Opportunities COVID 19 has presented for bovine practice

C. Chase, DVM, PhD
Department of Veterinary and Biomedical Sciences, South Dakota State University, Brookings, SD 57007

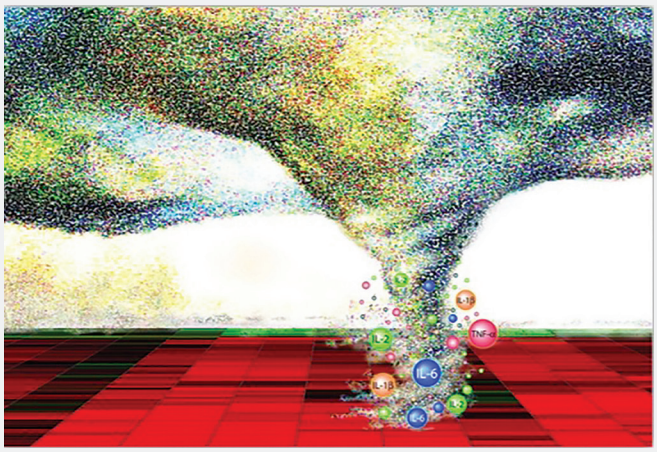
Abstract
The coronavirus Disease 2019 (COVID-19) pandemic has been a major disruptor of people's live since it went worldwide in spring of 2020. Although COVID-19 has resulted in personal inconvenience, economic impact and most importantly the loss of human life, there certainly have been advances made in medicine in response to the pandemic. Interestingly several of those were had already been instituted in veterinary medicine. The concept of the immune system going out of control i.e., cytokine storm, is a concept that we have hypothesized to occur with bovine respiratory disease (BRD) for almost a decade. The potent anti-inflammatory drugs i.e., dexamethasone, have been used as a supportive treatment for BRD for decades. Antiviral therapeutics which have been developed for COVID-19 have not been used extensively in veterinary medicine due to cost and concern about drug residues. Improvement of diagnostics using PCR for salivary samples has allowed easily collected samples to be examined. Lateral flow ELISA devices have allowed point of contact testing. In addition, devices like the Advance Animal Diagnostics flow cytometer developed for cattle prognostic testing have been applied to provide insight on human "cytokine storm" diseases. Although Covid-19 represented the first use of mRNA vaccines, other platform vaccines including baculovirus, Venezuelan equine encephalitis virus and DNA vaccines have been used in the veterinary market. The effect of probiotics both in the enteric and respiratory tract enhances mucosal immunity.

Key words: COVID-19, One-Health, bovine

Introduction
Coronavirus Disease 2019 (COVID-19) pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been a major disruptor of people's lives since it went worldwide in spring of 2020.[1] Although COVID-19 has resulted in personal inconvenience, economic impact and most importantly the loss of human life, there certainly have been advances made in medicine in response to the pandemic. Interestingly several of those had already been instituted in veterinary medicine. In this paper, I will review some of the major findings from COVID19 pathogenesis, therapy, diagnostics and prevention and how that relates to bovine medicine and One Health.

Pathogenesis
A cytokine storm (hypercytokinemia) is the systemic expression of a healthy and vigorous immune system resulting in the release of more than 150 known inflammatory mediators (cytokines, oxygen free radicals and coagulation factors) (Figure 1).[15] The innate immune system particularly macrophages, become activated and begin to produce interleukin-1 beta (IL-1b) interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF) and begins to recruit in inflammatory cells.[10] TNF then stimulates a kinase pathway, MLCK, that results in the breakdown of the tight junctions and the development of leaky gut which

Figure 1: Imagery of the "cytokine storm." Many different cytokines are activated and create "Cytokine Storm."[15]



can be a vicious circle-more bacteria and antigens leak through and a more severe inflammatory response develops (Figure 2).[10] Both pro-inflammatory cytokines [interferon gamma (INF-gamma),TNF, IL-1, and IL-6] and anti-inflammatory cytokines (such as interleukin 10 and interleukin 1 receptor antagonist) are elevated in the serum of people or animals experiencing a cytokine storm. This results in systemic spillover affecting other systems (Figure 3).[7,15] This is the primary reason for many deaths during the sudden acute respiratory syndrome (SARS) epidemic in 2003 in China and with current COVID-19 pandemic. To translate this to cattle, an animal with a systemic inflammatory response (cytokine storm) will have increased bovine respiratory disease, more severe mastitis, and metritis. The development of cytokine storms in people are believed to be responsible for many of the human deaths during the 1918 influenza pandemic, which killed a disproportionate number of young adults. In this case, a healthy immune system may have been a liability rather than an asset.

