Applications of Platform Technologies in Veterinary Vaccinology and the benefits for One Health

Applications of Platform Technologies in Veterinary Vaccinology and the Benefits for One Health

Gary Entrican[1] & Michael James Francis[2]

[1]The Roslin Institute at The University of Edinburgh, Easter Bush Campus, Midlothian, EH25 9RG, UK;

[2]BioVacc Consulting Ltd, The Red House, 10 Market Square, Amersham, Buckinghamshire, HP7 0DQ, UK

[1]Corresponding author, email: gary.entrican@roslin.ed.ac.uk

Abstract:

The animal-human interface has played a central role in advances made in vaccinology for the past two centuries. Many traditional veterinary vaccines were developed by growing, attenuating, inactivating and fractioning the pathogen of interest. While such approaches have been very successful, we have reached a point where they have largely been exhausted and alternative approaches are required. Furthermore, although subunit vaccines have enhanced safety profiles and created opportunities for combined discrimination between vaccinated and infected animal (DIVA) approaches, their functionality has largely been limited to diseases that can be controlled by humoral immunity until very recently. We now have a new generation of adjuvants and delivery systems that can elicit CD4+ve and/or CD8+ve T cell responses in addition to high-titre antibody responses. We review the current vaccine platform technologies, describe their roles in veterinary vaccinology and discuss how knowledge of their mode of action allows informed decisions on their deployment with wider benefits for One Health.

Keywords:

Veterinary vaccines, platform technologies, One Health, nanoparticle vaccines, viral vectors, nucleic acid vaccines

1. Introduction

Vaccinology is rooted in a series of empirical discoveries and technological innovations that have progressively changed the ways in which vaccines have been designed and delivered to prevent and control disease. The animal-human interface has been a fundamental element of the advances made in vaccinology, from Edward Jenner's use of cowpox as a vaccine for human smallpox, to the derivation of Bacillus Calmette-Guérin (BCG) from cattle tubercule bacilli as a vaccine for human tuberculosis, through to the creation of novel vaccine vectors by genetic manipulation of animal and human pathogens.

The history of vaccinology has been broadly separated into two phases based on the technological developments. The first of these, the empirical era, reflects trial-and-error approaches that were reliant on the ability to isolate, attenuate, inactivate and fraction the pathogen of interest to generate vaccine antigens [1]. Although these approaches were essentially 'informed guesswork', particularly when it came to knowledge of the protective host immune response, they have nevertheless been very successful, with the majority of human and veterinary vaccines currently in use having been developed this way. The advent of recombinant DNA technologies in the early 1970s was a major advancement that impacted all areas of biology [2]. This coincided with innovations in information technologies for processing data and marked the beginning of the so-called rational era of vaccine development, providing the ability to synthesise genetic sequences, manipulate the genome of pathogens and express recombinant proteins in formats that closely mimicked those expressed during natural infection, removing the reliance on pathogen growth for vaccine production [1].

We now find ourselves in an era where new vaccine technologies are being rapidly developed and tested in humans and a range of veterinary species, with opportunities to reduce our reliance on small rodents as biomedical models and understand more about vaccine safety and efficacy in

different species. This is particularly valuable for vaccination against zoonotic pathogens that cross species barriers. We still have much to learn about host-pathogen interactions and immune responses to zoonotic pathogens from both the animal and human perspective, but by addressing this we can generate knowledge that can be applied to novel vaccine development that benefits both animal and human health, the so called 'One Health Vaccinology' approach [3], [4]. Here, we review the key technological advances that have historically underpinned vaccine design, we set out the definition of vaccine platform technologies and discuss them in the context of veterinary vaccinology and the global One Health agenda.

2. Vaccinology at the animal-human interface

The experimental demonstration by Jenner that cowpox could serve as a safe and protective human smallpox vaccine in the late 18th century was a monumental milestone in medicine, leading to the certification of global eradication of smallpox by the World Health Organisation (WHO) in 1980 [5]. Almost 100 years passed between Jenner's experiment and the next major milestone in vaccinology, made by Louis Pasteur, also using an animal disease. In the late 19th century, Pasteur made the serendipitous discovery that accidentally-attenuated *Pasteurella multocida* could protect against chicken cholera. This led him to develop the first deliberately-attenuated vaccine, not for chicken cholera but for another animal disease that was also zoonotic, anthrax. He demonstrated that inoculation with temperature-treated anthrax bacilli could protect ruminants from experimental challenge with virulent bacilli. This was the first vaccine against a zoonotic pathogen that could cause fatal infection in both animals and humans [6].

Pasteur's discovery triggered a rapid expansion in the development of attenuated vaccines for bacterial diseases. This was facilitated by the ability to isolate and culture bacteria in the laboratory, which preceded the ability to grow viruses. The first rabies vaccine was a notable exception as it was based on homogenates of infected tissues, not culture [7]. This was soon followed by the first inactivated and sub-component toxoid-based vaccines for clostridial diseases. These had safety

advantages over live vaccines, but were less immunogenic and hence required the incorporation of adjuvants for activation of effective immune responses [8]. The advent of tissue culture technologies in the mid-20th century paved the way for the application of these approaches to the development of live-attenuated and killed vaccines for viral diseases. This led to one of the greatest achievements in veterinary vaccinology, namely the global eradication of rinderpest based on the Plowright tissue culture rinderpest vaccine (TCRV). Crucially, the vaccine eradication programme was supported by innovations in diagnostic capability and morbillivirus phylogenetics [9]. A summary of the major achievements in vaccinology at the animal-human interface is provided in Table 1.

3. One Health

The World Health Organisation (WHO) defines One Health as 'an approach to designing and implementing programmes, policies, legislation and research in which multiple sectors communicate and work together to achieve better public health outcomes (https://www.who.int/news-room/questions-and-answers/item/one-health). The COVID-19 pandemic has reinforced the importance of the One Health agenda and why we cannot view human health, animal health and environmental health in isolation [10].

