.E. Skin-associated B cells in health and inflammation. *J. Immunol.* **2019**, *202*, 1659–1666. [CrossRef] [PubMed]
48. Lawson, L.B.; Clements, J.D.; Freytag, L.C. Mucosal immune responses induced by transcutaneous vaccines. *Curr. Top. Microbiol. Immunol.* **2012**, *354*, 19–37. [PubMed]
49. Frey, C.F.; Bauhofer, O.; Ruggli, N.; Summerfield, A.; Hofmann, M.A.; Tratschin, J.D. Classical swine fever virus replicon particles lacking the Erns gene: A potential marker vaccine for intradermal application. *Vet. Res.* **2006**, *37*, 655–670. [CrossRef] [PubMed]
50. Le Luduec, J.B.; Debeer, S.; Piras, F.; Andréoni, C.; Boudet, F.; Laurent, P.; Kaiserlian, D.; Dubois, B. Intradermal vaccination with un-adjuvanted sub-unit vaccines triggers skin innate immunity and confers protective respiratory immunity in domestic swine. *Vaccine* **2016**, *34*, 914–922. [CrossRef]
51. Martelli, P.; Saleri, R.; Cavalli, V.; De Angelis, E.; Ferrari, L.; Benetti, M.; Ferrarini, G.; Merialdi, G.; Borghetti, P. Systemic and local immune response in pigs intradermally and intramuscularly injected with inactivated Mycoplasma hyopneumoniae vaccines. *Vet. Microbiol.* **2014**, *168*, 357–364. [CrossRef]
52. Vrdoljak, A.; Allen, E.A.; Ferrara, F.; Temperton, N.J.; Crean, A.M.; Moore, A.C. Induction of broad immunity by thermostabilised vaccines incorporated in dissolvable microneedles using novel fabrication methods. *J. Control. Release* **2016**, *225*, 192–204. [CrossRef]
53. Flynn, O.; Dillane, K.; Lanza, J.S.; Marshall, J.M.; Jin, J.; Silk, S.E.; Draper, S.J.; Moore, A.C. Low Adenovirus Vaccine Doses Administered to Skin Using Microneedle Patches Induce Better Functional Antibody Immunogenicity as Compared to Systemic Injection. *Vaccines* **2021**, *9*, 299. [CrossRef]
54. Zehrung, D.; Jarrahian, C.; Wales, A. Intradermal delivery for vaccine dose sparing: Overview of current issues. *Vaccine* **2013**, *31*, 3392–3395. [CrossRef] [PubMed]

55. Beraud, G. Shortages Without Frontiers: Antimicrobial Drug and Vaccine Shortages Impact Far Beyond the Individual! *Front. Med.* **2021**, *8*, 593712. [CrossRef] [PubMed]
56. Resik, S.; Mach, O.; Tejeda, A.; Jeyaseelan, V.; Fonseca, M.; Diaz, M.; Alemany, N.; Hung, L.H.; Aleman, Y.; Mesa, I.; et al. Immunogenicity of Intramuscular Fractional Dose of Inactivated Poliovirus Vaccine. *J. Infect. Dis.* **2020**, *221*, 895–901. [CrossRef] [PubMed]
57. Mashunye, T.R.; Ndwandwe, D.E.; Dube, K.R.; Shey, M.; Shelton, M.; Wiysonge, C.S. Fractional dose compared with standard dose inactivated poliovirus vaccine in children: A systematic review and meta-analysis. *Lancet Infect. Dis.* **2021**, *21*, 1161–1174. [CrossRef]
58. Resik, S.; Tejeda, A.; Mas Lago, P.; Diaz, M.; Carmenates, A.; Sarmiento, L.; Alemañi, N.; Galindo, B.; Burton, A.; Friede, M.; et al. Randomized Controlled Clinical Trial of Fractional Doses of Inactivated Poliovirus Vaccine Administered Intradermally by Needle-Free Device in Cuba. *J. Infect. Dis.* **2010**, *201*, 1344–1352. [CrossRef]
59. Anand, A.; Zaman, K.; Estívariz, C.F.; Yunus, M.; Gary, H.E.; Weldon, W.C.; Bari, T.I.; Steven Oberste, M.; Wassilak, S.G.; Luby, S.P.; et al. Early priming with inactivated poliovirus vaccine (IPV) and intradermal fractional dose IPV administered by a microneedle device: A randomized controlled trial. *Vaccine* **2015**, *33*, 6816–6822. [CrossRef]
60. Okayasu, H.; Sein, C.; Chang Blanc, D.; Gonzalez, A.R.; Zehrung, D.; Jarrahian, C.; Macklin, G.; Sutter, R.W. Intradermal Administration of Fractional Doses of Inactivated Poliovirus Vaccine: A Dose-Sparing Option for Polio Immunization. *J. Infect. Dis.* **2017**, *216* (Suppl. 1), S161–S167. [CrossRef]
61. Flynn, P.M.; Shenep, J.L.; Mao, L.; Crawford, R.; Williams, B.F.; Williams, B.G. Influence of Needle Gauge in Mantoux Skin Testing. *Chest* **1994**, *106*, 1463–1465. [CrossRef]
62. Lambert, P.H.; Laurent, P.E. Intradermal vaccine delivery: Will new delivery systems transform vaccine administration? *Vaccine* **2008**, *26*, 3197–3208. [CrossRef]
63. Pasteur, M.C.; Hall, D.R. The effects of inadvertent intramuscular injection of BCG vaccine. *Scand. J. Infect. Dis.* **2001**, *33*, 473–474.
64. Kis, E.E.; Winter, G.; Myschik, J. Devices for intradermal vaccination. *Vaccine* **2012**, *30*, 523–538. [CrossRef] [PubMed]
65. Resik, S.; Tejeda, A.; Mach, O.; Sein, C.; Molodecky, N.; Jarrahian, C.; Saganic, L.; Zehrung, D.; Fonseca, M.; Diaz, M.; et al. Needle-free jet injector intradermal delivery of fractional dose inactivated poliovirus vaccine: Association between injection quality and immunogenicity. *Vaccine* **2015**, *33*, 5873–5877. [CrossRef] [PubMed]
66. Barfoed, A.M.; Kristensen, B.; Dannemann-Jensen, T.; Viuff, B.; Bøtner, A.; Kamstrup, S.; Blixenkrone Møller, M. Influence of routes and administration parameters on antibody response of pigs following DNA vaccination. *Vaccine* **2004**, *22*, 1395–1405. [CrossRef] [PubMed]
67. Donadei, A.; Kraan, H.; Ophorst, O.; Flynn, O.; O'Mahony, C.; Soema, P.C.; Moore, A.C. Skin delivery of trivalent Sabin inactivated poliovirus vaccine using dissolvable microneedle patches induces neutralizing antibodies. *J. Control. Release* **2019**, *311–312*, 96–103. [CrossRef]
68. Lanza, J.S.; Vucen, S.; Flynn, O.; Donadei, A.; Cojean, S.; Loiseau, P.M.; Fernandes, A.P.S.M.; Frézard, F.; Moore, A.C. A TLR9-adjuvanted vaccine formulated into dissolvable microneedle patches or cationic liposomes protects against leishmaniasis after skin or subcutaneous immunization. *Int. J. Pharm.* **2020**, *586*, 119390. [CrossRef]
69. Momin, T.; Kansagra, K.; Patel, H.; Sharma, S.; Sharma, B.; Patel, J.; Mittal, R.; Sanmukhani, J.; Maithal, K.; Dey, A.; et al. Safety and Immunogenicity of a DNA SARS-CoV-2 vaccine (ZyCoV-D): Results of an open-label, non-randomized phase I part of phase I/II clinical study by intradermal route in healthy subjects in India. *eClinicalMedicine* **2021**, *38*, 101020. [CrossRef]
70. Hirobe, S.; Azukizawa, H.; Hanafusa, T.; Matsuo, K.; Quan, Y.S.; Kamiyama, F.; Katayama, I.; Okada, N.; Nakagawa, S. Clinical study and stability assessment of a novel transcutaneous influenza vaccination using a dissolving microneedle patch. *Biomaterials* **2015**, *57*, 50–58. [CrossRef]
71. Hirobe, S.; Azukizawa, H.; Matsuo, K.; Zhai, Y.; Quan, Y.S.; Kamiyama, F.; Suzuki, H.; Katayama, I.; Okada, N.; Nakagawa, S. Development and clinical study of a self-dissolving microneedle patch for transcutaneous immunization device. *Pharm. Res.* **2013**, *30*, 2664–2674. [CrossRef]
72. Ono, A.; Azukizawa, H.; Ito, S.; Nakamura, Y.; Asada, H.; Quan, Y.S.; Kamiyama, F.; Katayama, I.; Hirobe, S.; Okada, N. Development of novel double-decker microneedle patches for transcutaneous vaccine delivery. *Int. J. Pharm.* **2017**, *532*, 374–383. [CrossRef]
73. Frew, P.M.; Paine, M.B.; Rouphael, N.; Schamel, J.; Chung, Y.; Mulligan, M.J.; Prausnitz, M.R. Acceptability of an inactivated influenza vaccine delivered by microneedle patch: Results from a phase I clinical trial of safety, reactogenicity, and immunogenicity. *Vaccine* **2020**, *38*, 7175–7181. [CrossRef]
74. Fernando, G.J.P.; Hickling, J.; Jayashi Flores, C.M.; Griffin, P.; Anderson, C.D.; Skinner, S.R.; Davies, C.; Witham, K.; Pryor, M.; Bodle, J.; et al. Safety, tolerability, acceptability and immunogenicity of an influenza vaccine delivered to human skin by a novel high-density microprojection array patch (Nanopatch^{TM}). *Vaccine* **2018**, *36*, 3779–3788. [CrossRef] [PubMed]
75. Iwata, H.; Kakita, K.; Imafuku, K.; Takashima, S.; Haga, N.; Yamaguchi, Y.; Taguchi, K.; Oyamada, T. Safety and dose-sparing effect of Japanese encephalitis vaccine administered by microneedle patch in uninfected, healthy adults (MNA-J): A randomised, partly blinded, active-controlled, phase 1 trial. *Lancet Microbe* **2022**, *3*, e96–e104. [CrossRef] [PubMed]
76. Martelli, P.; Gozio, S.; Ferrari, L.; Rosina, S.; De Angelis, E.; Quintavalla, C.; Bottarelli, E.; Borghetti, P. Efficacy of a modified live porcine reproductive and respiratory syndrome virus (PRRSV) vaccine in pigs naturally exposed to a heterologous European (Italian cluster) field strain: Clinical protection and cell-mediated immunity. *Vaccine* **2009**, *27*, 3788–3799. [CrossRef] [PubMed]

77. Pandya, M.; Pacheco, J.M.; Bishop, E.; Kenney, M.; Milward, F.; Doel, T.; Golde, W.T. An alternate delivery system improves vaccine performance against foot-and-mouth disease virus (FMDV). *Vaccine* **2012**, *30*, 3106–3111. [CrossRef]
78. MSD Animal Health United Kingdom. IDAL Intradermal Vaccination [Internet]. Available online: https://www.msd-animal-health.co.uk/species/pigs/idal-intradermal-vaccination/ (accessed on 29 July 2022).
