STAR IDAZ | International Research Consortium on Animal Health

Roadmap Lead Summaries

Disease/pathogen	Coronavirus
Roadmap type	Vaccine Development

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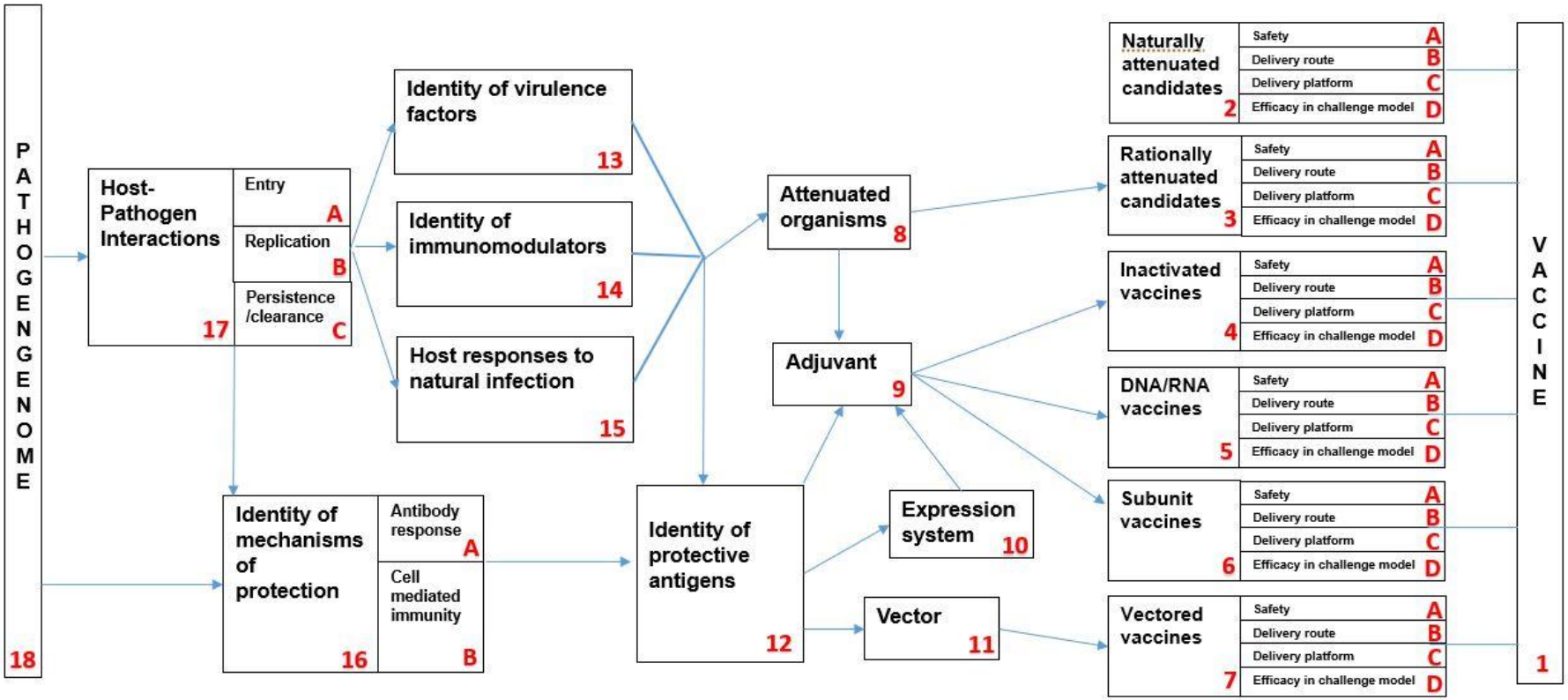
Roadmap for Vaccine Development



Lead Summary [1]- Vaccine

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

What are we trying to achieve?

Development of effective vaccines against the current most pathogenic animal health coronaviruses as well as future emerging viruses.

Problem:

Vaccine development is a multifactorial process - factors that need to be considered when designing a vaccine are which virus/antigen to target, which vaccine platform to use, animal model, safety, efficacy, cost, stability, demand etc. And whether there is a market!

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Platform and antigen selection: Identifying the most effective vaccine platform (e.g., mRNA, viral vector, protein subunit) and the right combination of antigens (e.g., spike, nucleocapsid) that can protect against a broad spectrum of coronavirus variants, including emerging ones, is a key challenge.

Broad protection against coronavirus variants: Developing a vaccine that offers protection not only against known coronaviruses but also against within-species variants is critical for long-term control of disease spread.

Testing vaccine platforms in challenge studies: Testing vaccines in natural hosts is preferred, and an advantage over human vaccine development but animal models may not always reflect the immune responses in the field, making it difficult to evaluate vaccine efficacy.

Balancing antibody and T-cell responses: Finding a platform that induces both strong antibody responses and long-term T-cell immunity is challenging. Both components are necessary for robust, durable protection. Most vaccines are targeted against the spike protein – there is a need to broaden the immune response to encompass cell-mediated and mucosal immunity. There is a requirement for protection against infection as well as disease.

Delivery routes: The ideal delivery route for vaccines (e.g., intramuscular, mucosal) remains uncertain. Mucosal delivery may enhance immunity as a first line of defence, particularly in the respiratory and gastrointestinal tracts, but requires further exploration.

Safety in production systems: Ensuring that vaccines are safe for widespread use in animal production systems, without adverse effects on animal health or product quality, is a key consideration.

Solution Routes

What approaches could/should be taken to address the research question?

Extensive testing with comparable methods: Vaccines should be tested using standardized methods and protocols (SOPs) across laboratories, allowing for reliable comparison of results. This ensures that the best vaccine platforms and delivery methods can be identified and optimized.

Specific funding calls for multidisciplinary approaches: Targeted funding opportunities should be established to encourage multi-disciplinary research, combining immunology, virology, genetics, and veterinary science to accelerate vaccine development.

Testing various vaccine platforms: A wide range of vaccine platforms should be tested, including those that can be delivered via mucosal

routes (e.g., viral vectors). This will help determine which platforms provide the best immunity and ease of administration.
Comparing delivery routes: Different delivery routes (e.g., intramuscular, mucosal, oral) should be compared, particularly exploring alternatives to intramuscular injections, to determine which route best stimulates immunity at key sites of viral entry.
Collaborative research: Collaboration between academia, industry, and government is crucial to speed up vaccine development. This could involve data sharing, joint trials, and cross-disciplinary partnerships to address both scientific and logistical challenges.
Accelerating universal platform approval: Regulatory agencies should focus on approving universal vaccine platforms, where new sequences can be rapidly inserted to adapt to emerging variants, reducing time to market for new vaccines. This should include the use of genetically modified organism (GMO) vaccines.
Safety testing across platforms: Comprehensive safety testing should be conducted across various vaccine platforms, comparing different immunogens to ensure wide-scale safety in different animal species and production environments.
Natural host studies: Wherever possible, vaccine testing should involve natural host species to ensure that the immune responses observed are reflective of those in the target species.
Epidemiological studies: unify the global efforts put into monitoring disease prevalence for difference coronaviruses.

Dependencies

What else needs to be done before we can solve this need?

A wholistic understanding of the pathogen, the disease, the immune response are absolutely essential. But also, an understanding that each outbreak has a unique environment and the human factors that might affect vaccine uptake and/or success might be very different. All these dependencies are likely needed to underpin vaccine delivery.

State of the Art

Existing knowledge including successes and failures

High-level biosecurity measures and vaccines remain the most effective strategies to prevent coronavirus diseases in both animals and humans. For many widespread animal coronavirus diseases—including those affecting bovines, dromedary camels, pigs, cats, dogs, and birds—successful commercial vaccines are available. These vaccines have historically been developed using either killed/inactivated virus or live/attenuated virus strategies.
While the majority of these vaccines are administered intramuscularly, some, such as those for infectious bronchitis (IB) in poultry, are delivered through drinking water, aerosol spray, or oculo-nasal routes. This highlights the potential role of local mucosal immunity in protecting against coronavirus diseases. Examples of available veterinary coronavirus vaccines include:

- **Avian infectious bronchitis:** Live attenuated virus delivered via drinking water, aerosol spray, or oculo-nasally.
- **Bovine coronavirus:** Inactivated whole virus vaccine administered intramuscularly, often combined with other vaccines.
- **Canine coronavirus (CCV):** Inactivated feline enteric coronavirus (FECV), which is antigenically similar to enteric CCV, given via injection to young puppies with a booster dose.
- **Feline infectious peritonitis:** Attenuated, temperature-sensitive strain administered intranasally.
- **Porcine transmissible gastroenteritis:** Live, attenuated virus delivered intramuscularly, either alone or in a regimen combining an oral priming dose with an intramuscular booster.

(Ref: https://www.rsb.org.uk/biologist-covid-19/coronavirus-veterinary-vaccines)

Projects *What activities are planned or underway?*

Lead Summary [2]- Naturally attenuated candidates

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

What are we trying to achieve and why?

To develop safe and effective live attenuated vaccines (LAVs) for animal coronaviruses, e.g. those that infect pets, livestock, and wildlife. Addressing coronavirus infections in animals is vital to safeguarding food security, biodiversity, and public health.

Problem:

Current vaccine strategies often lack broad protection across viral strains or genera, limiting effectiveness. Live attenuated vaccines offer a promising solution by providing durable immune responses but need to be developed and optimized for safe, cross-species use whilst avoiding reversion and other associated issues.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Genetic stability: Ensuring that the virus remains stable and does not revert to a virulent form.