Therapeutics
Anti-inflammatory drugs
The cattle industry has long used both steroidal (SAIDS) and nonsteroidal anti-inflammatory drugs (NSAIDS) for respiratory disease. The use of these drugs has been extensively reviewed[5] and their effect on febrile response is well accepted. Their effect on limiting lung damage has been more problematic.

Antiviral drugs
The use of antiviral drugs in food animals has been limited by cost and residue issues.[6] One antiviral that was first developed for influenza virus, molnupiravir, a ribonucleoside analogue, also inhibits SARS-CoV-2. Molnupiravir takes advantage of an inherent problem with all RNA viruses, they make mistakes on

Figure 2: Pathogenesis of leaky gut. The epithelial barrier normally restricts passage of luminal contents, including microbes and their products, but a small fraction of these materials do cross the tight junction. This diagram shows how dendritic cells (DC), macrophages (M), and T cells react to these materials. The naive T lymphocyte (T cell) responds to antigenic and other stimuli within the lamina propria, becoming a Th1-polarized cell (Th1), a T regulatory cell (Treg), or other differentiated T cell types. These innate and adaptive immune cells release cytokines that exert proinflammatory (TNF and IFN-γ) and anti-inflammatory (IL-10, TGF-β) effects. If proinflammatory signals dominate and signal to the epithelium, MLCK can be activated to cause barrier dysfunction, which would allow an increase in the amount of luminal material("leaky gut") presented to immune cells. In the absence of appropriate immune regulation, this activation may cause further proinflammatory immune activation, cytokine release, and barrier loss, resulting in a self-amplifying vicious cycle that can result in disease. Abbreviations: IL, interleukin; MLCK, myosin II regulatory light chain kinase; TGF, transforming growth factor; TNF, tumor necrosis factor.[10]

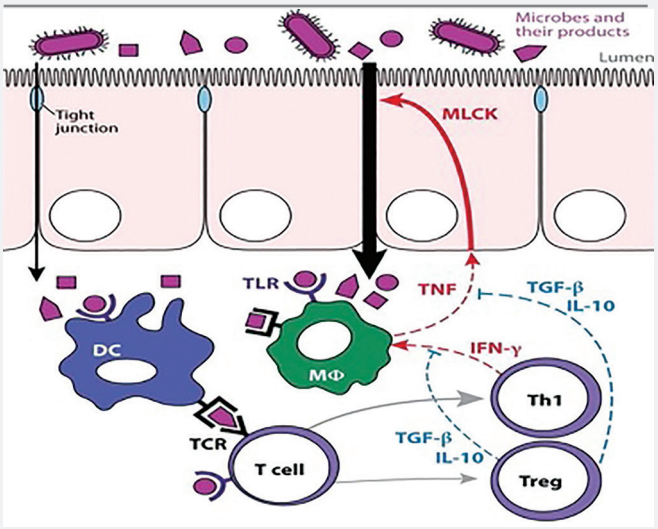
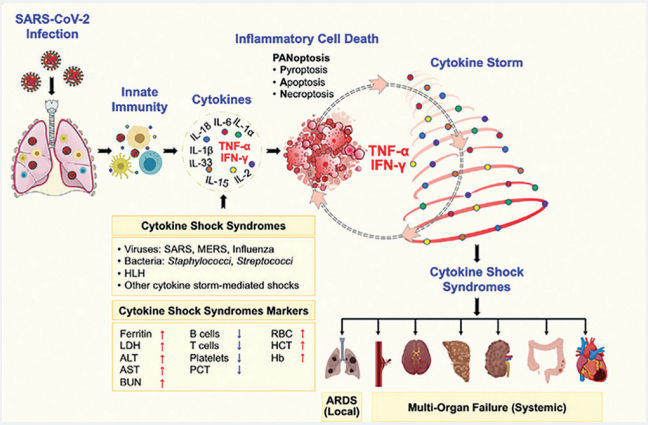