79. Hipradermic. Needle-free Device for Intradermal Vaccination [Internet]. Available online: https://hipradermic.com/hipradermic/ (accessed on 24 October 2022).
80. Gerdts, V.; Wilson, H.L.; Meurens, F.; van Drunen Littel-van den Hurk, S.; Wilson, D.; Walker, S.; Wheler, C.; Townsend, H.; Potter, A.A. Large Animal Models for Vaccine Development and Testing. *ILAR J.* **2015**, *56*, 53–62. [CrossRef]
81. Rajao, D.S.; Vincent, A.L. Swine as a model for influenza A virus infection and immunity. *ILAR J.* **2015**, *56*, 44–52. [CrossRef]
82. Meurens, F.; Summerfield, A.; Nauwynck, H.; Saif, L.; Gerdts, V. The pig: A model for human infectious diseases. *Trends Microbiol.* **2012**, *20*, 50–57. [CrossRef]
83. Ploemen, I.H.; Hirschberg, H.J.; Kraan, H.; Zeltner, A.; van Kuijk, S.; Lankveld, D.P.; Royals, M.; Kersten, G.F.; Amorij, J.P. Minipigs as an Animal Model for Dermal Vaccine Delivery. *Comp. Med.* **2014**, *64*, 50–54.
84. Swindle, M.M.; Makin, A.; Herron, A.J.; Clubb, F.J.; Frazier, K.S. Swine as models in biomedical research and toxicology testing. *Vet. Pathol.* **2012**, *49*, 344–356. [CrossRef]
85. Gutierrez, K.; Dicks, N.; Glanzner, W.; Agellon, L.; Bordignon, V. Efficacy of the porcine species in biomedical research. *Front. Genet.* **2015**, *6*, 293. [CrossRef]
86. Wernersson, R.; Schierup, M.H.; Jørgensen, F.G.; Gorodkin, J.; Panitz, F.; Stærfeldt, H.H.; Christensen, O.F.; Mailund, T.; Hornshøj, H.; Klein, A.; et al. Pigs in sequence space: A 0.66X coverage pig genome survey based on shotgun sequencing. *BMC Genom.* **2005**, *6*, 70. [CrossRef]
87. Rothkötter, H.J. Anatomical particularities of the porcine immune system—A physician's view. *Dev. Comp. Immunol.* **2009**, *33*, 267–272. [CrossRef] [PubMed]
88. Vreman, S.; Rebel, J.M.J.; McCaffrey, J.; Ledl, K.; Arkhipova, K.; Collins, D.; McDaid, D.; Savelkoul, H.F.J.; Skovgaard, K.; Moore, A.C.; et al. Early immune responses in skin and lymph node after skin delivery of Toll-like receptor agonists in neonatal and adult pigs. *Vaccine* **2021**, *39*, 1857–1869. [CrossRef] [PubMed]
89. Kong, R.; Bhargava, R. Characterization of porcine skin as a model for human skin studies using infrared spectroscopic imaging. *Analyst* **2011**, *136*, 2359–2366. [CrossRef] [PubMed]
90. Ranamukhaarachchi, S.A.; Lehnert, S.; Ranamukhaarachchi, S.L.; Sprenger, L.; Schneider, T.; Mansoor, I.; Rai, K.; Häfeli, U.O.; Stoeber, B. A micromechanical comparison of human and porcine skin before and after preservation by freezing for medical device development. *Sci. Rep.* **2016**, *6*, 32074. [CrossRef] [PubMed]
91. Summerfield, A.; Meurens, F.; Ricklin, M.E. The immunology of the porcine skin and its value as a model for human skin. *Mol. Immunol.* **2015**, *66*, 14–21. [CrossRef]
92. Romano, J.; Balaguer, L. Ultrastructural identification of Langerhans cells in normal swine epidermis. *J. Anat.* **1991**, *179*, 43–46.
93. Marquet, F.; Manh, T.P.V.; Maisonnasse, P.; Elhmouzi-Younes, J.; Urien, C.; Bouguyon, E.; Jouneau, L.; Bourge, M.; Simon, G.; Ezquerra, A.; et al. Pig Skin Includes Dendritic Cell Subsets Transcriptomically Related to Human CD1a and CD14 Dendritic Cells Presenting Different Migrating Behaviors and T Cell Activation Capacities. *J. Immunol.* **2014**, *193*, 5883–5893. [CrossRef]
94. Yang, H.; Parkhouse, R.M. Phenotypic classification of porcine lymphocyte subpopulations in blood and lymphoid tissues. *Immunology* **1996**, *89*, 76–83. [CrossRef]
95. Braun, R.O.; Python, S.; Summerfield, A. Porcine B Cell Subset Responses to Toll-like Receptor Ligands. *Front. Immunol.* **2017**, *8*, 1044. [CrossRef]
96. Takamatsu, H.H.; Denyer, M.S.; Stirling, C.; Cox, S.; Aggarwal, N.; Dash, P.; Wileman, T.E.; Barnett, P.V. Porcine γδ T cells: Possible roles on the innate and adaptive immune responses following virus infection. *Vet. Immunol. Immunopathol.* **2006**, *112*, 49–61. [CrossRef]
97. Saalmüller, A.; Reddehase, M.J.; Bühring, H.J.; Jonjić, S.; Koszinowski, U.H. Simultaneous expression of CD4 and CD8 antigens by a substantial proportion of resting porcine T lymphocytes. *Eur. J. Immunol.* **1987**, *17*, 1297–1301. [CrossRef] [PubMed]
98. Zuckermann, F.A.; Husmann, R.J. Functional and phenotypic analysis of porcine peripheral blood CD4/CD8 double-positive T cells. *Immunology* **1996**, *87*, 500–512. [PubMed]
99. Adisasmito, W.B.; Almuhairi, S.; Behravesh, C.B.; Bilivogui, P.; Bukachi, S.A.; Casas, N.; Cediel Becerra, N.; Charron, D.F.; Chaudhary, A.; One Health High-Level Expert Panel (OHHLEP); et al. One Health: A new definition for a sustainable and healthy future. *PLoS Pathog.* **2022**, *18*, e1010537.
100. Smith, G.J.D.; Vijaykrishna, D.; Bahl, J.; Lycett, S.J.; Worobey, M.; Pybus, O.G.; Ma, S.K.; Cheung, C.L.; Raghwani, J.; Bhatt, S.; et al. Origins and evolutionary genomics of the 2009 swine-origin H1N1 influenza A epidemic. *Nature* **2009**, *459*, 1122–1125. [CrossRef] [PubMed]
101. Mena, I.; Nelson, M.I.; Quezada-Monroy, F.; Dutta, J.; Cortes-Fernández, R.; Lara-Puente, J.H.; Castro-Peralta, F.; Cunha, L.F.; Trovão, N.S.; Lozano-Dubernard, B.; et al. Origins of the 2009 H1N1 influenza pandemic in swine in Mexico. *eLife* **2016**, *5*, e16777. [CrossRef] [PubMed]
102. Ito, T.; Couceiro, J.N.; Kelm, S.; Baum, L.G.; Krauss, S.; Castrucci, M.R.; Donatelli, I.; Kida, H.; Paulson, J.C.; Webster, R.G.; et al. Molecular basis for the generation in pigs of influenza A viruses with pandemic potential. *J. Virol.* **1998**, *72*, 7367–7373. [CrossRef]

103. Holzer, B.; Martini, V.; Edmans, M.; Tchilian, E. T and B Cell Immune Responses to Influenza Viruses in Pigs. *Front. Immunol.* **2019**, *10*, 98. [CrossRef]
104. EMA. European Medicines Agency. Medicines-Veterinary [Internet]. Available online: https://www.ema.europa.eu/en/medicines/field_ema_web_categories%253Aname_field/Veterinary (accessed on 22 September 2022).