Zoonoses are specifically highlighted by WHO as an area where a One Health approach is particularly relevant. Zoonotic pathogens account for 75% of emerging human infections and pose a major global threat to animal health, human health and food security [4]. The WHO works closely with the Food and Agriculture Organization of the United Nations (FAO) and the World Organization for Animal Health (OIE) to promote multi-sector approaches to public health threats at the animal-human interface. OIE is one of the partners of the Strategic Alliance for Research into Infectious Diseases of Animals and Zoonoses (STAR-IDAZ) International Research Consortium (IRC) on Animal Health which coordinates animal research globally to accelerate delivery of disease control tools and strategies (https://www.star-idaz.net/). This includes an interactive generic research roadmap to

guide the development of vaccines driven by gap analyses for priority diseases [11]. This can be used in conjunction with a publicly-available interactive online tool that is designed to identify bottlenecks in veterinary vaccine development from the identification of the vaccine Target Product Profile (TPP) through the processes of discovery, product development and registration [12]. A summary roadmap of the key steps in the research phase of vaccine development described in these interactive online tools is shown in Figure 1.

4. **Vaccine platform technologies**

Vaccine platform technologies are defined as technologies that utilise a common backbone or vector to deliver specific antigens for vaccines against different diseases. This includes (but is not limited to) protein-based (e.g. virus-like particles [VLPs]), vector-based (e.g. viruses, bacteria, protozoa), nucleic-acid based (e.g. DNA and RNA) and replicon-based (e.g. self-amplifying RNA) as defined by the European Medicines Agency (EMA) Committee for Medicinal Products for Veterinary Use (CMVP) (https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-data-requirements-vaccine-platform-technology-master-files-vptmf_en.pdf). This specific definition of vaccine platform technologies relates directly to the EMA CMVP strategy to include a Vaccine Platform Technology Master File (PTMF) in their veterinary medicine's legislation. The underlying aim of that strategy is to accelerate the development of immunological veterinary medicinal products (IVMPs) by reducing administrative regulatory burdens while ensuring the highest levels of human and animal health and environmental protection. This will be achieved by treating the PTMF as a stand-alone component of the dossier for an IVMP which will essentially be unchanged irrespective of the antigen(s) introduced. Consequently, once certified, the PTMF can be used in different vaccine submissions that exploit a common platform technology.

The guidelines on data requirements for vaccine PTMFs issued by EMA CMVP came into effect on 28 January 2022. The underlying principle of these guidelines had already been adopted by the

Zoonotic Anticipation and Preparedness Initiative (ZAPI). ZAPI is a European Union public-private partnership supported by the Innovative Medicines Initiative and have drafted a PTMF for a novel multimeric protein scaffold particles (MPSP) platform technology developed as part of their One Health Approach strategy [13]. The MPSP platform technology is being used to develop vaccines against Middle East Respiratory Syndrome (MERS) virus (zoonotic), Rift Valley Fever (RVF) virus (zoonotic) and Schmallenberg virus (zoonotic potential).

In this review we are working to the EMA CVMP definition of vaccine platform technologies that have been adopted in 2022. The United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) issued guidelines for the licencing of veterinary vaccines that incorporate antigens that have been expressed from genes of interest using common platforms in 2018 (https://www.aphis.usda.gov/animal_health/vet_biologics/publications/memo_800_213.pdf). The USDA APHIS guidelines are also designed to accelerate the regulatory processes for veterinary vaccines manufactured using established platforms but don't specify a PTMF in the same way as the EMA CVMP guidelines (Table 1).

Although veterinary vaccines typically take a shorter time to develop than human vaccines (3-6 years as opposed to 10-15 years), the COVID-19 pandemic has demonstrated how regulatory processes that employ platform technologies for human vaccine development can be streamlined when required and the adoption of a PTMF for veterinary vaccines will shorten the process further [10].

The starting point for vaccine development is the construction of a TPP (Figure 2). This serves as decision-making tool to ensure that the final product meets the expectations of end-users, otherwise the vaccine is unlikely to be deployed [11], [12]. Safety is always paramount and efficacy is required, but many other factors influence vaccine development. The relative influence of these factors depends on the impact of the disease in question, the species of interest and the existence of alternative disease control methods such as biosecurity and diagnosis. Alternative methods should

not be viewed in isolation from vaccination. Indeed, the ability to discriminate between vaccinated and immune animals (DIVA) can be a major factor in policy decisions for the deployment of vaccines against diseases such as bovine tuberculosis. The capability of the newer vaccine platform technologies to elicit high-level humoral and cellular immune responses and to deliver multiple defined antigens simultaneously creates exciting opportunities for combined DIVA approaches to the prevention and control of veterinary diseases, including pathogens that exist as multiple serotypes.

5. Protein-based vaccine platform technologies

(a). Expression systems

E. coli was the first expression system to be extensively studied for expression of recombinant proteins [2] and was the system of choice for the first prokaryote-expressed veterinary vaccine to protect cats against feline leukaemia virus (FeLV), a retroviral pathogen. The FeLV gp70 surface protein was expressed in *E. coli* and delivered with aluminium hydroxide and Quil A (a purified derivative of saponin) to successfully protect cats by eliciting a strong humoral and anamnestic response following challenge [14]. This vaccine is notable in many ways, not least because there are still no anti-retroviral human vaccines thirty years on.

E. coli has been a hugely successful protein expression system, but one of the drawbacks for vaccine antigens is that the expressed recombinant proteins may not be conformationally reflective of the native proteins and therefore not elicit protective responses. For antigens where conformation and glycosylation is important for protection, eukaryotic expression systems such as yeast, insect or mammalian cells are preferable. Yeast expression has been used for human vaccines (described in the section on nanoparticles but has not been extensively used for veterinary vaccine production to date. In contrast, ovarian insect cells infected with a baculovirus vector expressing the antigen of interest have resulted in a number of veterinary vaccines. Baculoviruses possess a strong promoter that results in high yields of recombinant protein within infected cells. Furthermore, insect cell-expressed recombinant proteins undergo post-translational modification including glycosylation,

phosphorylation and signal peptide cleavage. Although the glycosylation is different to that produced in mammalian cells, this platform has been successfully used to produce commercial vaccines against diseases such as porcine circovirus type 2 and classical swine fever [15], [16].

