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Using cross-species vaccination approaches to counter emerging infectious diseases

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Since the initial use of vaccination in the 18th century, our understanding of human and animal immunology has greatly advanced and a wide range of vaccine technologies and delivery systems have been developed. The COVID-19 pandemic response leveraged these innovations to enable rapid development of candidate vaccines within weeks of the viral genetic sequence being made available. Development of vaccines to tackle emerging infectious diseases is a priority for the World Health Organization and other global entities. More than 70% of emerging infectious diseases are acquired from animals, with some causing illness and death in both humans and the respective animal host. Yet the study of critical host–pathogen interactions and the underlying immune mechanisms to inform development of vaccines for their control are traditionally done in medical and veterinary immunology 'silos'. In this Perspective article we highlight a 'One Health vaccinology' approach and discuss some key areas of synergy in human and veterinary vaccinology that could be exploited to accelerate the development of effective vaccines against these shared health threats.

[H1] Introduction

Vaccination to control infectious diseases has had a major direct impact on human health and welfare directly and has also secured food supply by improving animal health and controlling zoonotic diseases. For instance, childhood vaccination averts over 2 million deaths every year, with vaccination coverage being a strong indicator of the incidence of vaccine-preventable diseases in humans (for example, measles, yellow fever and polio) [1]. Similarly, veterinary vaccination against endemic diseases improves survival and increases productivity in food-producing animals such as cattle and poultry, with net gains in disposable household income and access to protein-rich animal source foods improving human nutrition [2-5]. However, despite these and many other examples of vaccine impact, very little interaction occurs between human and animal vaccine developers and policymakers.

The development pipeline for human and animal vaccines is a similar process, including biological and scientific parallels in vaccine design and evaluation, as well as common bottlenecks that influence the success of vaccine development programmes [6] (**Figure 1**). However, there are differences in the complexity of the vaccine pipelines, largely due to the varying types of clinical data and regulatory requirements for licensure and the associated bottlenecks that are unique to the animal or human vaccine pipeline [6]. One example is the need for vaccine safety and efficacy data as assessed by experimental infection of vaccinated and unvaccinated target animal species in the veterinary field; in humans, phase 2 and phase 3 randomized controlled studies for estimation of vaccine efficacy against natural exposure are used, though human infection studies are now in use for some vaccine programmes [7]. Despite these differences, solutions to address bottlenecks in the animal and human vaccine development pipelines tend to be similar [6]. For instance, optimizing immunogenicity of vaccines, whether in animals or humans, involves iterative study of vaccination regimens or adjuvant combinations to inform ‘go’ or ‘no-go’ decisions with regards to subsequent development of promising vaccine candidates (**Figure 1**).

Most human infectious diseases have an animal origin, with more than 70% of emerging infectious diseases that affect humans initially crossing over from animals [8]. Generating wider knowledge of how pathogens behave in animals can give indications of how to develop control strategies for human diseases, and vice versa. ‘One Health vaccinology’, a concept in which synergies in human and veterinary immunology are identified and exploited for vaccine development, could transform our ability to control such emerging infectious diseases. Due to similarities in host–pathogen interactions, the natural animal hosts of a zoonotic infection may be the most appropriate model to study the disease and evaluate vaccine performance [9]. This could result in a scenario where a cross-species vaccine is feasible, such as Louis Pasteur's live attenuated

rabies vaccine that was protective in dogs and humans [10] — although different products are now in use for rabies vaccination in humans and dogs [11] — or our own group's Rift Valley Fever vaccine, which is in co-development for use in humans and multiple livestock species [12]. Effective control of zoonotic diseases may require vaccination within reservoir animal hosts to break transmission to humans, and in humans to prevent disease [13], making One Health vaccinology relevant for disease control policy. This strategy is already in use for prevention and elimination of rabies, where mass dog vaccination remains the most cost-effective strategy for breaking disease transmission to humans [14,15]. However, due to the difficulty in predicting spillover events of new infections from animals to humans [16], implementing a cross-species vaccination programme may only be feasible where the domestic animal reservoir of human infection is known (see **Table 1** for some examples), but a cost-benefit analysis would be necessary to inform implementation.