105. HPRA. Veterinary Medicines Information [Internet]. Available online: http://www.hpra.ie/homepage/veterinary/veterinary-medicines-information/find-a-medicine?query=porcilis&field= (accessed on 14 December 2022).
106. New World Encyclopedia. Pig [Internet]. Available online: https://www.newworldencyclopedia.org/p/index.php?title=Pig&oldid=687347 (accessed on 25 October 2022).
107. Ganderup, N.C. Chapter 3—Minipig models for toxicity testing and biomarkers. In *Biomarkers in Toxicology*; Gupta, R.C., Ed.; Academic Press: Boston, MA, USA, 2014; pp. 71–91.
108. Temple, D.; Jiménez, M.; Escribano, D.; Martín-Valls, G.; Díaz, I.; Manteca, X. Welfare Benefits of Intradermal Vaccination of Piglets. *Animals* **2020**, *10*, E1898. [CrossRef]
109. Bragstad, K.; Vinner, L.; Hansen, M.S.; Nielsen, J.; Fomsgaard, A. A polyvalent influenza A DNA vaccine induces heterologous immunity and protects pigs against pandemic A(H1N1)pdm09 virus infection. *Vaccine* **2013**, *31*, 2281–2288. [CrossRef]
110. Borggren, M.; Nielsen, J.; Karlsson, I.; Dalgaard, T.S.; Trebbien, R.; Williams, J.A.; Fomsgaard, A. A polyvalent influenza DNA vaccine applied by needle-free intradermal delivery induces cross-reactive humoral and cellular immune responses in pigs. *Vaccine* **2016**, *34*, 3634–3640. [CrossRef] [PubMed]
111. Hewitt, J.S.; Karuppannan, A.K.; Tan, S.; Gauger, P.; Halbur, P.G.; Gerber, P.F.; De Groot, A.S.; Moise, L.; Opriessnig, T. A prime-boost concept using a T-cell epitope-driven DNA vaccine followed by a whole virus vaccine effectively protected pigs in the pandemic H1N1 pig challenge model. *Vaccine* **2019**, *37*, 4302–4309. [CrossRef] [PubMed]
112. Cui, J.; O'Connell, C.M.; Costa, A.; Pan, Y.; Smyth, J.A.; Verardi, P.H.; Burgess, D.J.; Van Kruiningen, H.J.; Garmendia, A.E. A PRRSV GP5-Mosaic vaccine: Protection of pigs from challenge and ex vivo detection of IFNγ responses against several genotype 2 strains. *PLoS ONE* **2019**, *14*, e0208801.
113. Tassis, P.D.; Papatsiros, V.G.; Nell, T.; Maes, D.; Alexopoulos, C.; Kyriakis, S.C.; Tzika, E.D. Clinical evaluation of intradermal vaccination against porcine enzootic pneumonia (Mycoplasma hyopneumoniae). *Vet. Rec.* **2012**, *170*, 261. [CrossRef]
114. Beard, C.; Ward, G.; Rieder, E.; Chinsangaram, J.; Grubman, M.J.; Mason, P.W. Development of DNA vaccines for foot-and-mouth disease, evaluation of vaccines encoding replicating and non-replicating nucleic acids in swine. *J. Biotechnol.* **1999**, *73*, 243–249. [CrossRef]
115. Sisteré-Oró, M.; López-Serrano, S.; Veljkovic, V.; Pina-Pedrero, S.; Vergara-Alert, J.; Córdoba, L.; Pérez-Maillo, M.; Pleguezuelos, P.; Vidal, E.; Segalés, J.; et al. DNA vaccine based on conserved HA-peptides induces strong immune response and rapidly clears influenza virus infection from vaccinated pigs. *PLoS ONE* **2019**, *14*, e0222201. [CrossRef]
116. Opriessnig, T.; Madson, D.M.; Prickett, J.R.; Kuhar, D.; Lunney, J.K.; Elsener, J.; Halbur, P.G. Effect of porcine circovirus type 2 (PCV2) vaccination on porcine reproductive and respiratory syndrome virus (PRRSV) and PCV2 coinfection. *Vet. Microbiol.* **2008**, *131*, 103–114. [CrossRef]
117. Hernandez-Franco, J.F.; Mosley, Y.Y.C.; Franco, J.; Ragland, D.; Yao, Y.; HogenEsch, H. Effective and Safe Stimulation of Humoral and Cell-Mediated Immunity by Intradermal Immunization with a Cyclic Dinucleotide/Nanoparticle Combination Adjuvant. *J. Immunol.* **2021**, *206*, 700–711. [CrossRef]
118. Larsen, D.L.; Olsen, C.W. Effects of DNA dose, route of vaccination, and coadministration of porcine interleukin-6 DNA on results of DNA vaccination against influenza virus infection in pigs. *Am. J. Vet. Res.* **2002**, *63*, 653–659. [CrossRef]
119. Jacobs, A.A.C.; Harks, F.; Pauwels, R.; Cao, Q.; Holtslag, H.; Pel, S.; Segers, R.P.A.M. Efficacy of a novel intradermal Lawsonia intracellularis vaccine in pigs against experimental infection and under field conditions. *Porc. Health Manag.* **2020**, *6*, 25. [CrossRef]
120. Dortmans, J.C.F.M.; Loeffen, W.L.A.; Weerdmeester, K.; van der Poel, W.H.M.; de Bruin, M.G.M. Efficacy of intradermally administrated E2 subunit vaccines in reducing horizontal transmission of classical swine fever virus. *Vaccine* **2008**, *26*, 1235–1242. [CrossRef] [PubMed]
121. Choe, S.; Park, G.N.; Song, S.; Shin, J.; Le, V.P.; Nguyen, V.G.; Kim, K.S.; Kim, H.K.; Hyun, B.H.; An, D.J. Efficacy of Needle-Less Intradermal Vaccination against Porcine Epidemic Diarrhea Virus. *Pathogens* **2021**, *10*, 1115. [CrossRef] [PubMed]
122. Subramaniam, S.; Cao, D.; Tian, D.; Cao, Q.M.; Overend, C.; Yugo, D.M.; Matzinger, S.R.; Rogers, A.J.; Heffron, C.L.; Catanzaro, N.; et al. Efficient priming of CD4 T cells by Langerin-expressing dendritic cells targeted with porcine epidemic diarrhea virus spike protein domains in pigs. *Virus. Res.* **2017**, *227*, 212–219. [CrossRef] [PubMed]
123. Bustamante-Córdova, L.; Reséndiz-Sandoval, M.; Hernández, J. Evaluation of a Recombinant Mouse X Pig Chimeric Anti-Porcine DEC205 Antibody Fused with Structural and Nonstructural Peptides of PRRS Virus. *Vaccines* **2019**, *7*, E43. [CrossRef]
124. Vanderpooten, A.; Goddeeris, B.; De Roose, P.; Hendrickx, L.; Bironf, P.; Desmettre, P. Evaluation of parenteral vaccination methods with glycoproteins against Aujeszky's disease in pigs. *Vet. Microbiol.* **1997**, *55*, 81–89. [CrossRef]
125. Ferrari, L.; Borghetti, P.; Gozio, S.; De Angelis, E.; Ballotta, L.; Smeets, J.; Blanchaert, A.; Martelli, P. Evaluation of the immune response induced by intradermal vaccination by using a needle-less system in comparison with the intramuscular route in conventional pigs. *Res. Vet. Sci.* **2011**, *90*, 64–71. [CrossRef]
126. van der Leek, M.L.; Feller, J.A.; Sorensen, G.; Isaacson, W.; Adams, C.L.; Borde, D.J.; Pfeiffer, N.; Tran, T.; Moyer, R.W.; Gibbs, E.P. Evaluation of swinepox virus as a vaccine vector in pigs using an Aujeszky's disease (pseudorabies) virus gene insert coding for glycoproteins gp50 and gp63. *Vet. Rec.* **1994**, *134*, 13–18. [CrossRef]

127. Jørgensen, J.B. Experimental infection with Mycobacterium avium, serotype 2, in pigs. 5. The immunizing effect of BCG vaccine against M. avium infection. *Acta Vet. Scand.* **1978**, *19*, 430–440. [CrossRef]
128. van Gennip, H.G.P.; Bouma, A.; van Rijn, P.A.; Widjojoatmodjo, M.N.; Moormann, R.J.M. Experimental non-transmissible marker vaccines for classical swine fever (CSF) by trans-complementation of E(rns) or E2 of CSFV. *Vaccine* **2002**, *20*, 1544–1556. [CrossRef]