Mammalian or avian cells would arguably be the best means of expressing recombinant proteins for veterinary viral vaccines as they are most likely to mimic the antigenic structural and glycosylation patterns the host encounters during natural infection. However, many of the mammalian cell expression systems studied to date have suffered from technical difficulties relating to poor stability of expression and low antigen yields. Although Chinese hamster ovary (CHO) cells have tended to be the mammalian expression cell line of choice, several other cell lines have been employed such as baby hamster kidney (BHK) cells, human embryo kidney (HEK) cells and Vero cells [17].

Expression systems based on plants or plant cell culture have the advantage of rapid large-scale and inexpensive production of relatively large amounts of recombinant proteins. The first licensed vaccine to use this expression system in tobacco plants was against Newcastle Disease Virus (NDV) infection in poultry and has been investigated for other veterinary vaccine applications in a number of species, including Infectious Bronchitis Virus (IBV), Infectious Bursal Disease Virus (IBDV), ETEC, Bovine Viral Disease (BVD) and Bovine Herpes Virus [17].

(b). Adjuvants

The success of a protein subunit vaccine depends on formulation and delivery of those proteins in a manner that elicits safe and effective immune responses. Mineral salts-based adjuvants have been used extensively in human and veterinary vaccinology with excellent safety profiles and primarily appear to stimulate humoral immunity, although their exact mechanism of action is not known but appears to differ between animals and humans [18]. The newer generation of adjuvants being used in veterinary vaccines includes various emulsion formulations of water-in-oil (W/O), oil-in water (O/W) and water-in-oil-in-water (W/O/W), saponins, polymers, Toll-like receptor (TLR) agonists and various cytokines [19]. The introduction of these formulations is being supported by detailed

immunological knowledge on their modes of action. Of particular interest is the ability to stimulate cellular immune responses in multiple species a way that mineral salts-based adjuvants do not. For example, an immune-stimulating complex (ISCOM)-based vaccine has been shown to elicit cellular recall responses and Th-1-type immunity typified by IFN-γ production in horses [20], liposomal formulations elicit cytotoxic CD8+ T cell responses in pigs [21] and water-in-oil emulsions elicit Th-1-type immunity typified by production of IFN-γ in sheep [22], which were not achievable with traditional mineral-salts based adjuvants.

(c). Nanoparticles

An alternative system for effectively stimulating immune responses is to deliver recombinant proteins in particulate format in the size range of 20-100nm. This includes virus-like particles (VLPs) composed of molecules that self-assemble into structures that mimic the size and shape of a virus but lack genetic material, and biodegradable polymeric nanoparticles that can be constructed from organic or inorganic materials The first-ever licensed vaccine using recombinant DNA technology in 1986 also happened to be the first VLP vaccine, a remarkable technological achievement. Recombinant hepatitis B surface antigen (HBsAg) was expressed in yeast and assembled into VLPs 22nm in diameter [23]. The preceding hepatitis B vaccine was based on inactivated virus collected from the plasma of infected donors. The recombinant vaccine was not only much easier to produce and safer, but more immunogenic, eliciting higher titres of neutralising antibodies (https://www.nature.com/articles/d42859-020-00016-5). We now know that VLPs are taken up by dendritic cells, stimulating CD4+ and CD8+ T cells to induce both cellular and humoral immune responses to the target antigen, explaining their high immunogenicity [24]. VLPs can be produced using bacterial, yeast, plant and mammalian cells, but the most suitable system to date has been insect cell expression [25]. That was the system of choice for the porcine circovirus 2 (PCV2) VLP vaccine which is based on a recombinant capsid protein of PCV2 expressed in insect cells [15]. The immunostimulatory features of VLPs coupled with the choice of expression system make them a

popular option for vaccine design against a range of pathogens in veterinary species [26]. They have been shown to be effective in prime-boost strategies such as for PCV2 in pigs, but have also been shown to confer protection in chickens against a lethal challenge with H7N9 influenza following a single injection [27].

Polymeric nanoparticles can by synthesised from natural (chitosan) and synthetic (polyester, polyanhydride and poly[diamosulphide]) materials and used to deliver encapsulated antigens that reach the lymphatic system for presentation in draining lymph nodes and induce humoral and cellular immunity following a single shot [28]. This relatively new technology for veterinary vaccinology is being evaluated for disease caused by a range of viral and bacterial pathogens including Bovine Viral Diarrhea Virus (BVDV-1), Bovine Respiratory Syncytial Virus (BRSV), Bovine ParaInfluenza 3 (BPI3V), Bovine Adenovirus, Bovine Herpesvirus-1 (BHV-1), *Mycobacterium avium* subspecies *paratuberculosis, Brucella abortus* and *Anaplasma marginale* [29].

6. Vaccine vector platforms

The success of the vaccine development programmes initiated in response to the COVID-19 pandemic have demonstrated the adaptability and functionality of viral-vector vaccine platforms in human medicine. However, vaccines using vector technologies were first introduced in veterinary medicine over thirty years ago and now cover a range of viral, bacterial and protozoan vector platform technologies [17].

The most extensively used vector platform technologies are those based on viruses. The majority of early studies in veterinary vaccinology focussed on replicating large DNA viruses, in particular poxviruses, herpesviruses and adenoviruses [30]. These were chosen for their ability to incorporate foreign genes without affecting their infectivity or ability to replicate *in vivo*. In the 1980s a rabies vaccine was developed by inserting the viral glycoprotein G into the poxvirus vaccinia and delivered to wildlife in the form of oral bait (Table 1). This vaccine has led to the virtual eradication of wildlife rabies within both Europe and the US, a huge success for One Health by reducing transmission of

rabies to humans [31]. Vaccinia has also been explored (but not yet licenced) for a number of other veterinary diseases including hog cholera, transmissible gastroenteritis, swine influenza, avian infectious bronchitis, feline leukaemia and Newcastle disease [17].

In spite of these successes, there are a number of potential problems associated with the use of vaccinia as a vector, which include concerns of recombination with other pox viruses under field conditions and, in spite of its excellent track record in the smallpox eradication campaign, occasional adverse reactions. Modified Vaccinia Ankara (MVA) is a very well-characterised attenuated derivation of vaccinia which has lost its ability to replicate in mammalian cells through repeated passage in avian cells, increasing its safety profile as a vaccine vector [32]. An experimental MVA bivalent vaccine has been shown to protect sheep against Rift Valley Fever (RVF) and Bluetongue virus (BTV) [33].