Figure 3: Tumor necrosing factor alpha (TNF-a) and Interferon-gamma (IFN-g) Triggers Inflammatory Cell Death, Tissue Damage, and Mortality during a cytokine storm. Synergism of TNF-a and IFN-g induces PANoptosis, so-called because it involves the collective activation of pyroptosis, apoptosis, and necroptosis (PANoptosis). Pyroptosis is a highly inflammatory form of lytic programmed cell death associated with macrophages, apoptosis is cell death associated with little inflammation and necroptosis is a programmed form of necrosis, or inflammatory cell death. Conventionally, necrosis is associated with unprogrammed cell death resulting from cellular damage or infiltration by pathogens, in contrast to orderly, programmed cell death via apoptosis. PANoptosis perpetuates cytokine storm resulting in systemic shock seen with viral infections such as SARS-2 and bacterial sepsis.[7]



replication and molnupiravir accelerates this "mistake process" dramatically and causes "a catastrophe" for the virus by making the genome incorporate nucleosides that severely limits the replication of the virus.[9] Its use in SARS-CoV-2-infected people results in a dramatic reduction in shedding of SARS-CoV-2[4] (Figure 4). Antiviral therapy has been quite successful for hepatitis C virus (HCV) infection resulting in >90% cure rate for this chronic infection. Several antiviral therapies have been used both in vitro and in vivo for bovine viral diarrhea virus (BVDV), which is in the same family as HCV although there have been no commercial products.[3,6]

Monoclonal antibodies

Most of us are aware of friends or family who have received SARS- CoV-2 monoclonal antibodies (mAb) as a post-infection therapy. The first commercial use of mAb for use in prevention/treatment of infectious disease was developed in veterinary medicine in the 1980s for colibacillosis in calves.[13] One of the limiting factors to mAb commercialization was the expense of the production of mAb as B cells have a short life and various techniques were used to extend their life requiring expensive media and purification. The break through on mAb production occurred when Chinese Hamster Ovary (CHO) cells were engineered to produce antibody. They can be cultured continuously to produce recombinant mammalian proteins, in this case, antibodies in mg quantities at a much lower cost (Figure 5).[8,16]

Diagnostics

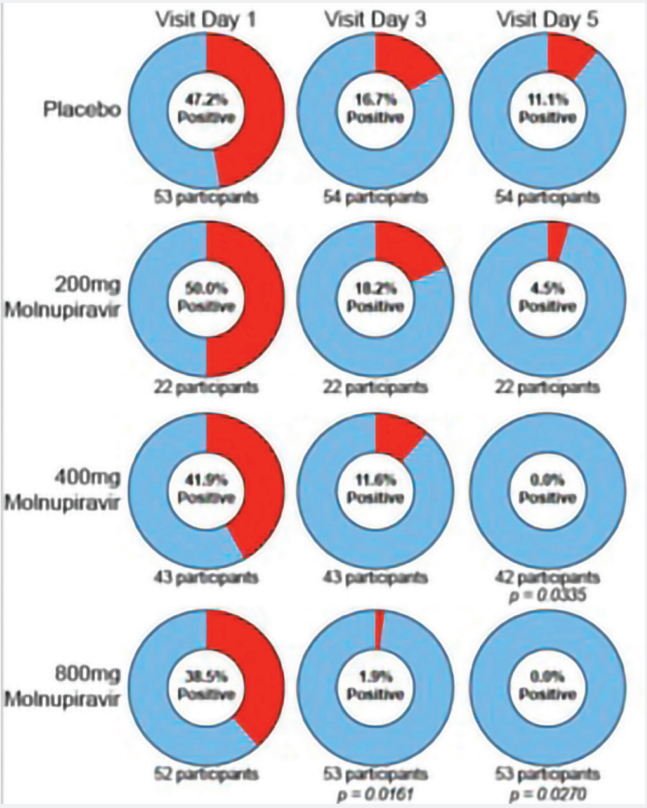
PCR and saliva

Multiple PCR tests are available for infectious diseases of cattle. Many require a blood sample or a swab (nasal or pharyngeal). The adaption of number of PCR extraction methods for testing SARS-Cov2 in saliva provides an opportunity to increase infectious disease surveillance in cattle. The utilization of ropes hanging in a pen or a feeder to collect salvia samples, a common sampling procedure in swine production, was first demonstrated in cattle for O157 E. coli and Salmonella spp testing for foodborne pathogens.[14] Being able to collect saliva as a surveillance tool with PCR detection provides a good opportunity for easy monitoring.

Point-of-care lateral flow devices

Covid-19 pandemic has resulted in the further development of lateral flow devices that are based on ELISA methodology. These devices have been available for home pregnancy tests in

Figure 4: SARS-2 Infectivity. Proportion of participants positive (red) for SARS-CoV-2 infectious virus; participants who are negative for SARS-CoV-2 infectious virus are in blue.[4]



humans and for milk progesterone in cows. The use of these devices for point of care for infectious disease has been limited to human medicine. The plethora of different lateral flow devices developed and optimized for the detection of SARS- CoV-2 can only help in the development of cost-effective point of care devices for animal health.

