

A Vaccine for Avian Influenza in Dairy Cattle

Moo U – May 8, 2025

Disclaimer

- A. Young is a Professor of Veterinary and Biomedical Sciences at South Dakota State University.
 - *Some diagnostic assays, reagents, and animal challenge studies have been performed at SDSU under contract with Medgene Labs.*
 - *Some core technology has been Licensed to Medgene Labs.*
- A. Young is a founder and Chief Technical Officer of Medgene Labs (2011).
 - *Medgene Labs has independently developed the various vaccine constructs and is the USDA licensed establishment for production of all vaccine technology discussed.*

Summary

- Alan Young Disclaimer
 - I am Chief Technology Officer of Medgene, an animal vaccine company.
 - I am also a Professor at South Dakota State University, Department of Veterinary and Biomedical Sciences.
 - I serve as Chair, USAHA Committee on Biologics and Biotechnology.
- Dairy Cattle Vaccines
 - What I've heard about.
 - What I've seen presented (Zoetis Vaccines).
 - What I know (Medgene vaccine status).

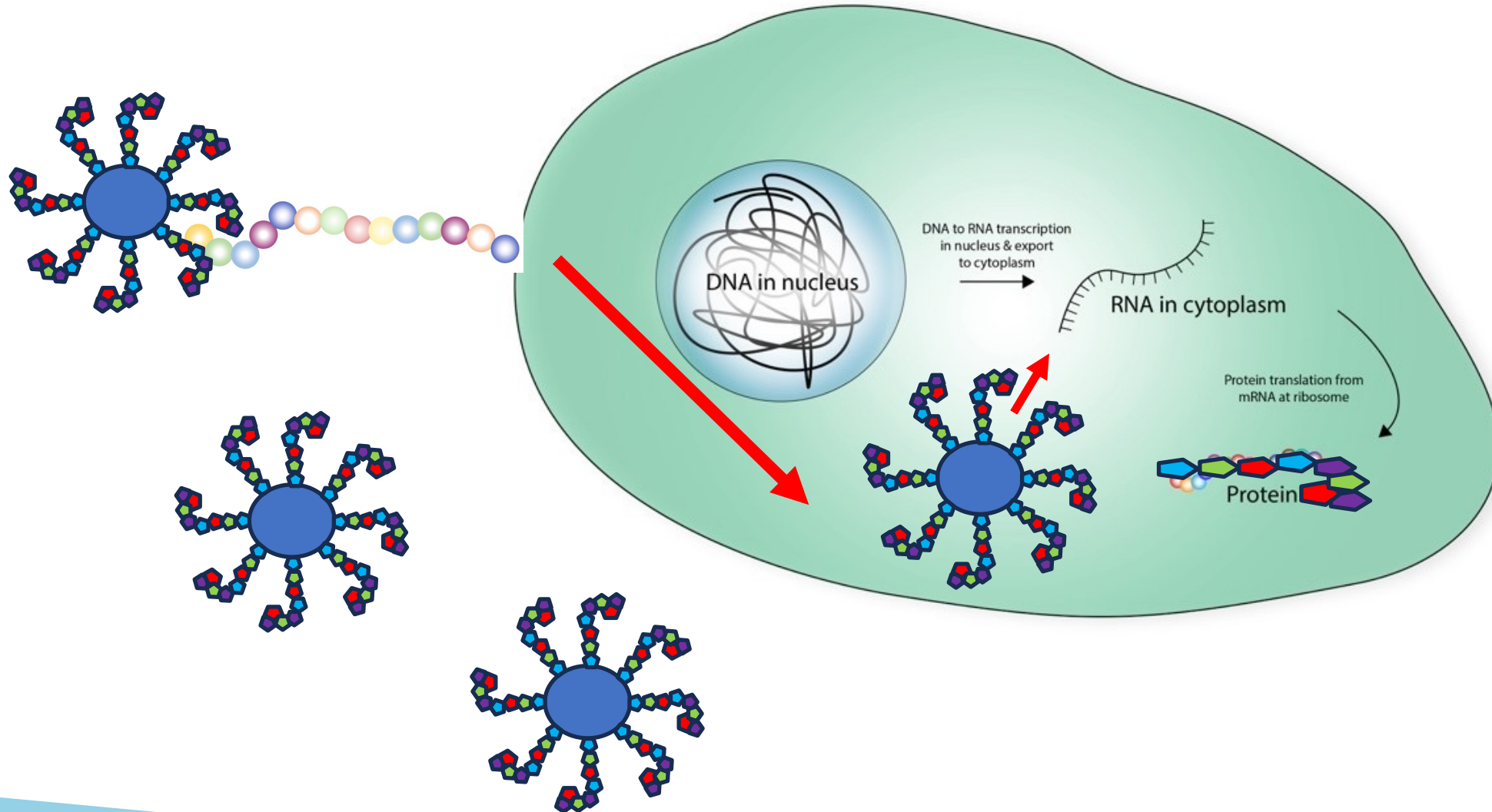
Outline

- How are vaccines made and regulated?
- What are Prescription Platform Vaccine Products?
- AND where are we with H5N1?



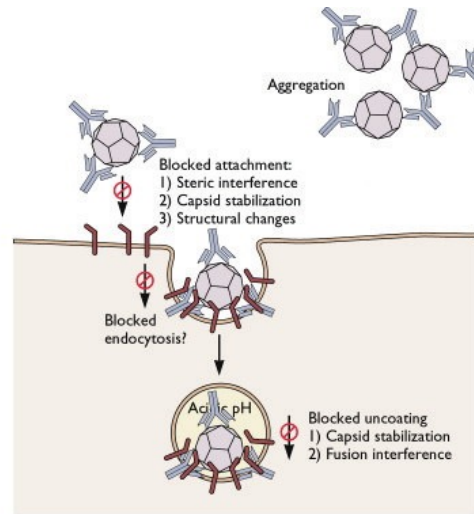
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Virus Infection