A number of other poxviruses have been evaluated as vectors for veterinary vaccinology including canarypox and fowlpox which are successfully used in a number of commercial avian and mammalian vaccines [16]. Despite their widespread use, relatively little is known about the precise immunological mechanisms by which poxvirus vectors induce immunity in different veterinary species. Most notably, although they are clearly efficient at inducing neutralising antibodies, their effects on cellular immunity are not well-defined.

Adenoviruses have been investigated intensely as potential vectors for gene delivery in humans, and as a result their properties are very well understood. They are highly-manipulable, allowing for the alteration of their replicative capacity, cell tropism, immunogenicity and the insertion of antigens of interest, making them a very portable vaccine platform technology. A range of human and animal viruses have been evaluated as potential vaccine vectors, perhaps the most notable being the replication-deficient chimpanzee adenovirus ChAdOx1 and human adenovirus serotype 26 (Ad26) vectors being used to vaccinate humans against SARS CoV-2 [34]. A particular feature of adenoviruses is their ability to infect or transduce myeloid cells, activate innate immune sensory

mechanisms and trigger adaptive immunity via efficient antigen processing and presentation via both MHC Class I and Class II, resulting in activation of CD8+ and CD4+ T cells and B cells for high antibody titres and effector cellular immunity against the antigen of interest [35]. A detailed understanding of the cellular effector mechanisms elicited by each platform can inform on their deployment depending on the disease in question. Notably, the adenoviral vectors being deployed to control the COVID-19 pandemic direct that cellular response towards a Th-1-type profile typified by production of IFN-γ, TNF-α and IL-2, associated with protective host responses to SARS CoV-2 infection [36].

Although adenoviral vectors have not been extensively used in veterinary species to date, the potential is clearly there and various vaccines are currently in development. Interestingly, the prime-boost strategy ultimately adopted for the SARS CoV-2 ChAdOx1 vaccine roll-out was partly based on immunological readouts from immunized pigs, a great example of One Health vaccinology in practice [37]. The ChAdOx1 vector has also been used to successfully protect ruminants against RVF abortion as a single-shot vaccine by eliciting neutralising antibodies [38]. That same vaccine Master Seed virus (the virus stock used to produce the vaccine) is also being used to develop a human vaccine to protect against RFV, another example of the benefits for One Health deriving from common vaccine design for controlling a zoonotic pathogen.

Among the many advantages of a single-shot immunization protocol for vector-based vaccines is the avoidance of anti-vector immunity. Several studies have demonstrated that pre-existing immunity to human Ad5 decreased the efficacy of its vector capacity to immunize humans against HIV and Ebola and that anti-Ad-neutralising antibodies and anti-Ad T cells can prevent transduction and kill transduced cells [34]. However, the effects of anti-vector immunity on responses to target antigens are by no means certain. The use of a homologous vector (Vesicular Stomatitis Virus [VSV]) to vaccinate humans against Lassa Fever and then Ebola resulted in protection against Ebola even though the recipients had detectable anti-VSV responses [39]. We are now witnessing the

emergence of data on anti-vector immunity as the SARS CoV-2 vaccine roll-out and the COVID-19 pandemic develop in tandem. There are some indications that anti-vector immunity could affect anti-SARS CoV-2 spike responses [36] but there are also data showing that detectable anti-vector responses to Ad26 do not correlate with anti-spike responses following vaccination [40], indicating that we still have much to learn regarding anti-vector immunity. Such knowledge will be very useful for informing on single-boost, prime-boost and multiple booster vaccine regimes.

Attenuated vaccines can be adapted to act as multivalent vaccine 'vectors' by inducing immunity to the primary target and an exogenous antigen of a different pathogen. This was the basis of the novel platform technology using the yellow fever vaccine as a backbone for insertion of exogenous genes to induce concomitant protection against dengue virus, West Nile virus and Japanese Encephalitis virus [41]. This approach has been used successfully in veterinary vaccinology. Herpesvirus of turkeys (HVT) is a ubiquitous, non-pathogenic virus of domestic turkeys, which is classified as the third serotype within the Marek's disease virus (MDV). HVT is also non-pathogenic in chickens, but it does induce a viremia which is associated with induction of protective immune responses against MDV1 and has been used as a live vaccine against Marek's disease since the late 1960s [30]. HVT has been modified to express antigens of other pathogens to create multivalent vaccines against Marek's and other diseases of poultry such as Newcastle Disease, infectious bursal disease and infectious laryngotracheitis [16]. These multivalent vector vaccines have established a new standard, offering end users ease of delivery and simultaneous protection against important poultry pathogens.

Bacteria and protozoa have also been investigated for their potential as veterinary vaccine vectors. They activate different arms of the immune response from viruses and can infect via different sites, such as mucosa. One such bacterium is *Salmonella typhimurium* which is related to *E. coli* and the systems for conjugation, transformation and transduction are therefore well-understood. Very recently, a vaccine utilising recombinant attenuated *Salmonella* vaccine technology has been developed to control necrotic enteritis caused by *Clostridium perfringens* Type A in chickens and can

be administered via drinking water or spray (https://www.huvepharma.com/news/article/huvepharma-inc-introduces-new-vaccine-to-combat-necrotic-enteritis/).

A further potential vector platform technology investigated for development of poultry vaccines is based on apicomplexan parasites, such as *Eimeria.* This approach is now being explored for the development of vaccines against *Campylobacter jejuni*, *Toxoplasma gondii*, Infectious Bursal Disease, Necrotic Enteritis and, potentially, other species of *Eimeria* [42]. Whilst there is currently no licenced vaccine available using this platform technology, the approach offers the possibility utilising of low-cost mass vaccine delivery methods (hatchery spray, in water and on feed) for endogenous and multiple exogenous antigen delivery against a variety of poultry pathogens.

