Genetic vaccines (mRNA/DNA) in animal use

Implications for animal health, human health, our combined microbiome and environment

Special considerations for use as “Countermeasures under Public Health Emergency”

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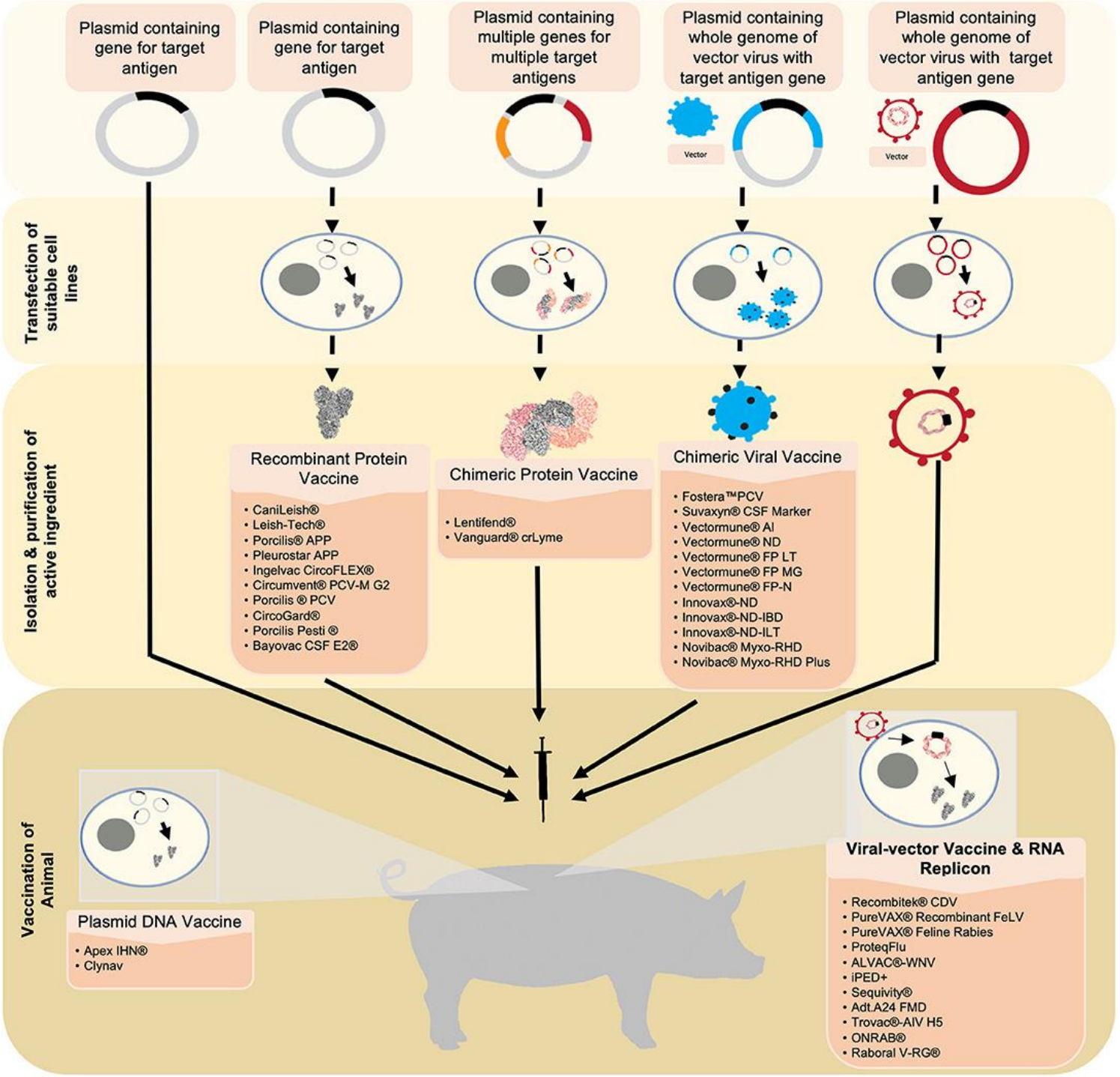
Summary

- Genetic biologics (DNA/RNA) have been licensed in the US for both human and veterinary vaccines, and as a new pesticide.
- Safety concerns include transfection of cells and genomes with non-self, non-species genetic codes, shedding risks, GMO status and transparent labeling.
- Ease of contamination and adulteration, difficulty of timely detection of same without highly specialized equipment and staff.
- Approval of “platform technologies” enables rapid production of biologics that are impossible to test for safety before mass deployment.



