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VACCINE INSIGHTS

SPOTLIGHT ON
Understanding immune responses

Guest Editor
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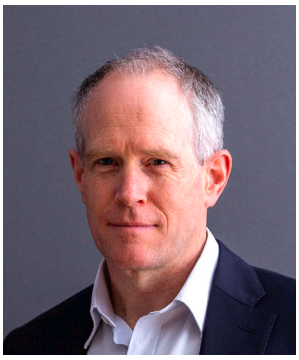
VACCINEINSIGHTS

UNDERSTANDING
IMMUNE RESPONSES

SPOTLIGHT

What type of vaccine immunity controls breakthrough COVID?

Stephen J Kent & Miles P Davenport



VIEWPOINT

"There is interest in the possibility of developing mucosally targeted vaccines in the hope these may increase mucosal antibody levels, improve protection and reduce shedding of virus..."

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A better understanding of immunity to either prevent COVID-19 infection or prevent severe disease should rationally allow improvements in vaccine design and schedules. Neutralizing antibodies (NAb) have emerged as a strong correlate of protection. This was first shown in studies making comparisons across eight vaccine platforms, where the level of NAb induced in phase 1/2 studies correlated with efficacy in phase 3 studies [1]. More recently, this has been confirmed by analysis of breakthrough infection in several phase 3 studies [2,3].

Although these initial trials addressed protection from the ancestral virus, more recently it has been shown that NAb titers predict protection across different SARS-CoV-2 variants and in the context of waning immunity [4,5]. Correlates of protection from severe disease are more difficult to assess as most randomized studies had low numbers of severe infections [1]. However, through analysis of a large number of observational studies with larger numbers of severe infections, it is clear NAb correlates strongly with protection from severe infection [5]. Studies of passive NAb administration demonstrate that antibodies are mechanistic in prophylaxis of symptomatic infection [6], and in a therapeutic setting are effective in reducing the progression from symptomatic to severe infection [7]. Indeed, the levels of NAb required for protection either induced by vaccination or administered passively are remarkably similar, adding further weight to the role of NAb in preventing and controlling COVID-19 [6,7].

As Omicron strains have become progressively more NAb-escaped, breakthrough COVID (infection despite vaccination) has become the norm. Since the level of NAb against a particular strain to prevent severe infection is much lower than the level to prevent infection altogether, protection from severe infection with Omicron strains has persisted, despite a reduction in protection from symptomatic infection [1,5]. Breakthrough COVID-19 in vaccinated individuals results in similar levels of virus at presentation (compared to unvaccinated individuals), which allows COVID-19 to continue to spread widely despite vaccination[8]. However, although peak viral levels are similar, viral levels decline more rapidly in vaccinated individuals. More rapid control of virus, whether through antiviral drugs or NAb administration, has recently been shown to correlate strongly with improved prognosis [9].

We recently assessed the immune responses that are recalled as the virus levels are brought under control. We serially sampled human cohorts—measuring both nasal virus levels and immune responses early after symptom onset. We found that recall of NAb responses in blood temporally coincide with control of virus levels [10,11], providing further weight to the role of NAb in preventing severe disease.

The contribution of T cell responses to COVID-19 immunity is more challenging to identify, at least in part due to the assays needed to assess this [12]. We initially found, using the activation-induced marker (AIM) assay, that T cell responses were more sporadic and tended to appear later as virus was cleared, suggesting a more modest role for T cells in controlling breakthrough COVID [10]. However, since the AIM assay measures *in vitro* activation following antigen simulation, any *in vivo* activation occurring during acute infection might obscure a better picture of the T cell response. Using HLA class 1 and 2 tetramers, we recently found that both CD4 and CD8 T cells are activated very early after symptom onset and also likely play a role in viral clearance [13]. A caveat of our studies was that our cohorts were generally healthy and younger, and therefore not susceptible to severe COVID. Detailed studies of breakthrough COVID in the elderly and other vulnerable groups should prove insightful regarding immune responses that prevent severe disease or not.

The data clearly identify NAb as the major factor mediating vaccine-induced and infection-induced protection from symptomatic

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SARS-CoV-2 infection, as well as protecting from progression to severe COVID-19. Both booster vaccination and breakthrough infection may act to broaden and maintain antibody levels over time, and the requirements for booster vaccination in different populations have yet to be elucidated. Updating of vaccine antigens to 'keep up' with circulating variants may provide marginally improved protection [14], although the current BA.1 or BA.5 bivalent vaccines have only a modest additional benefit over an ancestral-only booster vaccine [15]. There is interest in the possibility of developing mucosally targeted vaccines in the hope these may increase mucosal antibody levels, improve protection and reduce shedding of virus, although as yet this has not been demonstrated. Similarly, there is interest in vaccines that may enhance T cell responses, although proof of principle is yet to be shown.

In the current environment, most individuals have substantial immunity from COVID-19 resulting from previous exposure to infection and/or vaccination. It is clear that ongoing vaccination or breakthrough infection results in boosting of NAb over time and will provide reasoNAbly durable protection from the same or closely related variants [11,14]. Unfortunately, breakthrough infection leads to little immunity to infection with new variants that have significant neutralization escape. For example, the immune boosting of NAb after Omicron BA.1 or BA.2 infection in 2022 are predicted to provide <10% and <30% protection from current XBB strains [11]. The rapid evolution of further escaped variants poses a challenge to vaccine strain formulation and selection. Whether the Omicron-only monovalent XBB vaccines arriving later in 2023 can modify the pattern seen over the last 2 years is unclear.

BIOGRAPHIES

MILES DAVENPORT leads the Infection Analytics Program at the Kirby Institute at UNSW Sydney. His team of applied mathematicians incorporate statistical and computational approaches to understand infection and immunity. His research focus is on using modelling to analyse host-pathogen interactions in infections including SARS-CoV-2, HIV, and malaria. He has a wide variety of clinical and experimental collaborations both within Australia and overseas and his work aims to integrate experimental data and modeling. He is a past-President of the Australasian Society for Immunology, past Section Editor at *Journal of Immunology*, and current Senior Editor at *eLife*. His seminal work defining the correlates of protection from SARS-CoV-2 infection has helped inform vaccine policy for COVID-19 and understand immunity to viral variants.

STEPHEN KENT trained as an infectious diseases physician and viral immunologist in Melbourne and the USA. He is a National Health and Medical Research Council Investigator based at the Peter Doherty Institute for Infection and Immunity at the University of Melbourne. Stephen heads a lab studying immunity to HIV, Influenza, COVID-19 and other viruses that are difficult to target by vaccination.

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INTERVIEW

Unraveling systemic inflammatory responses to mRNA-LNPs

Charlotte Barker, Editor, *Vaccine Insights*, speaks with **Siri Tähtinen,** Principal Scientist at Genentech, a member of the Roche Group, about developing immunotherapies and vaccines for the treatment of autoimmune, inflammatory, and malignant diseases. They discuss inflammatory responses to mRNA-LNPs and the delicate balance between reactogenicity and immunogenicity.

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Q How did you get your start in immunology and how have your interests evolved?

ST: I did Bachelor's and Master's degrees in Biochemistry in my hometown of Turku, Finland, before moving to Helsinki to do a PhD in Translational Cancer Research. I joined a lab that was doing gene therapy with oncolytic viruses, which evolved into immunotherapy and vaccination. I have never had any official training in immunology but have learned on the job. It was an exciting time to join because the field was evolving from

simple cell biology into immunology and immunotherapy as we started understanding that oncolytic viruses hold vast immunostimulatory potential.

Over the course of my PhD and first postdoc, I was lucky enough to be part of the forefront of some revolutionary work. We submitted several patent applications and published multiple papers on immunotherapeutic and vaccine applications of oncolytic viruses. I received first-hand exposure to the industry as both my graduate student mentor and my first postdoc mentor founded spinout companies based on these seminal discoveries.

I wanted to keep working on vaccines as I was fascinated with this translational research, so when the opportunity presented itself, I accepted an offer to join Ira Mellman's lab at Genentech in South San Francisco. I focused on mechanism of action studies of cancer vaccine platforms, alongside Lélia Delamarre's group.

Q What are you working on right now?

ST: I am now working on non-viral gene delivery and vaccines; the biology is very similar to viruses. We are starting to appreciate that lipid-formulated RNA and DNA vaccines look a lot like enveloped viruses to the immune system and they induce many of the same antiviral pathways as viruses do.

The novel aspect that I have been focusing on is the fact that beyond RNA and DNA sensing, it appears that lipid nanoparticles (LNPs) themselves can activate various innate immune pathways, rather than simply being inert delivery vehicles. It feels like we are reliving the immunology discovery I previously experienced with oncolytic viruses.

Q How significant are the systemic inflammatory responses to mRNA-LNP therapies or vaccines?

ST: We know that both the BioNTech/Pfizer and the Moderna SARS-CoV-2 vaccines cause systemic inflammatory responses (reactogenicity) in many individuals, but we also know that these vaccines induced impressive antibody and T-cell responses (immunogenicity). When we consider innate immune activation or inflammation caused by vaccines, we have to remember that a certain level of innate immune stimulation or adjuvancy is required for a vaccine to induce adaptive immune responses [1].