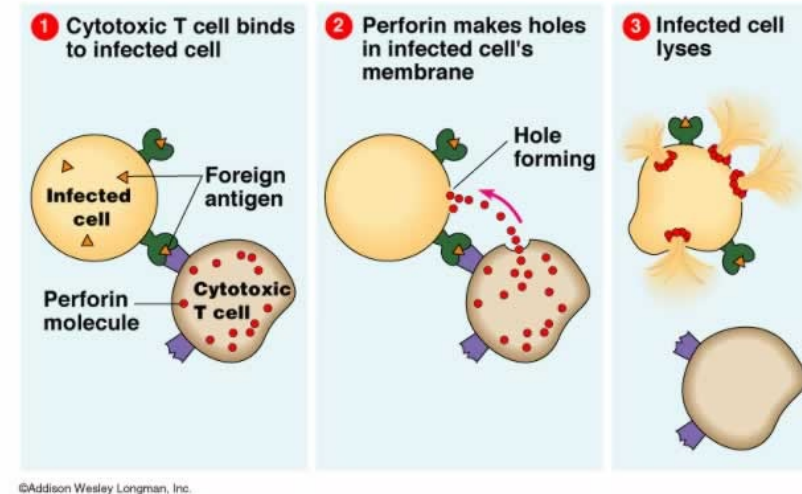


How does the Immune System Work?

Antibody-Based Immunity



Cellular Immunity



Or, more simply.....