7. Nucleic acid vaccine platforms

Nucleic acid vaccines are based on DNA or RNA encoding the antigen(s) of interest and can be delivered in various formats to induce immune responses. The first-ever licenced nucleic acid vaccine was a veterinary DNA vaccine, to protect Canadian Atlantic salmon against infectious haematopoietic necrosis virus disease in 2005 (Table 1) [43]. Although subsequent DNA vaccines have been licenced for use in fish and horses, they are yet to be widely deployed in veterinary species [44]. It has taken the COVID-19 pandemic for the first human DNA vaccine to be licenced, one which can be delivered using a high-pressure needle-free device pressed against the skin, offering practical advantages for mass vaccination [45]. In contrast to DNA vaccines, the focus has increasingly turned to RNA. There was a degree of scepticism regarding this approach due to the inherent instability of RNA. However, technological advances for stabilising and delivering RNA across cell membranes to allow translation of target antigens opened new avenues for vaccine design. The origins of this approach can be traced back to the late 1980s when strands of mRNA were mixed with fat droplets and delivered to human cells to synthesise proteins. This led to the concept that mRNA could be a vaccine platform technology, but uptake was limited initially [46].

Once again, it was the COVID-19 pandemic that accelerated the development and emergency approval of two new human mRNA vaccines. These vaccines are based on mRNA encoding SARS CoV-2 spike protein that is formulated within lipid nanoparticles for protected delivery to the cytosol. Both vaccines induce high titres of neutralising antibodies, with the prime-boost strategy inducing antibody titres exceeding those observed in COVID-19 convalescent sera. Like the adenoviral vector-based COVID-19 vaccines, the mRNA vaccines also induce CD4+ and CD8+ cellular immunity with a similar Th-1-type bias, although the magnitude of these cellular responses appear to be lower and more dependent on a booster dose [36]. Such knowledge supports informed decision-making on future deployment of these platforms, including their uptake for veterinary vaccinology. Of note, to date there are no licensed veterinary mRNA vaccines [47].

A related platform is self-amplifying(sa)RNA based on genetically engineered replicons that can be delivered as viral replicon particles (VRPs) with the saRNA packaged into the viral particle, or as a completely synthetic saRNA produced after *in vitro* transcription [48]. These saRNAs can express higher levels of antigen at lower doses compared to conventional mRNA, providing potential cost benefits. An alphavirus-based saRNA vector derived from Venezuelan equine encephalitis virus alphavirus has been evaluated for veterinary applications [44] and is the basis for two USDA-approved commercial pig vaccines for controlling infectious porcine endemic diarrhoea and swine influenza [16]. As for viral vectors, with saRNA there is a theoretical risk of induction of immunity against the products of genes involved amplification process of the platform itself.

8. Opportunities in veterinary vaccinology

The COVID-19 pandemic has rapidly accelerated the uptake of platform technologies in human medicine. The vaccine tracker operated by the London School of Hygiene and Tropical Medicine shows that there are more than 300 vaccines in development, with more than 100 in clinical trials (https://vac-lshtm.shinyapps.io/ncov_vaccine_landscape/). These include (but are not limited to) the

platform technologies described here (protein subunits, VLPs, viral vectors, DNA and RNA). An unprecedented scientific outcome of these efforts will be the detailed knowledge on how these different platforms perform against the same disease in terms of protection and associated immune responses. This will have enormous One Health benefits by allowing informed decisions to be made for future deployment of these platforms in human and veterinary vaccinology.

As we have seen, different platform technologies induce different types of immune response. Using this information, coupled with knowledge of the pathogen, functional knowledge of the protective host immune response and construction of the desired TPP, informed decisions can be made on novel vaccine design (Figure 2). Nevertheless, the availability of comprehensive data on immune responses to infection and vaccination in veterinary species remains an area where improvements can be made. Such research has been hampered by a lack of veterinary immunological reagents, but this is now being addressed through initiatives such as the Immunological Toolbox [49]. Ultimately, such studies will provide detailed knowledge on the comparative functionality of vaccine platform technologies across species, thereby benefitting One Health [4].

Above all, veterinary vaccines need to be practical for uptake and deployment by end-users. Practicality cannot be defined as one feature, rather it encapsulates a number of features that can directly influence each other (Figure 2). For example, the purchase cost of a vaccine will be influenced by the cost of goods for production, the number of shots required to elicit protective immunity and necessary infrastructure for delivery (such as cold chain). Thus, the possibility of an effective single-shot vaccine that could be delivered intranasally to induce protective mucosal immunity in livestock, such as the prototype BSRV polymeric nanoparticle vaccine, is a very important advancement in veterinary vaccine platform technologies [28].

9. Conclusion

Thus, it is clear from this review that there is a need for improved veterinary know-how and integration of knowledge on zoonotic threats that can be translated into global health policies for One Health benefits, including vaccination [50]. In addition, we need to be aware that non-zoonotic infectious diseases which only affect animals still have direct effects on animal welfare and food production systems. They can also exert environmental and societal effects that affect public health, well-being and the environment, such as climate change [51]. The timely introduction of proposed regulatory legislation with potential to accelerate registration of veterinary vaccines based on well-defined platform technologies offers exciting new opportunities to control animal diseases and have wider One Health benefits.

Acknowledgments

The authors thank Alex Morrow and Vish Nene from STAR-IDAZ IRC for critical review of the manuscript and Robert Taylor and Rachel Wood (CABI) for helping to prepare the manuscript for publication. GE is a member of STAR-IDAZ IRC Scientific Committee and The Secretariat for the STAR-IDAZ IRC (SIRCAH) is funded from the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement Number 727494.

Figure legends

Figure 1: Summary research roadmap for vaccine development

The route from disease identification to prototype vaccine development can be defined in a series of steps that involve characterisation of the pathogen, the host immune response, antigen production,

antigen delivery, evaluation of safety and efficacy. The multiple factors that determine the ultimate vaccine target product profile (TPP) and influence progression along the roadmap are described in Figure 2. Full, interactive research roadmaps for veterinary vaccine development can be found in references [11] and [12]. The pipeline for late-stage vaccine development and registration can also be found in reference [12].

Figure 2: Factors influencing the deployment of platform technologies in veterinary vaccinology

The construction of the vaccine target product profile (TPP) sets out the desired features for a successful vaccine and should be established at an early start of research and development. Safety and efficacy are essential over-arching criteria. Once the TPP is defined, multiple factors influence the design and ultimate uptake of a new vaccine by end-users. Abbreviations: TPP, Target Product Profile; DIVA, Discrimination Between Infected and Vaccinated Animals

References

[1] De Gregorio, E., Rappuoli, R. (2014). From empiricism to rational design: a personal perspective of the evolution of vaccine development. Nature Reviews Immunology, 14(7), 505–514.