DNA plasmids are a common starting raw material for “new generation” of genetic vaccines

DNA plasmids “transfect” cells – transfer genetic code into another cell’s genome

- Viral vectors utilize engineered viruses that express the gene of interest. VV vaccines release the recombinant genes into the host cells.
- RNA replicon vaccines utilize RNA segment that encodes the desired antigens encapsulated in a vesicle carrier

Forcing Animal to Express NON-Self Proteins

- DNA vaccines are pushed as a method to control the uncontrollable – illness/death due to intense commercial farming methods:
  - Overcrowding, unnatural stressful conditions
  - Pollution with biologic and chemical waste

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Genetic DNA/RNA Vaccines for Animals/Fish

- 2005, APEX-IHN (Novartis/Elanco) for Atlantic salmon against Infectious Hematopoietic Necrosis Virus (IHNV), British Colombia.
- West Nile Innovator - DNA (Fort Dodge Animal Health/Pfizer) for West Nile virus in condors and horses.
- Oncept (Merial) against dog melanoma.
- In 2017, CLYNAV (Elanco), a polyprotein-encoding DNA vaccine against Salmon Pancreas Disease Virus (SPDV) infection in Atlantic salmon was authorized by the European Medicines Agency (EMA).
- Sequivity (Merck) in swine (2017) – Emergency use in Canada, fully licensed in US (USDA, 2021). “Platform” for making farm-specific injections based on RNA-particle technology.

Risks to human genome/biome are not properly studied, waived off as “small chance”… claim rapid degradation of DNA plasmids (in mice)…

6. Safety aspects

Some potential risks have been associated with DNA vaccination. With respect to the vaccinated host, these include integration into genome and disruption of biological processes, and potential unwanted immune responses such as auto-immunity or tolerance to the pathogen [175,176]. Limited data is available for fish, but no significant adverse effects on the host have been identified in initial safety testing in humans [177].

The risks to the consumer concerns the potential ingestion of any residual plasmid from food products, containing elements such as human viral promoter regions (such as the CMV promoter) or antibiotic resistance genes that could potentially have harmful consequences if integrating into the consumers' genome or taken up by their gut microflora. However, this risk is considered negligible since the consumer is one step removed from the presentation of vaccine to the vaccinated animal, and at the site of vaccine injection there is a rapid degradation of the plasmid, within 90 min after vaccination in mice [178]. Fast degradation of the plasmid has also been observed in fish [82]. Con-

Collins et al, “DNA Vaccines in Fish and Aquaculture” https://doi.org/10.1016/j.fsi.2018.07.012

DNA Plasmids Found in Fish Muscle 320 Days Post Vaccination!

Table 8. Persistence of plasmids in epaxial muscle of rainbow trout collected at different days post-vaccination (dpv) during the field trial.

Trial	Time Point (dpv)	Plasmid Detection pVax1-vhsG-Positive	pVax1-ihnG-Positive
Potency test	90	5 /5	5/5
Field trial	120	1/5	1/5
	160	3/5	3/5
	180	3/5	2/5
	210	2/5	2/5
	230	3/5	3/5
	260	4/5	0/5
	280	0/5	0/5
	320	6/15	6/15

Efficacy of DNA Vaccines in Protecting Rainbow Trout against VHS and IHN under Intensive Farming Conditions

by Andrea Marsella [1,*], Francesco Pascoli [1], Tobia Pretto [1], Alessandra Buratin [1], Lorena Biasini [1], Miriam Abbadi [1], Luana Cortinovis [1], Paola Berto [1], Amedeo Manfrin [1], Marco Vanelli [2], Simona Perulli [2], Jesper S. Rasmussen [3], Dagoberto Sepúlveda [3], Niccolò Vendramin [3], Niels Lorenzen [3] and Anna Toffan [1]

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Vaccines **2022**, *10*(12), 2062; **https://doi.org/10.3390/vaccines10122062**

Both, vaccine or its recipients could become GMO, if genetic/biome integration is possible…

Vaccine products?