Neutrophil differentials

A hematological instrument developed for bovine respiratory disease (QScout BLD; Advanced Animal Diagnostics) has been adapted for use in COVID-19 and other human patients dealing with septicemia. The detection of band neutrophils is a prognostic tool for interventions. This is another case where One Health developments from animal health have had an impact on our testing and interventions for human health.

Prevention

Vaccines

Platform vaccines: These vaccines use a basic manufacturing process that does not change- the so-called "platform". These vaccine platforms, mRNA, DNA – plasmids, baculovirus, viral vectors – once approved by regulatory officials, use the same base components regardless of the antigen. The only thing that changes is the genetic code for the desired antigen. Such a change of only the sequence or antigen in question is much simpler and much faster than having to develop a complete vaccine from scratch.[2]

mRNA vaccines: Cells produce proteins that are the antigens that stimulate the immune system (Figure 6).[1,2] To produce proteins, they use DNA as the permanent template. The DNA is then transcribed into mRNA (transcription of DNA into mRNA). The mRNA is then translated into proteins (translation of mRNA into a protein by ribosomes, in the cytoplasm). What happens when mRNA vaccines are administered? The mRNA is introduced into the cells and the cell translates the mRNA into proteins and the mRNA is degraded. This construction of the protein based on the plan in the mRNA takes place outside the nucleus, so the mRNA does not come into contact with the DNA and can't become a permanent part of the cells. Since mRNA is not that stable and degrades quite rapidly in the cell, it is often "coated" in a synthetic envelope, a "liposome" to introduction the mRNA into the cell. To keep the mRNA-liposome complex stable during storage and transport, low temperatures may be necessary.

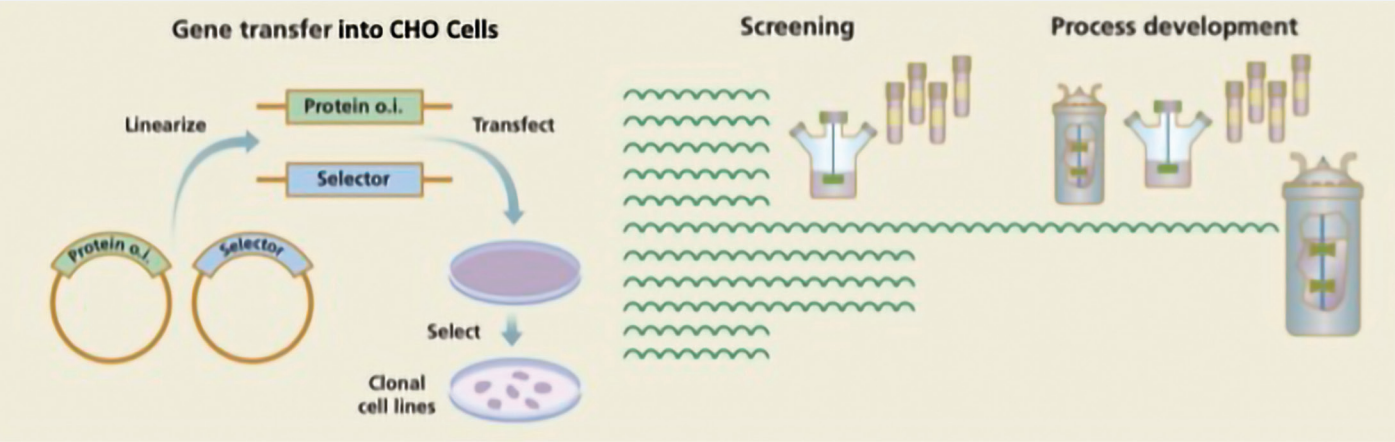
DNA vaccines: A DNA vaccine works in a similar way as mRNA in terms of its basic principle.[2] The vaccinated person receives a so-called plasmid – a ring-shaped piece of DNA that contains the genetic code for the desired antigen (virus protein). This migrates into the cell nucleus, where the normal process of transcription takes place, just as it does in the cell. Instead of receiving the transcript immediately, as is the case with the mRNA vaccine, the cell receives the template information and then makes mRNA transcript, and then the mRNA is transcribed in the cytoplasm into protein antigen. Plasmids are much more stable and resistant than RNA and therefore easier to produce and store. The first USDA-approved DNA vaccine was for West Nile Virus in horses. A DNA vaccine has been approved in Europe for salmon against contagious pancreatitis and is inoculated by intramuscular injection.

One advantage of DNA and RNA vaccines is their purity.[2] Since no virus is isolated and grown and no cell cultures are used, the risk of possible contamination with other viruses, other pathogens or cell residues and the extensive testing programs for these very contaminants normally required for vaccines are eliminated. This simplifies production. The finished vaccines do not require an adjuvant and generally contain few ingredients.