For non-zoonotic illnesses, the natural course of infection and acquisition of immunity against closely related pathogens may be similar between animals and humans allowing accelerated development of vaccines that target similar protective mechanisms. For example, bovine and human respiratory syncytial viruses (RSVs), which cause pneumonia in young calves and children, respectively, are closely related genetically and are targeted by the same types of immune mechanisms suggesting that vaccine strategies exploiting the same underlying mechanism of immunity may work for both species [17,18]. The most widely used human vaccine, bacille Calmette-Guerin (BCG) against tuberculosis, is essentially an attenuated strain of the cattle-infecting bacterium *Mycobacterium bovis* that is avirulent in a wide range of animal species [19]. BCG was developed through close collaboration between medical and veterinary practitioners over 100 years ago [19] and the extensive experience of its use in humans is now informing vaccination strategies to control tuberculosis in cattle [20]. A recent study has found that BCG vaccination in humans also confers protection against other non-tuberculous infections in early childhood [21]; we are aware of no studies of non-specific effects of BCG vaccination in cattle, but this clearly warrants future investigation. Edward Jenner's observation that milkmaids exposed to the cattle virus, cowpox, were protected against smallpox is perhaps the earliest example of exploiting pathogen relatedness for vaccine development and was the basis of the vaccine that was used for eradication of smallpox [22].

Early manufacture of Jenner's smallpox vaccine involved serial propagation of the cowpox virus in calves reared in 'vaccine farms' [23]. Vaccine manufacture has advanced considerably but animal-sourced materials are still in use for production of human vaccines; for example, embryonated chicken eggs are routinely used for manufacture of influenza and yellow fever vaccines [24,25]. New highly scalable platform technologies and delivery systems are accelerating vaccine development, such that it is now possible to go

from pathogen genetic sequence encoding an immunogen of choice to a vaccine candidate in a matter of weeks [26]. Bioinformatic analyses, X-ray crystallography and cryo-electron microscopy continue to be leveraged for the identification and optimization of protective antigens for human and veterinary vaccines [27-29]. These new technologies are being applied to emerging infectious diseases such as COVID-19 or stubborn persistent challenges, including malaria and brucellosis [30-33].

In this Perspective, we highlight some key areas of synergy in human and veterinary vaccinology that could be exploited to accelerate the development and deployment of effective vaccines against zoonotic diseases. We focus on comparative immunology, applications of current vaccine technologies, and regulatory and operational considerations for vaccine deployment.

[H1] Immune systems of different species

The overall structure and composition of the innate and adaptive immune systems of humans and animal species are broadly similar and comparing their responses to inoculation or infection with similar antigens or pathogens can inform vaccine development [34]. Allometric scaling is an important consideration and the body size and physiology of livestock species are more similar to humans than rodents. While rodents may be convenient for lab studies due to the ready availability of specific immunological reagents, lower purchase and maintenance costs and ease of handling, they may not reproduce the pathology and immunological attributes that would be observed in a natural animal host of infection [9]. The similarities between humans and livestock species may be most important when comparing the responses to aerosol delivery of antigens or pathogens [35]. Clearly, non-human primates are an ideal species to predict responses in humans, but their availability is limited and certainly not possible for field studies. Nevertheless, the differences between the immune systems of humans and animals are important and a cautious approach is justified when drawing detailed conclusions from animal studies.

Some of the most striking differences between the immune systems of humans and animals relate to their T cell populations and antibody structures (**Figure 2**). Pigs are increasingly used to study vaccine candidates, in particular influenza vaccines [36-38]. However, there are key differences between pigs and humans that should be kept in mind. For example, three distinct subpopulations of $CD8^+$ T cells have been identified in pigs by flow cytometry; a bright staining population that expresses the $CD8\alpha\beta$ heterodimer, a population that expresses the $CD8\alpha\alpha$ homodimer and a $CD8^+$ population that co-expresses CD4 [39-41]. Ongoing studies indicate that the majority of memory T cells in pigs are present in the double-positive

population and that this population is the predominant source of interferon-γ (IFNγ) in recall responses to live viral vaccines [39,42]. Peripheral $CD4^+CD8^+$ T cells have been characterized in many different species but the proportion of these cells in the total T cell population varies greatly, from 1-2% in humans to 10-20% in pigs. In humans the number and function of this sub-population changes in response to a range of infectious and neoplastic diseases [43]. Recently, these double-positive human T cells have been shown to exhibit a memory phenotype [44], similar to the double-positive T cells in pigs. The impact on responses to vaccination and infection due to the marked difference in the proportion of these double positive cells in different species is yet to be resolved.