129. Magiri, R.B.; Lai, K.J.; Mutwiri, G.K.; Wilson, H.L. Experimental PCEP-Adjuvanted Swine Influenza H1N1 Vaccine Induced Strong Immune Responses but Did Not Protect Piglets against Heterologous H3N2 Virus Challenge. *Vaccines* **2020**, *8*, E235. [CrossRef]
130. Eschbaumer, M.; Dill, V.; Carlson, J.C.; Arzt, J.; Stenfeldt, C.; Krug, P.W.; Hardham, J.M.; Stegner, J.E.; Rodriguez, L.L.; Rieder, E. Foot-and-Mouth Disease Virus Lacking the Leader Protein and Containing Two Negative DIVA Markers (FMDV LL3B3D A24) Is Highly Attenuated in Pigs. *Pathogens* **2020**, *9*, E129. [CrossRef] [PubMed]
131. Dory, D.; Rémond, M.; Béven, V.; Cariolet, R.; Zientara, S.; Jestin, A. Foot-and-Mouth Disease Virus neutralizing antibodies production induced by pcDNA3 and Sindbis virus based plasmid encoding FMDV P1-2A3C3D in swine. *Antivir. Res.* **2009**, *83*, 45–52. [CrossRef] [PubMed]
132. Cadenas-Fernández, E.; Sánchez-Vizcaíno, J.M.; van den Born, E.; Kosowska, A.; van Kilsdonk, E.; Fernández-Pacheco, P.; Gallardo, C.; Arias, M.; Barasona, J.A. High Doses of Inactivated African Swine Fever Virus Are Safe, but Do Not Confer Protection against a Virulent Challenge. *Vaccines* **2021**, *9*, 242. [CrossRef] [PubMed]
133. Martelli, P.; Saleri, R.; Andrani, M.; Cavalli, V.; De Angelis, E.; Ferrari, L.; Borghetti, P. Immune B cell responsiveness to single-dose intradermal vaccination against Mycoplasma hyopneumoniae. *Res. Vet. Sci.* **2021**, *141*, 66–75. [CrossRef]
134. Jiang, Y.; Li, X.; Yu, L.; Tong, W.; Chen, P.; Wang, S.; Zhao, K.; Tan, X.; Gao, F.; Yu, H.; et al. Immune efficacy of a candidate porcine reproductive and respiratory syndrome vaccine rHN-NP49 administered by a Needle-free intradermal delivery system in comparison with intramuscular injection. *Vaccine* **2021**, *39*, 5557–5562. [CrossRef]
135. Madapong, A.; Saeng-Chuto, K.; Chaikhumwang, P.; Tantituvanont, A.; Saardrak, K.; Pedrazuela Sanz, R.; Miranda Alvarez, J.; Nilubol, D. Immune response and protective efficacy of intramuscular and intradermal vaccination with porcine reproductive and respiratory syndrome virus 1 (PRRSV-1) modified live vaccine against highly pathogenic PRRSV-2 (HP-PRRSV-2) challenge, either alone or in combination with of PRRSV-1. *Vet. Microbiol.* **2020**, *244*, 108655.
136. Macklin, M.D.; McCabe, D.; McGregor, M.W.; Neumann, V.; Meyer, T.; Callan, R.; Hinshaw, V.S.; Swain, W.F. Immunization of pigs with a particle-mediated DNA vaccine to influenza A virus protects against challenge with homologous virus. *J. Virol.* **1998**, *72*, 1491–1496. [CrossRef]
137. Suter, R.; Summerfield, A.; Thomann-Harwood, L.J.; McCullough, K.C.; Tratschin, J.D.; Ruggli, N. Immunogenic and replicative properties of classical swine fever virus replicon particles modified to induce IFN-α/β and carry foreign genes. *Vaccine* **2011**, *29*, 1491–1503. [CrossRef]
138. Visser, N.; Egger, W.; Lütticken, D. Intradermal application of Aujeszky's disease virus strain Begonia with tocopherol-based adjuvant and a novel design injection device. *Acta Vet. Hung.* **1994**, *42*, 413–418.
139. Park, C.; Lee, M.S.; Baek, J.H.; Cho, S.H.; Hyun, B.H.; You, S.H.; Cha, S.H. Intradermal co-inoculation of codon pair deoptimization (CPD)-attenuated chimeric porcine reproductive and respiratory syndrome virus (PRRSV) with Toll like receptor (TLR) agonists enhanced the protective effects in pigs against heterologous challenge. *Vet. Microbiol.* **2021**, *256*, 109048. [CrossRef]
140. Eblé, P.L.; Weerdmeester, K.; van Hemert-Kluitenberg, F.; Dekker, A. Intradermal vaccination of pigs against FMD with 1/10 dose results in comparable vaccine efficacy as intramuscular vaccination with a full dose. *Vaccine* **2009**, *27*, 1272–1278. [CrossRef] [PubMed]
141. Ferrari, L.; Martelli, P.; Saleri, R.; De Angelis, E.; Cavalli, V.; Bresaola, M.; Benetti, M.; Borghetti, P. Lymphocyte activation as cytokine gene expression and secretion is related to the porcine reproductive and respiratory syndrome virus (PRRSV) isolate after in vitro homologous and heterologous recall of peripheral blood mononuclear cells (PBMC) from pigs vaccinated and exposed to natural infection. *Vet. Immunol. Immunopathol.* **2013**, *151*, 193–206. [PubMed]
142. Babiuk, S.; Baca-Estrada, M.E.; Foldvari, M.; Baizer, L.; Stout, R.; Storms, M.; Rabussay, D.; Widera, G.; Babiuk, L. Needle-free topical electroporation improves gene expression from plasmids administered in porcine skin. *Mol. Ther. J. Am. Soc. Gene Ther.* **2003**, *8*, 992–998. [CrossRef] [PubMed]
143. Hervé, P.L.; Descamps, D.; Deloizy, C.; Dhelft, V.; Laubreton, D.; Bouguyon, E.; Boukadiri, A.; Dubuquoy, C.; Larcher, T.; Benhamou, P.H.; et al. Non-invasive epicutaneous vaccine against Respiratory Syncytial Virus: Preclinical proof of concept. *J. Control. Release* **2016**, *243*, 146–159. [CrossRef] [PubMed]
144. Oreskovic, Z.; Kudlackova, H.; Krejci, J.; Nechvatalova, K.; Faldyna, M. Oil-based adjuvants delivered intradermally induce high primary IgG2 immune response in swine. *Res. Vet. Sci.* **2017**, *114*, 41–43. [CrossRef] [PubMed]
145. Maurer, R.; Stettler, P.; Ruggli, N.; Hofmann, M.A.; Tratschin, J.D. Oronasal vaccination with classical swine fever virus (CSFV) replicon particles with either partial or complete deletion of the E2 gene induces partial protection against lethal challenge with highly virulent CSFV. *Vaccine* **2005**, *23*, 3318–3328. [CrossRef] [PubMed]
146. Arrington, J.; Braun, R.P.; Dong, L.; Fuller, D.H.; Macklin, M.D.; Umlauf, S.W.; Wagner, S.J.; Wu, M.S.; Payne, L.G.; Haynes, J.R. Plasmid vectors encoding cholera toxin or the heat-labile enterotoxin from Escherichia coli are strong adjuvants for DNA vaccines. *J. Virol.* **2002**, *76*, 4536–4546. [CrossRef]
147. Melkebeek, V.; Verdonck, F.; Stuyven, E.; Goddeeris, B.; Cox, E. Plasmid-encoded GM-CSF induces priming of the F4(K88)-specific serum IgA response by FaeG DNA vaccination in pigs. *Vaccine* **2006**, *24*, 4592–4594. [CrossRef]

148. Gerdts, V.; Jöns, A.; Mettenleiter, T.C. Potency of an experimental DNA vaccine against Aujeszky's disease in pigs. *Vet. Microbiol.* **1999**, *66*, 1–13. [CrossRef]
149. Dincer, Z.; Jones, S.; Haworth, R. Preclinical safety assessment of a DNA vaccine using particle-mediated epidermal delivery in domestic pig, minipig and mouse. *Exp. Toxicol. Pathol.* **2006**, *57*, 351–357. [CrossRef]