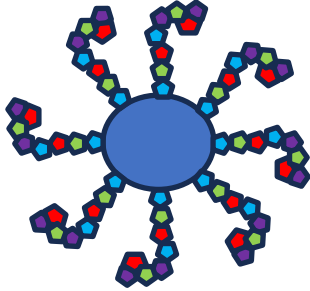
Antibody-Based Immunity



Cellular Immunity



Types of Vaccines



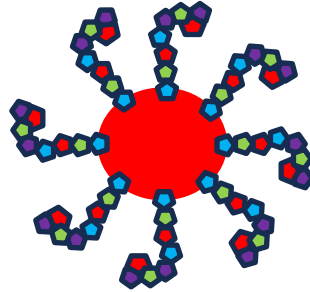
Live

1 Dose

Strong Cellular Response

Reversion

Danger in Some



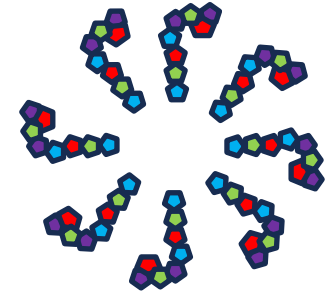
Killed

Safe

Easy to Make

2 Doses

Diversionary Responses



Subunit

Safe

Science-Based

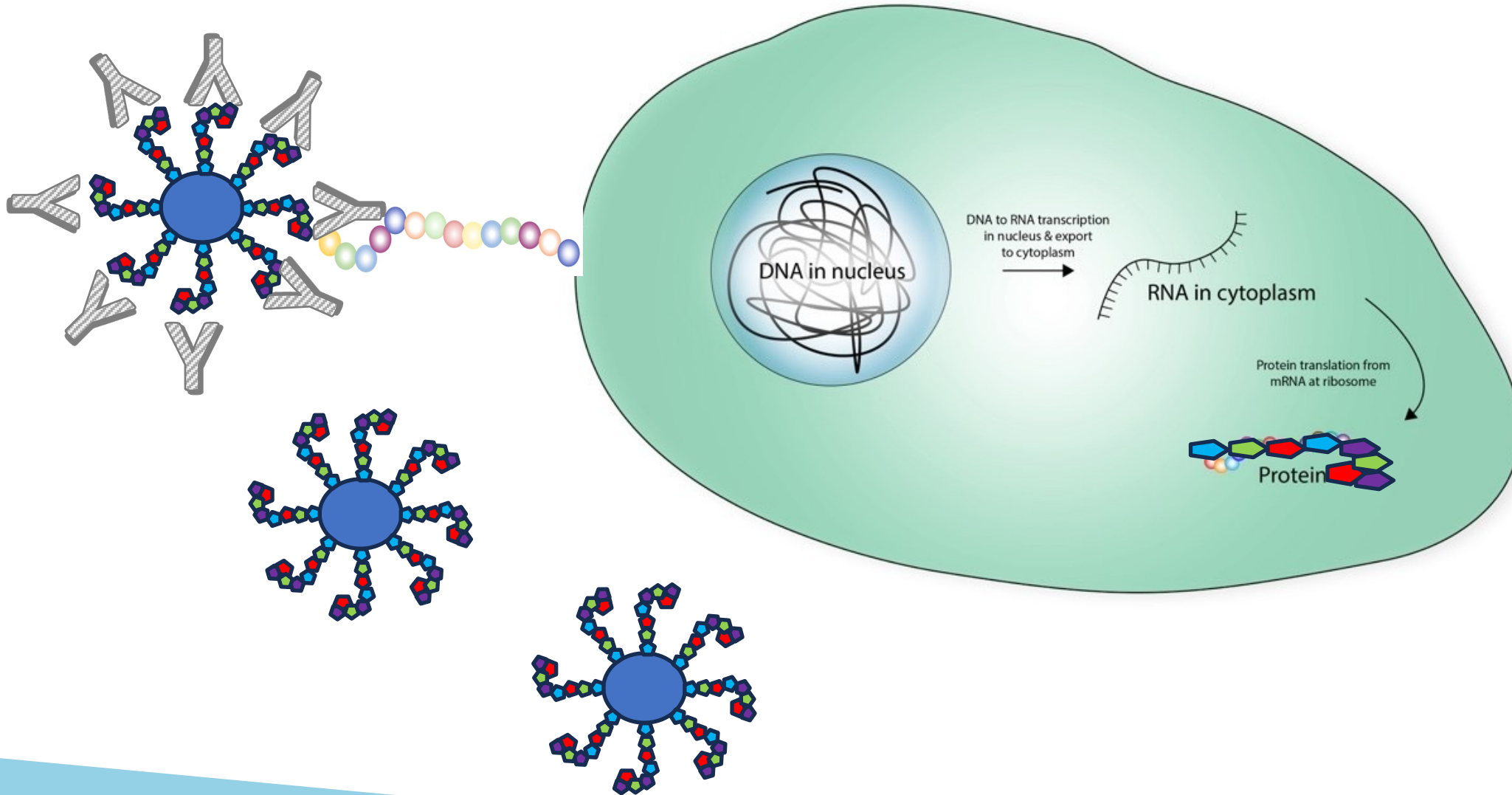
2 Doses

Need to know Target

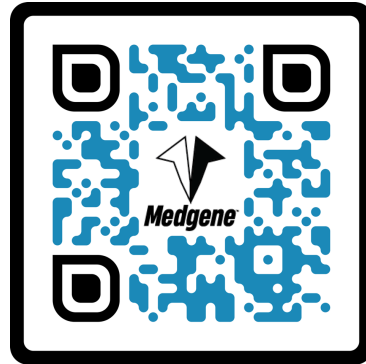
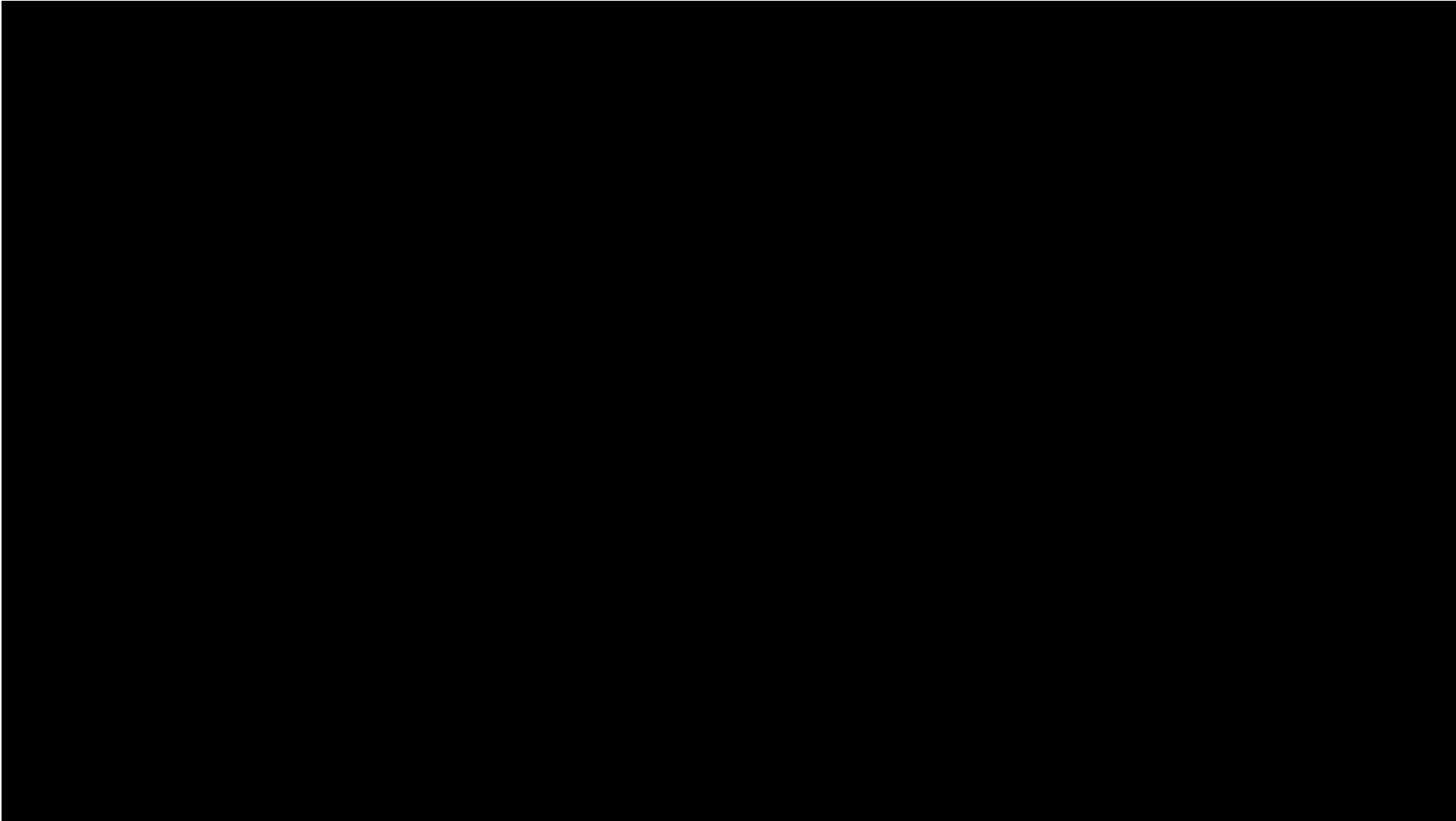