However, under EU legislation, DNA vaccines appear not to be considered as GMOs given the recent example of CLYNAV, a DNA vaccine against SPDV (see below). EU Directive 2001/18/EC defines "organisms" as any biological entity capable of replication or of transferring genetic material. GMOs are defined as organisms, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination. These definitions do not unambiguously exclude a plasmid, given that plasmids can replicate in bacterial cells and can transfer genetic material between bacteria, and that modified viral vectors, which also are incapable of replicating on their own, can be considered as GMOs. Nevertheless, the EU Commission has ratified the Cartagena Protocol (biosafety of GMOs in the environment) where it is stated that plasmids or naked genetic material are not considered as organisms [197] based on the criteria that the plasmid cannot replicate on its own. Given the decision that the DNA vaccine CLYNAV is not a GMO, then, unless a plasmid is deliberately modified to promote integration into a host genome, or to replicate in a eukaryotic host, it is unlikely to be considered a GMO under EU regulations.

Vaccinated animals? Humans?

The next consideration is whether DNA vaccinated animals are considered GMOs. Under Directive 2001/18/EC, Annex 1A, Part 1 lists techniques of genetic modification. Among others, this includes the insertion of nucleic acid material into plasmid vector systems, followed by administration of these into a host organism in which they do not naturally occur and where they are capable of continued replication. Secondly, techniques involving the direct introduction into an organism of replicating heritable material prepared outside the organism by micro- and macro-injection and microencapsulation. Therefore, the wording of EU directive 2001/18/EC does not specifically exclude the classification of DNA vaccinated fish as GMOs. However, in relation to DNA vaccines the plasmid will not replicate in the eukaryotic host, unless specifically modified to do so. Also, integration of the vaccine DNA into host cell (somatic or germinal) genomes is considered an unlikely event, as long as the plasmid is not specifically designed for this ([188]; Danish Medical Agency). Among European countries, only

Collins et al, "DNA Vaccines in Fish and Aquaculture" https://doi.org/10.1016/j.fsi.2018.07.012

Merck Sequivity RNA “platform” for pigs

- USDA approved for swine influenza in December 2021
- Synthetic (not-natural) RNA in nanoparticle
- No information available on the chemical composition of nanoparticle, nor its toxicities by itself:
  - No biodistribution studies available
  - No genotoxicity studies available
  - No carcinogenicity studies available
  - No published safety studies available in peer reviewed literature
- Collect and centralize genomic surveillance data from farms:
  - How is the data used? Who can access it? For what purposes?

MERCK Animal Health

SEQUIVITY®

USDA Label, Safety Summary (p.18)

Adverse Events Summary 21 days

VeDDRA Code	Total Animals	Percent of All Animals
No adverse events	525	70.20%
Anorexia	55	7.40%
Death	24	3.20%
Lameness	20	2.70%
Loss of Condition	12	1.60%
Diarrhea	11	1.50%
Unthrifty	7	0.90%
Anaphylaxis^	3	0.40%
Central Nervous System Disorder*	3	0.40%
Lethargy	3	0.40%
Respiratory Tract Infection*	3	0.40%
Arthritis	2	0.30%
Meningitis	2	0.30%
Musculoskeletal Disorder*	2	0.30%
Trauma*	2	0.30%
Abdominal Caviry Hernia	1	0.10%
Abscess*	1	0.10%
*Not otherwise specified		
^Related to IVP		

USDA Approval Date	December 13, 2021

30%!