In the case of RNA-LNP, the RNA is N1-pseudouridine modified and thus it is poorly recognized by Toll-like receptors (TLR) 7 and 8. We quickly started to understand that the adjuvanticity of the vaccine must be provided by other components. We and others have shown that LNPs, or more specifically the ionizable lipids in them, have significant intrinsic adjuvant activity in human cells and in mouse models [2,3]. Currently, we have identified molecular pathways that we know lead to the induction of pro-inflammatory cytokine IL-1. This IL-1 induction can trigger downstream cytokine cascades, leading to local and systemic inflammation.

These dose-dependent inflammatory responses were not predicted by preclinical trials using non-human primates or even mice due to differences in sensitivity and tolerability between species. Our work has been slowly trying to understand how different species react to these

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vaccines and the different components in them. There is a baseline difference in what pathways get triggered by the vaccines. It seems that mice are much more sensitive to the double-stranded RNA impurities in the vaccine, which are mainly detected by RIG-I and MDA5 pathways [4], whereas in humans, the predominant pathways activated by these vaccines are TLR8 and inflammasome [2].

> "We are starting to appreciate that lipid-formulated RNA and DNA vaccines look a lot like enveloped viruses to the immune system and they induce many of the same antiviral pathways..."

Moreover, one of our major discoveries was that pro-inflammatory IL-1 and anti-inflammatory IL-1 receptor antagonist (IL-1ra) are very differently induced by different species. Using non-human primate peripheral blood mononuclear cells (PBMCs), our preliminary data suggest that non-human primates resemble mice more than humans when it comes to IL-1 pathway activation by lipid-formulated RNA vaccines [2].

Q What do we know about the immunological mechanisms behind these responses?

ST: Prior to the completion of any human studies, our understanding of the immunological mechanisms of reactogenicity to lipid-formulated vaccines was limited. This was largely due to the species-specific differences in sensitivity and tolerability. When Genentech and BioNTech conducted a Phase 1 clinical trial with our joint mRNA-based cancer vaccine, we first started getting indications that humans are very sensitive to lipid-formulated RNA vaccines.

We got further clinical evidence of this when the SARS-CoV-2 vaccine clinical trials were published by BioNTech/Pfizer and Moderna. Moreover, the only previously approved LNP-formulated RNA therapeutic, Onpattro®, had also been associated with infusion-related adverse events, necessitating premedication with corticosteroids. These observations together gave rise to our efforts to understand what is driving this surprising reactivity to lipid-formulated RNA.

Q What has been your group's contribution to understanding these responses?

ST: Before the pandemic, we were working with BioNTech on the joint cancer vaccine program involving RNA-LPX, a slightly different lipid formulation from LNP but with very similar biology. We discovered that the 50 μg doses of RNA-LPX that are very well tolerated in mice caused transient mild to moderate flu-like symptoms in humans. We were baffled by the huge difference in tolerability.

Therefore, we set up a study to see what innate immune pathways are activated by RNA vaccines and how this differs between species. We found that both the lipid and RNA components

in the vaccine contribute to the activation of the inflammasome pathway, triggering the release of IL-1. IL-1 is the master regulator or upstream inducer of many downstream inflammatory cytokines leading to systemic and local inflammation by these vaccines.

Moreover, we realized quickly that mice differ from humans when it comes to IL-1 induction because mice preferentially upregulate IL-1ra, which is an endogenously expressed cytokine biologically meant to counteract the activity of IL-1. Mice upregulate this negative pathway regulator instead of IL-1.

We confirmed this by treating genetically deleted IL-1ra knockout mice with the RNA vaccine and seeing a massive decrease in tolerability to the vaccine characterized by transient hypothermia, body weight loss, and elevated serum cytokines. This indicated that the high levels of IL-1 receptor antagonist protected the mice from the uncontrolled systemic inflammation that was mediated by the vaccine-induced IL-1.

We also found that in assays with human PBMCs, monocytes are the main producer of IL-1. There are generally fewer monocytes and myeloid cells in mice or non-human primates than in humans, so we decided to treat the IL-1ra knockout mice with FMS-like tyrosine kinase 3 ligand (Flt3L), which expands the monocyte and myeloid compartment in those mice. When we treated these mice with RNA-LPX vaccine, we saw significant further sensitization to high doses of vaccines, indicating that both the IL-1 pathway and the levels of myeloid cells are driving these tolerability issues in humans.

One of our other key findings was that certain ionizable lipids in LNPs can activate this IL-1 pathway even in the absence of any RNA. This was a surprising finding, and we are working to further delineate why and how this happens.

Q How are you planning to expand on that work?

ST: We are working on several aspects of this. We want to establish if it is possible to fine-tune the delicate balance between reactogenicity and immunogenicity. Can we uncouple the two? Is it possible to have one without the other? As humans, we are evolutionarily hard-wired to sense and react to viruses. The key question is: can we get around these pathways by modifying the nucleic acid or lipid components in the vaccine?

IL-1 is important for the immunogenicity of vaccines. We and others have shown that IL-1 can affect the quality and quantity of T-cell responses [2]. There are also reports showing that IL-6, which is induced by IL-1, can drive T-cell and B-cell responses responsible for antibody generation by the vaccine [3]. IL-1 is important, but we want to fine-tune it to avoid negative systemic reactions.

Q You've worked on both cancer immunotherapies and prophylactic vaccines—what can these fields learn from each other?

ST: Conceptually, the two are not very far from each other. Both are aiming to induce antigen-specific immunity to either treat or prevent disease. Instead of segregating these fields, I'd like to encourage more collaboration and cross-functional discussion. The most

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groundbreaking science and innovation happens at the intersections of different scientific fields.

At Genentech, I have learned a lot by discussing nucleic acid chemistry, nanoparticle formulation, immunology, clinical science, bioinformatics, pharmacokinetics, and biomarker discovery. Our joint project meetings with colleagues from different backgrounds and departments allow the best science to happen, even though you may speak very different languages.

Q What's next for your work?

ST: Further work on the properties of LNPs and their immuno-stimulatory profiles is needed if we want to design safer and more effective RNA-based vaccines and therapeutics. There are many ongoing activities in understanding what base-level components are needed for optimal adjuvanted vaccines versus non-vaccine oligonucleotide delivery methods. People are working on many non-vaccine applications of LNPs, such as small interfering RNA, antisense oligonucleotides, gene editing, and protein replacement therapy.

It is important to distinguish what these LNPs are doing so we can either upregulate or downregulate whatever pathways are induced. We need a deeper understanding of how and why certain lipids activate innate immunity. Is it only lipid driven, or is there an RNA component to it? Many RNA-LNPs contain residual double-stranded RNA, which possibly contributes to innate stimulation.

Many of these things are going to be crucial, not just for vaccines but also for other applications of LNPs. We need better models, for example the IL-1ra knockout model for mice, but we also have to start thinking about using human primary immune cells or organoids instead of cell lines and question the relevance of non-human primates when it comes to reactogenicity studies with RNA-LNPs.

I would also like to see systemic studies using high throughput methods to assess what kind of lipid structures activate which innate immune sensors and in which species. If we just stick to mouse models, this may not be directly translational to humans.

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BIOGRAPHY

SIRI TÄHTINEN has over 10 years of experience in translational immunology both in academia and in industry, specializing in nanoparticle vaccines, immuno- and gene therapies and oncolytic viruses. Siri obtained her BSc and MSc in Biochemistry at University of Turku, Finland and her PhD in Biomedicine at the University of Helsinki, Finland. Siri recently completed her postdoctoral training at the Cancer Immunology department at Genentech, in the research group of Ira Mellman, while working in close collaboration with Lelia Delamarre's lab and other internal and external partners. She currently holds a position of Principal Scientist/Group Leader at the Department of Immunology Discovery at Genentech, Inc. Her lab is focused on investigating novel biology and potential therapeutic targets to treat autoimmune, inflammatory and malignant diseases by inducing antigen- and/or tissue-specific immunity. During her graduate and postgraduate work, Siri has received numerous awards and grants, has been a co-inventor in three patent applications and has been a first author or a co-author in over 25 scientific publications and manuscripts.

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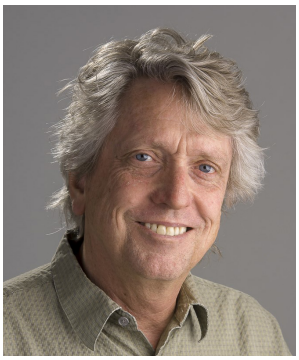
INTERVIEW

Establishing a B cell and T cell receptor data commons using next-generation sequencing



Charlotte Barker, Editor, *Vaccine Insights,* speaks to **Felix Breden,** Professor Emeritus at Simon Fraser University and founding Chair of the AIRR Community Executive Sub-committee. The Adaptive Immune Receptor Repertoire (AIRR) Community group is organizing and coordinating stakeholders in the use of next-generation sequencing technologies to establish B cell and T cell receptor data repertoires.

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Q How did the AIRR Community come about?

FB: My wife, Jamie Scott, and I both worked at Simon Fraser University for many years. She studied HIV vaccine development. In 2013, she organized a symposium in association with the Antibody Society on defining and delimiting clones in B cell repertoires. At the time, everybody defined those things differently (they pretty much still do).