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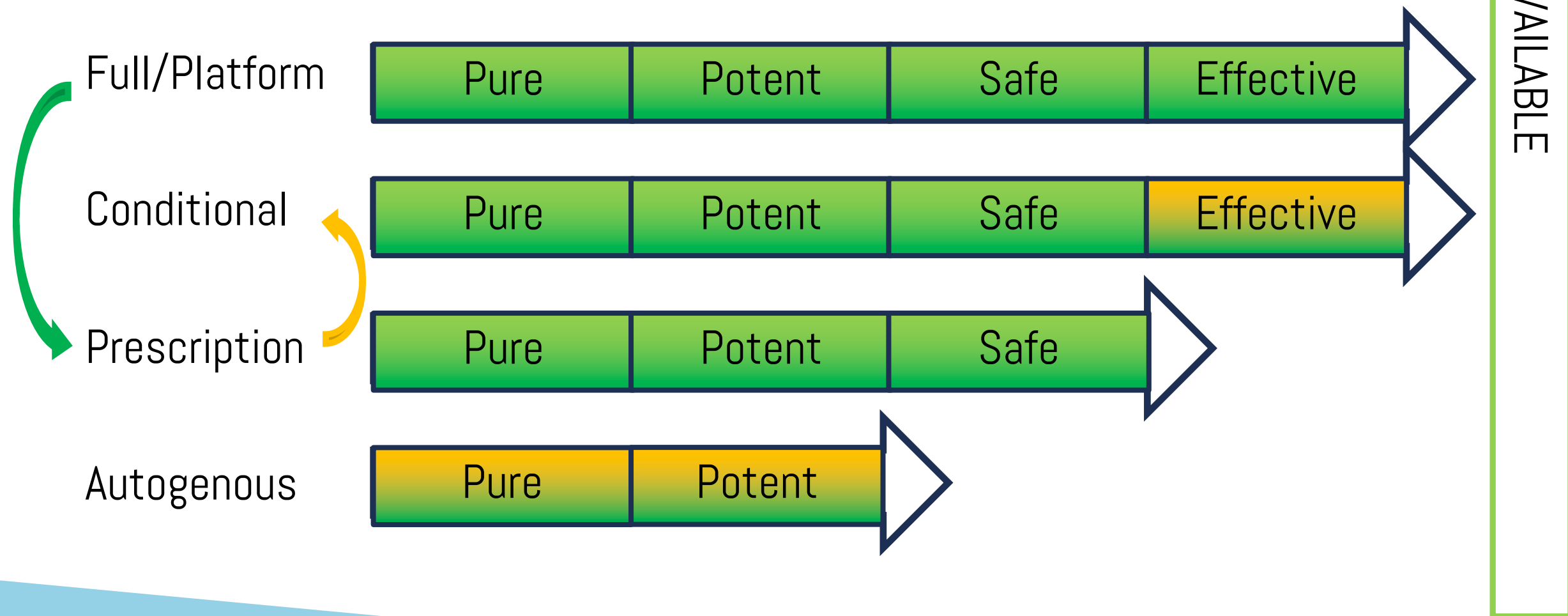
Subunit Vaccines Target Critical Proteins



A Modular Platform for Vaccine Production



USDA-Licensed Vaccine Pathways.



Flu in Cows is not New

PREVALENCE OF INFLUENZA A VIRUS (H1N1) ANTIBODIES IN BOVINE SERA

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Received July 23, 1997

Accepted September 26, 1997

SUMMARY

An H1 subtype-specific indirect ELISA was used to determine the prevalence of influenza A virus antibodies in a retrospective study of 2,345 bovine sera in Minnesota. Twenty-seven percent of the samples tested were positive, 31% were low-positive and 42% were negative. The prevalence of antibody appeared to peak during the months of September to November and then again from February to March. A subset of the above samples was examined by hemagglutination-inhibition (HI) to confirm the ELISA results. A 92% correlation was found between the ELISA and hemagglutination-inhibition assay. Western blot analysis on a subset of ELISA positive sera (n=50) confirmed the presence of antibodies to the nucleoprotein and H1 hemagglutinin protein of influenza A virus.

Evidence of influenza A virus infection in dairy cows with sporadic milk drop syndrome

R. F. GUNNING, I. H. BROWN,
T. R. CRAWSHAW

THE JOURNAL OF INFECTIOUS DISEASES • VOL. 135, NO. 4 • APRIL 1977
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Strains of Hong Kong Influenza Virus in Calves

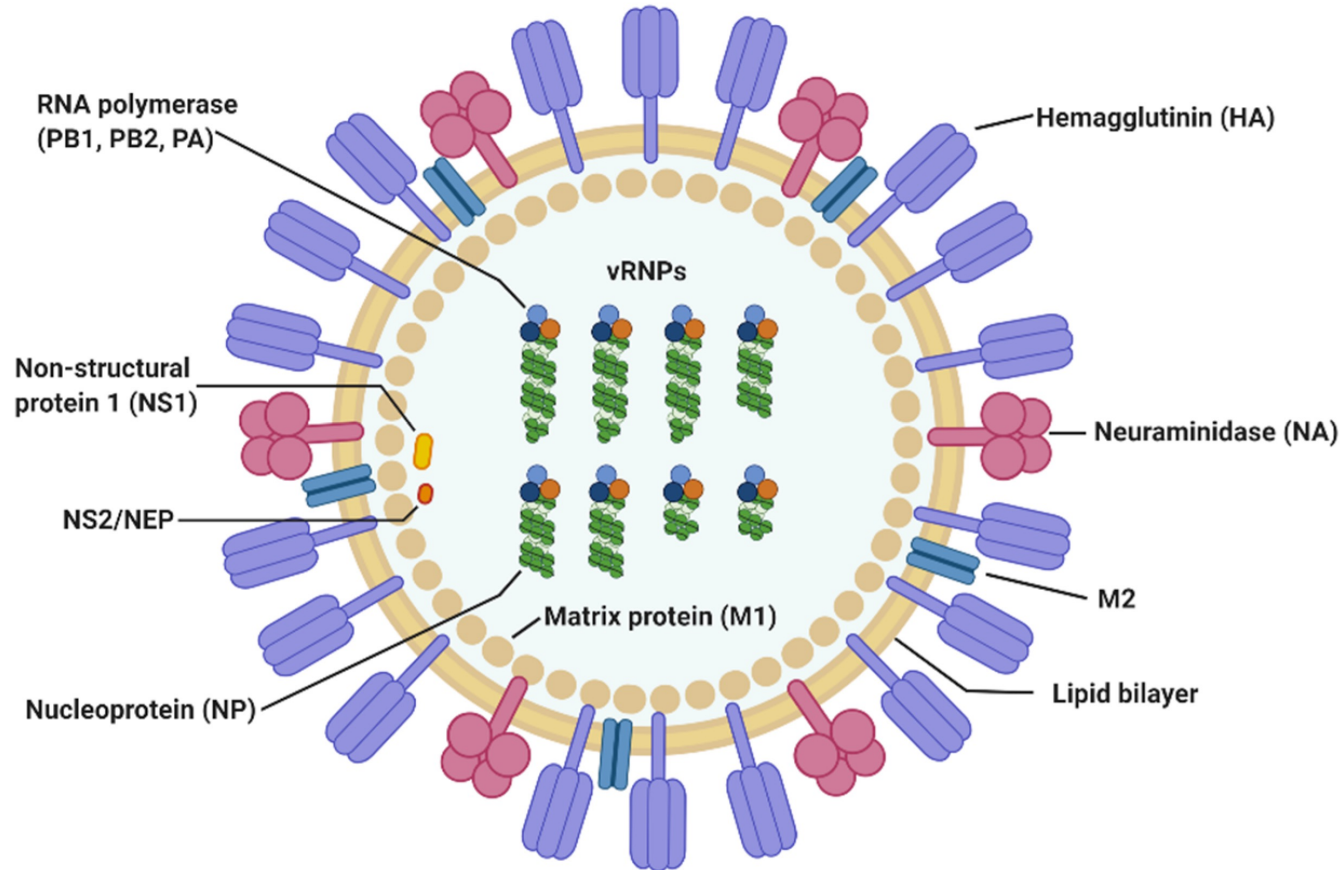
Charles H. Campbell, Bernard C. Easterday,
and Robert G. Webster