[2] Berg, P, Baltimore, D., Boyer, H. W., Cohen, S.N., Davis, R. W., Hogness, D. S., Nathans, D., Roblin, R., Watson, J. D., Weissman, S., Zinder, N. D. (1974). Potential Biohazards of Recombinant DNA Molecules. Science, 185(4148), 303.

[3] Francis, M. J. (2017) Vaccination for One Health. International Journal of Vaccines and Vaccination, 4(5), 00090.

[4] Warimwe, G. M., Francis, M. J., Bowden, T. A., Thumbi, S. M., Charleston, B. (2021). Using cross-species vaccination approaches to counter emerging infectious diseases. Nature Reviews Immunology, 1–8. Advance online publication.

[5] Henderson, D. A. (2011). The eradication of smallpox – An overview of the past, present, and future. Vaccine, 29 Suppl 4, D7–D9.

[6] Sternbach, G. (2003). The history of anthrax. The Journal of Emergency Medicine, 24(4), 463–467.

[7] Hicks, D. J., Fooks, A. R., Johnson, N. (2012). Developments in rabies vaccines. Clinical and Experimental immunology, 169(3), 199–204.

[8] Di Pasquale, A., Preiss, S., Tavares Da Silva, F., Garçon, N. (2015). Vaccine Adjuvants: from 1920 to 2015 and Beyond. Vaccines, 3(2), 320–343.

[9] Roeder, P., Mariner, J., Kock, R. (2013). Rinderpest: the veterinary perspective on eradication. Philosophical Transactions of the Royal Society B, 368(1623), 20120139.

[10] Francis, M. J. (2021). How has Covid-19 changed our views of the One Health agenda? Veterinary Record, 188(9), 361.

[11] Entrican, G., Charlier, J., Dalton, L., Messori, S., Sharma, S., Taylor, R., Morrow, A. (2021). Construction of generic roadmaps for the strategic coordination of global research into infectious diseases of animals and zoonoses. Transboundary and Emerging Diseases, 68(3), 1513–1520.

[12] Francis, M. J. (2020). A Veterinary Vaccine Development Process Map to assist in the development of new vaccines. Vaccine, 38(29), 4512–4515.

[13] Vandeputte, J., Saville, M., Cavaleri, M., Friede, M., Hacker, A., Mueller, S. O., Rizzi, R., Smith, D., Thirstrup, S., Wagner, R., Baay, M., Neels, P. (2021). IABS/CEPI platform technology webinar: Is it possible to reduce the vaccine development time? Biologicals, 71, 55–60.

[14] Marciani, D. J., Kensil, C. R., Beltz, G. A., Hung, C. H., Cronier, J., Aubert, A. (1991). Genetically-engineered subunit vaccine against feline leukaemia virus: protective immune response in cats. Vaccine, 9(2), 89–96.

[15] Crisci, E., Bárcena, J., Montoya, M. (2012). Virus-like particles: the new frontier of vaccines for animal viral infections. Veterinary Immunology and Immunopathology, 148(3-4), 211–225.

[16] Aida, V., Pliasas, V. C., Neasham, P. J., North, J. F., McWhorter, K. L., Glover, S. R., Kyriakis, C. S. (2021). Novel vaccine technologies in veterinary medicine: a herald to human medicine vaccines. Frontiers in Veterinary Science, 8, 654289.

[17] Francis M. J. (2018). Recent Advances in Vaccine Technologies. Veterinary Clinics of North America: Small Animal Practice, 48(2), 231–241.

[18] Del Giudice, G., Rappuoli, R., Didierlaurent, A. M. (2018). Correlates of adjuvanticity: A review on adjuvants in licensed vaccines. Seminars in Immunology, 39, 14–21.

[19] Burakova, Y., Madera, R., McVey, S., Schlup, J. R., Shi, J. (2018). Adjuvants for Animal Vaccines. Viral Immunology, 31(1), 11–22.

[20] Paillot, R., Grimmett, H., Elton, D., Daly, J. M. (2008). Protection, systemic IFNgamma, and antibody responses induced by an ISCOM-based vaccine against a recent equine influenza virus in its natural host. Veterinary Research, 39(3), 21.

[21] Overgaard, N. H., Frøsig, T. M., Jakobsen, J. T., Buus, S., Andersen, M. H., Jungersen, G. (2017). Low antigen dose formulated in CAF09 adjuvant Favours a cytotoxic T-cell response following intraperitoneal immunization in Göttingen minipigs. Vaccine, 35(42), 5629–5636.

[22] Begg, D. J., Dhungyel, O., Naddi, A., Dhand, N. K., Plain, K. M., de Silva, K., Purdie, A. C., Whittington, R. J. (2019). The immunogenicity and tissue reactivity of *Mycobacterium avium* subsp *paratuberculosis* inactivated whole cell vaccine is dependent on the adjuvant used. Heliyon, 5(6), e01911.

[23] Mulder, A. M., Carragher, B., Towne, V., Meng, Y., Wang, Y., Dieter, L., Potter, C. S., Washabaugh, M. W., Sitrin, R. D., Zhao, Q. (2012). Toolbox for non-intrusive structural and functional analysis of recombinant VLP based vaccines: a case study with hepatitis B vaccine. PLoS ONE, 7(4), e33235.

[24] Brisse, M., Vrba, S. M., Kirk, N., Liang, Y., Ly, H. (2020). Emerging Concepts and Technologies in Vaccine Development. Frontiers in Immunology, 11, 583077.

[25] Nooraei, S., Bahrulolum, H., Hoseini, Z. S., Katalani, C., Hajizade, A., Easton, A. J., Ahmadian, G. (2021). Virus-like particles: preparation, immunogenicity and their roles as nanovaccines and drug nanocarriers. Journal of Nanobiotechnology, 19, 59.

[26] Choudhury, S. M., Ma, X., Dang, W., Li, Y., Zheng, H. (2021) Recent development of ruminant vaccine against viral diseases. Frontiers in Veterinary Science, 8, 697194.