Vector vaccines: Vector vaccines use another virus as the backbone.[2] For canine vaccines, for example, modified pox viruses (canarypox) are used for canine distemper. Western equine encephalitis virus has been used for swine vaccines. These viruses are genetically modified so that they are harmless to the vaccinated animal and carry the required genetic information for the desired antigen. This means that when it enters the cell, i.e., "infects" it, it no longer initiates its own replication by the host cell, but instead the desired antigen is produced. Alternatively, the vector virus can be modified so that it carries the desired antigen, e.g., the protein of a coronavirus, on its surface. Then the body mistakes it for a coronavirus and produces appropriate antibodies. In poultry, there are some vector vaccines that can be used "twice"– if the vaccine is to protect against two different diseases or against two different variants of the same virus, the genetic code of the second pathogen is added to the vaccine virus and thus causes the cell to build the antigens for two viruses by means of infection by one virus.

Protein-based vaccines – recombinant nanoparticles or virus-like particles.[2] There are several different methods that are used to produce highly antigenic proteins – bacteria, yeast, baculovirus. The desired antigen is producing by inserting the

Figure 5: Production of Recombinant Monoclonal Antibodies in CHO cells. Chinese hamster ovary (CHO) cells development for cell culture processes for the generation of recombinant monoclonal antibodies (mAb). The wavy lines indicate CHO subcultivations of individual cell lines that are in a screening program to obtain the highest mAb producer. Vials indicate banks of cells frozen in liquid nitrogen. Spinner flasks represent scale-down systems for process optimization, and bioreactors represent large-scale production processes.[16]



genetic code for the desired antigen using genetic engineering methods and coupling it to a suitable protein or protein-lipid combination. In this case, the antigen is administered as in conventional vaccines, but in a highly purified form, since only this specific antigenic structure is contained and no other virus parts. In the course of the work on COVID vaccines, some optimized procedures have been developed, but the basic principle itself is not new; such vaccines have been in use for a long time in both the human and veterinary fields. These systems are also platform technologies since the same basic components are used for manufacturing and again the only changes is the genetic code for the desired antigen.