*From the Plum Island Animal Disease Center, U.S.
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Service, Northeastern Region, Greenport, New
York; The Department of Veterinary Science, University
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Children's Research Hospital, Memphis, Tennessee*

Three strains of Hong Kong influenza virus isolated from humans were compared with a strain isolated from a calf for their ability to cause disease in calves. One of the human strains, A/Aichi/2/68, was detected for five days in a calf, but all three failed to cause signs of disease. Strain A/calf/Duschanbe/55/71 could be detected for seven days and caused an influenza-like illness in calves.



H5N1



- As of this week, **3116 Complete Viral Sequences** of H5N1 in Dairy Cattle in the US.
- Some variation in internal proteins (PB2).
- All are **>96%** identical for H5 protein.

https://en.wikipedia.org/wiki/Influenza_A_virus

Use of Vaccine – Key Factors

- Based on California experience
 - Replacement Heifers provide a continuous “new source” of naïve animals for reinfection.
 - Abortion Storms in non-lactating dairy cattle have been observed.
 - Duration of natural immunity is unclear.
 - There is evidence for cross-infection of poultry facilities from dairy.
 - There is a need to permit continued animal movement.
 - There is a need to identify vaccinated from previously infected animals.

Medgene Background Data.

- Medgene is licensed in swine and cattle under Prescription Platform Guidelines (Licensed summer 2023).
 - *Animal component free, Protein-based vaccine using killed insect virus.*
- In 2022, when H5N1 began appearing in wild mammals/carnivores, Medgene added a specific H5 2.3.4.4b construct to its' library.
- Primary challenges to licensing include:
 - *Select Agent Status of H5N1 viruses.*
 - *BSL3 Requirement for H5N1 work.*
 - *No relevant animal model for H5N1 infection of dairy cattle.*
 - *Trade-associated restrictions on all animals used in vaccine licensing studies.*

USDA Guidance on Dairy H5N1 Vaccines

- CVB Notice 24-08 (April 29, 2024)
 - Outline of requirements for Diagnostics for Transboundary/FADs for consideration of use by NAHLN.
 - IDEXX NP-ELISA, *IDVet NP-ELISA*, *IDVet H5-ELISA*.
- CVB Notice 24-09 (May 1, 2024)
 - Survey of manufacturers on capacity to produce H5 Vaccine.
 - Large number of responses.
 - Unclear how many are actual established companies with capacity to produce versus interested researchers.
 - *Industry average among AHI members places average time to Conditional License at 5.2 years. There is currently no commercial market for this vaccine.*

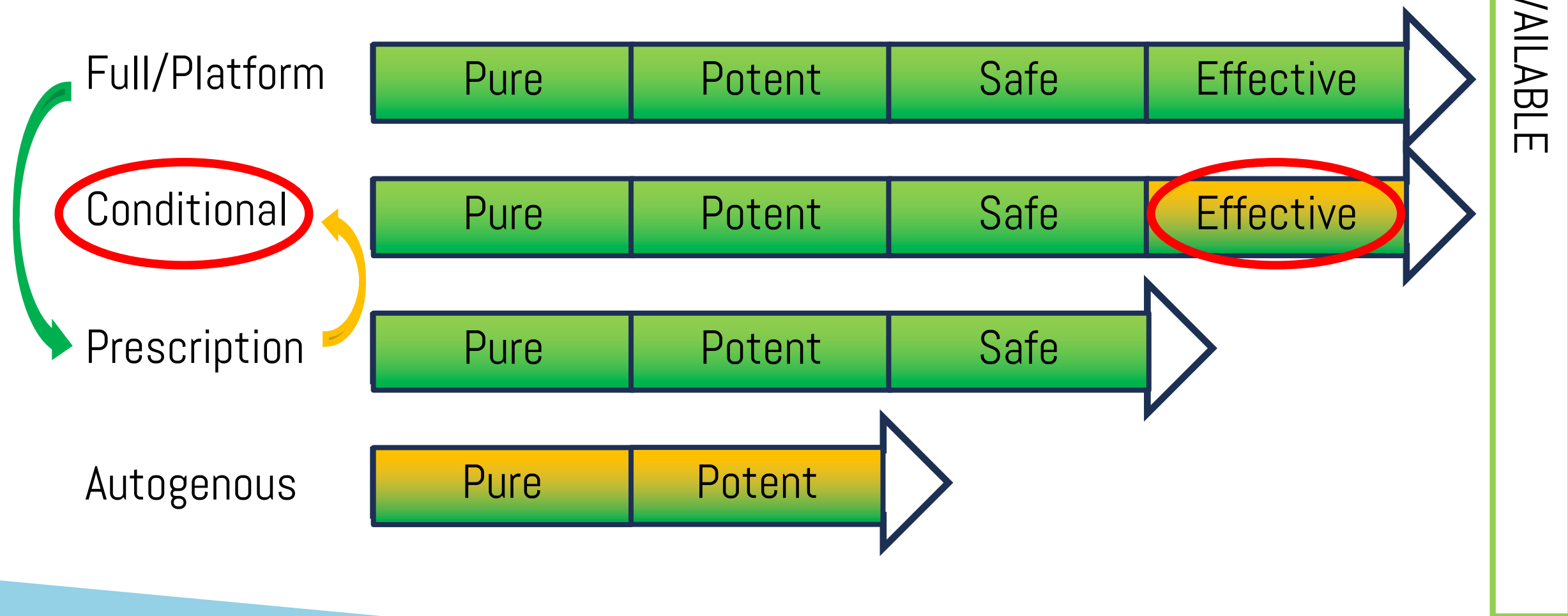
Initial Restrictions on H5N1 Vaccine Development for Animals

