A New Approach to Targeting Animal Diseases

Overview:

Protein-based prescription-platform vaccines represent a new approach to targeting diseases affecting animal health. In concept, the approach takes the sequences of field isolates to generate a library of ready-made vaccine targets that can be formulated into veterinarian-prescribed formulations to meet individual, regional, or national needs. In practice, the technology uses advanced field monitoring, computer-assisted predictive algorithms for vaccine design, and both computer-assisted and bench assay methods to ensure the effective delivery of appropriate structure and amount for immunogenic proteins.

What Are Platform-Based Vaccines?

The goal of any vaccine is to pre-expose the immune system to an infectious agent, to provoke a robust and protective immune response upon later encounters with that pathogen. The key to vaccination is to induce the correct protective antibodies and T cells to identify and eliminate the virulent infectious agent without the associated damage induced by actual infection. Traditional commercial and autogenous vaccines use weakened live pathogens or killed whole viruses or bacteria to generate that immunity. This exact approach was the basis of the original smallpox vaccine that started the field of immunology and has been highly successful despite some limitations. The main limitations arise in the extended time and cost to produce and license a commercial vaccine, and conversely the high specificity but limited broadspectrum of autogenous products. Simply put, these technologies have not kept up with science, largely due to regulatory barriers. Based on science, we can now identify specific components of the agents that are likely to confer protective immunity. Using current USDA guidelines, these vaccines can either be produced with 100% homology to farm-specific isolates like autogenous vaccines, or alternatively a library of vaccine antigens targeting circulating strains can be pre-developed for off-the-shelf usage by producers. Platform vaccines, therefore, share the advantages of traditional commercial vaccines allowing "off-the-shelf" solutions for many agents, while also facilitating the "custom design" characteristics of autogenous vaccines. Current USDA regulations set out the guidelines for this approach in their description of "prescription-platform vaccines".

DNA, RNA, or Protein?

To produce a platform-based vaccine, the entire production pathway is conserved but only the final target protein differs between vaccines. The key event for any of these methods is to introduce the immune system to the target protein in a form that promotes protective antibody and cellular responses. The difference between platforms is how that protein is generated and provided to the immune system. DNA and RNA vaccines create the nucleic acids that code for the target protein (for example, spike protein from coronaviruses) which is then injected into host cells following vaccination that, in turn, produce the target protein for recognition by the immune system. In contrast, protein-based vaccines, such as those produced by Medgene, use an animal-free cellculture system to produce and quantify the target protein which is then killed prior to formulating the final vaccine.

This allows Medgene to directly control and verify all aspects of the target production process, including not only the required dose but also the quality of the final protein delivered to the immune system.

What Are Protective Epitopes?

All viruses and bacteria contain many genes and proteins, only a few of which can be used as targets to limit infection and disease. We use a system to produce duplicates of those proteins in a harmless insect cell culture system. This is similar to cutting a new key at the hardware store as a substitute for the original. In this case, the key is the important regions on those proteins that stimulate protective antibodies, and the lock is the neutralizing antibodies. Our goal is to produce a duplicate of that key in our vaccines. To this end, the specific shape of the key blank is irrelevant – it can be the same as the original, but it doesn't have to look the same. The critical aspect is the shape of the notches and ridges – that must match, or it will not properly engage the lock (or induce the correct antibody).

What is Prescription Homology?

The key to a successful vaccine is targeting not only the correct proteins within the infectious agent to provide protection, but actually targeting the specific regions within those proteins. The region we need to duplicate within our antigen is the specific site of the viral or bacterial protein that interacts with the host, that when blocked eliminates or adversely affects the ability of the agent to cause infection. Returning to our key analogy above, everybody knows that if you need a new key cut, the part that matters is the blade, NOT the handle. The key blank that is selected at the store does not particularly matter, only the notches and ridges on the blade. At any hardware store, this analysis is done by computer, where the computer uses methods programmed into it to analyze the key and indicate an appropriate blank that can then be turned into a duplicate key. Medgene has adapted research developed over the past four decades to perform a similar analysis on target proteins, identifying critical regions for the immune response within those proteins, and then focusing on those regions to ensure precision homology to our vaccine targets. Those changes that affect the specific epitope regions are considered for inclusion in our antigen library, while pre-existing constructs can be used to immediately address industry needs quickly. This process allows us to create a comprehensive selection of target antigens that provide broad coverage across known agents circulating in the field, and update those targets as the field diversity increases. We have used this approach with success in addressing a number of pathogens ensuring that our vaccines, unlike fully licensed products, always address field strains of current importance.

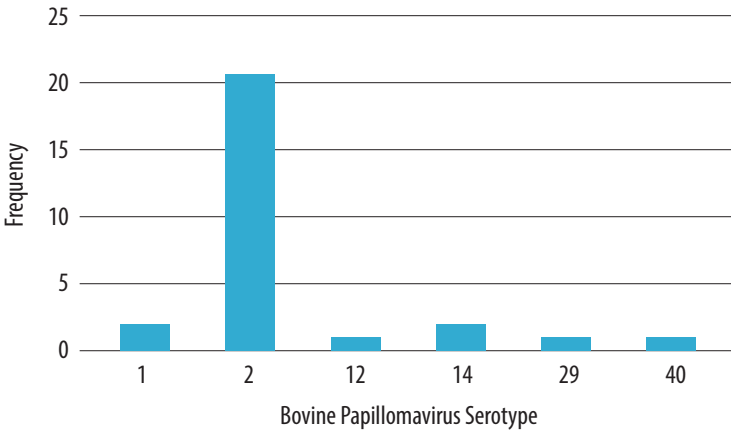
Bovine Papillomavirus

A family of circular, double-stranded DNA viruses known to infect both domestic and wild ruminants. Also infects equines (equine sarcoid).