150. Verfaillie, T.; Melkebeek, V.; Snoek, V.; Douterlungne, S.; Cox, E.; Verdonck, F.; Vanrompay, D.; Goddeeris, B.; Cox, E. Priming of piglets against enterotoxigenic E. coli F4 fimbriae by immunisation with FAEG DNA. *Vaccine* **2004**, *22*, 1640–1647. [CrossRef] [PubMed]
151. Martelli, P.; Cordioli, P.; Alborali, L.G.; Gozio, S.; De Angelis, E.; Ferrari, L.; Lombardi, G.; Borghetti, P. Protection and immune response in pigs intradermally vaccinated against porcine reproductive and respiratory syndrome (PRRS) and subsequently exposed to a heterologous European (Italian cluster) field strain. *Vaccine* **2007**, *25*, 3400–3408. [CrossRef]
152. Gerdts, V.; Jöns, A.; Makoschey, B.; Visser, N.; Mettenleiter, T.C. Protection of pigs against Aujeszky's disease by DNA vaccination. *J. Gen. Virol.* **1997**, *78 Pt 9*, 2139–2146. [CrossRef]
153. Lee, S.I.; Jeong, C.G.; Ul Salam Mattoo, S.; Nazki, S.; Prasad Aganja, R.; Kim, S.C.; Khatun, A.; Oh, Y.; Noh, S.H.; Lee, S.M.; et al. Protective immunity induced by concurrent intradermal injection of porcine circovirus type 2 and Mycoplasma hyopneumoniae inactivated vaccines in pigs. *Vaccine* **2021**, *39*, 6691–6699. [CrossRef] [PubMed]
154. Ko, E.Y.; Cho, J.; Cho, J.H.; Jo, K.; Lee, S.H.; Chung, Y.J.; Jung, S. Reduction in Lesion Incidence in Pork Carcass Using Transdermal Needle-free Injection of Foot-and-Mouth Disease Vaccine. *Korean J. Food Sci. Anim. Resour.* **2018**, *38*, 1155–1159. [CrossRef] [PubMed]
155. Madapong, A.; Saeng-Chuto, K.; Tantituvanont, A.; Nilubol, D. Safety of PRRSV-2 MLV vaccines administrated via the intramuscular or intradermal route and evaluation of PRRSV transmission upon needle-free and needle delivery. *Sci. Rep.* **2021**, *11*, 23107. [CrossRef] [PubMed]
156. Deloizy, C.; Fossum, E.; Barnier-Quer, C.; Urien, C.; Chrun, T.; Duval, A.; Codjovi, M.; Bouguyon, E.; Maisonnasse, P.; Hervé, P.L.; et al. The anti-influenza M2e antibody response is promoted by XCR1 targeting in pig skin. *Sci. Rep.* **2017**, *7*, 7639. [CrossRef] [PubMed]
157. Suradhat, S.; Wongyanin, P.; Sirisereewan, C.; Nedumpun, T.; Lumyai, M.; Triyarach, S.; Chaturavittawong, D.; Paphavasit, T.; Panyatong, R.; Thanawongnuwech, R. Transdermal delivery of plasmid encoding truncated nucleocapsid protein enhanced PRRSV-specific immune responses. *Vaccine* **2016**, *34*, 609–615. [CrossRef] [PubMed]
158. Vannier, P.; Cariolet, R. Vaccination of pigs against Aujeszky's disease by the intradermal route using live attenuated and inactivated virus vaccines. *Vet. Microbiol.* **1991**, *26*, 11–23. [CrossRef]
159. Melgoza-González, E.A.; Reséndiz-Sandoval, M.; Hinojosa-Trujillo, D.; Hernández-Valenzuela, S.; García-Vega, M.; Mata-Haro, V.; Tepale-Segura, A.; Bonifaz, L.C.; Perez-Torres, A.; Hernández, J. Antigen Targeting of Porcine Skin DEC205(+) Dendritic Cells. *Vaccines* **2022**, *10*, 684. [CrossRef]
160. Puig, A.; Bernal, I.; Sabaté, D.; Ballarà, I.; Montané, J.; Nodar, L.; Angelats, D.; Jordà, R. Comparison of effects of a single dose of MHYOSPHERE®PCV ID with three commercial porcine vaccine associations against Mycoplasma hyopneumoniae (Mhyo) and porcine circovirus type 2 (PCV2) on piglet growth during the nursery period under field conditions. *Vet. Res. Commun.* **2022**, *46*, 1167–1173. [CrossRef] [PubMed]
161. Lim, J.; Jin, M.; Yoon, I.; Yoo, H.S. Efficacy of bivalent vaccines of porcine circovirus type 2 and Mycoplasma hyopneumoniae in specific pathogen-free pigs challenged with porcine circovirus type 2d. *J. Vet. Sci.* **2022**, *23*, e49. [CrossRef] [PubMed]
162. The Pig Site. Assessment of the Economic Impact of PRRSV on United States Pork Producers [Internet]. Available online: https://www.thepigsite.com/articles/assessment-of-the-economic-impact-of-prrsv-on-united-states-pork-producers (accessed on 13 October 2022).
163. Pig333. PRRS Cost for the European Swine Industry [Internet]. Available online: https://www.pig333.com/articles/prrs-cost-for-the-european-swine-industry_10069/ (accessed on 26 October 2022).
164. HIPRA. UNISTRAIN®PRRS Vaccine against PRRS [Internet]. Available online: https://www.hipra.com/portal/en/hipra/animalhealth/products/detail/unistrain-prrs (accessed on 26 October 2022).
165. MSD Animal Health HUB. Porcilis®PRRS Lyophilisate and Solvent for Suspension for Injection for Pigs [Internet]. Available online: https://www.msd-animal-health-hub.co.uk/Products/Porcilis-PRRS (accessed on 26 October 2022).
166. Nan, Y.; Wu, C.; Gu, G.; Sun, W.; Zhang, Y.J.; Zhou, E.M. Improved Vaccine against PRRSV: Current Progress and Future Perspective. *Front. Microbiol.* **2017**, *8*, 1635. [CrossRef] [PubMed]
167. Tizard, I.R. Porcine vaccines. *Vaccines Vet.* **2021**, 225–242.e1. [CrossRef]
168. Temple, D.; Escribano, D.; Jiménez, M.; Mainau, E.; Cerón, J.J.; Manteca, X. Effect of the needle-free 'intra dermal application of liquids' vaccination on the welfare of pregnant sows. *Porc. Health Manag.* **2017**, *3*, 9. [CrossRef]
169. Van Reeth, K.; Vincent, A.L. Influenza Viruses. In *Diseases of Swine*; John Wiley & Sons, Ltd.: Hoboken, NJ, USA, 2019; pp. 576–593.
170. Mancera Gracia, J.C.; Pearce, D.S.; Masic, A.; Balasch, M. Influenza A Virus in Swine: Epidemiology, Challenges and Vaccination Strategies. *Front. Vet. Sci.* **2020**, *7*, 647. [CrossRef]
171. Paules, C.; Subbarao, K. Influenza. *Lancet* **2017**, *390*, 697–708. [CrossRef]
172. World Health Organization. Estimating Disease Burden of Influenza [Internet]. Available online: https://www.who.int/europe/activities/estimating-disease-burden-of-influenza (accessed on 13 October 2022).

173. Putri, W.C.W.S.; Muscatello, D.J.; Stockwell, M.S.; Newall, A.T. Economic burden of seasonal influenza in the United States. *Vaccine* **2018**, *36*, 3960–3966. [CrossRef]
174. Janke, B.H. Clinicopathological features of Swine influenza. *Curr. Top. Microbiol. Immunol.* **2013**, *370*, 69–83.
175. Baudon, E.; Peyre, M.; Peiris, M.; Cowling, B.J. Epidemiological features of influenza circulation in swine populations: A systematic review and meta-analysis. *PLoS ONE* **2017**, *12*, e0179044. [CrossRef]
176. World Health Organization. Gobla Influenza Programme Vaccines [Internet]. Available online: https://www.who.int/teams/global-influenza-programme/vaccines (accessed on 27 October 2022).