- Select Agent Status
- BSL3 Status of virus
- Even killed vaccines must be used in BSL2 facilities
- Full challenge experiments in BSL3 Biocontainment
- No understanding of natural route of infection
- All animals being used must be disposed of following vaccination

Specific Guidance on Vaccines

- CVB Notice 24-11 (July 22, 2024)
 - **Conditional Licensure** – CVB will consider **serological response** in vaccinated target species as data to support a reasonable expectation of efficacy. Serological testing should focus on antibodies to the H5 clade 2.3.4.4b hemagglutinin using an assay such as or similar to **the hemagglutination inhibition assay**. Study protocols including proposed serologic testing details should be submitted to CVB for review.
 - **Full Licensure** – Currently CVB will only consider studies using **vaccination-challenge** with H5 HPAI in the target species, along with all other licensure requirements, to obtain full licensure. Note the recent **Select Agent Exemption for H5 HPAI**. Study protocols should be submitted to CVB for review.
 - CVB strongly encourages applications for licensure, with the caveat that U.S. licensure is distinct from authorization for U.S. use. At the time of this CVB Notice, USDA has not authorized the use of H5 vaccines in the United States. **Any products that are licensed will carry a restriction prohibiting U.S. sale, distribution, and use until further notice.**

USDA-Licensed Vaccine Pathways.



Specific Guidance on Vaccines

• CVB Notice 24-13 (August 28, 2024)

- Removal of BSL2 containment requirements for killed vaccine products.
 - 2 year tracking of all animals used*
- BSL3 containment still required for challenge experiments.
- USDA Veterinary approval for slaughter within 2 years.
- Efficacy assessment based on HI assay to H5 or similar.

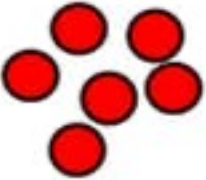

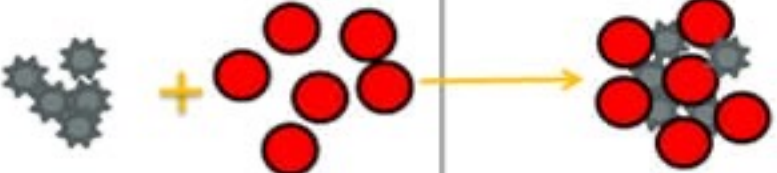



Zoetis Vaccine Status From Nov 2024

- Zoetis developed a recombinant influenza killed whole-virus vaccine (H5N2) during the 2013-2014 outbreak that was added to the stockpile.
- Following recurrence of H5N1 in 2022, Zoetis developed a companion animal updated H5N2 vaccine for dogs and cats.
- Upon appearance in dairy cattle, Zoetis pivoted and demonstrated serological immunity in cattle.
 - 9 animals, HI effective titers in vaccinated calves.
 - DIVA based on differentiation of N1/N2.
- As of November 2024, they reported continuing to proceed towards licensing.
 - Field Safety, Potency, etc.
 - Shared concerns over regulations and requirements over "safe in pregnant" and "tracking of all vaccinates for 2 years or until death."

Medgene Vaccine

- Medgene built their avian influenza vaccine on the exact same platform as existing products in use (*Coronavirus, Rotavirus, Influenza D, Papillomavirus, Hornfly, Ticks, etc*).
 - If this were a common cattle disease (ie Influenza D), the vaccine would be released and available as a Prescription Product under our existing license
- It contains the H5 protein homologous to the circulating 2.3.4.4b strain produced in an animal-component free system.
- The vaccine differentiates from natural infection
 - NP, N1 proteins absent, therefore only infected animals have antibody
 - Undetectable by PCR – no interference with shipping, trade, surveillance

Medgene's Flu Protein Mimics H5N1

	Components	Interaction	Microtiter Results
A	RBCs		No Reaction 
B	Virus + RBCs		Hemagglutination 
C	Virus + Antibody + RBCs		Hemagglutination Inhibition 

- *The assay uses Red Blood Cells (RBCs) as an indicator of Hemagglutinin (H1, H3, H5) function.*
- *The H-protein on the virus OR Medgene H5 binds RBCs, and they stick together in a "mat."*
- *Antibodies from vaccinated animals bind the H-protein, blocking the ability to mat the RBCs and they pellet instead.*

CDC:<https://www.cdc.gov/flu/about/professionals/antigenic.htm>

Antibody levels in Convalescent Cows

Assay	Negative Cows	Convalescent Serum
*HAI (H5 Antigen)	1:19	1:234
ELISA (NP) OD Value	0.31	0.68

**Note: In all species to date, >1:40 is 50% protective antibody titer.*

Medgene H5 Influenza Vaccine in Cattle

**H5 Competitive ELISA (IDVet), S/N Average*

Description	Pre-Vax	21 Days post 1 st dose	14 Days post 2 nd dose
H5N1 Vaccine	93.1	25.1	3.7
Placebo	88.8	90.1	89.2