Genus, Deltapapilloma is the most common and contains types 1, 2, 13, and 14 with BPV type 2 most common. At Medgene, all the penile papillomas from AL, KS, MO, NE, SD, TX have been type 2 with a mixed infection of type 1 on two submissions. We have tested over 30 submissions since August 2022 and with pooling that accounts for over 100 penile papillomas tested. BPV types 12, 14, 29 and 40 have been identified from cutaneous warts at Medgene.

BPV type 2 (and other DeltaPVs) replicate in lymphocytes and chorionic epithelium of the placenta. Type 2 can be found in blood, urine, semen, and milk as well as the ovary, oocytes, cumulus cells and uterine lavage fluid. There is evidence of vertical transmission and the virus may also be spread by vectors and needles. A study in 2021 in clinically healthy sheep in five locations in Italy showed a prevalence of 68% BPV in the blood by digital PCR.

Bovine Papillomavirus Diversity



▲ **Figure 1.** In a survey of 28 diagnostic submissions by veterinarians across the country, the frequency of bovine papilloma serotypes can be seen in Figure 1. The majority of the samples came from penile warts, however there were also samples collected from cutaneous and vaginal warts as well. Serotype 2 was the predominant type found.

Bovine Papillomavirus Pathogenesis - Historically thought to be transmitted only by cutaneous or mucocutaneous contact that may include microtrauma to the area. We now understand, with more sophisticated testing, that it may be transmitted in many different ways. Veterinarians may need to reconsider BPV pathogenesis and modify vaccination protocols accordingly, as it may be prudent to vaccinate early in life.

BPV Vaccine - Medgene BPV vaccine is made the same or similar to human vaccine. All human vaccines contain the L1 capsid, which are made in either baculovirus or yeast expression systems; Medgene vaccine is made using baculovirus. HPV vaccines tend to produce robust antibody responses, often higher than natural infections.

Bovine Coronavirus

"While strong humoral responses are elicited against the S, M, N and HE proteins following natural infection, the predominant antigens involved in virus neutralization are located in the S and HE proteins." (Fulton, Ridpath and Burge, 2013.)

Commercial BCoV vaccines contain genotype 1 based on spike (S) protein phylogeny. All coronaviruses identified at KSVDL since 2020 have been genotype 14, whether enteric isolates, and current Medgene vaccines include both genotype 14 S or respiratory HE.

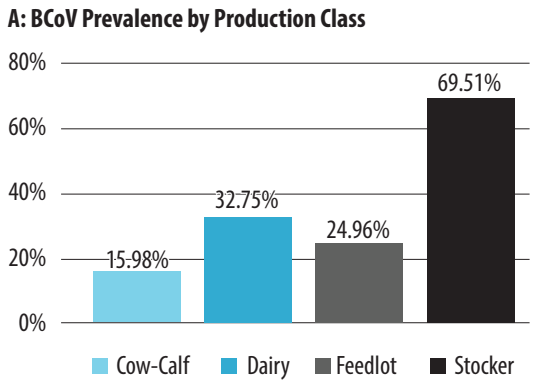
Bovine Influenza D Virus - Medgene has bovine influenza D (IDV) in combination with BCoV. There are many sequences, but OK and 660 are the most common. We have numerous isolates of both clades

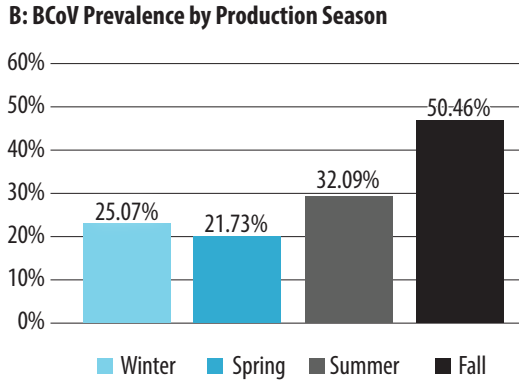
Bovine Rotavirus - Vaccine is made with VP4 and VP7antigens, with multiple P serotypes and G serotypes included, respectively.

BRD Mortality and Associated Economics

	Mortality	Cost
All Cattle	1.5% Total Mortality	$907.8 Million
Beef Cow Operations	15.9%: Cow Mortality	$370.8 Million
	23.0%: Calf Mortality	
Feedlots/Stocker	55.0%: Cattle	274.84 Million
	36.3%: Calves	
Dairy Operations	16.0%: Cattle	$197.89 Million
	32.7%: Calves	

▲ **Figure 3.** Associated mortality and subsequent economics of bovine respiratory disease (BRD), in which BCoV can be a leading coinfection (adapted from Peel, 2020).





◄ **Figure 2.** Kansas State Veterinary Diagnostic Laboratory reported from 3,215 samples associated with BRD that BCoV was most present in stocker operations (A) and during the fall (B; Adapted from Lubbers et al., 2017).