We recognized a need for a common language for these huge datasets that were just starting to be produced, thanks to next-generation high-throughput sequencing being applied to B cell or T cell repertoires. In 2015 in Vancouver, we had our first international meeting to bring together immunologists, computer scientists, and experts in ethics of data sharing, and

the Adaptive Immune Receptor Repertoire (AIRR) Community was born. Now, our eighth international meeting is planned for June of next year in Europe.

One of the first things we did was come up with minimal standards for curating these types of data, as these datasets are huge, often including 1 million or even 10 million sequences for each sample. We wanted to establish the minimal data, or metadata, needed to accompany a dataset so that a researcher would know how the samples were processed, sequenced, and analyzed bioinformatically. 80 columns of minimal standards were established as a common way of describing the samples. These minimal standards would make it easy to share these kinds of data among researchers, which was a key motivation for establishing the AIRR community.

Sometimes it is hard to get people to agree. But by remaining a grass-roots, community initiative all these years, rather than taking a top-down approach with a few leaders dictating how things should be done, we have been able to establish community-approved protocols for curation and sharing of these data.

Q What is the goal and structure of the AIRR Community?

FB: The overarching goal is to be able to share data effectively. The main work of the AIRR Community is accomplished by seven working groups that anyone can join, which meet about once a month. We work by consensus. Once a standard has been developed, we publish it in a scientific publication, and everybody in the community has a chance to vote to approve that publication. That is one way we maintain community control of the standards that are produced. The working group I am most involved in is the common repository working group, which is building the AIRR Data Commons. Other working groups focus on aspects such as software and immunoglobulin and T cell receptor germline genes.

The AIRR community is an open community. Anybody can join the working groups and membership is free for students and postdocs. If *Vaccine Insights* readers would like to get involved, they can find us here [1].

Q How is the AIRR Data Commons helping researchers?

FB: One part of the vision is to be able to compare big vaccine studies easily. This has previously required downloading studies from the sequence read archive (SRA) as raw data. These files are often not in very good shape—there might be bits missing or unexplained quirks. These raw data then must be annotated against germline immunoglobulin and T cell receptor genes, using several different algorithms with various assumptions.

In the AIRR Data Commons, we store data in a usable form, with comprehensive annotations and metadata in a common format; for example, gender should always appear in column fourteen. If you store the data according to the minimal standards in an AIRR-compliant manner, then anyone can access and query these data from different repositories.

The AIRR Data Commons has always embraced a distributed repository model. The data sets are huge and often have data risk constraints, so it often would be best to keep data at the home institution. However, if it is all in the same format, you can either write a program to do queries or use a science gateway, such as iReceptor [2], which does those queries for you,

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across the distributed repositories. Researchers can also access the AIRR Data Commons in a similar fashion through VDJServer [3]. Having to re-annotate the data and reformat the metadata so you can do statistics on it can take a long time. We are not doing anything that researchers cannot do on their own, but we are facilitating it so it can be done in a few hours rather than 6 months. The vision of the AIRR Data Commons is to facilitate that work, sharing immunological data in a common format.

"The vision of the AIRR Data Commons is to facilitate that work, sharing immunological data in a common format."

Q What is the biggest challenge with this work?

FB: It can be hard to convince people to take that extra step to make their data more easily shareable within the whole community. We need to establish a data-sharing culture. Researchers talk about wanting to share their data, but sometimes it can be too hard to do in practice. The AIRR Community has developed these tools to easily add data into the AIRR Data Commons in a common format. With COVID, it was good to see researchers excited about making their data publicly available through the AIRR Data Commons.

We have also seen scientists at commercial organizations starting to use the AIRR data-compliant format. They might not put the data into the AIRR Data Commons and make it public, but this common format makes it easier for their researchers to query the public data and compare it to their own.

Q How are these large data sets curated and made accessible to researchers around the world?

FB: There are about 5 billion receptor sequences in the AIRR Data Commons from around 80 studies. Although the goal is to have geographically distributed repositories, most of those 5 billion are in two different repositories–the iReceptor Public Archive at Simon Fraser University and VDJServer, an NIH-funded group at the University of Texas Southwest Medical Center. These two groups have curated the data from public sources, reformatted the metadata, and re-annotated it according to an annotation program. Motivated by the pandemic, a large amount of data from COVID studies was curated in collaboration with COVID researchers. We also have a repository at the German Cancer Research Center (Deutsches Krebsforschungszentrum, DKFZ) and one at the University of Münster, Germany. We are pushing for different groups to develop their own repositories.

On the iReceptor gateway, the researcher will not see that these data are coming from different repositories. It is all federated into an integrated display. You can do a quick search on a CDR3, one of the important pieces of the receptor molecule, for example, and see whether that CDR3 shows up in other diseases or other individuals, or you can search the metadata for repertoires from specific diseases, such as HIV or flu.

Q What projects are you excited about right now?

FB: We are collaborating with Monica Westley, Founder of the(sugar)science, who is working tirelessly on getting type 1 diabetes researchers to share their data. She has convinced a lot of large labs to share their data publicly, and iReceptor is working with them to put those into the AIRR Data Commons. You could compare public versus private clonotypes at the push of a button or determine whether patients with worse outcomes are characterized by particular clones. To be able to do that, you have to look at a large group of diverse individuals and compare data beyond type 1 diabetes to other autoimmune diseases.

Another aspect is that single-cell work is becoming popular. Right now, most data sets are based on bulk sequencing of 1 million to 10 million sequences per sample. The AIRR Data Commons web Application Programming Interface (API) now allows for single-cell immune profiling, which includes sequences of the two chains of the receptor, the gene expression data, and some of the phenotypic markers from every single cell. We only have three single-cell datasets in the AIRR Data Commons right now, but with these, you could search the different samples to find those expressing certain genes at a high level, and then correlate these with particular immune receptors.

With single-cell data, each cell has a 25,000-count matrix associated with it. It is going to be a big challenge to curate that much data for each study, but it holds exciting potential. The ability to look at different types of cells producing a receptor and the physiological state of each of those cells can help determine the important groups of cells for diagnostics or therapeutics. We expect such single-cell studies to be a growth area for the AIRR Data Commons and for immunogenetics researchers

Q What are your hopes for the future of AIRR?

FB: I want to share and integrate datasets, get more data faster, and get everybody to agree that gender goes in column fourteen!

We are working to integrate information in the AIRR Data Commons with databases that curate germline genes. The information in each individual's expressed B cell and T cell receptor repertoires can be used to infer genetic polymorphisms in their immunoglobulin (for B cells) and T cell receptor germline genes. We are also working to link with the Immune Epitope Database (IEDB), which is a large database of epitopes and antigen specificity data for many of these B and T cell receptors. The big dream is to integrate from germline polymorphisms all the way up to phenotype, including disease phenotype, in order to predict propensity for diseases and understand the molecular underpinnings of disease.

We are working with the International Union of Immunological Societies to expand the view of the AIRR Community initiative for shared metadata to other immunological data types, such as flow and microbiome. The real vision is to make it easier to share and analyze all of these data types and get a complete picture, rather than having them in silos or difficult-to-navigate data lakes.

We have also talked about having some sort of digital object identifier (DOI) or stamp on each data set, to make it possible to count how many times your data has been downloaded

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or used in an AIRR-compliant analysis program. Right now, if somebody uses your data, you might not know about it for 2–3 years, until it is used in a publication. There is great value in other people using your data, and it can be useful to know and be rewarded (e.g., tenure committees, funding institutions) when it is happening.

Q What keeps you motivated?

FB: I am an evolutionary geneticist by training; I worked in beetles, toads, and guppies until about 15 years ago when my wife got me interested in human immunogenetics. Working in an area that could have positive outcomes for human health and patient care is both exciting and satisfying.

We are currently in the Wild West phase in terms of the analysis of these immune cell receptor repertoires. We do not know what information is in there, or how important that information will be, but there is a lot of potential. It is exciting to be working with one of the first groups to look at this in a very systematic way, combining data across labs, institutions, and diseases.

BIOGRAPHY

FELIX BREDEN is trained broadly in evolutionary genetics, including population genetics, behavioral analysis, and molecular genetics. In 2003, Dr Jamie Scott introduced him to the wonderful world of immunology and immunogenetics, and since then much of his effort has been dedicated to developing community and bioinformatic resources for studying both the fascinating evolutionary dynamics of the adaptive immune system, and of course how understanding these systems can lead to new therapies and diagnostics. The resources he is developing for curating, analyzing and sharing immunogenetic data, through the iReceptor Project and the Adaptive Immune Receptor Repertoire (AIRR) Community, will facilitate research in cancer, and autoimmune and infectious diseases.