177. Ma, W.; Kahn, R.E.; Richt, J.A. The pig as a mixing vessel for influenza viruses: Human and veterinary implications. *J. Mol. Genet. Med. Int. J. Biomed. Res.* **2008**, *3*, 158–166. [CrossRef]
178. Wang, J.; Shah, D.; Chen, X.; Anderson, R.R.; Wu, M.X. A micro-sterile inflammation array as an adjuvant for influenza vaccines. *Nat. Commun.* **2014**, *5*, 4447. [CrossRef] [PubMed]
179. Engert, J.; Anamur, C.; Engelke, L.; Fellner, C.; Lell, P.; Henke, S.; Stadler, J.; Zöls, S.; Ritzmann, M.; Winter, G. A pilot study using a novel pyrotechnically driven prototype applicator for epidermal powder immunization in piglets. *Int. J. Pharm.* **2018**, *545*, 215–228. [CrossRef] [PubMed]
180. Bernelin-Cottet, C.; Deloizy, C.; Stanek, O.; Barc, C.; Bouguyon, E.; Urien, C.; Boulesteix, O.; Pezant, J.; Richard, C.A.; Moudjou, M.; et al. A Universal Influenza Vaccine Can Lead to Disease Exacerbation or Viral Control Depending on Delivery Strategies. *Front. Immunol.* **2016**, *7*, 641. [CrossRef]
181. Grodeland, G.; Fredriksen, A.B.; Løset, G.Å.; Vikse, E.; Fugger, L.; Bogen, B. Antigen Targeting to Human HLA Class II Molecules Increases Efficacy of DNA Vaccination. *J. Immunol.* **2016**, *197*, 3575–3585. [CrossRef]
182. Sisteré-Oró, M.; Vergara-Alert, J.; Stratmann, T.; López-Serrano, S.; Pina-Pedrero, S.; Córdoba, L.; Pérez-Maillo, M.; Pleguezuelos, P.; Vidal, E.; Veljkovic, V.; et al. Conserved HA-peptide NG34 formulated in pCMV-CTLA4-Ig reduces viral shedding in pigs after a heterosubtypic influenza virus SwH3N2 challenge. *PLoS ONE* **2019**, *14*, e0212431. [CrossRef]
183. Wang, J.; Li, B.; Wu, M.X. Effective and lesion-free cutaneous influenza vaccination. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 5005–5010. [CrossRef]
184. Eriksson, E.; Yao, F.; Svensjö, T.; Winkler, T.; Slama, J.; Macklin, M.D.; Andree, C.; McGregor, M.; Hinshaw, V.; Swain, W.F. In vivo gene transfer to skin and wound by microseeding. *J. Surg. Res.* **1998**, *78*, 85–91. [CrossRef]
185. Wei, J.C.; Cartmill, I.D.; Kendall, M.A.; Crichton, M.L. In vivo, in situ and ex vivo comparison of porcine skin for microprojection array penetration depth, delivery efficiency and elastic modulus assessment. *J. Mech. Behav. Biomed. Mater.* **2022**, *130*, 105187. [CrossRef]
186. Magiri, R.; Lai, K.; Chaffey, A.; Zhou, Y.; Pyo, H.M.; Gerdts, V.; Wilson, H.L.; Mutwiri, G. Intradermal immunization with inactivated swine influenza virus and adjuvant polydi(sodium carboxylatoethylphenoxy)phosphazene (PCEP) induced humoral and cell-mediated immunity and reduced lung viral titres in pigs. *Vaccine* **2018**, *36*, 1606–1613. [CrossRef]
187. Wang, J.; Li, P.; Wu, M.X. Natural STING Agonist as an 'Ideal' Adjuvant for Cutaneous Vaccination. *J. Invest. Dermatol.* **2016**, *136*, 2183–2191. [CrossRef] [PubMed]
188. Karlsson, I.; Borggren, M.; Rosenstierne, M.W.; Trebbien, R.; Williams, J.A.; Vidal, E.; Vergara-Alert, J.; Foz, D.S.; Darji, A.; Sisteré-Oró, M.; et al. Protective effect of a polyvalent influenza DNA vaccine in pigs. *Vet. Immunol. Immunopathol.* **2018**, *195*, 25–32. [CrossRef] [PubMed]
189. Lee, J.Y.S.; Wilson, M.R. A Review of Pseudorabies (Aujeszky's Disease) in Pigs. *Can. Vet. J.* **1979**, *20*, 65–69. [PubMed]
190. Wong, G.; Lu, J.; Zhang, W.; Gao, G.F. Pseudorabies virus: A neglected zoonotic pathogen in humans? *Emerg. Microbes Infect.* **2019**, *8*, 150–154. [CrossRef]
191. Liu, Y.; Chen, Q.; Rao, X.; Diao, X.; Yang, L.; Fang, X.; Hogeveen, H. An economic assessment of pseudorabies (Aujeszky' disease) elimination on hog farms in China. *Prev. Vet. Med.* **2019**, *163*, 24–30. [CrossRef]
192. Wang, D.; Tao, X.; Fei, M.; Chen, J.; Guo, W.; Li, P.; Wang, J. Human encephalitis caused by pseudorabies virus infection: A case report. *J. Neurovirol.* **2020**, *26*, 442–448. [CrossRef]
193. Maes, D.; Boyen, F.; Devriendt, B.; Kuhnert, P.; Summerfield, A.; Haesebrouck, F. Perspectives for improvement of Mycoplasma hyopneumoniae vaccines in pigs. *Vet. Res.* **2021**, *52*, 67. [CrossRef]
194. World Health Organization. Enterotoxigenic Escherichia coli (ETEC) [Internet]. Available online: https://www.who.int/teams/immunization-vaccines-and-biologicals/diseases/enterotoxigenic-escherichia-coli-(etec) (accessed on 27 October 2022).
195. Dubreuil, J.D. Pig vaccination strategies based on enterotoxigenic Escherichia coli toxins. *Braz. J. Microbiol. Publ. Braz. Soc. Microbiol.* **2021**, *52*, 2499–2509. [CrossRef]
196. Jongert, E.; Melkebeek, V.; De Craeye, S.; Dewit, J.; Verhelst, D.; Cox, E. An enhanced GRA1-GRA7 cocktail DNA vaccine primes anti-Toxoplasma immune responses in pigs. *Vaccine* **2008**, *26*, 1025–1031. [CrossRef]
197. Tian, F.; Lin, D.; Wu, J.; Gao, Y.; Zhang, D.; Ji, M.; Wu, G. Immune events associated with high level protection against Schistosoma japonicum infection in pigs immunized with UV-attenuated cercariae. *PLoS ONE* **2010**, *5*, e13408. [CrossRef]
198. Chen, H.; Nara, T.; Zeng, X.; Satoh, M.; Wu, G.; Jiang, W.; Yi, F.; Kojima, S.; Zhang, S.; Hirayama, K. Vaccination of domestic pig with recombinant paramyosin. against Schistosoma japonicum in China. *Vaccine* **2000**, *18*, 2142–2146. [CrossRef] [PubMed]
199. Oreskovic, Z.; Nechvatalova, K.; Krejci, J.; Kummer, V.; Faldyna, M. Aspects of intradermal immunization with different adjuvants: The role of dendritic cells and Th1/Th2 response. *PLoS ONE* **2019**, *14*, e0211896. [CrossRef] [PubMed]

200. Chen, Y.H.; Lai, K.Y.; Chiu, Y.H.; Wu, Y.W.; Shiau, A.L.; Chen, M.C. Implantable microneedles with an immune-boosting function for effective intradermal influenza vaccination. *Acta Biomater.* **2019**, *97*, 230–238. [CrossRef]
201. Chen, X.; Kositratna, G.; Zhou, C.; Manstein, D.; Wu, M.X. Micro-fractional epidermal powder delivery for improved skin vaccination. *J. Control. Release* **2014**, *192*, 310–316. [CrossRef]
202. McKay, P.F.; King, D.F.L.; Mann, J.F.S.; Barinaga, G.; Carter, D.; Shattock, R.J. TLR4 and TLR7/8 Adjuvant Combinations Generate Different Vaccine Antigen-Specific Immune Outcomes in Minipigs when Administered via the ID or IN Routes. *PLoS ONE* **2016**, *11*, e0148984. [CrossRef]
203. Bernardy, J.; Nechvatalova, K.; Krejci, J.; Kudlacková, H.; Brazdova, I.; Kucerova, Z.; Faldyna, M. Comparison of different doses of antigen for intradermal administration in pigs: The Actinobacillus pleuropneumoniae model. *Vaccine* **2008**, *26*, 6368–6372. [CrossRef]
204. Schautteet, K.; De Clercq, E.; Jönsson, Y.; Lagae, S.; Chiers, K.; Cox, E.; Vanrompay, D. Protection of pigs against genital Chlamydia trachomatis challenge by parenteral or mucosal DNA immunization. *Vaccine* **2012**, *30*, 2869–2881. [CrossRef]
205. D'apice, M.; Penha, A.M.; Cury, R. Vaccination against hog cholera with crystal violet vaccine by the intradermic route. *J. Am. Vet. Med. Assoc.* **1948**, *112*, 230–233.
206. Melkebeek, V.; Verdonck, F.; Goddeeris, B.M.; Cox, E. Comparison of immune responses in parenteral FaeG DNA primed pigs boosted orally with F4 protein or reimmunized with the DNA vaccine. *Vet. Immunol. Immunopathol.* **2007**, *116*, 199–214. [CrossRef]
207. Melkebeek, V.; Van den Broeck, W.; Verdonck, F.; Goddeeris, B.M.; Cox, E. Effect of plasmid DNA encoding the porcine granulocyte-macrophage colony-stimulating factor on antigen-presenting cells in pigs. *Vet. Immunol. Immunopathol.* **2008**, *125*, 354–360. [CrossRef]
208. Melkebeek, V.; Sonck, E.; Verdonck, F.; Goddeeris, B.M.; Cox, E. Optimized FaeG expression and a thermolabile enterotoxin DNA adjuvant enhance priming of an intestinal immune response by an FaeG DNA vaccine in pigs. *Clin. Vaccine Immunol. CVI* **2007**, *14*, 28–35. [CrossRef] [PubMed]
209. Benvenisti, L.; Rogel, A.; Kuznetzova, L.; Bujanover, S.; Becker, Y.; Stram, Y. Gene gun-mediate DNA vaccination against foot-and-mouth disease virus. *Vaccine* **2001**, *19*, 3885–3895. [CrossRef] [PubMed]
210. Anderson, E.C.; Masters, R.C.; Mowat, G.N. Immune response of pigs to inactivated foot-and-mouth disease vaccines. Response to emulsion vaccines. *Res. Vet. Sci.* **1971**, *12*, 342–350. [CrossRef] [PubMed]
211. Hwang, J.H.; Lee, K.N.; Kim, S.M.; Lee, G.; Moon, Y.; Kim, B.; Lee, J.S.; Park, J.H. Needleless intradermal vaccination for foot-and-mouth disease induced granuloma-free effective protection in pigs. *J. Vet. Sci.* **2019**, *20*, e29. [CrossRef]
212. Bergamin, F.; Saurer, L.; Neuhaus, V.; McCullough, K.C.; Summerfield, A. Porcine B-cell activating factor promotes anti-FMDV antibodies in vitro but not in vivo after DNA vaccination of pigs. *Vet. Immunol. Immunopathol.* **2007**, *120*, 115–123. [CrossRef]
213. Gosser, H.S.; Olson, L.D. Failure to immunize swine against streptococcic lymphadenitis with autogenous bacterins. *Am. J. Vet. Res.* **1973**, *34*, 129–130.