**Note: Lower Numbers = Higher Antibody.*

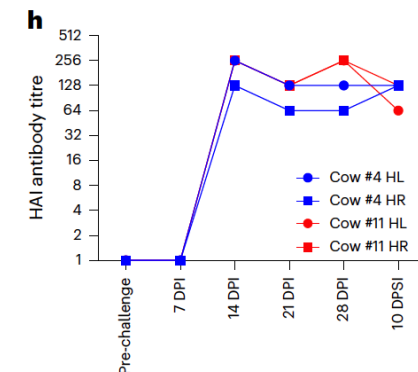
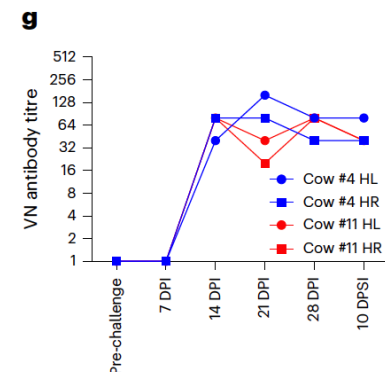
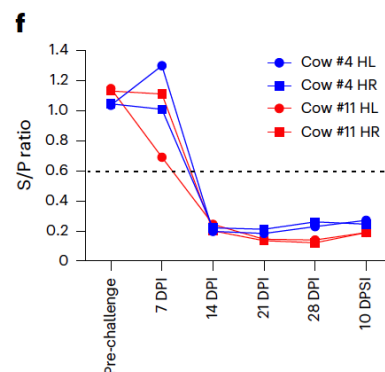
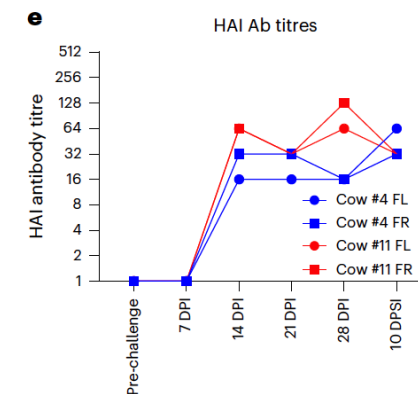
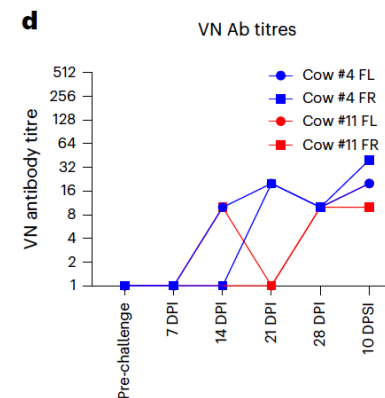
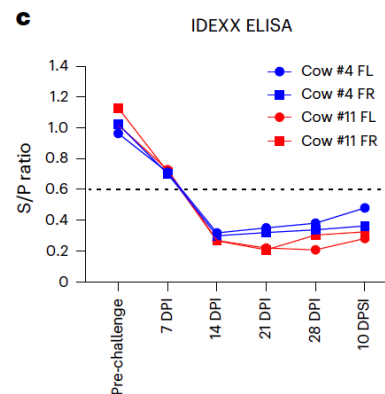
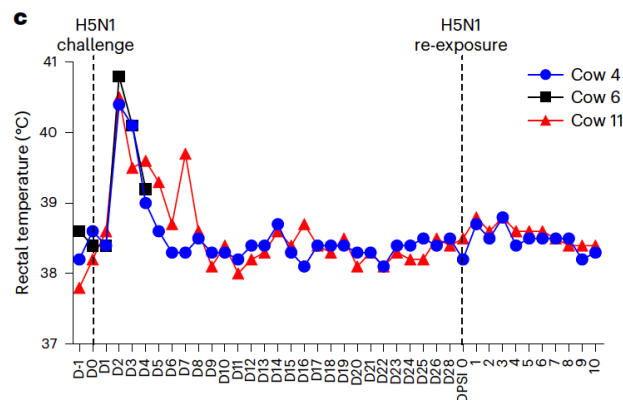
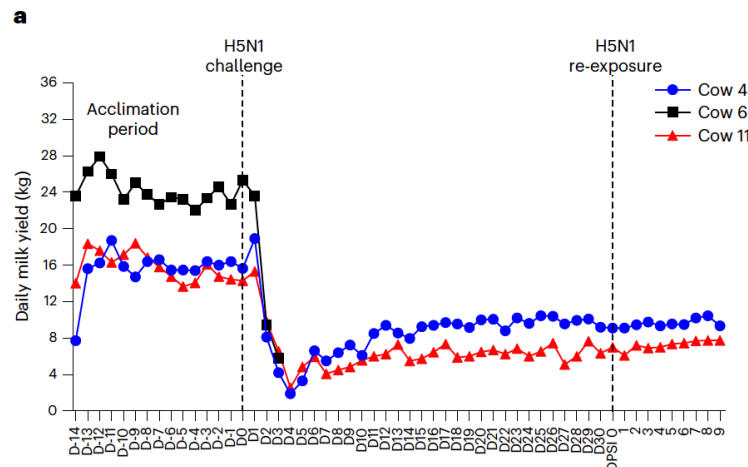
Medgene H5 Influenza Vaccine in Cattle

**Serum Hemagglutination Inhibition Titer (HAI)*

Description	Pre-Vax	21 Days post 1 st dose	14 Days post 2 nd dose
H5N1 Vaccine	1:7	1:31	1:279
Placebo	1:7	1:7	1:9

**Note: In all species to date, >1:40 is 50% protective antibody titer.*

So how much antibody do we need?