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AUTHORSHIP & CONFLICT OF INTEREST

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VACCINE INSIGHTS

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REVIEW

Veterinary coronavirus vaccines: successes, challenges & lessons learned for SARS-CoV-2 control

Anna M Hassebroek & Xiang-Jin Meng

Members of the *Coronaviridae* family infect a large number of animal species, including humans. Coronaviruses of clinical significance in veterinary species include severe and fatal vasculitis in cats caused by feline infectious peritonitis virus (FIPV), and highly contagious and economically devastating diseases in livestock, including porcine epidemic diarrhea virus (PEDV), transmissible gastroenteritis virus (TGEV) in pigs, bovine coronavirus (BCoV) in dairy and beef cattle, and avian infectious bronchitis virus (IBV) in chickens. Knowledge of the viral replication, pathogenesis, protective immunity, and genomic mutations and evolution has led to the development of a variety of licensed vaccines against these veterinary coronaviruses. Some of the licensed animal coronavirus vaccines have been in commercial use for decades and have demonstrated many of the same challenges in veterinary species that are being faced today with the relatively new SARS-CoV-2 vaccines. Here we review the coronaviruses of high clinical impact in veterinary species, identify common themes regarding the challenges and successes of animal coronavirus vaccines that have been in commercial use for decades, and offer potential insights and lessons learned for SARS-CoV-2 vaccine and vaccination programs.

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Since 2002 three novel coronaviruses, Severe Acute respiratory syndrome-associated coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), and most recently, SARS-CoV-2, have caused deadly diseases in humans with immense social, economic and health impacts worldwide [1,2]. The SARS-CoV-2 pandemic introduced key concepts of virology into the consciousness of the world's populace, providing lessons on the importance of coronavirus vaccine development, 'herd health', and biosecurity measures as a means of disease control. In veterinary medicine, these concepts have been put to use for decades in many veterinary species to prevent and control deadly animal coronaviruses that have been the cause of clinically significant disease, increased treatment costs

in companion animals and livestock, and led to economic losses across several livestock and poultry industries worldwide.

The *Coronaviridae* family are enveloped, positive-sense, single-strand RNA viruses which are divided into four distinct genera that infect avian and mammalian species [1,3], including *Alpha-*, *Beta-*, *Gamma-*, and *Delta*-coronaviruses [4]. The *Alphacoronavirus* genus includes viruses that generally cause gastrointestinal disease in porcine, feline and canine hosts; the *Betacoronavirus* genus includes several viruses that cause respiratory diseases in humans, including SARS-CoV-1, MERS-CoV, and SARS-CoV-2, as well as the bovine coronavirus, which causes both enteric and respiratory disease [1,4]. The *Gammacoronavirus* genus includes an important virus in avian species, and the genus *Deltacoronavirus* infects mammalian and avian hosts [4].

Veterinarians and producers have been using licensed commercial vaccines for decades to prevent and control coronavirus infection in various animal populations (Table 1). A review of these veterinary coronavirus vaccines demonstrates the importance of understanding the pathogenesis of the virus, elucidates many important aspects of mucosal immunity, and illustrates the challenges faced specific to development of effective coronavirus vaccines. While there are many coronaviruses that infect a wide range of veterinary species, this review focuses only on a few selected animal coronaviruses with significant clinical and/or economic impact, and the efficacy of vaccines in controlling infection caused by these viruses. We hope that the knowledge gained during decades of use of these animal coronavirus vaccines will offer potential insight to the field of SARS-CoV-2 vaccine and vaccination programs.

ALPHACORONAVIRUSES

Members in the genus *alphacoronavirus* cause animal diseases of great economic importance, including porcine epidemic diarrhea virus (PEDV) and transmissible gastroenteritis virus of swine (TGEV). The highly contagious but relatively clinically quiet, feline enteric coronavirus (FeCV) and canine coronavirus (CCoV) also belong to this genus. Feline infectious peritonitis virus (FIPV) is a, unique, randomly mutated FeCV with severe and fatal clinical consequences in cats [4]. The economic impact of PEDV, TGEV, and the fatal consequences of FIPV, have led to considerable interest in developing vaccines against these animal alphacoronaviruses.

Feline infectious peritonitis virus (FIPV)

Feline coronaviruses (FCoV) consist of two serotypes: type I FCoV and type II FCoV [5]. Either of these types can take one of two different pathotypes, remaining an enteric coronaviruses (FeCV) or mutating into the highly pathogenic and fatal FIPV [5]. The majority of circulating FeCV are type I, which are ubiquitous within feline populations, especially in group-housed settings such as catteries or shelters, and tend to be asymptomatic or cause mild enteric disease [6]. FeCV initially infects enterocytes and can lead to viremia by infecting monocytes [7]. A small percentage (5–20%) of FeCV mutate to FIPV by gaining the ability to replicate rapidly within monocytes and macrophages [7–9]. The mechanism for this adaptation is not well understood, but mutations in the Spike protein are thought to play at least a partial role in altering cell tropism [10,11]. FIPV causes phlebitis, peritonitis, and serositis, as well as granulomatous inflammation in almost any organ system; it can present with ('wet', 'effusive' form) or without ('dry', 'non-effusive' form) body effusions, and is almost always fatal [12,13].

Vaccine development against FIPV has been ongoing since the 1980s and has involved a myriad of vaccine platforms and approaches, with early studies focusing mainly on live-attenuated FIPV [6,14–17] and heterologous live vaccines (canine coronavirus, transmissible gastroenteritis of swine,

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TABLE 1

Examples of licensed commercial vaccines in the United States against selected economically important animal coronaviruses in various veterinary species.

Animal coronavirus	Genus	Clinical diseases	Licensed vaccine (United States)	Vaccine type	Recommended vaccination route
Feline infectious peritonitis (FIPV)	*Alphacoronavirus*	Phlebitis Peritonitis +/ – effusion Serositis High mortality	Vanguard® FIP/ Felocell® FIP (Zoetis)	Modified-live	Intranasal
Transmissible gastroenteritis virus of swine (TGEV)	*Alphacoronavirus*	Diarrhea Vomiting Severe disease in neonatal piglets High neonatal mortality	USDA licensed	Modified-live	
Porcine epidemic diarrhea virus (PEDV)	*Alphacoronavirus*	Diarrhea Vomiting Anorexia Severe dehydration Severe disease and high mortality in neonatal piglets	PED vaccine (Zoetis) SEQUIVITY (personalized vaccine, Merck)	Killed vaccine RNA particle vaccine	• IM • Administer 2 doses to pregnant sow/ gilt prior to farrowing
Bovine coronavirus (BCoV)	*Betacoronavirus*	Calf diarrhea Winter dysentery (adults) Respiratory disease Generally mild disease unless there are comorbidities	Bovilis (Merck) Bovilis Guardian (Merck) Scourguard 4KC (Zoetis)	Modified live Killed virus vaccine	• Intranasal • Administer to neonatal calves • IM or SQ in pregnant cattle • Administer 2 doses prior to calving
Infectious bronchitis virus (IBV)	*Gammacoronavirus*	Primarily mild upper respiratory disease, complications with co-infections Pneumonia & airsacculitis Nephritis Decreased egg production and quality Stunted growth	**Merck** Massachusetts serotype **Zoetis** Massachusetts serotype Vaccines targeting one or more of the following IBV serotypes: **Merck** • Massachusetts • Connecticut • Arkansas • GA-08 • Mildvac-MA5™ • Mildvac™ Mass+Conn • NEWHATCH-C2-M® • NEWHATCH-C2-MC® **Zoetis** • Massachusetts • Connecticut • GA08 • GA98 • Arkansas **Elanco** • Arkansas • Massachusetts • Connecticut **Boehringer Ingelheim** • Arkansas • Connecticut • Massachusetts	Inactivated virus vaccine Live virus vaccine	• IM or SQ vaccination • Best used to booster chickens previously immunized with live vaccine of the same strain (Merck) • Administered in drinking water or as coarse spray • Periodic re-vaccinations may be needed

IBV: Infectious bronchitis virus ; IM: Intramuscular ; SQ: Subcutaneous.

and human coronavirus 229E) [18–20]. A licensed, modified-live, intranasal FIPV vaccine is available in the United States (Vanguard® FIP, formerly: Felocell® FIP; Zoetis). However, vaccination against FIPV proved challenging from the onset, with vaccines unable to protect against disease, and frequent reports of vaccinated cats appearing to develop FIPV infection earlier compared to controls when challenged [6,19,21,22]. These results were caused by antibody-dependent enhancement (ADE) following vaccination, and were mediated by an imbalance between humoral and cell-mediated immune (CMI) responses [22–24] and by antibodies directed against specific regions within the FIPV S protein [24,25]. Subsequent studies developed vaccines that meant to stimulate CMI but not humoral immunity, using recombinant vaccines containing one or both of the FIPV nucleocapsid (N) or membrane (M) proteins [26,27]. While these vaccines avoided FIPV S protein-associated ADE, they had varying results in protecting against disease [26,27].

The route of transmission for FeCV is fecal-oral and as such, induction of mucosal immunity is an important vaccination strategy to prevent FIPV infection. Several studies utilize either an intranasal or oral route of immunization in attempts to induce a mucosal immune response [14,17,19,21]. Two of these studies reported protection against challenge with homologous FIPV and no ADE [14,17]. One study documented neutralizing mucosal IgA responses and a CMI response in addition to protection [14]. This live-attenuated vaccine consisted of a temperature-sensitive FIPV DF2 (type II FIPV) and was successfully licensed for commercial use (Vanguard® FIP, formerly: Felocell® FIP; Zoetis) [14,28]. Several large post-marketing follow-up studies of this commercial vaccine concluded that the vaccine was safe, with no evidence of ADE under field conditions [15], and can protect cats that have no or low serum FCoV antibodies at the time of first vaccination [16]. However, in cats that had previous exposure or current infection at the time of vaccination, the vaccine showed no protection against disease [16]. The absence of ADE following vaccination may be attributed to the low infectious dose in a natural setting compared to high doses of virus during experimental challenge [16,29].