Probiotics/prebiotics

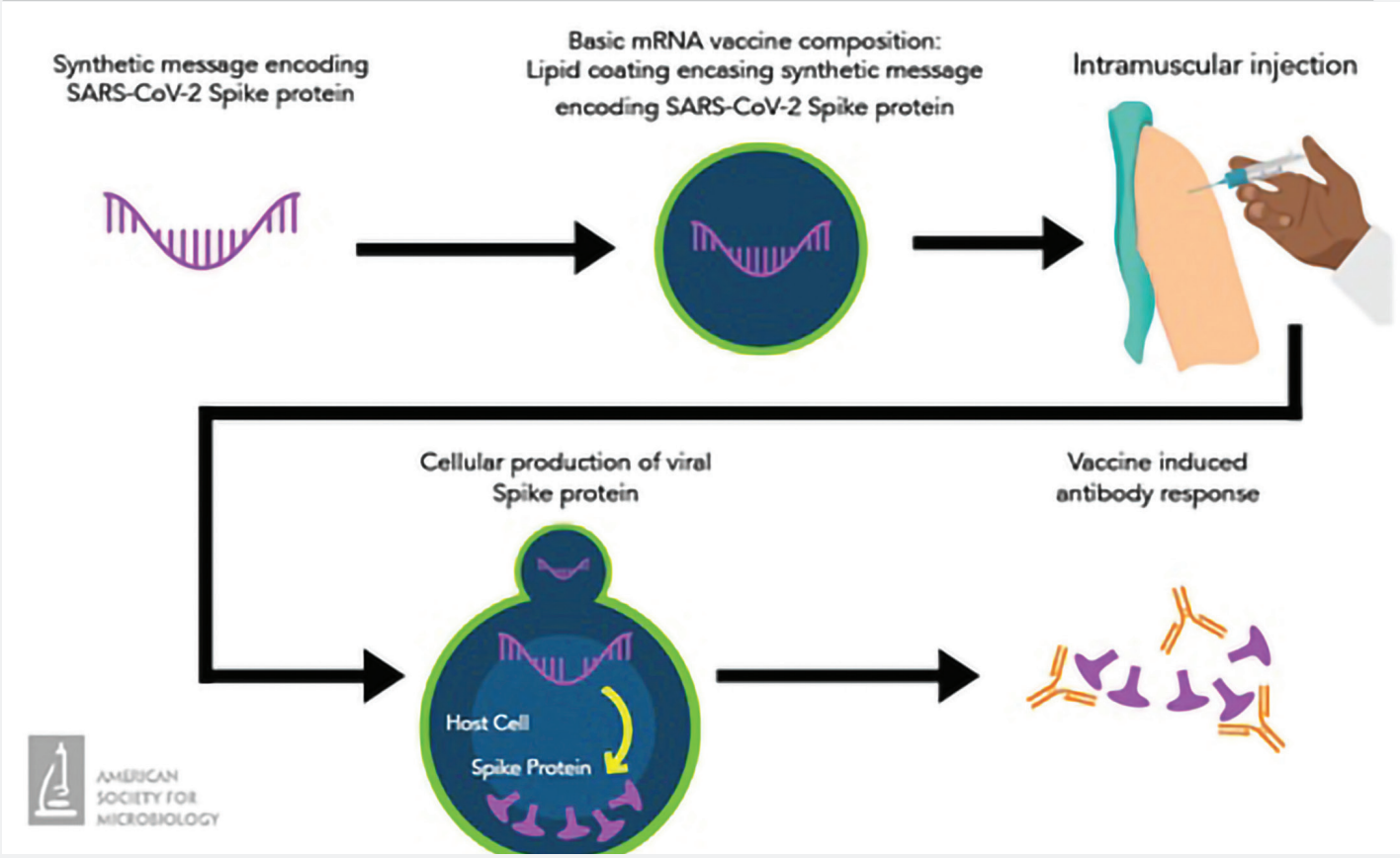
Probiotics have a preventative role in COVID-19.[11] Although we have been using prebiotics, probiotics, essential oils and/or organic acids in animal production for years, the approaches have often been empirical and based on one or two components with little understanding of the mechanism of action. In looking at human medicine and the prevention and treatment of inflammatory bowel disease, there has been a more holistic multipronged approach developed (Figure 7).[12] Like veterinary medicine, the initial approaches for prevention and/or treatment of gastrointestinal (GIT) disease were pharmaceutical-based with antibiotics being a major tool. Using a multipronged approach in humans has been aimed at reducing the use of exogenous corticosteroids and/or antibiotics (Figure 7, circle lower left). There are several GIT health goals from these multipronged approaches. First, maintain a healthy "kill zone" and mucosa and block specific pathogen attachment (Figure 7, center green box). Second, correct dysbiosis and restore normal microbial function (Figure 7, upper left blue box), and normalize the immune dysfunction and repair barrier defects (Figure 7, upper right lavender box). These approaches may be accomplished by using traditional approaches (probiotics, organic oils, high fiber diets, or combinations of these), cutting edge methods (fecal microbial transplants, synthetic mixtures of defined microbes personalized for an individual's specific microbiota profile, and personalized diets). Then there are novel experimental approaches (bacteriophages targeting key aggressive bacteria, using synthetic microbial metabolites or recombinant bacterial species) that also have promise.

In livestock, we have several other unique approaches to improving GIT health in addition to the traditional approaches (probiotics, organic oils, high fiber diets, or combinations of these). These approaches including prebiotics (refined functional carbohydrates {RFC}; inhibiting bacterial attachment, promoting a more anaerobic environment; blocking bacterial receptors; stimulating protective mammalian pathways); mixtures of defined microbes based on culture and sensitivity testing that are herd and/or region specific and hen egg IgY antibodies against specific organisms. With ruminant housing and pasture management exposure to feces (and rumen content transplants), there is an on-farm "microbial transplant" opportunity.

Conclusion

COVID-19 illustrates an important application of the One Health Concept by incorporating concepts of pathogenesis, therapy and prevention from animal health to human health. "Too much of a good thing" – the over-response of the innate response is an underlying issue for disease pathogenesis in human and animals. Several technologies have been developed in veterinary medicine that improved the outcomes of COVID-19 in people. These include anti-inflammatory therapeutics, mAb and diagnostic testing (neutrophil differentials). The special feature of the platform vaccines (mRNA, DNA, vector, recombinant proteins) is that the animal is not administered the "live" antigen (as we know it from conventional MLV vaccines), but either the transcript or the protein. The antigens produced/incorporated by these vaccine uses mechanisms that are present in the cell to provide a more specific response and are more safe than conventional MLV vaccines. Probiotics may have a role both when fed directly or when administered in the nasal cavity.