Understanding host-specific immunity: Different animal species, such as livestock (e.g., pigs, cattle), pets (e.g., cats, dogs), and wildlife (e.g., bats, ferrets), have varying immune responses to coronaviruses and it is not clear how much we need to understand these to design LAVs across species.

Strain diversity: Animal coronaviruses exhibit significant genetic diversity, so it is difficult to design a universal LAV. Indeed, vaccine candidates may need to be strain- or species-specific, limiting their generalizability.

Safety concerns: While LAVs can often provide strong immunity, they may pose safety risks, particularly in immunocompromised animals. Since they are LAVs, there is also the need to address if there is potential for unintended transmission to non-target species (including humans).

Mucosal immunity: For almost all vaccines, inducing strong mucosal immunity (e.g., in the nasal or gastrointestinal tracts) is crucial. It is unclear where LAVs stand within the spectrum of responses (are they the best?).

Solution Routes

What approaches could/should be taken to address the research question?

Rational attenuation through genetic engineering: Using reverse genetics to target specific viral genes or cis/trans-acting sequences involved in pathogenicity (e.g., non-structural proteins or accessory proteins).

Cold-adaptation or temperature-sensitive mutants: LAVs that only replicate at lower temperatures (such as those found in the upper respiratory tract) may reduce the risk of systemic infection and virulence, particularly for animals like pigs and cattle.

Targeting mucosal immunity: Targeting LAVs to induce both systemic and mucosal immunity by focusing on routes of administration (e.g., intranasal or oral vaccines) might improve the situation.

Using corona LAVs as multivalent vaccine candidates: Could a single vaccine targeting multiple viral strains or species-specific coronaviruses be developed, either by combining multiple attenuated strains into a single formulation or engineering multi-valency?

Dependencies

What else needs to be done before we can solve this need?

Enhanced surveillance and sequencing: Continuous surveillance of circulating coronaviruses in animal populations (including genetic sequencing) is essential to monitor emerging strains and assess the potential for cross-species transmission. This is critical for updating and refining live attenuated vaccine strains.

Animal models: Establishing reliable animal models for studying coronavirus infections in pets, livestock, and wildlife is necessary for preclinical vaccine testing. These models need to reflect the physiological responses of different species to coronavirus infection and vaccine response.

Safety testing protocols: Development of standardized protocols for evaluating the safety and genetic stability of live attenuated vaccines, particularly in terms of reversion risk and potential transmission to non-target species.

Cross-species immune response studies: Detailed research into how different species' immune systems respond to coronavirus infection and vaccination is required. This includes understanding differences in innate, mucosal, and adaptive immune responses.

Regulatory frameworks: Clear regulatory pathways for the approval and deployment of live attenuated vaccines in animals, particularly for wildlife species, need to be established.

Rational attenuation through genetic engineering: Using reverse genetics to target specific viral genes or cis/trans-acting sequences involved in pathogenicity, such as for non-structural proteins or accessory proteins, requires knowledge of what these are.

State of the Art

Existing knowledge including successes and failures

LAVs have been successfully developed for porcine epidemic diarrhoea virus (PEDV) in pigs, and BCoV for cattle, leading to widespread use. However, issues with incomplete protection and viral shedding remain concerns. Failures in cross-protection between strains highlight the need for updated and multivalent approaches. LAVs for cats have shown some promise, but issues with strain-specific immunity and adverse reactions persist. People are exploring LAV candidates for controlling SARS-CoV-2 in animals, e.g. cats/mink that are known reservoirs or spillover risks. Bivalent CoV and rotavirus vaccine (Calf-Guard) has been shown to be effective for use in cattle against betacoronavirus.
A multivalent (IBV, NDV, egg drop syndome virus) inactivated vaccine has been produced against gammacoronavirus in poultry (Nobilis IB + ND + EDS).
A bivalent (TGE virus and rotavirus) vaccine has been shown to be effective against alphacoronavirus in pigs (ProSystem TGE/Rota).

A multivalent (CoV, adenovirus, parainfluenza, parvovirus) vaccine has been shown to be effective against alphacoronavirus in dogs (Solo-Jec 6).
A monovalent vaccine has been shown to be effective against alphacoronavirus (FIP) in cats.

What failures do we know about?
Attenuated virus would most likely only work in immune naive individuals, so a non-replicating platform would be better and also safer.

Projects

What activities are planned or underway?

Lead Summary [3] - Rationally attenuated candidates

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

The goal is to develop stable, rationally attenuated vaccines for animal coronaviruses that are safe, effective, and most importantly do not revert to virulent forms, which is a problem with older-LAVs. These vaccines should induce strong immune responses while minimizing the risks of viral shedding and recombination, both of which are particular challenges with LAVs.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Risk of shedding, reversion and reassortment: LAVs, while effective, carry the risk of reversion and/or shedding, whereby animals can also spread the virus. Connected to this is reassortment, where recombination occurs with circulating strains. This could lead to the emergence of virulent or immune escape variants, complicating control efforts.

Attenuation genetic stability: Identifying, characterizing and validating the genetic changes that stably attenuate candidate virus vaccines without the risk of reversion is critical. There is also a need to understand how conserved they are across different coronavirus strains, species and also susceptible hosts.

Outdated technology: Current vaccines are successful but rely on passage of virus, which is an outdate technology and continually needs to be adapted when new virus strains emerge. Efforts needs to be made to advance technology for example, using live attenuated vectored vaccines. Considerations will need to be given to issues such as affordability.

Solution Routes

What approaches could/should be taken to address the research question?

As above for LAVs we can use genetic engineering techniques, such as reverse genetics, to introduce stable attenuating mutations in key viral genes. These mutations need to strike the right balance between attenuating the virus but preserving its immunogenicity.

Integrate attenuation with DIVA capabilities: Develop DIVA vaccines that allow for the simultaneous use of diagnostic tests. These rationally attenuated vaccines could also add markers that allow DIVA, aiding in surveillance and disease control.

Improving understanding and modelling of immune escape: Develop better data sets and build predictive models to monitor (and predict) viral evolution and recombination events in the field that might bypass vaccine-induced immunity.

Dependencies

What else needs to be done before we can solve this need?

Diagnostic tools for DIVA vaccines: Reliable and sensitive diagnostic tools must be developed to accompany DIVA vaccines.
Surveillance systems: Surveillance systems are needed to monitor for vaccine strain reversion and recombination with circulating strains, particularly in regions with high viral diversity. This is essential for ensuring the long-term safety and efficacy of attenuated vaccines.

State of the Art

Existing knowledge including successes and failures

Rational attenuation of live virus for vaccines for coronaviruses could provide strong immunity, more than inactivated vaccines, for example. However, they carry the risk of reversion. From an animal coronavirus perspective they are cost-effective for mass vaccinations in livestock and pets.

Projects

What activities are planned or underway?

Defining Antagonism Hierarchy of Porcine Epidemic Diarrhoea Virus for Live Vaccines Design. 2024-2026 Lager Kelly, Deng, Xufang et all. USDA/ NIFA 5030-32000-230-080-R
https://www.ars.usda.gov/research/project/?accnNo=445534
The specific objectives of this proposal are: I) Validate and invalidate immune antagonists and investigate the antagonism hierarchy of Porcine Epidemic Diarrhoea Virus (PEDV). II) Identify an optimal combination of immune antagonists that profoundly attenuates PEDV upon inactivation but allow it to induce a strong antibody response. III) Evaluate the candidacy (antibody response, protective lactogenic immunity, genetic stability, etc.) of PEDV mutants as live-attenuated vaccines in porcine models.

Lead Summary [4] - Inactivated vaccines

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

To examine whether killed virus represents a realistic route to immunity and regulatory approval, especially in the post mRNA, viral vector landscape. The case may be stronger for animal vaccines than human vaccines, since multivalent inactivated vaccines are popular already in this space.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Inactivated vaccines are still widely used and have established efficacy, e.g. for influenza, Rotavec, and SARS-CoV-2. It remains to be seen if their use and practicality can be replaced by a next generation of vaccines, and whether this is a globally applicable scenario or restricted to certain countries.
Improved adjuvants would help tackle the relatively poorer cell-mediated immune response.
There are multivalent formulations, e.g. Rotavec - could this be expanded to other hosts?

Solution Routes

What approaches could/should be taken to address the research question?

Side-by-side comparison of efficacy between different vaccine platforms

Cost benefit analysis of production pipelines to understand the barriers to implementation of newer technologies.
More research on adjuvants and/or how to boost cellular immunity.

Dependencies

What else needs to be done before we can solve this need?

State of the Art

Existing knowledge including successes and failures

Inactivated virus vaccines designed to combat canine coronavirus seem to be a good example of long-lasting protective immunity, generally eliciting protective immunity 21 days after the primary vaccination and lasting one year. However, it has proved difficult to induce prolonged strong immunity with some other animal coronavirus vaccines.
From past experience in SARS-CoV, dengue virus, measles and respiratory syncytial virus vaccines, inactivated vaccines are associated with concerns of antibody-dependent enhancement (ADE).
(Xu L., Ma Z., Li Y., Pang Z., Xiao S. Antibody dependent enhancement: Unavoidable problems in vaccine development. Adv Immunol. 2021;151:99–133)
China used an inactivated SARS2 vaccine extensively, Sinovac, to manage the pandemic. There are extensive studies comparing vaccine efficacy between this vaccine and other vaccines, e.g. PMID: 35412612.
While safer in terms of reversion, inactivated vaccines often require adjuvants to boost immune responses. Examples include trivalent vaccines like *Rotavec Corona* for cattle (administered to pregnant cows to provide passive immunity to calves through colostrum) and *Nobivac*

Canine1-Cv for dogs (vaccine is particularly useful in multi-dog environments, such as shelters and breeding facilities, where outbreaks of CCoV can spread rapidly). However, these vaccines may not induce the same level of cellular immunity as live attenuated vaccines.