As of 2020, the feline vaccination guidelines from the American Animal Hospital Association (AAHA)/American Association of Feline Practitioners (AAFP) do not recommend vaccination for FIPV. The vaccines currently available are labeled for administration at 16 weeks of age, however, it is assumed that most cats have already been exposed and/or infected by the virus before this age [16,30].

The potential for ADE following natural infection or vaccination with SARS-CoV-2 has also been reported [31–35], however, currently this does not seem to be clinically problematic. Certainly, this will need to be monitored for future variants of concern. Regardless, the importance of CMI in the control of viral infection is well-known and is illustrated in the case of FIPV. Strategies targeting non-surface viral proteins for FIPV vaccine development can help guide future coronavirus vaccine designs to augment humoral immune responses and produce a more well-rounded and robust T cell response.

Transmissible gastroenteritis virus of swine (TGEV)

TGEV causes gastrointestinal disease in pigs worldwide [36] and disease epidemiology depends on the herd's immune status. In naïve herds, older pigs exhibit inappetence, mild diarrhea and vomiting, and have a low mortality rate [37]. In contrast, neonatal piglets less than 2 weeks of age experience severe disease, including diarrhea, vomiting and dehydration, and mortality rates can be as high as 100% [38–40]. In herds that are persistently infected with TGEV, disease and the highest mortality occurs in piglets of newly introduced, naive animals, and to a lesser extent, in 2–3-week-old piglets as

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protection from maternal antibodies wanes [37,40].

TGEV infects enterocytes that line the surface of the small intestinal villi; infection is initiated by binding between the TGEV spike protein and host cell Aminopeptidase N receptors [41,42]. A decrease in severity of TGEV-associated disease and mortality is seen in neonatal suckling piglets due to lactogenic immunity from sow to piglet in either colostrum or milk [38,43]. Effectiveness of passive immunity requires that piglets regularly receive and maintain adequate levels of neutralizing IgA within the small intestine [38,43,44]. These neutralizing antibodies target the antigenically and immunogenically important spike protein of TGEV [45,46]. Therefore, the goal of developing an effective TGEV vaccine is to elicit the production of mucosal, anti-spike IgA antibodies in the small intestine of neonatal pigs. Modified-live TGEV vaccines in combination with other enteric pathogens (rotavirus, *Clostridium perfringens* Type C, *E. coli* bacterin-toxoid) are currently licensed in the United States (United States Department of Agriculture (USDA), Current Veterinary Biologics Product Catalog, Feb 2, 2023).

The two most used vaccine platforms for TGEV are live-attenuated and inactivated vaccines, which are administered to pregnant sows by various routes. Whereas natural TGEV infection induces protective IgA antibodies in colostrum and milk, the majority of orally-administered, live-attenuated TGEV vaccine studies report IgG antibody secretion in milk [47]. Although not as protective as natural infection, these studies do report less severe disease and lower mortality rates in neonatal pigs born to vaccinated sows when challenged with TGEV, compared to unvaccinated controls [47,48]. Interestingly, these vaccines do not seem to produce protective immunity in vaccinated sows, as some developed clinical signs and began secreting IgA in milk when their nursing piglets were experimentally challenged [47]. The ineffectiveness of oral administration of a live-attenuated TGEV vaccine may be due to several factors, including degradation and loss of replicative ability as the vaccine moves through the stomach [43]. Other routes of inoculation of live-attenuated TGEV vaccines also report IgG antibodies in milk, decreased severity of disease, and variable levels of protection upon challenge, with a moderate level of protection against mortality by intramammary routes [38,49], and varying levels of protection by intramuscular (IM) or IM+oral administration [38,48,49]. The most promising finding of the IM vaccines reported high levels of protection comparable to the then-available vaccine [50]. Inactivated TGEV vaccines have also struggled to replicate the effective lactogenic immunity noted in natural TGEV infection, reporting varying degrees of protection against challenge regardless of administration route, as well as stimulation of IgG (but not IgA) antibodies in colostrum and milk [41,51,52].

Although IgA antibodies at the mucosal surface following natural infection elicit the best protective efficacy, high levels of IgG in colostrum and milk after vaccination with either live-attenuated or inactivated TGEV appear to be capable of lowering mortality and severity of disease [38]. Licensed live-attenuated TGEV vaccines have historically been available in the United States. However, when post-marketing evaluation was performed on two such vaccines, survival in piglets from sows that received the live-attenuated TGEV vaccine was no different from unvaccinated sows [53].

One development that may have led to natural immunologic protection against TGEV disease came not from TGEV vaccines, but from the emergence of another coronavirus, porcine respiratory coronavirus (PRCV). PRCV, a deletion mutant in the S gene of TGEV, is antigenically and genetically related to TGEV but has altered tropism, infecting tonsillar and respiratory epithelial cells rather than small intestinal enterocytes, and is generally asymptomatic in infected pigs [40,54]. Pigs exposed to PRCV antigen by natural and experimental infection, or immunization,

have been shown to shed infectious TGEV for shorter periods of time compared to PRCV seronegative pigs, and these exposures induce varying degrees of protection against challenge with TGEV [40,45,54–56]. The prevalence of TGEV has decreased since the emergence of PRCV and this may be due to partial protection from natural PRCV exposure, increased biosecurity measures, or both [36,45]. This decrease in TGEV prevalence has likewise decreased the demand for a vaccine, although live-attenuated and killed TGEV vaccines are currently licensed by the USDA.

Porcine epidemic diarrhea virus (PEDV)

PEDV is antigenically distinct from TGEV, however, the two cannot be differentiated based on clinical disease or histological lesions in affected pigs [57]. PEDV can infect as many as 50% of small intestinal enterocytes, leading to acute necrosis of infected cells and contributing to malabsorptive diarrhea, severe dehydration, electrolyte imbalances, and death [57]. Disease patterns during PEDV outbreaks also depend on the herd's immune status. In naïve herds, the clinical picture includes disease in pigs of all age groups, with more severe clinical signs in neonates, in which mortality can reach 95% [57]. Clinical disease on an affected farm experiencing an epidemic typically lasts up to 4 weeks as animals either succumb to disease or develop immunity [57]. In contrast, in facilities with endemic PEDV, diarrhea is more severe in newly introduced and naïve gilts or piglets, while clinical disease is mild to absent in nursing neonatal piglets [57]. Low morbidity and mortality in this latter group is attributed to passive transfer of lactogenic immunity from sows [57].

PEDV initially was identified in Europe in the 1970s and over the next two decades, spread to many Asian countries [58]. Despite vaccine and vaccination programs in these countries, PEDV epidemics continued to occur, and in 2013, PEDV suddenly emerged for the first time in the United States, where it caused immense economic losses to the swine industry [59]. Two conditionally licensed vaccines became available within a year of the initial outbreak in the United States [60] but epidemics continue to occur. As with other coronaviruses, the most important PEDV immunogenic antigen is the S protein, which binds to the host cell aminopeptidase N receptor on small intestinal enterocytes and induces neutralizing antibodies [57]. There are two genotypes of PEDV: genotype 1 (subtypes G1a and G1b) and genotype 2 (subtypes G2a, G2b, G2c) [61]; the circulating genotypes play a role in vaccine effectiveness and escape, with G2 strains contributing to epidemics [62].

Like TGEV, the ideal vaccine against PEDV would induce a mucosal immune response targeting the S protein. The majority of early PEDV vaccines in Asia were live-attenuated vaccines administered by various routes, including orally administered to neonatal piglets [63], and oral and/or IM administration to pregnant sows/gilts pre-farrow [62,64,65]. While none of these vaccine strategies were completely protective against PEDV infection, piglets born to, and nursing from vaccinated pregnant sows that received an oral vaccine reported the best outcomes, with decreased severity of disease and decreased mortality when challenged with homologous virus [64]. PEDV vaccines have been on the market in Asia for many decades, and yet epidemics continue to occur, largely due to recombination between variants and vaccine strains, emergence of mutations leading to vaccine escape, incomplete protection provided by vaccines, and potentially increased virulence in newly emerging variants [57,66–70]. Many of the earlier vaccines targeted G1 strains, which have only partial to no cross protection against G2 epidemic strains [67,71].

After the sudden emergence of PEDV in 2013 in the United States, two vaccines were quickly developed and both received conditional licenses from the USDA, including a killed-virus vaccine and an RNA particle

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vaccine [60]. The RNA particle vaccine was composed of the PEDV spike gene in a replication deficient Venezuelan equine encephalitis virus (VEEV) vector, and was tested on neonatal piglets as well as naïve and previously exposed pregnant sows pre-farrow, by both oral and IM administration [60]. Decreased disease severity and mortality but incomplete protection against infection were reported [60]. The killed vaccine consisted of an inactivated, whole-virus plus adjuvant, and was administered IM to sows pre-farrow and reported higher antibody titers in both the vaccinated sows and their piglets but no mortality data [60]. Field studies of each of these two conditionally-licensed vaccines yielded disappointing results, reporting increased IgG antibodies in the colostrum of vaccinated sows (RNA particle vaccine) but no protection against mortality between piglets born to sows vaccinated with either the RNA particle or inactivated vaccine [60].