Another striking difference is the large percentage of circulating γδ T cells in young pigs and ruminants. γδ T cells comprise up to 60% of circulating lymphocytes in young cattle [45] and pigs [46]. Even in adulthood, 30% of the peripheral blood mononuclear cells (PBMCs) found in these species are γδ T cells [47], whereas in humans only approximately 4% of PBMCs are γδ T cells[48]. Despite this difference between humans and ruminants the results of protection studies in ruminants can provide valuable evidence to support development of human vaccines; for example, the protection of calves from bovine RSV by a stabilized prefusion F protein vaccine may guide human vaccines against RSV [49]. There is a case to be made that the results of vaccine efficacy and safety studies in large animals can provide important information to help shape vaccine development programmes in humans, but the precise immune mechanisms conferring that resistance may be different between species.

The similarities between human and bovine tuberculosis offer a potential opportunity for cross-species development of novel vaccines against the diseases. A BCG challenge model has been used to investigate the protective immune response in humans. Genes linked to protective responses included *IFNG* and *IL17F*, together with other genes associated with these two cytokines, such as *NOD2, IL22, IL23A*, and *FCGR1B* [50]. A recent review highlights the potential role of IL-22 in the protective response to *Mycobacterium tuberculosis* infection in cattle and humans [51]. In cattle, IL-22 and IFNγ produced by protein purified derivative (PPD)-stimulated PBMCs were identified as the primary predictors of vaccine-induced protection in a *M. bovis* challenge model [52]. Further, BCG vaccination in children and in young calves provides protection against tuberculosis, with activation of natural killer cells being a key immune mechanism in the induction of protective immunity in both species [53,54]. Therefore, there are elements of the protective immune response to tuberculosis that are consistent between cattle and humans, including humoral responses [55]. The similarities of immune responses in different species to closely related pathogens may help identify protective vaccine responses.

Another example where studying comparative immunology may improve our understanding of protective immune responses is in determining the role of antibodies with long heavy chain complementarity-determining region 3 (CDR H3) gene segments. Human antibody CDR H3 range between 8 to 16 amino acids (aa) in length, though antibodies with longer CDR H3 have been observed (for example, 18aa [56] and 28aa [57]) and seem to play an important role in cross-protective immune responses to HIV-1 [56,57]. However, such long CDR H3 antibodies are rare in humans, which makes some investigations more challenging [58]. In contrast, cattle have a larger population of antibodies (>10% of antibodies) with long and ultra-long (>70 aa) CDR H3 gene segments [59] and the study of these ultra-long cattle antibodies may prove useful for development of human interventions. For instance, immunizing cattle with HIV envelope glycoprotein results in rapid induction of broadly neutralizing ultra-long CDR H3 antibodies, whereas it takes many years for such broadly neutralizing antibodies to develop in humans following HIV infection [60]. These bovine antibodies may be engineered for prophylactic or therapeutic use and, in addition, determining the immune mechanisms that underlie their induction could inform vaccine design.

On the other hand, the antibody repertoire in dromedary camels is composed of both conventional and heavy-chain only IgG antibody (HCAb) molecules, with the latter accounting for >70% of the repertoire [61]. These smaller HCAb molecules are better adapted for binding cryptic epitopes on pathogens that may be inaccessible to conventional IgG molecules [62], allowing their use in diverse applications in diagnostics, therapy and research [62]. However, very little is known about the relative contribution of HCAbs to naturally acquired immunity to infections and whether vaccines could be tailored to elicit immune responses solely focused on either HCAbs or conventional IgG molecules. Dromedary camels are susceptible to infection with a wide range of pathogens that are also able to infect humans and domestic livestock such as cattle, sheep and goats; examples include Rift Valley fever virus, *Brucella* species (which cause brucellosis) and Crimean-Congo hemorrhagic fever (CCHF) virus, among others [63]. They are also reservoirs of Middle East respiratory syndrome (MERS) coronavirus, which emerged in 2012 and is associated with high case fatality in humans [63]. Understanding how different species are able to mount protective immunity against common pathogens, despite profound differences in IgG structures, could transform approaches to vaccine design and development. These differences in antibody structure can be exploited to identify different mechanisms of protection, for example, long CDR H3 antibodies penetrating viral glycan shields [64]. Importantly a single vaccine platform can induce protection across multiple species [12], including humans [65], despite these differences in immune response. Veterinary vaccinology has made a significant contribution to the broad knowledge base to develop vaccines and understand how they work, but there may be a difference in the