214. Poirier, D.; Renaud, F.; Dewar, V.; Strodiot, L.; Wauters, F.; Janimak, J.; Shimada, T.; Nomura, T.; Kabata, K.; Kuruma, K.; et al. Hepatitis B surface antigen incorporated in dissolvable microneedle array patch is antigenic and thermostable. *Biomaterials* **2017**, *145*, 256–265. [CrossRef]
215. Andrianov, A.K.; DeCollibus, D.P.; Gillis, H.A.; Kha, H.H.; Marin, A.; Prausnitz, M.R.; Babiuk, L.A.; Townsend, H.; Mutwiri, G. Poly[di(carboxylatophenoxy)phosphazene] is a potent adjuvant for intradermal immunization. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 18936–18941. [CrossRef]
216. Pilling, A.M.; Harman, R.M.; Jones, S.A.; McCormack, N.A.M.; Lavender, D.; Haworth, R. The assessment of local tolerance, acute toxicity, and DNA biodistribution following particle-mediated delivery of a DNA vaccine to minipigs. *Toxicol. Pathol.* **2002**, *30*, 298–305. [CrossRef]
217. Li, Y.P.; Kang, H.N.; Babiuk, L.A.; Liu, Q. Elicitation of strong immune responses by a DNA vaccine expressing a secreted form of hepatitis C virus envelope protein E2 in murine and porcine animal models. *World J. Gastroenterol.* **2006**, *12*, 7126–7135. [CrossRef]
218. Grubor-Bauk, B.; Yu, W.; Wijesundara, D.; Gummow, J.; Garrod, T.; Brennan, A.J.; Voskoboinik, I.; Gowans, E.J. Intradermal delivery of DNA encoding HCV NS3 and perforin elicits robust cell-mediated immunity in mice and pigs. *Gene Ther.* **2016**, *23*, 26–37. [CrossRef] [PubMed]
219. Christiansen, D.; Earnest-Silveira, L.; Grubor-Bauk, B.; Wijesundara, D.K.; Boo, I.; Ramsland, P.A.; Vincan, E.; Drummer, H.E.; Gowans, E.J.; Torresi, J. Pre-clinical evaluation of a quadrivalent HCV VLP vaccine in pigs following microneedle delivery. *Sci. Rep.* **2019**, *9*, 9251. [CrossRef] [PubMed]
220. Horsington, J.; Witvliet, M.; Jacobs, A.A.C.; Segers, R.P.A.M. Efficacy of Simultaneous Intradermal Vaccination of Swine against Porcine Circovirus 2, Porcine Reproductive and Respiratory Syndrome Virus, Mycoplasma hyopneumoniae and Lawsonia intracellularis. *Animals* **2021**, *11*, 2225. [CrossRef] [PubMed]
221. Renshaw, H.W.; Gessner, J.W.; Woodard, L.F.; Everson, D.O. Delayed-type skin hypersensitivity and in vitro lymphocyte immunostimulation responses of swine following inoculation with Mycobacterium avium cell walls and a mycobacterial immunopotentiating glycolipid. *Vet. Microbiol.* **1983**, *8*, 281–291. [CrossRef]
222. Beffort, L.; Weiß, C.; Fiebig, K.; Jolie, R.; Ritzmann, M.; Eddicks, M. Field study on the safety and efficacy of intradermal versus intramuscular vaccination against Mycoplasma hyopneumoniae. *Vet. Rec.* **2017**, *181*, 348. [CrossRef]

223. Bruffaerts, N.; Pedersen, L.E.; Vandermeulen, G.; Préat, V.; Stockhofe-Zurwieden, N.; Huygen, K.; Romano, M. Increased B and T Cell Responses in M. bovis Bacille Calmette-Guérin Vaccinated Pigs Co-Immunized with Plasmid DNA Encoding a Prototype Tuberculosis Antigen. *PLoS ONE* **2015**, *10*, e0132288. [CrossRef]
224. Imada, T.; Kawamura, H.; Nishimori, T.; Murata, H.; Narita, M.; Honda, Y.; Ishigaki, K. Evaluation of the pathogenicity and immunogenicity of vaccinia virus to piglets. *Nihon Juigaku Zasshi Jpn. J. Vet. Sci.* **1989**, *51*, 96–104. [CrossRef]
225. Dalmau, A.; Sánchez-Matamoros, A.; Molina, J.M.; Xercavins, A.; Varvaró-Porter, A.; Muñoz, I.; Moles, X.; Baulida, B.; Fàbrega, E.; Velarde, A.; et al. Intramuscular vs. Intradermic Needle-Free Vaccination in Piglets: Relevance for Animal Welfare Based on an Aversion Learning Test and Vocalizations. *Front. Vet. Sci.* **2021**, *8*, 715260. [CrossRef]
226. Bernelin-Cottet, C.; Urien, C.; McCaffrey, J.; Collins, D.; Donadei, A.; McDaid, D.; Jakob, V.; Barnier-Quer, C.; Collin, N.; Bouguyon, E.; et al. Electroporation of a nanoparticle-associated DNA vaccine induces higher inflammation and immunity compared to its delivery with microneedle patches in pigs. *J. Control. Release* **2019**, *308*, 14–28. [CrossRef]
227. Vreman, S.; Stockhofe-Zurwieden, N.; Popma-de Graaf, D.J.; Savelkoul, H.F.J.; Barnier-Quer, C.; Collin, N.; Collins, D.; McDaid, D.; Moore, A.C.; Rebel, J.M.J. Immune responses induced by inactivated porcine reproductive and respiratory syndrome virus (PRRSV) vaccine in neonatal pigs using different adjuvants. *Vet. Immunol. Immunopathol.* **2021**, *232*, 110170. [CrossRef]
228. Vreman, S.; McCaffrey, J.; Popma-de Graaf, D.J.; Nauwynck, H.; Savelkoul, H.F.J.; Moore, A.; Rebel, J.M.J.; Stockhofe-Zurwieden, N. Toll-like receptor agonists as adjuvants for inactivated porcine reproductive and respiratory syndrome virus (PRRSV) vaccine. *Vet. Immunol. Immunopathol.* **2019**, *212*, 27–37. [CrossRef] [PubMed]
229. Bernelin-Cottet, C.; Urien, C.; Stubsrud, E.; Jakob, V.; Bouguyon, E.; Bordet, E.; Barc, C.; Boulesteix, O.; Contreras, V.; Barnier-Quer, C.; et al. A DNA-Modified Live Vaccine Prime-Boost Strategy Broadens the T-Cell Response and Enhances the Antibody Response against the Porcine Reproductive and Respiratory Syndrome Virus. *Viruses* **2019**, *11*, E551. [CrossRef] [PubMed]
230. Bernelin-Cottet, C.; Urien, C.; Fretaud, M.; Langevin, C.; Trus, I.; Jouneau, L.; Blanc, F.; Leplat, J.J.; Barc, C.; Boulesteix, O.; et al. A DNA Prime Immuno-Potentiates a Modified Live Vaccine against the Porcine Reproductive and Respiratory Syndrome Virus but Does Not Improve Heterologous Protection. *Viruses* **2019**, *11*, 576. [CrossRef] [PubMed]
231. Gagnon, C.A.; Lachapelle, G.; Langelier, Y.; Massie, B.; Dea, S. Adenoviral-expressed GP5 of porcine respiratory and reproductive syndrome virus differs in its cellular maturation from the authentic viral protein but maintains known biological functions. *Arch. Virol.* **2003**, *148*, 951–972. [CrossRef]
232. Maragkakis, G.; Korou, L.M.; Chaintoutis, S.C.; Christodoulopoulos, G.; Dovas, C.I.; Perrea, D.; Athanasiou, L.V.; Konstantopoulos, P.; Maes, D.; Papatsiros, V.G. Investigation of Fas (APO-1)-Related Apoptosis in Piglets Intradermally or Intramuscularly Vaccinated with a Commercial PRRSV MLV. *Viral Immunol.* **2022**, *35*, 129–137. [CrossRef]
233. Stadler, J.; Naderer, L.; Beffort, L.; Ritzmann, M.; Emrich, D.; Hermanns, W.; Fiebig, K.; Saalmüller, A.; Gerner, W.; Glatthaar-Saalmüller, B.; et al. Safety and immune responses after intradermal application of Porcilis PRRS in either the neck or the perianal region. *PLoS ONE* **2018**, *13*, e0203560. [CrossRef]
234. Ferrari, M.; Brack, A.; Romanelli, M.G.; Mettenleiter, T.C.; Corradi, A.; Dal Mas, N.; Losio, M.N.; Silini, R.; Pinoni, C.; Pratelli, A. A study of the ability of a TK-negative and gI/gE-negative pseudorabies virus (PRV) mutant inoculated by different routes to protect pigs against PRV infection. *J. Vet. Med. B Infect. Dis. Vet. Public Health* **2000**, *47*, 753–762. [CrossRef]
235. van Rooij, E.M.; Haagmans, B.L.; de Visser, Y.E.; de Bruin, M.G.; Boersma, W.; Bianchi, A.T. Effect of vaccination route and composition of DNA vaccine on the induction of protective immunity against pseudorabies infection in pigs. *Vet. Immunol. Immunopathol.* **1998**, *66*, 113–126. [CrossRef]
236. Mikulska-Skupień, E.; Szweda, W.; Procajło, Z. Evaluation of specific humoral immune response in pigs vaccinated intradermally with deleted Aujeszky's disease vaccine and challenged with virulent strain of Herpesvirus suis type 1. *Pol. J. Vet. Sci.* **2005**, *8*, 11–16.