One strategy for improving the immune response to the currently licensed vaccines is to utilize a prime-boost vaccination schedule. Several studies report increased IgA levels in milk, and colostrum, and protection against disease in piglets from sows that received a pre-farrow IM booster dose of killed PEDV vaccine following either natural infection or priming with an initial live-attenuated PED vaccine [72,73]. Due to the evolving mutations of PEDV, emerging PEDV variants are likely to continue to cause vaccine escape and outbreaks in regions where PEDV remains endemic, including in Asian countries and in the United States. Currently existing vaccines, such as the licensed killed vaccine (Zoetis, Inc.) in the United States, may need to be updated periodically in order to offer better protection against contemporary circulating strains.

BETACORONAVIRUS

Betacoronaviruses are known to cause deadly respiratory diseases in humans, including SARS-CoV-1, MERS-CoV, and SARS-CoV-2. One clinically and economically important betacoronavirus in veterinary species is the bovine coronavirus (BCoV), which causes both enteric and respiratory disease [1,4].

Bovine coronavirus (BCoV)

BCoV infects both the gastrointestinal and respiratory tracts and causes three distinct clinical syndromes: calf diarrhea, winter dysentery in adults, and respiratory disease at any age [74,75]. In calves, enteric BCoV infects the enterocytes throughout the small and large intestine, causing malabsorptive diarrhea [74,75]. Disease can be mild but co-infections with other pathogens such as rotavirus and enterotoxigenic *E. coli* can lead to severe dehydration and mortality [74,75]. In adults, enteric BCoV presents clinically as hemorrhagic diarrhea, with lesions mainly in the colon [74]. Mortality is low in adults but infection does cause economic loss in the form of a prolonged decrease in milk production [74]. Respiratory BCoV mainly infects the epithelium of the upper respiratory tract and can contribute to bovine respiratory disease complex (BRDC), which is a major cause of death and economic loss in feedlot cattle [75,76]. Importantly, no antigenic difference has been found between the BCoV causing these three syndromes; different serotypes of BCoV have good cross-protection and any serotype can cause either enteric or respiratory disease [74–78]. There are currently two licensed BCoV vaccines in the United States: a modified live vaccine and a killed vaccine (USDA, Current Veterinary Biologics Product Catalog, Feb 2, 2023).

BCoV is unique in that it has two structural proteins involved with attachment to the host cell: the prototypical spike (S) protein and the hemagglutinin-esterase (HE) protein [76,79]. The S protein is responsible for binding to sialic acid containing receptors on host cells and contains the main neutralizing epitopes, while the HE reverses hemagglutination [76]. Efficient binding to, and release from, host cells is thought to occur through

the right balance in the activities of these two proteins [80].

BCoV vaccine development has focused mainly on establishing protection against diarrhea in neonatal calves. 'Scours' in calves is often caused by mixed infections composed of not only BCoV, but other enteric pathogens as well such as rotavirus, enterotoxigenic *E. coli* (K99) and *C. perfringens* Types C and D [74], and as such, many of the historical and current BCoV vaccines are formulated together with immunogens from these other pathogens [81–83]. Experimental and field studies have reported increased antibody responses in serum, colostrum, and milk of cows and/or heifers that were vaccinated (IM or subcutaneous) with an inactivated vaccine plus adjuvant [84–86], and, in pregnant cows, a corresponding increase in antibody responses in their calves' serum [85]. Many studies on BCoV vaccination were performed in the field, and few studies report virus challenge results due to the difficulties in assessing clinical disease in the field and in the face of mixed infections. Initial field studies reported little to no protection following vaccination, however, more recent vaccination studies have shown some promise, with increased antibody responses in cows vaccinated with a single dose of inactivated (IM) vaccine prior to calving, and decreased diarrhea after challenge in their calves [87]. Calves have also been directly vaccinated with an orally administered, modified-live BCoV vaccine; after virus challenge, vaccinated calves remained clinically normal, had a faster rate of gain, and had no fecal viral shedding, compared to unvaccinated controls [88].

There is evidence that respiratory shedding of BCoV may be a source of continual exposure of a herd or to newly mingled animals on feedlots [74,89]. However, the current vaccines are directed against enteric disease and only a few studies have addressed efficacy in preventing respiratory disease. There is evidence that intranasal vaccination with a modified-live BCoV vaccine upon entry into a feedlot decreases the risk of being treated for respiratory disease [90]. Development of a live-attenuated BCoV vaccine targeting respiratory disease reported a high safety profile and high antibody titers but no virus challenge was performed [91]. Unfortunately, no experimental virus challenge studies on respiratory protection following vaccination have been reported, and to date there is no licensed vaccine targeting the respiratory disease associated with BCoV infection.

The relative paucity of research on BCoV vaccines is likely due to several challenges, including: (1) the virus has a wide range of cell tropism and establishing mucosal immunity in multiple sites is unlikely to be accomplished with a single vaccine; (2) the benefits of enteric mucosal immunity are well understood due to the extensive research in coronaviruses of pigs, however, passive, lactogenic immunity is more challenging to practically achieve in large beef herds and in dairy herds in general; and (3) both the enteric and respiratory syndromes experienced with BCoV infection are often seen in conjunction with co-infections with one or more other pathogens, further complicating experimental methods and assessment of outcomes associated with the protective efficacy of vaccines. Nonetheless, multiple modified live and killed virus vaccines are licensed and in use in the United States.

GAMMACORONAVIRUS

The gammacoronaviruses include an economically important veterinary virus infecting poultry, the infectious bronchitis virus (IBV), and many commercial vaccines have been licensed and are in use.

Avian infectious bronchitis virus (IBV)

IBV is primarily a disease of the upper respiratory tract and infects the ciliated and mucous-secreting epithelial cells, causing clinical signs such as gasping, snicking, sneezing, coughing, and nasal discharge in birds [92,93].

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IBV infection compromises mucociliary function, and predisposes animals to secondary infections that are often the actual cause of death [93,94]. While the upper respiratory tract is the primary site of virus infection, IBV is capable of infecting many other organ systems, including the lower respiratory tract, the gastrointestinal tract, kidneys, and reproductive tract [92]. As a result, IBV can be isolated from both respiratory secretions and feces, and disease can include pneumonia and airsacculitis, decreased egg production and decreased egg quality, including soft and deformed shells [92]. Nephropathogenic strains of IBV target renal tubular epithelial cells and cause severe, acute, necrotizing nephritis, renal failure, and increased mortality in birds [92,94]. When exposed at a young age, IBV can cause enteritis, stunted growth, and chronic cystic oviduct that prevents egg formation [94]. IBV is extremely contagious: morbidity in an affected flock is typically 100% and mortality is usually low, unless the IBV strain is nephropathogenic or there are coinfections with other pathogens [92,93].

The IBV S protein mediates host cell binding and is cleaved into the S1 and S2 subunits. S1 is responsible for cell tropism and binding to a sialic acid receptor on host cell membranes, as well as inducing virus neutralizing antibodies [95–97]. There are many different serotypes of IBV and neutralizing antibodies for each type have poor cross-protection for other serotypes [93]. As such, there are many different licensed IBV vaccines in the United States that are composed of a variety of serotypes, in live-attenuated virus, and killed vaccine platforms, and often in combination with other avian pathogens, including Newcastle disease virus and infectious bursal disease virus (USDA, Current Veterinary Biologics Product Catalog, Feb 2, 2023).

IBV was first described in the 1930's; the first vaccine became available in the 1950's, and vaccination has been practiced worldwide ever since [93]. As with other animal coronaviruses, mucosal and local immunity is important for anti-IBV immunity [98–105]. Many commercial IBV vaccines are administered either in drinking water or in coarse spray, both methods of delivery are efficient for producers and target local mucosal immune responses. Many of the first vaccines used were live-attenuated virus vaccines administered within a few weeks of hatch. Several studies reported protection against homologous challenge, with less severe clinical disease, maintained ciliary activity in the trachea, and decreased viral shedding [106,107]. Protection afforded by live-attenuated IBV vaccines was short-lived and declined about 9 weeks post-vaccination but could be prolonged with a booster dose [108,109]. The live-attenuated IBV vaccines have been shown to be capable of infecting susceptible contact chickens, undergoing recombination with circulating virulent strains, and/or mutating into virulent strains themselves [110,111]. While inactivated IBV vaccines have had mixed results on their own, they can be used in combination to extend immune protection. When administered as multiple doses by aerosol or subcutaneous routes, or as an aerosol-subcutaneous prime-boost schedule, inactivated vaccines could produce a virus neutralizing antibody response and decrease virus isolation from the trachea [112,113].