response to similar vaccine platforms in different species. It may also be the case that some platforms may work well in humans and not some other species. However, within each species, individual heterogeneity in mounting immune responses does occur and this might be due to factors such as chronic underlying illnesses, genetics, age, among others [66]. Carrying out comparative studies to identify the common protective mechanisms across species, while accounting for individual within-species heterogeneity, will move the field beyond identifying correlates of protection to defining protective mechanisms.

[H1] Vaccine deployment in different species

The goals of vaccination programmes in humans and animals are similar and range from global disease eradication (permanent worldwide reduction of incidence of a specific disease to zero), elimination of target diseases from a specified region with deliberate measures to prevent re-establishment, prevention of epidemic cycles, and minimizing mortality and morbidity associated with infectious diseases. To date, vaccination has resulted in the eradication of smallpox in humans and rinderpest in cattle [22,67]. Although focused on two separate species, the two eradication programmes employed similar vaccine deployment strategies combining mass vaccination campaigns to achieve herd immunity, intensive surveillance systems to identify and contain outbreaks promptly, and surveillance reporting and sharing of new knowledge that allowed eradication in stubborn pockets of each disease [67,68]. The endgame for these programmes required innovative strategies that included house-to-house case searches for smallpox and containment of outbreaks, and participatory community-based surveillance approaches to identify the hidden rinderpest disease pockets and deploy the vaccines. The key similarities in the deployment of vaccines and cross-learning from the medical and veterinary fields go beyond these disease eradication programmes to the control and elimination of current vaccine-preventable diseases.

Vaccination for the control of zoonoses has dual benefits for both human and animal health. A good example is the control of rabies, a viral zoonosis transmitted to humans through dog bites, which is responsible for about 60,000 human deaths annually [15]. A global elimination goal for human deaths from rabies has been set for 2030 [14]. The key strategies for achieving this goal are mass dog vaccinations to break the dog-dog and dog-human transmission cycles, prompt provision of post-exposure prophylaxis (PEP) to prevent clinical rabies among bite patients, and enhanced surveillance systems to detect areas where the virus circulates and targeting the rabies interventions [14]. Whereas provision of rabies PEP prevents clinical disease and death in people, elimination of human deaths from rabies is only cost-effective when combined with mass dog vaccinations [69,70]. Similarly, animal vaccination against brucellosis (a

bacterial zoonosis transmitted to humans by livestock) reduces the incidence of human brucellosis whilst improving milk production and other production indices among vaccinated livestock [71].

Animal vaccination against epidemic zoonoses is a key strategy to limit human illness. The design and implementation of vaccine programmes for this purpose requires interaction between veterinary and public health personnel. Ideally, such collaboration needs to be in place before the occurrence of an epidemic, rather than be reactive [72]. The zoonotic diseases unit (ZDU) in Kenya, a national entity co-led by medical and veterinary epidemiologists for the purpose of zoonotic disease surveillance, may provide an exemplar framework through which vaccine programmes to tackle endemic/epidemic zoonoses can be implemented [73]. For instance, through extant surveillance for key disease syndromes in livestock, the most recent Rift Valley fever outbreak in Kenya was detected in humans within a fortnight of confirmed livestock cases [74]. In such a scenario, Rift Valley fever vaccination could be implemented among susceptible animals (licensed vaccines are already available) and humans (when a vaccine is available) within a radius in proximity to the initial cases. Such a 'ring vaccination' approach has its roots in the control of disease outbreaks in livestock [75] and has been used successfully to control Ebola virus disease epidemics [76].