Projects
What activities are planned or underway?

Project: Development and evaluation of MERS-CoV killed and attenuated vaccines for camels in Abu Dhabi, UAE.

Lead Summary [5] - DNA/RNA vaccines

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

Develop nucleic acid-based vaccines that are efficacious, safe and usable in animal production systems, whilst establishing cost effective mechanisms for product delivery.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Mucosal immunity: DNA/RNA vaccines typically induce systemic immunity, but their ability to generate strong mucosal immunity remains limited. This is critical for respiratory and gastrointestinal coronaviruses in animals.

Cold chain maintenance: Currently mRNA vaccines, in particular, require stringent cold chain conditions, which complicates distribution in remote or field conditions, especially problematic for livestock and wildlife.

Affordability: Despite their high efficacy, RNA and DNA vaccines may be more expensive to produce and distribute, particularly in low-income regions or for large-scale animal vaccination campaigns, as has been seen for COVID-19 access at a global level.

Sterilizing immunity: Related to the mucosal immunity, there is uncertainty over whether these vaccines can induce sterilizing immunity. Will non-sterilizing immunity will be enough to control viral transmission in large herd/flocks etc?

Breadth of immunity: mRNA vaccines tend to be species-specific, raising questions about their ability to protect across different animal species.

Response to variants: While DNA and RNA vaccines can be quickly updated to target new variants, it remains a challenge to predict how new variants will emerge and escape immune responses, although this is true of all vaccines.

Public perception: Public opinion on the safety and efficacy of novel vaccine platforms (DNA/RNA) remains mixed, especially in agriculture, where concerns about the safety of animal products from vaccinated animals can affect acceptance.

Solution Routes

What approaches could/should be taken to address the research question?

Non-sterilizing immunity: Research should investigate how non-sterilizing immunity driven by DNA/RNA vaccines could impact immune selection and recombination of viral variants, as well as its role in disease control.

Alternative administration routes: Investigating alternative routes of administration, such as aerosolized RNA vaccines, may enhance mucosal immunity and provide better protection at the sites of viral entry.

Broadening immunity: DNA vaccines may offer broader immunity across species. Studies should explore how these and mRNA vaccines can be utilized in different hosts to create cross-protective immunity.

Vaccine-therapeutic combinations: Testing the combination of vaccines with therapeutic agents (e.g., antiviral drugs or

immunomodulators) could improve outcomes by enhancing immune responses and viral clearance.
Antigen selection for mRNA vaccines: Research is needed to determine the best combinations of antigens (e.g., spike, nucleocapsid) to include in DNA/RNA vaccines, aiming to elicit a balanced T-cell and antibody response.
Investigating immune tolerance: The increasing IgG4 levels observed with multiple mRNA doses suggests the possibility of immunotolerance, which should be further investigated to ensure long-term efficacy of these vaccines.
T-cell responses: Studies are required to assess how DNA/RNA vaccines induce long-lasting T-cell responses, which are critical for durable protection and viral clearance.
Maternal immunity: The role of maternal immunity should be explored in developing vaccination plans for production systems with animals of different ages, particularly in livestock where passive immunity from mother to offspring is important.
Public education campaigns: To address concerns around DNA/RNA vaccines, educational campaigns should focus on the safety and efficacy of these platforms, particularly in the context of safe animal products entering the food chain.

Dependencies

What else needs to be done before we can solve this need?

Vaccine administration strategies: A comprehensive vaccine administration plan must account for vaccine durability, animal age, and the type of production system to optimize outcomes across species.
Public communication plans: Clear communication strategies are needed to improve public acceptability of products from vaccinated animals, addressing concerns about safety and marketability.
Emergency financing and commitment: Investments are required to ensure vaccines are produced and available for immediate use during variant emergencies, with commitments from governments and organizations to purchase vaccines in the event of outbreaks in affected animals.
Regulatory acceleration: Regulatory pathways need to be streamlined to allow for the rapid approval and deployment of new vaccines in the event of emerging variants, similar to what was done during the COVID-19 pandemic.

State of the Art

Existing knowledge including successes and failures

mRNA vaccines: mRNA vaccines have demonstrated efficacy in humans (e.g., COVID-19) but are generally species-specific, which raises challenges in developing cross-species vaccines for animals. However, the speed of R&D during the COVID-19 pandemic showed that these platforms could be rapidly adapted in emergencies.
T-cell responses: The majority of studies on DNA/RNA vaccines have shown relatively low T-cell responses, which may limit their ability to provide long-term protection without further optimization.
Immune tolerance: There is emerging evidence that repeated mRNA vaccinations increase IgG4 levels, suggesting the possibility of immune tolerance, which could reduce vaccine effectiveness over time. This phenomenon warrants further study.

Projects

What activities are planned or underway?

VetCoVac- F2022/IOF-StarTT024 (2022-2024) Niek Sanders et al. Ghent University https://research.ugent.be/web/result/project/23c4c299-f6df-11ec-aa48-53788b68d579/details/en
MucRNAVax (CNRS) - Design and evaluation of a LipoParticulate mRNA vaccine carrier, mucus penetrating, able to induce mucosal immune responses against respiratory infectious diseases 2.
mRNA-based vaccines against MERS and rift valley

Lead Summary [6] - Subunit vaccines

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

What are we trying to achieve?

To develop safe and effective subunit vaccines for animal coronaviruses to safeguard animal health.

What is the problem we are trying to solve?

Other vaccine strategies employ live or attenuated vaccines, which may result in reversion, infection or transmission in vaccinated animals, so subunit vaccines provide a safe and relatively stable alternative, where the technology is already well-established.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Weaker immune response: Antigens used to elicit immunes responses may lack molecular structures (e.g., PAMPs), so do not infect cells. Therefore, these antigens mainly only trigger antibody-mediated immune responses, with weaker cell-mediated T cell responses.

Longevity of immune response: Adjuvants and boosters may be required to enhance the immune response, or boost a waning response over time, which may not be ideal for use in animals. This would also require the development of suitable adjuvants for animals in which subunit vaccines are not routinely used.

Purity: Subunit vaccines are made in bacteria/yeast/insect/mammalian expression systems, so require substrates to grow them and can become expensive, particularly if purifying and concentrating vaccine preparations. This method also requires care to avoid contamination with other organisms.

Speed: Determining the correct antigen, or part of antigen to use as the immunogen can take time to design and synthesise, so may be a slow process if a vaccine is required urgently.

Strain diversity: Animal coronaviruses exhibit significant genetic diversity, so it is difficult to design a universal subunit vaccine based on the spike protein of one coronavirus. Different subunit vaccines may need to be produced that are virus strain or animal species specific, which limits their universality and will be more expensive long-term to manufacture.

Solution Routes

What approaches could/should be taken to address the research question?

Expression system: Mammalian expression system are generally cleaner and produce fewer contaminants than other expression systems, so may be used as a preference. These expression systems also allow the production of large amounts of protein, which is ideal for generating high yields of vaccine quickly.

More specific immunogens: Target conserved components of the coronavirus genome to develop vaccines (e.g., RBD, S1), that may elicit broader immune responses across several coronaviruses

Multivalent vaccines: As subunit vaccines only contain a small amount of virus genetic material, which is usually synthetic, a multivalent approach could be taken to incorporate the spike of more divergent coronaviruses and help to elicit a broader immune response.

Dependencies

What else needs to be done before we can solve this need?

Animal models: Establishing animal models in less well studied animals that act as reservoirs for coronaviruses – this is important for understanding virus transmission, pathology and developing species-specific adjuvants

Determining immunogens: The spike protein is generally used to develop coronavirus subunit vaccines, as it is responsible for attachment and entry and is generally where most of the neutralising antibodies are targeted to. However, other structural proteins could also be used in combination with S to enhance immunity (e.g., N to induce cellular immunity), which should be researched further.

Diagnostics: The immunogens used for subunit vaccines are also important when considering diagnostics for emerging animal coronaviruses. Generally, diagnostics are based on the N-protein so using structural proteins other than N in vaccine design allows for development of DIVA vaccines. Spike-based vaccines would allow for DIVA.

State of the Art

Existing knowledge including successes and failures

Subunit vaccines have been developed and licensed for other animal pathogens, for example the Hendra virus G subunit vaccine for use in horses (EquiVac).

An S-based subunit vaccine has been described for use in piglets against porcine deltacoronavirus (PDCoV) : A novel recombinant S-based subunit vaccine induces protective immunity against porcine deltacoronavirus challenge in piglets | Journal of Virology (asm.org)

Subunit vaccines of either full-length spike, S1 or RBD have been shown to experimentally elicit antibody responses in experimental animal models, against SARS-CoV-1, MERS, SARS-CoV-2 and other human CoVs (mice, hamsters, palm civet, rhesus macaque) Frontiers | Subunit Vaccines Against Emerging Pathogenic Human Coronaviruses (frontiersin.org)

Cross neutralisation of human and civet SARS-CoV variants has been seen, induced by S RBD-subunit vaccines. Cross-Neutralization of Human and Palm Civet Severe Acute Respiratory Syndrome Coronaviruses by Antibodies Targeting the Receptor-Binding Domain of Spike Protein | The Journal of Immunology | American Association of Immunologists (aai.org)

Projects

What activities are planned or underway?

Nanovaccin 1 (Université de Tours /INRAE): Validation in a K18-hACE2 mouse model of a vaccine candidate mucosal anti-SARS-Cov2 for immune responses and protective.