The main obstacle for successful IBV control is that vaccines offer limited to no protection against challenge with heterologous serotypes. IBV has a high mutation rate and only a few point mutations in the spike protein are thought to be necessary before commercial vaccines lose their protective efficacy [92]. For this reason, IBV continues to have a huge economic impact on poultry industries worldwide as newly emerging IBV strains are not protected by commercially available vaccines. Efforts at addressing waning protective efficacy and increasing cross-protection have focused on utilizing prime-boost vaccine strategies utilizing different IBV strains in combination to broaden protective coverage. When inactivated IBV vaccines were used as a booster following natural exposure or live-attenuated IBV vaccination, vaccinated

chicks experienced increased antibody titers in serum, protection against drop in egg production, and/or experienced a decrease in viral load in tissues (i.e., trachea, kidney) [101,114–117]. Currently many IBV vaccines are commercially available, the majority of which are live-attenuated virus vaccines, along with a few inactivated virus vaccines. Vaccine protocols for these IBV vaccines recommend booster doses after natural infection or primary vaccination.

TRANSLATION INSIGHTS

This concise review touches on a few of the important highlights of vaccine development for each of the selected clinically important veterinary coronaviruses, with a focus on licensed commercial animal vaccines. Individual and more detailed reviews could certainly be written on each of the selected viruses, as a variety of vaccine platforms, expression systems, administration routes, and many different adjuvants and co-stimulatory molecules, have been studied *in vitro* and in experimental models for each virus. Thus, the knowledge in searching for effective veterinary vaccines for these selected coronaviruses has the potential to inform future coronavirus vaccine development in any species, including humans. Across the animal coronaviruses discussed in this brief review, three common themes stand out: the battle to control disease in the face of constantly mutating coronaviruses, the importance of establishing mucosal immunity in mitigating the viral life cycle, and the challenge faced with the short-term and incomplete protection provided by these veterinary coronavirus vaccines.

Coronaviruses have one of the largest RNA genomes, thereby providing ample opportunity for mutations and recombination to occur [118], and enabling the emergence of new variants. This challenging scenario is illustrated in many of the animal coronaviruses discussed here, including IBV and PEDV. Despite decades of vaccination programs, disease associated with IBV infection is still a major concern for the global poultry industry due to newly emerging variants, often with poor or no cross-protection by existing licensed vaccines. Establishing the predominant circulating virus strains on a geographical basis is necessary for planning the vaccination program for a specific region and consideration must be given to 'regionalization' of available vaccine products based on these data. Surveillance and tracking of the emerging mutations across the globe may help identify areas of improvement for biosecurity measures or potential trends that contribute to important vaccine updates and modifications. Similarly, molecular epidemiologic methods have been widely used for SARS-CoV-2 surveillance, with many open access systems available to track number of cases, as well as genomic and phylogenetic trends of variants worldwide [119].

Emergence of variants also necessitates either periodic production of updated vaccines, as seen with development of the bivalent SARS-CoV-2 mRNA vaccines, or ideally, development of a broadly-protective or pan-coronavirus vaccines. The decrease noted in TGEV infection rates following emergence of PRCV provides some hope for pan-coronavirus vaccine development. Studies evaluating cross-protection between SARS-CoV-2 and other coronaviruses in either human (i.e., OC43, HUK1, NL63, 229E) or animal populations (i.e., bats) generally indicate that antibodies do not have significant cross-neutralizing effects and there is variable evidence for protection against severe disease [120]. However, there is evidence of pre-existing, cross-reactive CD4 and CD8 T cells to non-S1 structural and non-structural proteins [120–122]. Studies on cross-protection among coronaviruses have the potential to identify important humoral and cell-mediated immune mechanisms of protective immunity for developing broadly-protective vaccines in both veterinary and human medicine.

The potential impact and importance of a mucosal immune response for efficient

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protection is a strong and common theme throughout all of the animal coronaviruses discussed in this review. Many *in vitro*, *in vivo* experimental, and field studies consistently establish the beneficial effects that neutralizing mucosal IgA antibodies, as well as CMI responses, have on controlling coronavirus infection at the initial site of infection. Mucosal immunization routes across different animal species have a multitude of options, including vaccines administered via oral or intranasal routes, in eye drops or drinking water, or even applied as a coarse spray. In most instances, these routes of administration produce a better local mucosal immune response when compared to parenteral vaccines. TGEV and PEDV vaccines helped characterize the mucosal, lactogenic, passive immunity from dam to their neonates. Studies in livestock and poultry pushed the vaccine field to look at immunity in a new light, as serum antibodies, the outcome of focus for many coronavirus vaccines, were less predictive of protection against disease compared to IgA antibodies at mucosal sites and/or in mucosal secretions or fecal matter. The benefit of lactogenic immunity is driven home by the struggles with establishing protection against coronavirus in bovine species, where continuous exposure to milk containing adequate neutralizing antibodies is not practically possible. There have been many published reports across different veterinary coronaviruses on the inclusion of various mucosal adjuvants and co-stimulatory molecules to target mucosal cells and induce homing of immune cells to mucosal sites in order to induce or boost a mucosal immune response, and these strategies could be applied to future SARS-CoV-2 vaccine as well.

This review focuses mainly on experimental vaccines that were either integral to vaccine development in the respective species, and/or licensed and commercially available vaccines that have been used in a vaccination program. Many other vaccine platforms, from DNA to viral- or bacterial-vectored to virus-like-particles, have been studied for each of the veterinary coronavirus species reviewed here but are not detailed in this brief review. To date, the majority of vaccines on the market for veterinary species are modified-live or inactivated virus vaccines. With the honing of mRNA technology for successful vaccine development during the SARS-CoV-2 pandemic, it will be interesting to see how the field of veterinary vaccines evolves and whether this new mRNA platform can be applied cost-effectively to veterinary species to benefit patients of any species.

The potential benefits of vaccine-induced mucosal immunity in combating SARS-CoV-2 have been reviewed elegantly elsewhere [123–125]. As with other parenterally delivered vaccine platforms, the currently available mRNA vaccines induce strong protective neutralizing antibodies in serum but low levels of the same in respiratory mucosal secretions (nasal swab or bronchoalveolar lavage) [126,127]. Recent studies have begun to investigate the potential of mucosal vaccines for SARS-CoV-2 [127–129], and combined with the benefits consistently described in coronavirus vaccines for veterinary species, this approach provides hope for vaccine improvements in prevention of infection and further transmission in humans as well.

The final common theme from these animal coronavirus vaccines is that licensed vaccines for coronaviruses across veterinary species can decrease disease severity and lower mortality rates, but rarely confer complete protection. Observed benefits are usually short-lived, thereby requiring repeated booster immunizations. In most veterinary species, even natural infection induces only short-lived protective immunity. The closest mimic of this immune response is induced by live-attenuated coronavirus vaccines administered at the mucosal site where natural infection initially occurs. The same challenges are also known with SARS-CoV-2, as re-infection does occur following either natural infection or vaccination, further underscoring the importance of boosters and vaccine updates.

These decades of experience from animal coronavirus vaccines in veterinary species illustrate the shared challenges faced when developing long-lasting protective vaccines for coronaviruses in any species. One approach in animal coronavirus vaccination programs that seems to improve upon the incomplete protective efficacy, prolong duration of protection, improve mucosal immune responses, and provide better cross-protection, is the use of a prime-boost vaccination schedules. There is some evidence that this approach may also be beneficial in SARS-CoV-2 disease control. Neutralizing antibodies in the nasal mucosa have been isolated after natural breakthrough infection with SARS-CoV-2 in already vaccinated individuals [126]. This has been replicated experimentally, in which immunization of mice first with a SARS-CoV-2 IM mRNA vaccine followed by an IN vaccine induced strong neutralizing antibodies in the respiratory mucosa [127]. Collectively, the available data from veterinary coronavirus vaccines and available SARS-CoV-2 data support the recommendation for periodic SARS-CoV-2 vaccine update and booster doses, and for vaccination after recovery from SARS-CoV-2 infection.

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VACCINEINSIGHTS

COMMENTARY

Testing in times of COVID-19: legacy & unfinished agenda

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Fast and effective testing is a critical part of pandemic preparedness and response; however, during the COVID-19 pandemic, there have been major disparities in access to diagnostic tests. Here, we outline barriers and progress toward equitable access to diagnostics during the COVID-19 pandemic and highlight important lessons learned for the future.

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The COVID-19 pandemic has adversely affected the world in unparalleled ways, reversing decades of progress in health, social and educational gains, leading to severe disruption to the achievement of the 2030 Sustainable Development Goals [1,2].

As of December 2022, 3 years after the first SARS-CoV-2 pneumonia cases were reported in Wuhan, China, there have been over 645 million COVID-19 cases and over 6.6 million deaths officially reported globally [3]. Since the virus was first identified, it has continuously evolved, leading to the emergence of distinct variants of concern (VOCs) that have caused surges and challenged existing public health structures. The deaths and disability from COVID-19 are expected to rise significantly when the full toll of the Omicron VOC and its return to China in early 2023 are fully considered and evaluated.

Diagnostics are a cornerstone for pandemic response and preparedness, as they provide essential intelligence to guide public health decisions. Effective access to testing can help

identify outbreaks, monitor trends in infections, detect variations in incidence, evaluate community-level immunity and measure the impact of vaccination programs. Diagnostics can also inform policy and strategy development, resource allocation, risk communication, and evaluation of interventions.