However, not all zoonoses of public health importance cause clinical disease in animals. For instance, domestic ruminants such as sheep and goats are key animal hosts of CCHF virus, which only results in asymptomatic infection in these species [77]. In contrast, CCHF is a highly fatal disease in humans and is among the diseases prioritized by the WHO for urgent development of countermeasures [72]. Investigating the pathophysiology and immunology of CCHF virus in ruminant species may provide clues towards identifying therapeutic targets and aid the development of vaccines against human CCHF. Further, assessment of vaccine efficacy against CCHF virus infection in livestock field trials could support development of human CCHF vaccines by providing a stringent test for ranking the performance of candidate vaccines outside of a high-containment laboratory environment. Due to the lack of clinical disease in animals, estimation of vaccine efficacy following natural exposure could rely on serological detection of responses to virus components that are not part of a vaccine, thereby allowing distinction of infected from vaccinated animals (DIVA), a concept well-known in veterinary vaccinology. Following widespread COVID-19 vaccine use, a similar serological monitoring strategy could be useful for tracking population-level SARS-CoV-2 exposure, based on detection of antibodies against antigens absent from approved vaccines. There are several licensed veterinary vaccines against coronavirus infections in domestic animals (**Box 1, Table 2**); the experience with licensure and use of these products provides insights on the likely performance of

241 vaccines against COVID-19 and other coronavirus infections in humans but has rarely been discussed in the
242 medical debate on vaccine development.

243

244 **[H1] Operational considerations**

245 The target product profile for any vaccine, whether human or veterinary, needs to incorporate an efficient
246 manufacturing strategy, design of optimal vaccination regimens and consideration for deployment
247 requirements very early in the development pipeline. All these factors influence the final cost per vaccine
248 dose and, after considering the potential benefit of using the product, inform go versus no-go decisions on
249 vaccine implementation programmes and policy. The business case for development of vaccines against
250 many of the known zoonotic pathogens with epidemic potential is poor [78]. This is largely due to the sporadic
251 nature of the epidemics they cause — for example, there are typically intervals of 5-15 years between
252 epidemics of Rift Valley Fever, and even longer intervals between epidemics of CCHF and other diseases —
253 in addition to their restricted geography and poor data on their economic costs, which make the design of
254 cost-effective vaccine implementation plans challenging. The costs associated with vaccine development
255 and manufacture (**Figure 1**) mean that returns on investments made by vaccine developers on such
256 diseases are unlikely to be high.

257 Initiatives such as the coalition for epidemic preparedness innovations (CEPI) are de-risking the
258 human vaccine development process through provision of funding to support early to late-stage development
259 of vaccines against epidemics, including clinical evaluation that is crucial for licensure [79]. Funding schemes
260 to advance veterinary vaccine development are also available, though none exclusively target epizootic
261 diseases. The global alliance for livestock veterinary medicines (GALVmed) was founded in 2004 with a
262 primary focus on supporting the development and eventual registration of control interventions for a wide
263 range of livestock diseases in low- and middle-income countries (LMICs) [80]; CEPI now plays a similar role for
264 human vaccines.

265 For veterinary vaccines, the ideal cost per dose for ruminants should be less than $0.5 to allow cost-
266 effective use in LMICs [81]. The per dose cost of human vaccines tends to be much higher and highly variable
267 from product-to-product [82]. However, though higher human vaccine costs can be tolerated, especially in
268 high-income countries, vaccine cost remains a key factor that underlays the demand, affordability and
269 implementation of immunization programs in LMICs [83]. To further reduce deployment costs, most veterinary
270 vaccines are multivalent, composed of different immunogens co-formulated to target two or more diseases

with a single vaccination (see **Table 2** for coronavirus vaccines as an example). Co-administration of different immunogens in a single product (for example, the childhood pentavalent vaccine that targets diphtheria, tetanus, pertussis, hepatitis B and *Haemophilus influenzae* type b) is commonplace for the expanded programme for immunization (EPI) in children, which accounts for the bulk of global vaccine use. Cross-learning between the veterinary and medical fields from their respective experiences relating to vaccine development and implementation of programmes based on multivalent or co-administered products could be of mutual benefit.