Nanoparticle-based swine vaccine (USDA NIFA/VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY) Zhang, Chenming 2022-2025

Lead Summary [7] - Vectored vaccines

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

To develop safe and effective vectored vaccines for coronaviruses in pets, livestock, and wildlife.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Vector safety and stability: Ensuring that vaccine vectors are safe, non-pathogenic, and stable in diverse animal species.

Pre-existing immunity to viral vectors: Pre-existing immunity to viral vectors (like adenoviruses or measles viruses) in animals can reduce the efficacy of vectored vaccines.

Efficient vaccine delivery to wildlife: Delivering vaccines to large populations of livestock or free-ranging wildlife is logistically challenging.

Cross-species transmission: The risk of vaccine vectors spreading from vaccinated animals to non-target species, including humans, poses a safety risk.

Cost and scalability: The cost of producing vectored vaccines and scaling them for large populations, especially in wildlife or low-resource livestock farming environments, can be prohibitive.

Understanding host-specific immunity: The immune systems of pets, livestock, and wild animals, vary significantly. A vaccine vector that works well in one species might not elicit the same immune response in another.

Ecological and evolutionary impacts in wildlife: The mass vaccination of wildlife with vectored vaccines may have unforeseen ecological consequences, such as altering predator-prey dynamics or influencing viral evolution in non-target species

Solution Routes

What approaches could/should be taken to address the research question?

Determine the most suitable vectors.
Develop next-generation vector platforms, such as novel or less common viral vectors that avoid pre-existing immunity.
New approaches are needed for vaccine/ immunogen design to achieve robust protection.
Engineer vectors to be more thermostable, enabling easier storage and transport for use in remote or resource-poor environments.
Educational campaigns about new vaccine strategies and platforms.

Dependencies

What else needs to be done before we can solve this need?

Funding: Increased funding is needed for comparative immunology studies across species, particularly wild animals.

Immune system response to infection:Detailed research into how different species' immune systems respond to coronavirus infection and vaccination is required. This includes understanding differences in innate, mucosal, and adaptive immune responses.

Route of delivery: Evaluate alternate routes of vaccine delivery (intranasal, oral, topical).

Assay techniques and endpoints: Interlaboratory variability in assay techniques and assay endpoints limits comparison among measurements of immunogenicity. Standardized and harmonised assays and protocols are required.

Animal models: Establishment of reliable animal models for studying coronavirus infections in pets, livestock, and wildlife for preclinical vaccine testing.

State of the Art

Existing knowledge including successes and failures

Several well-known vectors offer the possibility of protection against coronavirus infection (e.g. poxvirus, adenovirus, measles, and togavirus). If these vectors are reasonably safe, they are limited to presenting one or a reduced number of coronavirus antigens to the immune system (Enjuanes, L., et al. "Molecular basis of coronavirus virulence and vaccine development." *Advances in virus research* 96 (2016): 245-286.)

ChAdOx1 and MVA based vaccines have been used against MERS in camels https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9413082/ https://www.nature.com/articles/d42473-018-00392-7 https://www.thelancet.com/journals/langlo/article/PIIS2214-109X%2823%2900164-X/fulltext

Other vectors of interest are vesicular stomatitis virus (VSV) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7359519/; https://www.sciencedirect.com/science/article/pii/S1931312820303619; https://www.vet.cornell.edu/research/awards/202105/two-vaccine-platforms-prevent-feline-coronavirus-disease used for feline infectious peritonitis (FIP)

Newcastle Disease Virus (NDV): NDV vectors have been used in poultry vaccines with success and are now being studied for potential use against avian coronaviruses. https://www.sciencedirect.com/science/article/pii/S2352396420305089; https://www.nature.com/articles/s41467-021-26499-y

Venezuelan equine encephalitis virus (VEE): https://pubmed.ncbi.nlm.nih.gov/20980507/

Rabies virus vectors are well-established in veterinary medicine, particularly for wild animal vaccination programmes. https://jdc.jefferson.edu/mifp/119/; https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9229041/

Parainfluenza virus (PIV): https://pubmed.ncbi.nlm.nih.gov/15220033/

Projects

What activities are planned or underway?

What other projects are underway?
Adenovirus-based MERS vaccine in camels

Dr Matthias Tenbusch at University Hospital Erlangen is conducting vaccine trials in non-human primates primed with mRNA vaccine Comirnaty, followed by a mucosal booster of adenovirus serotype 5 vector encoding spide or nucelocapsid protein (Ad5-S/N) or a LAV via oropharyngeal spray. Adenoviral vector immunisation was successful in boosting systemic and mucosal immunity.

Lead Summary [8] - Attenuated organisms

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

What are we trying to achieve?

The goal is to generate livestock (companion animals possibly) that are genetically engineered to be refractory to coronavirus infections. By modifying host genetics to prevent viral entry, replication, or transmission, we could create disease-resistant animal populations thus negating the need for vaccines.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Genetic modifications and unintended consequences: Genetic engineering for virus resistance must ensure that the modifications do not lead to unintended health effects or compromised immune responses in livestock. Identifying the right gene targets that block coronavirus infection without affecting the animal's overall health or performance is crucial. Weigh up the risk of using GMO with benefits. Focus on resilient animals at the immune level, rather than making animals highly resistant to a virus at the entry level, which could instead drive selection of more resistant strains or of different pathogens (e.g. bacteria/parasites). Also, it is important to determine how resistant to drought or high temperatures, changes in climate these animals will be. Also important to determine the stability of the genetically modified organisms (GMOs).

Restrictions in use: Will use be restricted to big commercial companies? What does this mean for small holders?

Ethical and regulatory concerns: The use of GMOs in food-producing animals raises ethical and regulatory challenges, including consumer acceptance and marketability of genetically engineered livestock products. Regulatory frameworks may need to be updated to accommodate these advancements.

Viral escape mechanisms: There is a potential risk that coronaviruses could evolve to bypass the engineered resistance mechanisms, rendering the genetic modifications ineffective over time. Continuous monitoring and adaptation would be necessary.

Lack of field data: While genetic engineering for viral resistance has been successful in model systems, there is a lack of large-scale field data for coronavirus resistance in livestock. Long-term studies are needed to validate the durability and safety of this approach.

Solution Routes

What approaches could/should be taken to address the research question?

Gene editing for receptor modification: One of the most promising strategies involves using CRISPR or other gene-editing technologies to modify or delete host receptor genes (e.g., ACE2 or DPP4).

Genome-wide association studies (GWAS): Conduct GWAS to identify naturally occurring genetic variants that confer resistance to coronaviruses in livestock populations. These findings can be used to guide targeted gene editing efforts.

Synthetic biology: Advanced synthetic biology approaches could be used to introduce antiviral proteins or pathways into livestock genomes, allowing animals to detect and destroy coronaviruses before they can establish an infection.

Epigenetic modifications: Explore whether heritable epigenetic changes can be induced to create viral resistance without altering the underlying DNA sequence. This might be a more flexible and reversible approach compared to permanent genetic engineering.

Dependencies

What else needs to be done before we can solve this need?

Regulatory approval for genetically modified livestock: Streamlined and updated regulatory pathways are required for the approval of genetically engineered livestock. Regulations must address safety concerns, potential environmental impacts, and ethical issues surrounding the use of GMOs in agriculture.

Public acceptance: Educational campaigns and transparent communication about the safety and benefits of genetically modified livestock are necessary to gain public acceptance, particularly concerning animal welfare and food safety.

Surveillance for viral escape: Once genetically engineered livestock are in use, surveillance systems must be put in place to monitor for any viral adaptations or escape mechanisms that could render the modifications ineffective. And this could be selected for in the viral population if the resistance is not absolute.

State of the Art

Existing knowledge including successes and failures

PRRS-resistant pigs (not coronavirus): One of the most successful examples of genetic engineering for viral resistance is the development of pigs that are resistant to porcine reproductive and respiratory syndrome virus (PRRSV). These pigs were engineered to lack the CD163 receptor, which PRRSV uses for cell entry, making them refractory to infection. This model has paved the way for similar strategies targeting coronaviruses in livestock. Research is ongoing into modifying ACE2 and DPP4 receptors in animal models to prevent infection by coronaviruses like SARS-CoV-2 and MERS-CoV.

Projects

What activities are planned or underway?

What other projects are underway?

Roslin Institute has made a recombinant pig with human ACE2 for SARS-CoV-2 studies and they are more permissive than normal pigs.

Lead Summary [9] - Adjuvant

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

What are we trying to achieve?

Identify and develop optimal adjuvants that could be used in different animal models and for different vaccine platforms to enhance the immune response induced by vaccines against animal coronaviruses

What is the problem?

Deciding which adjuvants are suitable in different animals, their formulation, their effectiveness, and any possible off-target effects are all factors that need to be considered when choosing a suitable adjuvant. Also, there may be a lack of suitable adjuvant (either it does not exist, or it has not been validated) for less-well characterized animal models

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Mechanisms of action: Although adjuvants are widely used in vaccines to enhance the immune response, the mechanisms by which they do this has not been well characterised (i.e., we know they promote activation of PRRs/PAMPs, but not specific pathways). Therefore, there is the possibility of off-target effects, adverse reactions and non-specific immune responses that could be triggered by adjuvants.

Formulation: Many different formulations of adjuvant exist, including alum-based, oil emulsions, water-in-oil nanoemulsions, lipid nanoparticles, mineral salts, microbial products, saponins, polymers, liposomes and synthetic small molecular agonists. Deciding on the most appropriate adjuvant may then rely on biased preference or on knowledge of pre-existing usage, which may not be the most optimal adjuvant.