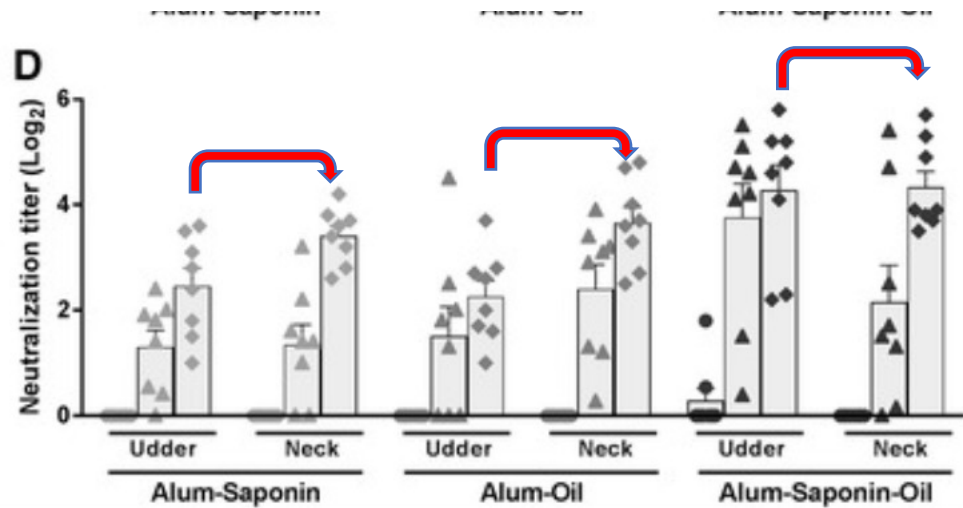


A local titer of >1:16 appears to be protective with direct intramammary injection

Facciolo et al., 2025. <https://doi.org/10.1038/s41564-025-01998-6>

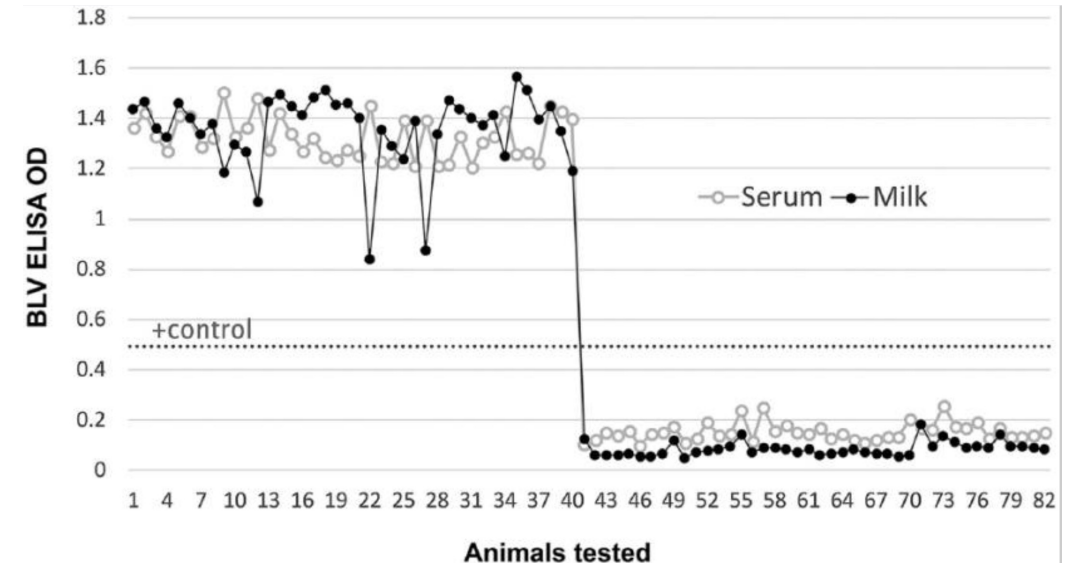
Milk/serum antibodies correlate post-vax in cows.

Staph. Aureus antibody



Boerhout et al., 2018. *Vet Res.* 49:25-35

BLV antibody



Evermann et al., 2019. *J. Vet Diag. Invest.* 31(4):598-600



	Serum Antibody for H5N1 Virus		Serum DNA
<u>Vaccination Status</u>	<u>H5 Protein Assay Result</u>	<u>*NP Protein Assay Result</u>	<u>PCR Identification</u>
No infection No vaccine	Negative	Negative	Negative
Cleared infection No Vaccine	Positive	Positive	Negative
Active Infection	Positive	Positive	Positive
Medgene Vaccine	Positive	Negative	Negative
Whole Virus Vaccine	Positive	Positive	?
Autogenous Vaccine (Not Permitted)	Positive	Positive	?

**Used to differentiate active or previous infection (double positive for H5 and NP) from vaccinated (H5 positive, NP negative).*

Safety

- This formulation is identical to our commercial vaccines, with the exception of the H5 protein instead of Rotavirus, Coronavirus, etc.
 - *Full safety study completed on formulation during licensing.*
 - *Over 500,000 doses in use in the field.*
- No significant safety issues observed in Efficacy Study.
 - *Some transient injection site reactions were observed, as expected.*
- 9CFR USDA Safety study in calves currently underway.
 - *Study expected to satisfy conditional license requirements.*

Customer Driven Milk Drop Study

Treatment	5 days Pre to 5 days Post Vaccination (total milk drop dollars)	10 days Pre to 10 days Post Vaccination (total milk drop dollars)
No Vaccination	(\$7.04)	(\$16.92)
Negative Control - Saline	(\$6.10)	(\$18.24)
Positive Control - Commercial Vax	(\$18.32)*	(\$44.80)*
Medgene 1 mL	(\$5.64)	(\$15.84)
Medgene 2 mL	(\$6.90)	(\$23.04)



Milk valued at \$20 cwt

<https://www.cmegroup.com/markets/agriculture/dairy/class-iii-milk.html#venue=globex>

*significance of $p=0.05$

Take home message on H5N1

- Medgene respects and supports USDA and Industry Policies regarding vaccination.
 - *We are awaiting Conditional Licensing to exceed requirements but provide additional confidence for use, if such use is warranted and needed.*
- The USDA regulatory pathway and business model of Medgene was developed for this eventuality, and has already been applied to other Foreign Animal Disease outbreaks.
 - *The vaccine is available in production and passed all steps used to ensure safety, quality, and consistency commensurate with all our vaccine products.*
- Medgene is prepared with a matched vaccine to the current outbreak (H5N1 2.3.4.4b) compatible with both DIVA and surveillance assays if needed.
 - *Demonstrated protective titers with Influenza A (H5) vaccine in cattle.*
 - *Safety defined from >500,000 doses of non-H5 vaccine in cattle.*
 - *Compatible with both surveillance and movement-restriction assays.*
 - *Conditional licensing process underway.*

Questions?

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Or

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