Where a single vaccine is developed for use in both animals and humans, the same vaccine master seed stock could be used to generate bulk material that is then processed in parallel for human and animal use in accordance with the respective manufacturing requirements to derive a livestock product and one for use in humans from the same manufacturing run. Bulk material could be stockpiled with downstream processing initiated as soon as the need for vaccination arises. However, this strategy would only be successful if the bulk material was stable in the long-term and a regulatory approval strategy incorporating veterinary and human considerations were in place. Harmonized regulatory processes allowing mutual recognition of vaccine registration procedures between countries are already in place, aiming to address operational bottlenecks that limit rapid access to licensed human and veterinary vaccines [84,85].

[H1] Conclusions

Studying animal pathogens, diseases and protective immune responses has had a major impact on controlling human diseases over the past century It is usually the case that new vaccine platforms are deployed more rapidly in veterinary species than in humans. Veterinary vaccines commonly undergo rigorous safety and efficacy testing in the target hosts using challenge models before registration and widespread use. Safety testing and pharmacovigilance are especially important in food-producing animals. Also, developing cost effective manufacturing at scale is essential for animal vaccines. In the past few decades, the widespread use of safe and effective vaccines in livestock has given confidence to develop the same platforms or indeed the same active substances for use in human vaccines. However, there remain untapped opportunities to leverage advances in human and veterinary immunology for development of vaccines, as well as operational experiences to inform vaccine deployment. Effective control of zoonotic infections, which account for the bulk of public health emergencies, require One Health approaches with complementary interventions in both animal hosts and humans. The success of the One Health approach in eliminating disease burden also requires attention to the challenges associated with eradication of zoonotic

diseases in natural reservoirs. Indeed, the elimination of the risk associated with other natural hosts such as bats, rodents, and arthropods constitute a long-standing challenge, which may only be addressed through an improved understanding of reservoir species immunobiology and epidemiology. Research strategies and funding priorities need to be realigned in order to improve interactions between animal and human health communities.

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Author contributions

All authors researched data for the article and contributed substantially to discussion of the content. G.M.W, M.J.F, T.A.B, S.M.T & B.C. wrote the article. All authors reviewed and/or edited the manuscript before submission.

Competing interests

The authors declare no competing interests.

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543 **Table 1: Key diseases where cross-species vaccination programmes may be feasible**

Human disease	Key domestic animal hosts	Licensed human vaccines available?	Licensed veterinary vaccines available?
Rabies	Dogs	Yes	Yes
Rift Valley Fever	Sheep, goats, cattle, camels	No	Yes
Brucellosis	Sheep, goats, cattle, camels	No	Yes
Crimean-Congo haemorrhagic fever	Sheep, goats, cattle, camels	No	No
Middle East respiratory syndrome	Camels	No	No
Tuberculosis	Cattle	Yes	No
Q fever	Sheep, goats, cattle, camels	Yes	No
Nipah virus infection	Pigs	No	No
Hendra virus infection	Horses	No	Yes

544

545 **Table 2: Examples of licensed coronavirus (CoV) vaccines for veterinary use**
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Target species	CoV genus targeted by vaccine	CoV-induced disease	Licensed product	Technology	Formulation
Cattle	Betacoronavirus	Gastroenteritis, neonatal calf diarrhoea	Rotavec™Corona	Inactivated plus adjuvant	Trivalent (CoV, Rotavirus, *E. coli*)
			Bovigen® Scour	Inactivated plus adjuvant	Trivalent (CoV, Rotavirus, *E. coli*)
			Calf-Guard®	Live attenuated	Bivalent (CoV and Rotavirus)
Poultry	Gammacoronavirus	Respiratory disease, reduced egg yields	Nobilis® IB+ND+EDS	Live attenuated	Multivalent (Infectious bronchitis virus, Newcastle disease virus and Egg drop syndrome virus)
Pigs	Alphacoronavirus G	Gastroenteritis	ProSystem® TGE/Rota	Live attenuated	Bivalent (TGE virus and Rotavirus)
Dogs	Alphacoronavirus G	Gastroenteritis	Solo-Jec® 6	Live attenuated plus inactivated plus Adjuvant	Multivalent (CoV, adenovirus, parainfluenza virus, parvovirus)

			Nobivac® Canine 1-Cv	Inactivated plus adjuvant	Monovalent (CoV)
Cats	Alphacoronavirus G	Peritonitis	Felocell® FIP	Live attenuated	Monovalent (FIP virus)

Abbreviations: CoV, coronavirus; FIP, feline infectious peritonitis; TGE, transmissible gastroenteritis.