Side effects/toxicity: Freud's adjuvant is not licensed for use in human vaccine because of its toxicity.

Specificity: Because of the lack of systemic response and immune effectiveness, it is difficult to match and design appropriate adjuvants for specific vaccines, which may pose a barrier in deciding the most suitable candidate.

Licensing/approvals: If novel adjuvants are formulated, or have been less well-characterized for a specific vaccine, then getting the regulatory approvals for use in animals may provide a hurdle (cost, time). Considerations of using adjuvants that bind you with a specific organization – limits use of other adjuvants, may mean you do not have an adjuvant if that company stops making it.

Longevity of immune response: Generally, adjuvanted vaccines require booster vaccinations as they do not provide long-lived immune responses. Consideration needs to me given when deciding whether repeated vaccination with this adjuvant is beneficial, or whether it can be withdrawn for safe livestock production.

Adjuvants for mucosal delivery: Are there adjuvants for mucosal delivery?

Solution Routes

What approaches could/should be taken to address the research question?

Monitoring: Clinical development stage of promising adjuvants should be monitored to understand what is available for use.
Safety: Testing for safety in animals and animal products to understand adverse or off-target effects of adjuvants.
Optimisation: Studying the optimal ratio of adjuvant required for desired impact on immune response
Longitudinal assessments: As immunity wanes over time with adjuvanted vaccines, longitudinal assessment of the generated immune response is important to understand when titres start to decline to know when boosters may be required, and it should be determined whether there is longevity in response with different adjuvants.

Dependencies

What else needs to be done before we can solve this need?

Animal models: Understanding which animals are at risk from infection by coronaviruses, and therefore will require vaccination + adjuvants.

Type of antigen: Deciding what type of antigen (e.g., subunit or mRNA) and the immune response required will help with deciding which adjuvant may be more appropriate.

Considerations of using a combination of adjuvants and delivery systems: To achieve higher immune activations.

State of the Art

Existing knowledge including successes and failures

Promising adjuvants have been tested or are testing in preclinical models. A better knowledge about mechanisms of action could result in more adjuvants entering a clinical development stage, but perhaps this is less of a problem when designing adjuvants for animal vaccines. (Castrodeza-Sanz, Javier, Iván Sanz-Muñoz, and Jose M. Eiros. "Adjuvants for COVID-19 Vaccines." *Vaccines* 11.5 (2023): 902.)

Projects

What activities are planned or underway?

Novel water-in-oil nanoemulsion: mechanism of action is the formation of an antigen factory with slow release to prolong the bioavailability of antigens (Montanide ISA 51 and Montanide ISA 720) - clinical trials NCT00199836, NCT01008527, NCT01585350

Lead Summary [10] - Expression system

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

What are we trying to achieve?

Development of efficient expression system for recombinant viral glycoproteins, live attenuated virus cell lines or viral vector production.

What is the problem?

Deciding suitable expression systems for different vaccine platforms, their efficiency and whether they would work in different animal models, particularly when developing a new animal model.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Universality: Not all viral glycoproteins will be as easily expressed, stabilised, pseudotyped, and mono-serotypic as SARS2 spike. We need to turn our attention to more difficult orthologues whose expression and immunization does not always lead to the provision of immunity, e.g. Lassa, HIV. Will spike stabilization strategies like HexaPro be applicable to all coronaviruses?

Cell culture system: Choosing the appropriate cell culture (mammalian, yeast, bacteria, plant-based, insect) system to obtain best expression, optimal yield, purity, minimise contaminants etc.

Immune response: Understanding whether different expression systems will result in differential immune response, and whether this is sufficient to provide protection, stop onward transmission etc. This is also important when considering comparing different coronavirus vaccines made using different expression systems – what do we define as a correlate of protection?

Cost: Mammalian expression systems are more costly than e.g., insect expression systems – is one preferable over the other when generating animal vaccines? More vaccines could be produced, the requirement for purity is less stringent than in human vaccines, and it is cheaper to make animal vaccines.

Yield: Will one expression system return a greater yield of vaccine over another? Is this as important if the vaccine is not needed urgently? An economical vaccine that requires less investment and can be easily scaled up is of highest priority when designing animal vaccines.

Solution Routes

What approaches could/should be taken to address the research question?

Finding appropriate cell or tissue cultures to study coronaviruses in vitro that best mimic in vivo replication.

Alternative methods: Use of ex-vivo tissue and organoid cultures from natural host species when possible.

Availability: Broaden access to validated expression systems that are compatible with the first steps in clinical trial.

Dependencies

What else needs to be done before we can solve this need?

There needs to be a better understanding of coronavirus glycoprotein biology to recognise which expression systems may be appropriate for

spike without continuous rounds of trial and error, particularly the more difficult orthologues.

State of the Art

Existing knowledge including successes and failures

Modified live attenuated vaccine for PEDV generated through passage through Vero cells

- PEDV recombinant vaccines – mammalian and yeast expression system
  - Immunogenicity and protective efficacy of recombinant S1 domain of the porcine epidemic diarrhea virus spike protein - PMC (nih.gov)
  - S1 domain of the porcine epidemic diarrhea virus spike protein as a vaccine antigen - PMC (nih.gov)
- Corn-based delivery system for TGEV
  - A corn-based delivery system for animal vaccines: an oral transmissible gastroenteritis virus vaccine boosts lactogenic immunity in swine - PMC (nih.gov)
- Bacteria expression system for PEDV and TGEV :
  - Construction of a bivalent DNA vaccine co-expressing S genes of transmissible gastroenteritis virus and porcine epidemic diarrhea virus delivered by attenuated Salmonella typhimurium - PubMed (nih.gov)

Projects

What activities are planned or underway?

Nanoparticle-based swine vaccine (PEDV)- Zhang, Chenming et all. USDA-NIFA Project- Viginia Polytechnic Institute & State University https://portal.nifa.usda.gov/web/crisprojectpages/1027851-nanoparticle-based-swine-vaccine.html

Lead Summary [11] - Vector

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

Identification of the appropriate vector for different animal species to express animal coronavirus antigens to develop vaccines.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Appropriate vector: Several viral vectors have successfully been used in the past for various viruses to develop vaccines (e.g., paramyxoviruses, canarypox, adenoviruses, VSV [summarised in 7]), but understanding which vector would be most suitable for the target antigen is also important e.g., do the viral vectors have the same route of entry and replication?

Immunity: Viral vectored vaccines are effective at generating cellular immune responses, but this may come at the expense of a strong antibody response. Therefore, understanding the CoP for an animal coronavirus is important, and how both the humoral and cellular immune response can be maximized. Also, whether boosters will be required.

Contraindications: Does the viral vector itself induce any immune response in the host? Is there any pre-existing immunity in the host against the suggested vector that may mean it is ineffective at delivering the target antigen?

Production: What is the efficiency of the production methods? How expensive are viral vectors compared to recombinant proteins? Do you want to use a non-replicating or replicating vector?

Solution Routes

What approaches could/should be taken to address the research question?

Cost: Could bivalent vaccines be produced that cover multiple animal pathogens, and that make the vaccine more cost effective and favourable to vets/farmers etc. for immunisation of their animals?

Targeted modulation of viruses: Either by targeting host factors (cellular factors and pathways that are essential for the virus to survive) or by targeting the virus itself by developing compounds that selectively bind to the virus capsid – ensure vector is safe for use in animals.

Dependencies

What else needs to be done before we can solve this need?

Routes of administration: Which route of administration will deliver the most robust immune response? Will one route hinder the effectiveness of a vector?

Vector biology: Understanding the route of entry and replication of the vector, and mechanisms of action that would then impact the immune response triggered when used as a vaccine delivery system.

State of the Art

Existing knowledge including successes and failures

Imbokodo phase II/b HIV vaccine clinical trial (ClinicalTrials.gov Identifier: NCT03060629) ChAdOx1 for SARS-CoV-2, MERS-CoV VSV for SARS

What activities are planned or underway?

Projects

Lead Summary [12]- Identity of protective antigens

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

Determine which antigens (or combination of antigens) give better protective and long-term immunity. In particular, we aim to identify protective antigens beyond the spike (S) protein for animal coronavirus vaccines, e.g. envelope (E), membrane (M), and nucleocapsid (N) proteins. The goal is to explore whether these alternative proteins can induce robust T-cell responses or non-neutralizing immune responses, such as antibody-dependent cellular cytotoxicity (ADCC), enhancing the breadth and durability of vaccines and whether they should be included with S in novel vaccines?
Identification of the appropriate protective antigen is probably the most important factor above vaccine platforms, mechanism of delivery, etc.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Was SARS2 a soft target? Need to develop a better understanding of expression strategies for more difficult/less amenable viral S glycoproteins – are all coronaviruses likely to be suitable and will all evolve in the way SARS2 has under vaccine selection (without evolving into an entirely new serotype).
Limited knowledge on non-spike proteins: While the spike protein is well studied, the protective potential of other viral proteins (E, M, N) is less understood, especially in generating cellular immunity. Research role on ADCC on non-spike proteins (but also non-neutralising Abs induced by spike?)
T-cell response: Current vaccines often focus on neutralizing antibodies, but inducing strong T-cell responses may be critical for long-term protection. Are the alternative antigens suitable T-cell targets?
Cross-species variation: Non-spike antigens may elicit varied immune responses across different species, complicating vaccine design for diverse animal hosts.
Stability of antigen: Industry does not have the time/capability to study the best formulation of antigen so more basic research is needed for this – antigen structure, stability, optimal nucelotides/amino acids/UTRs etc.
Antigen combinations: Multivalent antigens present a challenge.

Solution Routes

What approaches could/should be taken to address the research question?