[27] Li, J., Li, R., Zhang, Q., Peng, P., Wang, X., Gu, M., Hu, Z., Jiao, X., Peng, D., Hu, J., Liu, X. (2021). H7N9 influenza virus-like particle based on BEVS protects chickens from lethal challenge with highly pathogenic H7N9 avian influenza virus. Veterinary Microbiology, 258, 109106.

[28] McGill, J. L., Kelly, S. M., Kumar, P., Speckhart, S., Haughney, S. L., Henningson, J., Narasimhan, B., Sacco, R. E. (2018). Efficacy of mucosal polyanhydride nanovaccine against respiratory syncytial virus infection in the neonatal calf. Scientific Reports, 8, 3021.

[29] Maina, T. W., Grego, E. A., Boggiatto, P. M., Sacco, R. E., Narasimhan, B., McGill, J. L. (2020). Applications of nanovaccines for disease prevention in cattle. Frontiers in Bioengineering and Biotechnology, 8, 608050.

[30] Baron, M. D., Iqbal, M., Nair, V. (2018). Recent advances in viral vectors in veterinary vaccinology. Current Opinion in Virology, 29, 1–7.

[31] Maki, J., Guiot, A. L., Aubert, M., Brochier, B., Cliquet, F., Hanlon, C. A., King, R., Oertli, E. H., Rupprecht, C. E., Schumacher, C., Slate, D., Yakobson, B., Wohlers, A., Lankau, E. W. (2017). Oral

vaccination of wildlife using a vaccinia-rabies-glycoprotein recombinant virus vaccine (RABORAL V-RG®): a global review. Veterinary Research, 48, 57.

[32] Volz, A., Sutter, G. (2017). Modified Vaccinia Virus Ankara: History, value in basic research, and current perspectives for vaccine development. Advances in Virus Research, 97, 187–243.

[33] Calvo-Pinilla, E., Marín-López, A., Moreno, S., Lorenzo, G., Utrilla-Trigo, S., Jiménez-Cabello, L., Benavides, J., Nogales, A., Blasco, R., Brun, A., Ortego, J. (2020). A protective bivalent vaccine against Rift Valley fever and bluetongue. NPJ Vaccines, 5, 70.

[34] Mendonça, S. A., Lorincz, R., Boucher, P., Curiel, D. T. (2021). Adenoviral vector vaccine platforms in the SARS-CoV-2 pandemic. NPJ Vaccines, 6, 97.

[35] Coughlan, L. (2020). Factors which contribute to the immunogenicity of non-replicating adenoviral vectored vaccines. Frontiers in Immunology, 11, 909.

[36] Sadarangani, M., Marchant, A., Kollmann, T. R. (2021). Immunological mechanisms of vaccine-induced protection against COVID-19 in humans. Nature Reviews Immunology, 21, 475–484.

[37] Graham, S. P., McLean, R. K., Spencer, A. J., Belij-Rammerstorfer, S., Wright, D., Ulaszewska, M., Edwards, J. C., Hayes, J., Martini, V., Thakur, N., Conceicao, C., Dietrich, I., Shelton, H., Waters, R., Ludi, A., Wilsden, G., Browning, C., Bialy, D., Bhat, S., Stevenson-Leggett, P., Hollinghurst, P., Gilbride, C., Pulido, D., Moffat, K., Sharpe, H., Allen, E., Mioulet, V., Chiu, C., Newman, J., Asfor, A. S., Burman, A., Crossley, S., Huo, J., Owens, R. J., Carroll, M., Hammond, J. A., Tchilian, E., Bailey, D., Charleston, B., Gilbert, S. C., Tuthill, T. J., Lambe, T. (2020). Evaluation of the immunogenicity of prime-boost vaccination with the replication-deficient viral vectored COVID-19 vaccine candidate ChAdOx1 nCoV-19. NPJ Vaccines, 5, 69.

[38] Stedman, A., Wright, D., Wichgers Schreur, P. J., Clark, M., Hill, A., Gilbert, S. C., Francis, M. J., van Keulen, L., Kortekaas, J., Charleston, B., Warimwe, G. M. (2019). Safety and efficacy of ChAdOx1 RVF vaccine against Rift Valley fever in pregnant sheep and goats. NPJ Vaccines, 4, 44.

[39] Poetsch, J. H., Dahlke, C., Zinser, M. E., Kasonta, R., Lunemann, S., Rechtien, A., Ly, M. L., Stubbe, H. C., Krähling, V., Biedenkopf, N., Eickmann, M., Fehling, S. K., Olearo, F., Strecker, T., Sharma, P., Lang, K. S., Lohse, A. W., Schmiedel, S., Becker, S., VSV-Ebola Consortium (VEBCON), Addo, M. M. (2019). Detectable vesicular stomatitis virus (VSV)-specific humoral and cellular immune responses following VSV-Ebola virus vaccination in humans. The Journal of Infectious Diseases, 219(4), 556–561.

[40] Sadoff, J., Le Gars, M., Shukarev, G., Heerwegh, D., Truyers, C., de Groot, A. M., Stoop, J., Tete, S., Van Damme, W., Leroux-Roels, I., Berghmans, P. J., Kimmel, M., Van Damme, P., de Hoon, J., Smith, W., Stephenson, K. E., De Rosa, S. C., Cohen, K. W., McElrath, M. J., Cormier, E., Scheper, G., Barouch, D. H., Hendriks, J., Struyf, F., Douoguih, M., Van Hoof, J., Schuitemaker, H. (2021). Interim results of a phase 1-2a trial of Ad26.COV2.S Covid-19 vaccine. New England Journal of Medicine, 384(19), 1824–1835.

[41] Guy, B., Guirakhoo, F., Barban, V., Higgs, S., Monath, T. P., Lang, J. (2010). Preclinical and clinical development of YFV 17D-based chimeric vaccines against dengue, West Nile and Japanese encephalitis viruses. Vaccine, 28(3), 632–649.

[42] Francis, M. J. (2021). Spotlight on avian pathology: the importance of recombinant vector platform technologies in poultry vaccination. Avian Pathology, 50(2), 109–111.

[43] Tonheim, T. C., Bøgwald, J., Dalmo, R. A. (2008). What happens to the DNA vaccine in fish? A review of current knowledge. Fish and Shellfish Immunology, 25(1-2), 1–18.