However, stark inequities in diagnostics access have been reported since the onset of the pandemic, mainly in low- and middle-income countries (LMICs) where lack of testing infrastructure, weak supply chains, and high dependency on imported tests have left them vulnerable to the unfair supply-demand dynamics, especially in the early days of this global crisis [4].

Despite significant disparities, major progress has been made in COVID-19 diagnostics, particularly with robust investments to build in-country capacity to scale up availability. In addition, ongoing strategic initiatives spurred by the pandemic to improve policies and accelerate access to essential diagnostics at national and regional levels are paramount to support pandemic preparedness efforts, enhance healthcare delivery, and enable universal health coverage [5].

Among the main COVID-19 testing and surveillance milestones is the significant expansion in genomic sequencing capacity globally, especially in LMICs. This generated data that was instrumental to track viral evolution, identify variants, and provide evidence to guide public health measures, offering key opportunities for other disease control efforts, especially those of potential pandemic importance. Another achievement is the increased awareness and literacy around testing as a crucial element for an effective public health response.

Furthermore, the rapid acceptance of self-testing offers precedents for innovative public health strategies beyond SARS-CoV-2, in alignment with the increasing trends in uptake reported for human immunodeficiency virus and other infectious conditions in recent years [6]. In many LMICs, modern diagnostics and surveillance practices around COVID-19 may serve as the basis for strengthening their health systems but such gains need to be sustained and scaled as part of a fundamental change in the global health architecture [7].

POINT OF CARE DIAGNOSTICS

The initial response to the coronavirus pandemic was hampered by health systems' heavy dependence on laboratory-based diagnostics. With COVID-19 lockdown measures and mobility limitations, point of care (POC) diagnostics experienced a steep growth in adoption as they enabled improved access to individuals, including via home tests. Coronavirus POC tests' easy-to-use applicability shifted the perception of their usefulness by healthcare providers and the general population. Furthermore, the potential applicability of these tools beyond COVID-19, including for infectious and non-communicable diseases has been documented as a means to bring diagnostics closer to patients and empower users [8,9].

The urgency to further invest in the development of novel POC multi-pathogen testing has also emerged. These platforms offer crucial opportunities to center health systems around patient needs, improve surveillance, and transform diagnostics and management of multiple infectious threats, including pandemic-prone diseases. Some of the molecular platforms developed for SARS-CoV-2 can be re-engineered for other pathogens and pivoted to address unmet diagnostics needs [9,10]. Sustained investments, collaborations, and concerted actions will be required for this approach to reach its full potential, mostly in LMICs where these tools can fill public health gaps, to address major global concerns such as tuberculosis (TB), arboviruses, and endemic conditions such as Ebola, Mpox, [11,12] and others. In addition, manufacturers of POC diagnostics cannot sustain a 'feast or famine' demand. Single pathogen diagnostics will continue

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to face ups and downs in demand, but multiplex diagnostic platforms can be a better mechanism to stabilize demand.

TEST & TREAT APPROACHES

The development of novel antiviral treatment and/or prophylactic options for SARS-CoV-2 has been one of the great biomedical milestones of the pandemic [13]. It also offers an opportunity to halt the impact of infection in individuals, especially those with a higher risk for severe disease, as well as an opportunity to mitigate the burden on the most fragile health systems. The short window for treatment initiation required by novel antivirals calls for timely decentralized access to testing, including standardized self-testing approaches [14].

Testing rates remain low in LMICs and represent an important barrier to the successful implementation of test-and-treat (T&T) approaches [15]. The COVID Treatment Quick Start Consortium was recently founded to expand access to novel antiviral drugs in LMICs [16].

In order to maximize the public health potential benefit of novel antiviral drugs, major strategic investments will be required to scale up availability and access to diagnostics and healthcare infrastructure, technical capacity, resourcing, and staffing. Strong advocacy and community engagement at the country level are essential to co-design strategies and campaigns, as well as to tailor messages to the general public, opinion leaders, and high-risk groups. UNITAID and FIND are working with local stakeholders and advocacy partners in LMICs to coordinate programs to boost the potential impact of this initiative in these settings [17]. Experience in high-income countries that launched T&T programs for COVID-19 demonstrated that including pharmacies and other healthcare facilities closest to the patient is an imperative for T&T to succeed. Future preparedness to deploy T&T in LMICs requires a model that involves private pharmacies in LMICs with the technical capability and data-sharing infrastructure [18].

REGIONAL MANUFACTURING CAPACITY

The urgency to expand regional manufacturing capacity for COVID-19 tools has been a recurrent theme since the onset of the pandemic. Multi-stakeholder partnerships and robust investments in technology transfer happened in response to COVID-19 and beyond [19]. Such investments in local testing capacity offer crucial opportunities for the creation of flexible platforms that can prepare health systems to respond to future pandemic threats. Furthermore, investments in local manufacturing capacity will contribute to more robust primary healthcare systems to better respond to the evolving needs of populations in LMICs [20]. It is promising that strengthening regional manufacturing of diagnostics has been taken up alongside other medical countermeasures by India as part of the agenda for their G-20 presidency this year [21]. The business model for local manufacturing of diagnostic tests may require advanced commitments from purchasers, or price premiums in the short term, but these are important for jump-starting a local industry for diagnostics whose benefits go beyond just resilience in supply. This will lead to new avenues for developing and manufacturing of test kits for diseases with small market sizes.

GENOMIC SEQUENCING

The COVID-19 pandemic has catalyzed the rapid expansion of genomic sequencing capacity worldwide. Genomic surveillance has been crucial to monitor pandemic evolution and has enabled the identification of various viral lineages that fueled multiple pandemic waves, including the emergence of VOCs. By providing an accurate landscape of viral evolution and distribution, it also has the potential to guide public health responses,

and optimize treatment, vaccines, and molecular test development [22].

Before the pandemic, genomic sequencing activities were primarily limited to research initiatives and a few other vertical disease programs, especially in LMICs. In Africa for example, 38 countries have built next-generation sequencing infrastructure to address the coronavirus crisis, a significant increase from only seven countries in 2018 [23]. The Africa Centre for Disease Control and Prevention (CDC) has spearheaded the Africa Pathogen Genomics Initiative (Africa PGI) which aims to increase disease surveillance and public health partnerships to leverage genomic sequencing technologies [24]. Similar initiatives have taken place in Latin America and Asia [23].

Despite the fast expansion of sequencing capabilities at the country level, it has not yet reached its full public health benefit and major disparities persist, following similar inequity trends of the pandemic. During the first two years of COVID, 78% of high-income countries (HICs) sequenced more than 5% of their COVID cases, whereas, in LMICs, only 42% of countries reached that level. Similar disparities have been observed with regard to turnaround time (TAT), with around 25% of HICs submitting samples for sequencing within 21 days, while only 5% of LMICs achieved that level [25]. Despite major achievements triggered by the pandemic, sustained investments are needed to achieve equity in access and wider availability of genomic sequencing capacity. PGS labs face uncertainty in future demand as the demand for COVID-19 sequencing fades away. Standardized end-to-end workflows for different pathogens and applications, NGS laboratory and bioinformatics training, supply chain optimization and other such efforts have to be put in place to make PGS sustainable in the long term.

The new WHO rapid communication issued this July for the use of sequencing for the diagnosis of drug-resistant tuberculosis represents a landmark move for sequencing from a tool for research and surveillance to a tool to improve clinical care for patients [26]. Strong genomic sequencing capacity can improve pandemic preparedness, strengthen surveillance efforts, supporting countries at higher vulnerability for emerging threats and current epidemics (for example, improve TB clinical management and pave the way to achieve global targets for TB), among others. Investing now in expanding local genomics capacity will benefit not only individual countries but global health security more broadly.

CONCLUSION

While being one of the most devastating health challenges of our time, the coronavirus pandemic did spur unprecedented progress in biomedical innovations, including diagnostics. However, innovations have not reached lower-income countries at the pace seen in high-income countries. Ultimately, the impact of the crisis has been disproportionately higher among the world's most vulnerable, primarily due to unequal access to novel tools and systemic barriers to health care. Peeling *et al.* have highlighted how the innovations and investments in diagnostics triggered by the pandemic can be leveraged to improve health outcomes, strengthen health systems, and advance global health security [27].

There is growing consensus that strong pandemic preparedness and resilient health systems should be grounded in equity principles. While much of the priority has been directed at global equity for vaccines and vaccinations, diagnostics also demand our attention [28]. This will be true not only for COVID-19, but as highlighted above for many neglected diseases including TB and malaria. The diagnostic equity gap is especially glaring for neglected tropical diseases of regional importance, such as Buruli ulcer, leishmaniasis, or Chagas disease, that fall below the radar screen of multinational companies.

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In addition, there is an urgent need for well-coordinated R&D policies based on public health needs, not market demands. These policies need to be translated into timely access, with emphasis on stronger regional capacity, especially in LMICs where the reliance on philanthropy and development assistance has historically left their populations vulnerable to lengthy timelines in innovation adoption and fragile supply chains. Maintaining and expanding investments in diagnostics development and access that were triggered by the pandemic offers important opportunities to respond not only to COVID-19 but to other current crises, infectious and non-communicable diseases, and prepare for future epidemic threats, and in the worst case, pandemics.

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