Immunogenicity studies: Investigate the T-cell and Ab (e.g. ADCC-inducing potential) of E, M, N proteins through immunization studies and models.
Multivalent vaccine design: Incorporate these alternative antigens into vaccine candidates to boost both humoral and cellular immunity. Combining antigens may broaden the response against different strains including more conserved structural proteins/epitopes, and may induce greater levels of protection and more cell-mediated immune responses. Also, it would be beneficial to have multivalent vaccines across different coronaviruses.
Adjuvants and delivery systems: Use adjuvants or viral vector systems to enhance the presentation of non-spike proteins to the immune system.

Dependencies *What else needs to be done before we can solve this need?*
Vaccine strategies should be tailored based on immune responses we would like to induce. Mapping epitopes on non-spike proteins from animal coronaviruses that are recognized by T cells or involved in non-neutralizing responses could help underpin vaccine design (what is known from SARS2 and is it applicable?). Study of immune responses to non-spike proteins across species would be required to ensure cross-protection.

State of the Art *Existing knowledge including successes and failures*
M and N are the most promising candidates, but also ADCC data for E.

Projects *What activities are planned or underway?*

Lead Summary [13] - Identity of virulence factors

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

To identify the key virulence factors in animal coronaviruses that contribute to disease severity, infectivity, and transmissibility. By mapping these factors to various components of the viral genome (RNA sequences/ proteins/ alternative transcripts) and understanding their role in pathogenesis, we hope to develop better vaccines and antiviral strategies to mitigate outbreaks in animals. Understanding virulence determinants is crucial for quick understanding of viral variants if they emerge mid-epidemic.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Zoonotic risk: For novel viruses there is the large outstanding question of how to understand zoonotic risk from sequence alone?
Understanding of virulence factors: Broadly we must identify virulence factors that contribute to viral infectivity, transmissibility and immune evasions of severe animal coronavirus diseases and understand their conservation across species/hosts. Coronaviruses have large genomes with multiple non-structural and accessory proteins whose contributions to virulence remain unclear. In addition, virulence factors may behave differently in various host species, affecting disease outcomes and transmissibility.

Emergence of new variance: As with SARS2 VOCs, the emergence of new variants with altered virulence necessitates constant monitoring and characterization of these changes in animal populations, especially mid-epidemic.

Solution Routes

What approaches could/should be taken to address the research question?

Genomic approaches to understand variant emergence: Study factors and determinants that give rise to emerging variants of interest (pathogenic and/or zoonotic) using the same genomic approaches applied to SARS2 to understand variant emergence. Feasible to, in tandem, use reverse genetics to create mutations in suspected virulence factors (e.g., ORF3, ORF8 and their orthologies) and assess their impact on infectivity, replication etc.
Use of predictive animal models: Animal models (preferable in a relevant host – easier for animal coronas?) to predict virulence and transmissibility of variants with different virulence factors would be good.

Dependencies

What else needs to be done before we can solve this need?

Understanding which animal coronaviruses we have detailed pathogenesis studies for, and which needs more preliminary work to enable the solutions detailed above.
Understanding which viruses have the most detailed genomic screening at the moment (in the field), and which need improving.
Identifying orthologues across species – is there the possibility of coronaviruses targeting the same pathways in slightly different ways, or are they all very different?

Existing knowledge including successes and failures

Coronavirus recombination contributes to the emergence of more virulent variants, but predicting these shifts remains challenging.

Projects

What activities are planned or underway?

Pirbright and University of Surrey has a proteomics project to understand which pathways are universally upregulated by coronavirus infection, and which are unique to certain species. Also, looking at more viruses outside of the coronas at the same time.
PorCoV single cell RNAseq in addition to measuring Ab and T cell responses

State of the Art

Lead Summary [14] - Identity of immunomodulators

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

To identify the coronavirus genes or proteins that modulate the immune response during infection or vaccination in pets, livestock, and wildlife. Understanding how these work can add crucial information for designing effective vaccines (for example by targeting them for knockout) that overcome viral immune-evasion and/or improving protective immunity.

What approaches could/should be taken to address the research question?

In vitro biology: Use reverse genetics to identify and characterize coronavirus immunomodulatory genes, and/or protein-protein interaction studies to map interactions between viral proteins and host immune components to understand their mechanism, also dipping into comparative studies to allow cross-species analysis. Immune-modulating proteins may have conserved and species-specific effects, which may also help to understand determinants of spillover.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Immune evasion mechanisms: Coronaviruses encode various proteins (e.g., non-structural, accessory proteins from the 3' of the genome) that modulate innate immune responses, including interferon signalling and cytokine production. However, these may have species-specific effects, i.e. behaving differently across species, complicating vaccine design for diverse animal populations.

Characterization of immunomodulator functions: Many of the immunomodulatory functions remain poorly characterized, particularly in non-human coronaviruses - can these functions be easily inferred from viruses like SARS-CoV-2. In addition, there is often a range of different proteins made by the different coronaviruses and so it can be difficult to assign the same function to orthologues.

Dependencies

What else needs to be done before we can solve this need?

Better animal models could help to study coronavirus-host immune interactions across species. What can be learned from SARS2 in hamsters here?

Elsewhere the development of high throughout assays for rapid identification of viral immune modulators across diverse species could speed up our understanding – is there a role for AI here?

Solution Routes

State of the Art

Existing knowledge including successes and failures

SARS2 accessory proteins (e.g., ORF3, ORF6, alternative N transcripts/proteins) are known to inhibit interferon responses – what

is the current knowledge for PEDV/ FCV/ BoCV/ IBV and how might this knowledge affect vaccine development (rational attenuation etc.)	Immunomechanism of coronavirus of livestocks (LVRI, CAAS- China)
Projects *What activities are planned or underway?*	

Lead Summary [15] - Host response to natural infection

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

What are we trying to achieve?

Gain a better understanding of host response to natural infection to inform vaccine development strategies and understand disease pathology in animal models

Problem?

Generally, the same/similar model systems are used to understand pathology, transmission and response to infection to novel pathogens, based on established models for similar viruses. However, this may not be the most physiologically relevant model. There is a lack of knowledge and research on animal immunology for e.g., birds, pets, wildlife – some work has been done to advance understanding of porcine and bovine immunology, but not as much as in mice and humans.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Accuracy: Not all animal models mimic natural infection well, and may be exaggerated or inaccurate because the virus being used may not usually be able to infect that host. Also, inbred vs outbred animals may yield varied immune responses

Immunity: There is a need to test long-term immunity to both natural infection and after vaccination. Measuring more than just nAb responses – T-cell response, muscosal, non-neutralizing Ab responses (ADCC, complement etc). Can we identify immune response markers that are freely/widely available (that provide broad protection)?

Selecting the correct animal model: Deciding which animal model to use that will support virus infection and replication to study disease is important as this may not be the same for all coronaviruses, e.g., change to hamster model for SARS-CoV-2 due to lack of productive infection in ferret model.

Other factors: Understanding the role of pre-exposure to a pathogen, co-infections of other pathogens, the role of innate immunity and age to host response. All these factors will impact a host's response to natural infection. Quality control is not available from a lot of commercial companies.

Lack of resources to measure immune response: There are many resources available for mouse models to look at, for e.g., cytokine responses, setting up large flow cytometry panels to look at immune cell profiles; however, these resources are less available or established for other animal models, reducing the amount of information we are able to obtain. Lack of, for e.g. pig interferons so that human interferons have to be used.

Solution Routes

What approaches could/should be taken to address the research question?

Resources: Developing tools and resources to broaden the immune response that can be characterised in less –well-established animal models

Challenge studies: Experimental challenge studies on long-term immunity in both natural infection and after vaccination - best addressed initially in natural host systems.

More bioinformatics needed: Predicting similarity at sequence/protein level using whole animal genome study data to resources that could be broad-acting

Dependencies

What else needs to be done before we can solve this need?

Establishing animal models in natural host systems for where this is not yet done.
Requirement of developing more specific reagents. Is there a way for industry (animal vaccine producers) and academia to work together to support the development of non-human immunological toolboxes? Why have these not succeeded at a global level previously? Could this be something that IVVN could lead on?
Understand innate and adaptive immune pathways in animal models and accurately document what disease and transmission looks like in these animals.
Better One-Health collaboration: Needs to be more exchange of knowledge/experience from researchers working on vet vs human vaccines – vet field were immunising animals for decades against coronaviruses, but this prior knowledge is largely ignored when developing human vaccines.

State of the Art

Existing knowledge including successes and failures

SARS-CoV-2: Ferret model did not show productive infection when infected with SARS-CoV-2 (modelled off what was done for SARS-CoV-1), so there was a transition to use the hamster model instead.
Models established for PDV/TGEV to study infection and to test vaccine efficacy in pigs.
Previous toolboxes established in Europe that have not been successful – what can we learn from these and what approaches do work? More success seen from individual groups.

Projects

What activities are planned or underway?

Immunological Toolbox (The Pirbright Institute) to develop species-specific antibodies/reagents - can academia work more closely with animal vaccine companies to develop these reagents?

Lead Summary [16] - Identity of mechanisms of protection

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

The goal is to understand the immune mechanisms responsible for long-lasting protection against animal coronaviruses. This will help tailor vaccines that induce both robust humoral and cell-mediated immunity, ensuring durable protection for pets, livestock, and wildlife. An improved understanding of immune responses, especially specific correlates of protection, will optimize vaccine efficacy and provide insights into which immuno-correlates (e.g. the balance of cellular vs. Humoral response) are key for different animal species and viral strains.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Mechanisms underpinning long-lasting protection: A key challenge is defining the immunological mechanisms responsible for long-term protection, particularly in distinguishing when and for which virus cell-mediated immunity is more crucial than humoral responses. Understanding these correlates will inform vaccine design.