Figure legends

Figure 1: Vaccine development pipeline.

The typical vaccine development pipeline is shown, starting from target product profiling to licensure and deployment. The respective stages and approximate costs for veterinary and human vaccines are shown. Though presented as a linear chronological process, some of the different stages of the pipeline for a 'multi-species' vaccine can occur in parallel. For instance, the candidate ChAdOx1 RVF vaccine against Rift Valley Fever [12] will soon undergo evaluation in human clinical trials in parallel with the veterinary development, having been made using the same manufacturing starting material.

Figure 2: The heavy chains of bovine antibodies can encode a very long CDR H3, which contrasts the equivalent CDRs of human, mouse, and heavy chain camelid antibodies. Structures of antigen binding fragment regions from (A) bovine (BLV1H12, PDB ID 4K3D [58]), (B) human (PG9, PDB ID 3U2S [86]), (C) mouse (93F3, PDB ID 1T4K [87]), and (D) camelid (VHH-5, PDB ID 5U65 [88]) antibodies (shown in cartoon representation). Heavy chains are colored blue and light chains are colored green. The CDR H3 (or CDR3 in the case of the camelid antibody) for each structure is colored orange. Note, PG9 contains a relatively long CDR H3 for human antibodies. Structures were rendered using PyMOL (The PyMOL Molecular Graphics System, Version 1.8.6.0, Schrödinger, LLC).

Box 1: Experience and lessons from the use of coronavirus vaccines in animals

All major domestic animal species are susceptible to coronavirus (CoV) infection, typically resulting in clinical symptoms involving the respiratory or gastrointestinal systems. Several licensed veterinary vaccines against CoV-associated disease are available (see **Table 2** for examples) and these are predominantly composed of live-attenuated CoV or whole inactivated virions that are administered in adjuvant. However, subunit, viral-vectored, and other types of recombinant vaccines are in development. Some examples of licensed animal

CoV vaccines and some key immunological observations are summarized below, with further details available in recent reviews of animal CoV vaccines [89-91].

Key observations from veterinary use of coronavirus vaccines [89-91]:

- Virus-neutralizing antibodies directed to the surface spike (S) protein play a major role in protective immunity.

- The duration of vaccine-induced immunity is variable but can last for at least 12 months with annual boosters required to maintain protective levels of immunity.

- Vaccines can be highly protective against severe CoV illness yet show limited protection against mild disease or infection.

- Passive transfer of maternal antibodies from vaccinated dams can provide protective immunity against both enteric and respiratory CoV infections, as has been demonstrated in cattle.

- T cell responses play an active role in the control of CoV infections. For instance, adoptive transfer of $CD8^+$ T cells from immune chickens into unvaccinated chicks provides protection from acute infectious bronchitis, with epitopes mapped on the nucleocapsid and spike protein.

- Different routes of administration can be used for CoV vaccination. Some veterinary vaccines have been deployed for use orally (for example, infectious bronchitis in poultry), intranasally (for example, bovine CoV vaccines in calves) or as an oral prime followed by an intramuscular boost (for example, TGE in pigs). Induction of mucosal immunity, mediated by IgA, is thought to improve protective efficacy of vaccines.

- Emergence of CoV spike protein variants may impact vaccine performance, resulting in insufficient protection and necessitating updates to vaccine immunogens. Strategies used to improve the breadth of the protective immune response against different CoV variants include; one, prime–boost regimens using vaccines incorporating different CoV variants, that is, vaccinating with one strain and boosting with another, and two, inclusion of multiple CoV strains within a single vaccine.

- Antibody-dependent enhancement (ADE) of CoV infection following vaccination and virus exposure can be readily demonstrated in cats. This may provide a useful model to understand the ADE phenomenon.

- Monitoring of CoV antibody seroprevalence in poultry has been used to inform decisions on whether to implement a vaccination programme based on the levels of flock immunity. This is primarily aimed at achieving a cost-efficient disease control programme but could also be used in a scenario where vaccine supply is limited.