Figure 6: mRNA vaccine-induced antibody response against SARS-CoV-2 spike proteins. Vaccination delivers a genetic message that signals host cells to produce copies of this antigen. Ribosomes translate the mRNA and initiate host production of spike protein copies. Then antigen-presenting cells display the antigen on their surfaces, triggering the immune system to produce antibodies and T-cells in response to the foreign protein. Source: American Society for Microbiology[1]



References

1. Anonymous 2020. COVID-19 Vaccine FAQs. December 4, 2020. **https://asm.org/Articles/2020/December/COVID-19-Vaccine-FAQs Accessed October 18, 2021.**

2. Anonymous. 2021. Modern Vaccines and Platform Technologies - Explained in an Understandable Way. April 2.2021. **https://www.gmp-compliance.org/gmp-news/modern-vaccines-and-platform-technologies-explained-in-an-understandable-way** Accessed October 18, 2021.

3. Finkielsztein, L.M., Moltrasio, G.Y., Caputto, M.E., Castro, E.F., Cavallaro, L.V., Moglioni, A.G., 2010. What is Known About the Antiviral Agents Active Against Bovine Viral Diarrhea Virus (BVDV)? Curr Med Chem 17, 2933–2955. **https://doi.org/10.2174/092986710792065036**

4. Fischer, W. et al., 2021. Molnupiravir, an Oral Antiviral Treatment for COVID-19. Medrxiv 2021.06.17.21258639. **https://doi.org/10.1101/2021.06.17.21258639**

5. Francoz, D., Buczinski, S., Apley, M., 2012. Evidence Related to the Use of Ancillary Drugs in Bovine Respiratory Disease (Anti-Inflammatory and Others): Are They Justified or Not? Vet Clin North Am Food Animal Pract 28, 23–38. **https://doi.org/10.1016/j.cvfa.2011.12.003**

6. Givens, M.D., et al., 2003. Detection of Inhibition of Bovine Viral Diarrhea Virus by Aromatic Cationic Molecules. Antimicrob Agents Ch 47, 2223–2230. **https://doi.org/10.1128/aac.47.7.2223-2230.2003**

7. Karki, R., et al. 2021. Synergism of TNF-α and IFN-γ Triggers Inflammatory Cell Death, Tissue Damage, and Mortality in SARS-CoV-2 Infection and Cytokine Shock Syndromes. Cell 184, 149-168.e17. **https://doi.org/10.1016/j.cell.2020.11.025**

8. Kunert, R., Reinhart, D., 2016. Advances in recombinant antibody manufacturing. *Appl Microbiol Biot* 100, 3451–3461. **https://doi.org/10.1007/s00253-016-7388-9**

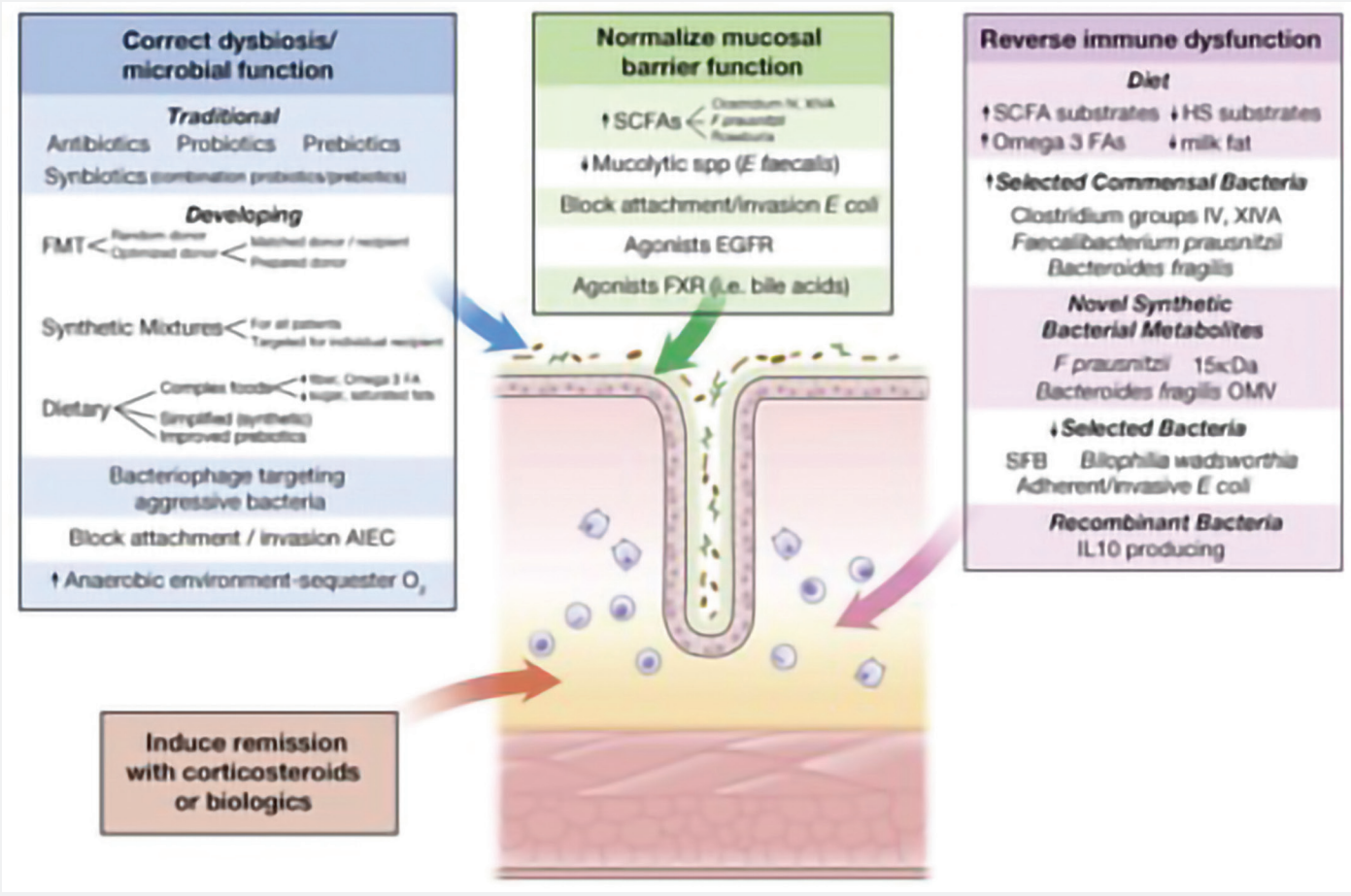
9. Malone, B., Campbell, E.A., 2021. Molnupiravir: coding for catastrophe. *Nat Struct Mol Biol* 28, 706–708. **https://doi.org/10.1038/s41594-021-00657-8**