237. Artursson, K.; Lindersson, M.; Varela, N.; Scheynius, A.; Alm, G.V. Interferon-alpha production and tissue localization of interferon-alpha/beta producing cells after intradermal administration of Aujeszky's disease virus-infected cells in pigs. *Scand. J. Immunol.* **1995**, *41*, 121–129. [CrossRef]
238. Ferrari, M.; Gualandi, G.L.; Corradi, A.; Monaci, C.; Romanelli, M.G.; Losio, M.N.; Cantoni, A.M.; Pratelli, A. The response of pigs inoculated with a thymidine kinase-negative (TK-) pseudorabies virus to challenge infection with virulent virus. *Comp. Immunol. Microbiol. Infect. Dis.* **2000**, *23*, 15–26. [CrossRef] [PubMed]
239. Wang, Y.; Vlasova, A.; Velasquez, D.E.; Saif, L.J.; Kandasamy, S.; Kochba, E.; Levin, Y.; Jiang, B. Skin Vaccination against Rotavirus Using Microneedles: Proof of Concept in Gnotobiotic Piglets. *PLoS ONE* **2016**, *11*, e0166038. [CrossRef]
240. Opriessnig, T.; Mattei, A.A.; Karuppannan, A.K.; Halbur, P.G. Future perspectives on swine viral vaccines: Where are we headed? *Porc. Health Manag.* **2021**, *7*, 1. [CrossRef]
241. Redding, L.; Werner, D.B. DNA vaccines in veterinary use. *Expert Rev. Vaccines* **2009**, *8*, 1251–1276. [CrossRef] [PubMed]
242. Ebrahimi, M. DNA Vaccine and its Importance in Veterinary. *J. Dairy Vet. Sci.* **2019**, *11*, 1–2. [CrossRef]
243. Hobernik, D.; Bros, M. DNA Vaccines—How Far from Clinical Use? *Int. J. Mol. Sci.* **2018**, *19*, 3605. [CrossRef] [PubMed]
244. Accensi, F.; Bosch-Camós, L.; Monteagudo, P.L.; Rodríguez, F. DNA Vaccines in Pigs: From Immunization to Antigen Identification. *Methods Mol. Biol.* **2022**, *2465*, 109–124. [PubMed]
245. Gary, E.N.; Weiner, D.B. DNA vaccines: Prime time is now. *Curr. Opin. Immunol.* **2020**, *65*, 21–27. [CrossRef] [PubMed]

246. Dey, A.; Chozhavel Rajanathan, T.M.; Chandra, H.; Pericherla, H.P.R.; Kumar, S.; Choonia, H.S.; Bajpai, M.; Singh, A.K.; Sinha, A.; Saini, G.; et al. Immunogenic potential of DNA vaccine candidate, ZyCoV-D against SARS-CoV-2 in animal models. *Vaccine* **2021**, *39*, 4108–4116. [CrossRef] [PubMed]
247. Mallapaty, S. India's DNA COVID vaccine is a world first–more are coming. *Nature* **2021**, *597*, 161–162. [CrossRef] [PubMed]
248. European Medicines Agency. Adjuvants in Vaccines for Human Use [Internet]. 2018. Available online: https://www.ema.europa.eu/en/adjuvants-vaccines-human-use (accessed on 28 July 2022).
249. Madera, R.; Burakova, Y.; Shi, J. Emulsion Adjuvants for Use in Veterinary Vaccines. *Methods Mol. Biol.* **2022**, *2412*, 247–253.
250. Burakova, Y.; Madera, R.; McVey, S.; Schlup, J.R.; Shi, J. Adjuvants for Animal Vaccines. *Viral Immunol.* **2018**, *31*, 11–22. [CrossRef] [PubMed]
251. Fukanoki, S.; Iwakura, T.; Iwaki, S.; Matsumoto, K.; Takeda, R.; Ikeda, K.; Shi, Z.; Mori, H. Safety and efficacy of water-in-oil-in-water emulsion vaccines containing Newcastle disease virus haemagglutinin-neuraminidase glycoprotein. *Avian Pathol. J. WVPA* **2001**, *30*, 509–516. [CrossRef] [PubMed]
252. Buonavoglia, D.; Greco, G.; Quaranta, V.; Corrente, M.; Martella, V.; Decaro, N. An oil-emulsion vaccine induces full-protection against Mycoplasma agalactiae infection in sheep. *New Microbiol.* **2008**, *31*, 117–123. [PubMed]
253. Aucouturier, J.; Dupuis, L.; Ganne, V. Adjuvants designed for veterinary and human vaccines. *Vaccine* **2001**, *19*, 2666–2672. [CrossRef]
254. Vogelbruch, M.; Nuss, B.; Körner, M.; Kapp, A.; Kiehl, P.; Bohm, W. Aluminium-induced granulomas after inaccurate intradermal hyposensitization injections of aluminium-adsorbed depot preparations. *Allergy* **2000**, *55*, 883–887. [PubMed]
255. SEPPIC. Animal Species and Veterinary Vaccines [Internet]. Available online: https://www.seppic.com/en/animal-health/animal-species (accessed on 21 December 2022).
256. van Doorn, E.; Liu, H.; Huckriede, A.; Hak, E. Safety and tolerability evaluation of the use of Montanide ISA^{TM}51 as vaccine adjuvant: A systematic review. *Hum. Vaccines Immunother.* **2016**, *12*, 159–169. [CrossRef]
257. Nanishi, E.; Dowling, D.J.; Levy, O. Toward precision adjuvants: Optimizing science and safety. *Curr. Opin. Pediatr.* **2020**, *32*, 125–138. [CrossRef]
258. Vreman, S.; Auray, G.; Savelkoul, H.F.J.; Rebel, A.; Summerfield, A.; Stockhofe-Zurwieden, N. Neonatal porcine blood derived dendritic cell subsets show activation after TLR2 or TLR9 stimulation. *Dev. Comp. Immunol.* **2018**, *84*, 361–370. [CrossRef]
259. Di Pasquale, A.; Preiss, S.; Tavares Da Silva, F.; Garçon, N. Vaccine Adjuvants: From 1920 to 2015 and Beyond. *Vaccines* **2015**, *3*, 320–343. [CrossRef]
260. FDA. Route of Administration [Internet]. Available online: https://www.fda.gov/drugs/data-standards-manual-monographs/route-administration (accessed on 28 July 2022).
261. Sattler, T.; Pikalo, J.; Wodak, E.; Schmoll, F. Ability of ELISAs to detect antibodies against porcine respiratory and reproductive syndrome virus in serum of pigs after inactivated vaccination and subsequent challenge. *BMC Vet. Res.* **2016**, *12*, 259. [CrossRef]
262. Hingson, R.A.; Hughes, J.G. Clinical studies with jet injection; a new method of drug administration. *Curr. Res. Anesth. Analg.* **1947**, *26*, 221–230. [CrossRef] [PubMed]
263. Hingson, R.A.; Davis, H.S.; Bloomfield, R.A.; Brailey, R.F. Mass inoculation of the Salk polio vaccine with the multiple dose jet injector. *GP* **1957**, *15*, 94–96. [PubMed]
264. Plotkin, S.A.; Orenstein, W.A.; Offit, P.A.; Edwards, K.M. 9—General Immunization Practices. In *Plotkin's Vaccines*, 7th ed.; Elsevier: Amsterdam, The Netherlands, 2018; pp. 96–120.e6.
265. Chase, C.C.L.; Daniels, C.S.; Garcia, R.; Milward, F.; Nation, T. Needle-free injection technology in swine: Progress toward vaccine efficacy and pork quality. *J. Swine Health Prod.* **2008**, *16*, 8.
266. Kale, T.R.; Momin, M. Needle free injection technology—An overview. *Innov. Pharm.* **2014**, *5*, 192–199. [CrossRef]
267. Corporate Home Page—MSD Animal Health. The IDAL®Way, 20 Years and Counting [Internet]. Available online: https://www.msd-animal-health.com/the-idal-way-20-years-and-counting/ (accessed on 17 August 2022).
268. Diotti, R.A.; Caputo, V.; Sautto, G.A. 12—Conventional and nontraditional delivery methods and routes of vaccine administration. In *Practical Aspects of Vaccine Development*; Kolhe, P., Ohtake, S., Eds.; Academic Press: Cambridge, MA, USA, 2022; pp. 329–355.
269. Godbey, W.T. Chapter 13—Gene Delivery. In *An Introduction to Biotechnology*; Godbey, W.T., Ed.; Woodhead Publishing: Southton, UK, 2014; pp. 275–312.
270. Su, F.; Patel, G.B.; Hu, S.; Chen, W. Induction of mucosal immunity through systemic immunization: Phantom or reality? *Hum. Vaccines Immunother.* **2016**, *12*, 1070–1079. [CrossRef] [PubMed]
271. Hervé, C.; Laupèze, B.; Del Giudice, G.; Didierlaurent, A.M.; Tavares Da Silva, F. The how's and what's of vaccine reactogenicity. *NPJ Vaccines* **2019**, *4*, 39. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.