How pathogens determine the correlates of protection: Currently, it is unclear how much the different animal coronavirus pathogens (PEDV, CCOV, PRCV, FCV etc.) differ, i.e. is vaccine efficacy more closely linked to humoral immunity rather than cell-mediated and is there a general and predictable pattern for such pathogens/vaccines? Measuring more than just nAb responses – T-cell response, mucosal, non-neutralizing Ab responses (ADCC, complement etc). Sometimes see protection without serological response. Considerations of ADE.

Tools for cross-species study: How lacking are we in this area for the main pathogens? Do we have good, validated challenge models for these viruses? Holistic approach to analyzing the immune system.

Antigen conservation across intra-species variants: Identifying conserved viral antigens that can provide protection across all variants, especially in the context of rapid evolution.

Solution Routes

What approaches could/should be taken to address the research question?

Memory response research: Improved understanding of memory immune responses, particularly which factors drive long-lasting T-cell and B-cell immunity across different species.

Benchmarking vaccine immunity: Develop standardized benchmarks to assess vaccine efficacy in generating both cell-mediated and humoral immune responses. These benchmarks can be used to compare vaccine platforms across laboratories under consistent conditions.

Host response studies: Investigate the immune responses to both virulent and attenuated strains of coronaviruses to understand the specific immune mechanisms at play. This includes exploring how viral load, antigen presentation, and immune activation differ between

strains and host species and will help to determine what sort of immune response is important for vaccine development.

Comparative vaccine platform testing: A wide range of vaccine platforms should be tested using standardized tests and shared SOPs across labs. This should ensure that direct comparisons can be made between vaccines, helping identify which platforms best elicit the desired immune response.

Correlates of protection research: Do we have the necessary tools and models to study correlates of protection in animal species other than humans and mice, which can include studying the balance between neutralizing antibodies and T-cell responses. There is ongoing work on this at places like Pirbright to develop porcine/bovine/chicken tools.

New tools: Expand the use of new computational tools, multiplexing, system immunology using innate signatures, to evaluate immune responses in pets, livestock and wildlife

Dependencies

What else needs to be done before we can solve this need?

Development of research tools: i.e. for studying immune responses to coronaviruses in animals like livestock and wildlife away from classic models. This includes generating new animal models or adapting existing ones to facilitate cross-species immune studies.

Data on conserved antigens: Confirming target antigens so that the relevant tools can be made.

State of the Art

Existing knowledge including successes and failures

Humoral and cellular immunity: It is well established now that virus-neutralizing antibodies targeting the spike protein are very important for protective immunity following vaccination. However, immune responses, particularly involving T cells, enhance viral clearance and durability of immunity. There are also emerging roles for non-neutralizing mAbs.

Challenges in animal models: For the COVID field there have been some issues, e.g. wild-type mice are less/not permissive to infection by SARS-CoV due to weak binding affinities between murine ACE2 and spike. Is this an issue for animal coronaviruses? Do some host breeds provide a different vaccine responses to others and thus complicate the development of correlates of immunity or protection.

Projects

What activities are planned or underway?

Immunomechanism of Coronavirus of livestocks. Huazhong Agricultural University, China (2021-2024)

Lead Summary [16a] - Antibody Response

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

Understand the role of antibody response to protect from infections.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Understanding of other species: Lack of comprehensive understanding of pets, livestock and wildlife immunology limits coronavirus vaccine research and development.

Differences in antibody responses: Differences in antibody responses to coronavirus infection versus vaccination are not adequately understood.

Immune mechanism: Understanding of immune mechanism of non-neutralising antibodies (ADCC, complement) and their potential for immune protection versus disease enhancement – can these be induced with different routes of vaccine administration?

Mechanism of humoral immunity: Differences in mechanism of humoral immunity between protection against infection (sterilising immunity) and protection from severe disease.

Spike immunodominance: The spike is immunodominant over more conserved viral antigens, which may create obstacles for developing vaccines targeting more conserved domains of the virus.

Immune imprinting: The role of immune imprinting and prior exposure to CoV infection or immunization.

Measuring antibody response: Lack of qualified and harmonized methods to measure antibody responses other than ELISA. Lack of reagents.

Pan-antibody identification: Identification of a pan-antibody with a broad spectrum of actions against different sarbecoviruses is challenging.

Solution Routes

What approaches could/should be taken to address the research question?

Understanding generation of antibody responses: Conduct basic research to understand generation of antibody responses in pets, livestock and wildlife. Comparison of antibody responses for different species.

Mucosal immunity: Improve understanding of mucosal immunity.

Identify B cell response: Identify how distinct types of B cell responses (neutralizing antibodies against the spike and ADCC antibodies against

non-neutralizing conserved epitopes) synergize or compete to result in a protective response.

Route of vaccine administration: Assess how the route of vaccine administration affects the protective immune response in the different immunologic compartments.

Boosting strategies: Boosting strategies based on antigenic distance to avoid 'back-boost' due to immune imprinting.

Pan-monoclonal antibodies: Identification and characterization of pan monoclonal antibodies (mAbs).

Dependencies

What else needs to be done before we can solve this need?

Develop functional assays to determine the range and breadth of protective antibody responses other than virus neutralisation.

State of the Art

Existing knowledge including successes and failures

Virus-neutralising antibodies directed against the coronavirus spike protein are critical in providing protective immunity following vaccination. Highly potent mAbs targeting the receptor binding domain (RBD) of huACE2-dependent sarbecovirus have been isolated and characterised from a SARS-CoV survivor vaccinated (Chia *et al.*, 2023, *Sci. Adv.* **9**, eade3470).

Some studies suggests a unique strategy for selecting booster vaccines based on antigenic distance, which may be useful in designing future booster vaccines as new SARSCoV-2 variants emerge (Hu YF et al., Rational design of a booster vaccine against COVID-19 based on antigenic distance. Cell Host Microbe. 2023 Aug 9;31(8):1301-1316.e8. doi: 10.1016/j.chom.2023.07.004)

Projects

What activities are planned or underway?

Livestock Antibody Hub Pirbright
https://www.immunologicaltoolbox.co.uk/hub
Immunological Toolbox https://www.pirbright.ac.uk/specialist-science-facilities/immunological-toolbox

Lead Summary [16b] - Cell Mediated Immunity

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

Understand the role of cellular immune response to protect form infections.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

T-cell mediated immune response: Understanding the protective roles of T-cell mediated immune responses. A robust T cell response to CoV may protect against severe disease, however can also have the potential to lead to excessive pro-inflammatory response and enhanced immunopathology.

MHC complex variation: The variation in MHC (Major Histocompatibility Complex) molecules between species makes it difficult to generalize findings across animals, as T cell recognition relies heavily on these molecules.

Environmental differences: Livestock and pets are often housed in controlled environments, but wildlife are exposed to a broad range of pathogens. This makes it harder to predict how their T cell responses will behave in the wild.

Cost and scalability: In livestock, cost and scalability are significant barriers to developing T cell-focused vaccines or treatments.

T cell memory and longevity: The duration of T cell memory is hard to quantify, especially in species where long-term studies have not been conducted

Effect of stress and overcrowding: In livestock, animals often experience stress, overcrowding, and poor nutrition, all of which can suppress T cell function and overall immunity, making it difficult to predict how this group will respond to infections?????

Solution Routes

What approaches could/should be taken to address the research question?

Identification of epitopes: Identify epitopes recognized by CD4 and CD8 T cells in different species that elicit broadly reactive T cell immunity.

Role of mucosal immunity: Identify how routes of immunization (internals oral, sublingual, or other mucosal routes) affect the degree of mucosal immunity.

Escape from T cell immunity: Explore methods to circumvent virus escape from T cell immunity (optimize peptide presentation or boost responses to subdominant epitopes).

Use of emerging technologies: Efforts to use emerging technologies to reduce reliance on animal models.

Multi pathogen vaccines: Develop multi pathogen vaccines as animal are exposed to broad range of pathogens.

Memory T cell response: Promote development of memory T cell responses at the site of entry of coronavirus.

Better protection from disease: Correlate of protection from disease is most likely the CD8 T cell response, but with more T cell antigens (like N) in the vaccine, would we get a better protection?

Dependencies

What else needs to be done before we can solve this need?

Improved, affordable and standardized T cell assays or markers for T cell function are needed. Reagents to characterize pets, livestock and wildlife T cell subsets.
Characterise MHC epitopes in pets, livestock and wildlife to better allow assessment of T cell-based vaccines.
Basic research to understand T cell responses at different anatomic locations.

Eliciting a cell-mediated immune response in addition to an antibody response is likely to improve both protective immunity and viral clearance.
Cytotoxic T lymphocytes are critical in the control of infectious bronchitis virus in poultry. (Collisson, E. W. et al. Cytotoxic T lymphocytes are critical in the control of infectious bronchitis virus in poultry. Dev. Comp.Immunol. 24(2–3), 187–200 (2000)

Projects
What activities are planned or underway?

State of the Art
Existing knowledge including successes and failures

Lead Summary [17] - Host-pathogen interaction

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

To improve understanding of the detailed mechanisms of host-pathogen interactions that unfold during the animal coronavirus life cycle, from entry to replication, and eventual persistence or clearance. How much can be applied from SARS-CoV-2 and what is clearly different. Ultimately, this knowledge would be crucial for improving vaccine designs by allowing the targeting of key stages of viral entry, replication, and immune evasion etc. To allow the prevention of severe disease outcomes. Additionally, what drives resistance to infection and rapid clearance in some hosts, which can guide future antiviral therapies and spillover prevention.
Also, we need a better understanding of recombination and the speed of recombination?