10. Marchiando AM, Graham WV, Turner JR. Epithelial barriers in homeostasis and disease. *Annu Rev Pathol*. 2010;5:119-144.

11. Mirzaei, R., Attar, A., Papizadeh, S., Jeda, A.S., Hosseini-Fard, S.R., Jamasbi, E., Kazemi, S., Amerkani, S., Talei, G.R., Moradi, P., Jalalifar, S., Yousefimashouf, R., Hossain, M.A., Keyvani, H., Karampoor, S., 2021. The emerging role of probiotics as a mitigation strategy against coronavirus disease 2019 (COVID-19). Arch Virol 166, 1819–1840. **https://doi.org/10.1007/s00705-021-05036-8**

12. Sartor RB, Wu GD. 2017. Roles for Intestinal Bacteria, Viruses, and Fungi in Pathogenesis of Inflammatory Bowel Diseases and Therapeutic Approaches. *Gastroenterol*. 152(2):327–339.e4

Figure 7: Targeting the mucosa with nutraceuticals that specifically enhance the microbiota and improve barrier and immune function. AIEC, attaching and effacing E. coli; EGFR, epidermal growth factor receptor; FA, fatty acid; FXR, farnesoid X receptor; FMT, fecal microbial transplant; HS, hydrogen sulfide; IL-10, interleukin 10; OMV, outer membrane vesicles; SCFA, short chain fatty acids; SFB, segmented filamentous bacteria.[12]



13. Sherman, D.M., Acres, S.D., Sadowski, P.L., Springer, J.A., Bray, B., Raybould, T.J., Muscoplat, C.C., 1983. Protection of calves against fatal enteric colibacillosis by orally administered Escherichia coli K99-specific monoclonal antibody. Infect Immun 42, 653–658. **https://doi.org/10.1128/iai.42.2.653-658.1983**

14. Smith, D.R., Gray, J.T., Moxley, R.A., Younts-Dahl, S.M., Blackford, M.P., Hinkley, S., Hungerford, L.L., Milton, C.T., Klopfenstein, T.J., 2004. A diagnostic strategy to determine the Shiga toxin-producing Escherichia coli O157 status of pens of feedlot cattle. *Epidemiology Amp Infect* 132, 297–302. **https://doi.org/10.1017/s0950268803001699**

15. Tisoncik, J.R., Korth, M.J., Simmons, C.P., Farrar, J., Martin, T.R., Katze, M.G., 2012. Into the eye of the cytokine storm. *Microbiol Mol Biol R* 76, 16–32. **https://doi.org/10.1128/mmbr.05015-11**

16. Wurm, F.M., 2004. Production of recombinant protein therapeutics in cultivated mammalian cells. *Nat Biotechnol* 22, 1393–1398. **https://doi.org/10.1038/nbt1026**