[44] Dory, D. Jestin, A. (2021). Vaccins à ADN et à ARN: des technologies également utilisées en vaccinology vétérinaire [DNA and RNA vaccines: technologies also used in veterinary vaccinology]. Bulletin de l'Académie Vétérinaire de France, https://doi.org/10.3406/bavf.2021.70927

[45] Momin, T., Kansagra, K., Patel, H., Sharma, S., Sharma, B., Patel, J., Mittal, R., Sanmukhani, J., Maithal, K., Dey, A., Chandra, H., Rajanathan, C. T., Pericherla, H. P., Kumar, P., Narkhede, A.,

Parmar, D. (2021). 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, 38, 101020.

[46] Pardi, N., Hogan, M. J., Porter, F. W., Weissman, D. (2018). mRNA vaccines - a new era in vaccinology. Nature Reviews Drug Discovery, 17(4), 261–279.

[47] Fomsgaard, A., Liu, M. A. (2021). The Key Role of Nucleic Acid Vaccines for One Health. Viruses, 13(2), 258.

[48] Tregoning, J. S., Brown, E. S., Cheeseman, H. M., Flight, K. E., Higham, S. L., Lemm, N. M., Pierce, B. F., Stirling, D. C., Wang, Z., Pollock, K. M. (2020). Vaccines for COVID-19. Clinical and Experimental Immunology, 202(2), 162–192.

[49] Mwangi, W., Maccari, G., Hope, J. C., Entrican, G., Hammond, J. A. (2020). The UK Veterinary Immunological Toolbox Website: promoting vaccine research by facilitating communication and removing reagent barriers. Immunology, 161(1), 25–27.

[50] Bowsher, G., McNamara, T., Bernard, R., Sullivan, R. (2021). Veterinary intelligence: integrating zoonotic threats into global health security. Journal of the Royal Society of Medicine, 1410768211035355. Advance online publication. https://doi.org/10.1177/01410768211035355

[51] Vermeulen, S. J., Campbell, B. M., Ingram, J. S. I. (2012). Climate Change and Food Systems. Annual Review of Environment and Resources, 37, 195-222.

Table 1: Landmark technological advances in vaccinology at the animal-human interface with One Health benefits

Date	Technological achievements in veterinary vaccinology and impacts	References
1796	Edward Jenner conducts experimental human vaccination with cowpox followed by challenge infection with smallpox, ultimately leading to the declaration of global eradication of smallpox in 1980.	[5]
1881	Louis Pasteur attenuates anthrax bacilli and demonstrates protection in a successful vaccination/challenge experiment in cattle, the first deliberately-attenuated live vaccine.	[6]
1960	Sir Walter Plowright develops the TCRV that ultimately results in the declaration of the global eradication of rinderpest in 2011.	[9]
1987	Incorporation of an exogenous protein into a viral vector to protect a veterinary species, employed for wildlife vaccination resulting in the eradication of rabies in Europe.	[17]
1991	The first prokaryotic-expressed protein subunit veterinary vaccine to protect cats against FeLV, remains a leader in retroviral vaccine development.	[14]
2005	A DNA vaccine to protect fish against IHNV disease, the first ever genetic vaccine to be licensed.	[43]
2006	A plant-based vaccine is licensed to protect chickens against NDV, the first ever vaccine incorporating a plant-expressed recombinant protein.	[17]
2018	USDA APHIS issues guidelines for the licencing of veterinary vaccines that incorporate antigens that have been expressed from genes of interest using common platforms.	[*]
2022	EMA CMVP introduces guidelines for incorporation of a PTMF into regulatory processes thereby accelerating the route to market for novel vaccines based on validated platform technologies.	[13], [**]

Major technological advances and achievements in veterinary vaccinology leading to the inclusion of a Platform Technology Master File (PTMF) in veterinary vaccine legislation. For a full history of vaccine development see reference [1]. For information on currently-licenced commercial veterinary vaccines utilising different platform technologies see reference [16]. Abbreviations: APHIS: Animal and Plant Health Inspection Service; EMA: European Medicines Agency; Committee for Medicinal Products for Veterinary Use; FeLV: Feline Leukaemia Virus; IHNV: Infectious Haematopoietic Necrosis Virus; TCRV: Tissue Culture Rinderpest Vaccine; USDA: United States Department of Agriculture.

*(https://www.aphis.usda.gov/animal_health/vet_biologics/publications/memo_800_213.pdf);

**(https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-data-requirements-vaccine-platform-technology-master-files-vptmf_en.pdf)

Figure 1

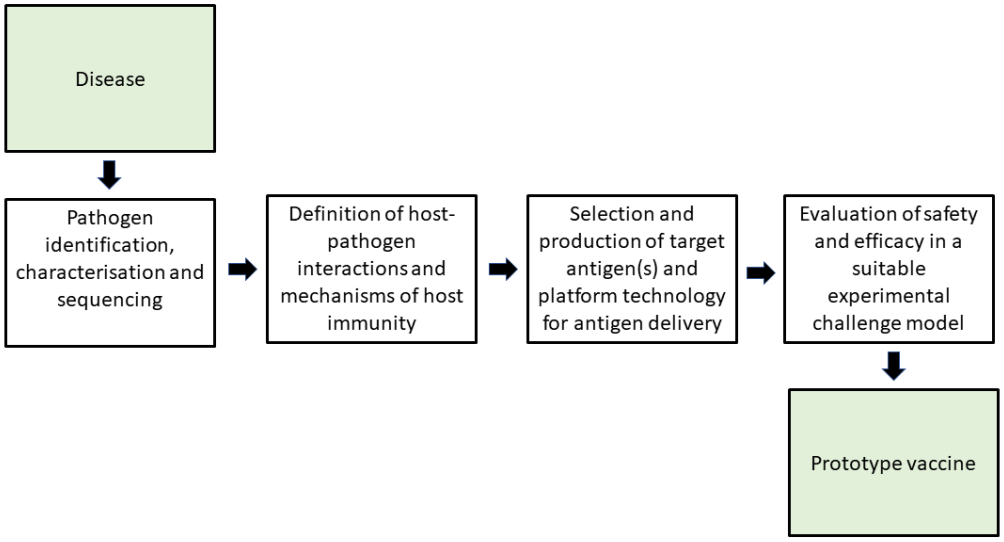

Figure 2