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Systemic infection mechanisms: It is unclear why some coronaviruses become systemic in certain cases, leading to severe disease and why some have gastrointestinal or respiratory tropism (or both). Understanding these mechanisms is critical for targeting vaccine responses to prevent systemic spread, e.g. by driving route of administration decisions. Deletion in NTD of spike (PRCV/TGEV), but also PRCV has partial deletion in accessory proteins – explore more differences outside of Spike too.

Innate immunity, e.g. interferon-stimulated genes (ISGs): The role of ISGs in restricting coronavirus replication is not fully understood, limiting our ability to exploit these pathways for antiviral strategies and/or for enhanced rational design of vaccines. In addition, knowing how ISG variability between species affects outcome could also help.

Cell entry pathways: The entry pathway of several animal coronaviruses, including infectious bronchitis virus (IBV), bovine coronavirus (BCoV), and swine acute diarrhoea syndrome coronavirus (SADS-CoV), is poorly characterized. Mapping these entry pathways is crucial for understanding viral tropism and improving vaccine efficacy as well as for the development of easy-to-use accompanying tests.

Non-structural and accessory proteins: The functions of many non-structural proteins (NSPs) and accessory proteins in the viral life cycle are still unknown. These proteins could be potential targets for therapeutic interventions and vaccine development (see other sections on immunomodulators etc.)

In vitro study limitations: Some coronaviruses are difficult to propagate in cell culture, limiting their study in vitro and making it challenging to explore the full life cycle or test antiviral therapies, as well as the development of cheap LAVs and other easy to use tools.

Understanding animal reservoirs: Animal-animal spread of coronaviruses and themechanism needed to get to humans requires understanding, as well as the need to better understand emergence of new pandemic coronaviruses.

Solution Routes

What approaches could/should be taken to address the research question?

Investigating severe disease factors: Focus on identifying factors (e.g., viral genes or host conditions) that contribute to the severity of coronavirus infections, including how viral load, immune evasion strategies, and host responses differ between mild and severe cases.

Innate immune response to infection: Focus on studying the innate response, e.g. ISGs and their roles in restricting animal coronavirus replication. This can be potentially exploited to develop therapeutic strategies that enhance these natural host defences during vaccination (additional PAMPS, vectored cytokines etc.)

Mapping cell entry pathways: Further research on cell entry pathways, especially for under studied animal coronaviruses, is critical in particular the identification of novel entry factors.

Organoid and ex vivo models: To study coronaviruses that are difficult to propagate in continuous cell cultures, organoid and ex vivo tissue culture models could be developed. These systems provide better simulation of natural sites of viral replication and enable deeper investigation into viral life cycles and can potentially be augmented with immune cells to even understand vaccine responses or correlated of protection.

Functional omics of coronaviruses: Use omics to determine the functions of NSPs and accessory proteins, to identify conserved mechanisms of pathogenesis and potential targets for antiviral drugs or vaccines. Basic research should focus on the roles of these proteins in immune evasion, viral replication, and host cell manipulation.

Dependencies

What else needs to be done before we can solve this need?

Cross-species viral research: Comparative studies of different coronavirus species and their host could be needed to determine if all viral families share the same risk of zoonotic spillover and identify any unique host-pathogen interactions that contribute to higher risk. This would support other aspects and make sure the questions asked do not become too big, so that they become impossible to answer.

Advanced viral modeling: Development of more accurate animal and cell culture models that can simulate the full life cycle of coronaviruses is critical. Improved modeling systems will allow for better testing of antiviral treatments and vaccine candidates.

State of the Art

Existing knowledge including successes and failures

Entry mechanisms: Coronaviruses enter host cells via spike (S) protein binding to cell surface receptors such as ACE2, DPP4, and APN. This is well established for some animal coronaviruses but behind the human field for others. For example, knowing the IBV receptor would be a game changer for that field.

Innate pathways: It has been shown that certain interferon-stimulated genes (ISGs) can restrict coronaviral replication, but how this extends to animal viruses is not very clear.

Disease severity factors: Comorbidities and age have been shown to increase the probability of severe disease, but it is not clear how this applies to other animals. For example, the situation in cats is very complicated, with overlapping vaccination etc. Is it the same for other species?

Projects
What activities are planned or underway?
Immunomechanism of coronavirus of livestock (LVRI, CAAS- China)

Lead Summary [17a]- Entry

Currently blank as comments integrated into Lead Summary 17

Research Question *What are we trying to achieve and why? What is the problem we are trying to solve?*

Challenge(s) *What are the scientific and technological challenges (knowledge gaps needing to be addressed)?*

Solution Routes *What approaches could/should be taken to address the research question?*

Dependencies *What else needs to be done before we can solve this need?*

State of the Art *Existing knowledge including successes and failures*

Projects *What activities are planned or underway?*
Versatile (INRAE)- Emerging SARS-CoV-2 variants: intersection of entry routes and antiviral responses.

Lead Summary [17b] - Replication

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Research Question *What are we trying to achieve and why? What is the problem we are trying to solve?*

Challenge(s) *What are the scientific and technological challenges (knowledge gaps needing to be addressed)?*

Solution Routes *What approaches could/should be taken to address the research question?*

B

Dependencies *What else needs to be done before we can solve this need?*

State of the Art *Existing knowledge including successes and failures*

Projects *What activities are planned or underway?*

Lead Summary [17c] - Persistence/clearance

Currently blank as comments integrated into Lead Summary 17

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Solution Routes

What approaches could/should be taken to address the research question?

Dependencies

What else needs to be done before we can solve this need?

State of the Art

Existing knowledge including successes and failures

Projects

What activities are planned or underway?

Lead Summary [18]- Challenge models

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

Develop optimal challenge models to understand disease dynamics, transmission, and immune responses in animals to improve vaccines and treatments.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Developing challenge models that accurately represent how different species react to coronavirus infections in terms of immune response, viral clearance, and immunopathology.

Creating a standardized challenge model that can be applied to multiple species – there may already be good models available, but there is a lack of tools/assays for good readouts of them. Creating challenge models that can effectively evaluate vaccine efficacy in diverse species, given differences in immune system function, viral evolution, and vaccine response. Is there an available bat challenge model?

Risk of coronaviruses mutating in animal hosts and generating new variants with potential zoonotic spillover or enhanced transmission.

Solution Routes

What approaches could/should be taken to address the research question?

Standardized challenge models that allow easy comparative vaccine performance study.

Standardizing certain aspects of the challenge model (e.g., virus dose, route of administration) while adapting others (e.g., species-specific endpoints and biomarkers).

More cross-species vaccine trials and multi-pathogen vaccines that target conserved viral components across species.

Research into alternative or more representative animal models, including genetically modified animals (bovinized or porcinized mice) or organ-on-a-chip technologies.

Developing safe, field-compatible challenge models that can be performed without spreading the virus into animal populations or ecosystems.

Dependencies

What else needs to be done before we can solve this need?

More research into the immune responses of various species is needed, particularly in wildlife species that are less well studied. Cross-species studies that explore viral infection patterns in a variety of animals are necessary to determine which species are susceptible and the disease progression in these species

State of the Art

Existing knowledge including successes and failures

Projects

What activities are planned or underway?

Lead Summary [19] - Pathogen genome

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

Increasing our knowledge of the pathogen genome to identify genetic factors that contribute to virulence, transmission, and immune evasion. Essentially improving of genotype-to-phenotype linkages.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Big data management: The sheer volume of genomic data generated through high-throughput sequencing makes it challenging to manage, store, and analyze. Advanced bioinformatics tools are needed to handle large datasets efficiently.

Predictability of mutations and pathogenesis: There is limited understanding of how specific mutations in the coronavirus genome correlate with changes in pathogenicity and transmission (genotype to phenotype). This lack of predictability hinders the development of vaccines and treatments for emerging variants.

Early detection of variants of concern: Timely identification of genetic changes that could lead to more transmissible or virulent variants is critical for preventing outbreaks in animal populations. However, current surveillance and sequencing efforts often lag behind the rapid evolution of coronaviruses.

Envelope (E) protein knowledge gap: The role of the E protein, which is involved in viral assembly and release, remains poorly understood. There is a need to investigate whether the E protein also contributes to viral pathogenesis, as recombinant coronaviruses lacking the E protein show reduced viral titres.

Solution Routes

What approaches could/should be taken to address the research question?

Modelling for variant emergence: Based on COVID data, advanced computational models, including those using artificial intelligence, could be developed to predict the emergence of variants of concern. These models could help identify mutations that may lead to higher pathogenicity or immune escape or predict how coronaviruses might evolve in response to environmental pressures or vaccination campaigns.

Study genetic differences in variants: Comparative genomic studies are needed to identify and analyze genetic differences between coronavirus strains and/or species. This correlative research will provide additional insights into which mutations are associated with changes in virulence, transmission, and host specificity.

Genome sequencing for mutation analysis: Continuous genome sequencing of circulating animal coronavirus strains is essential to monitor ongoing mutations and get an overall idea of the in-field situation.

Dependencies

What else needs to be done before we can solve this need?

Bioinformatics capacity: The ability to store, process, and analyze vast amounts of genomic data requires investment in bioinformatics infrastructure. Increased computational power, skilled personnel, and advanced software are necessary to handle high-throughput sequencing data efficiently.

State of the Art

Existing knowledge including successes and failures

COVID has highlighted what can be done in this space with significant investment. Is there enough financial support for this in the animal pathogen space.

Projects

What activities are planned or underway?