https://www.aphis.usda.gov/wcm/connect/

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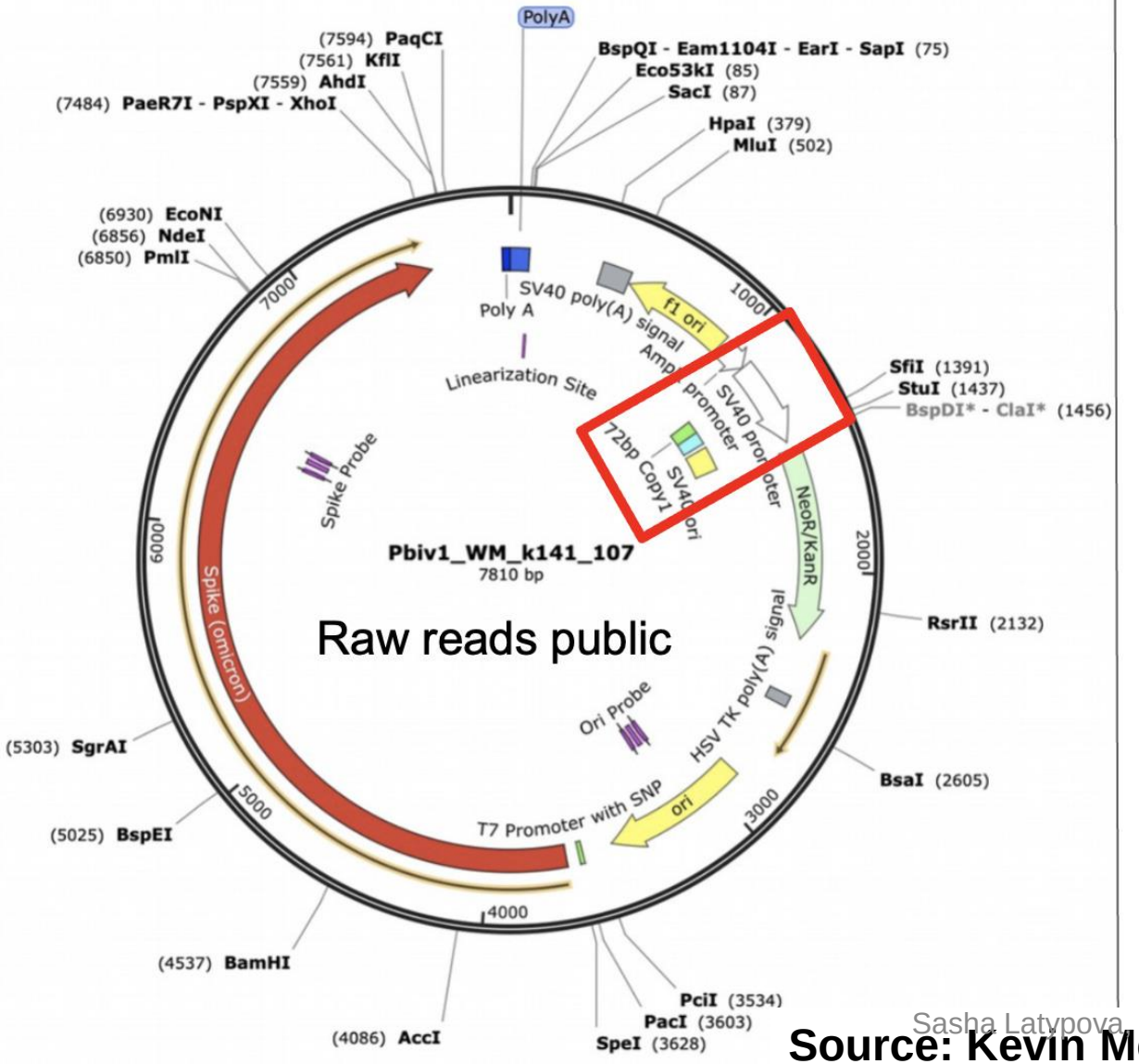
EPA Fast Tracked Ledprona – RNAi Pesticide

- Novel pesticide based on RNA interference (RNAi) technology - mechanism used by plants and insects to regulate gene expression.
- The EPA granted Ledprona an Experimental Use Permit (EUP), allowing GreenLight Biosciences 2 years to gather data from limited test plots.
- **Astonishingly, the agency also gave Ledprona 3 years of commercial use—before the standard testing period is even complete!**
- The pesticide could trigger unintended immune responses in humans. **Environmental risks: harm off-target insect species, disrupting ecosystems in unforeseen ways.**

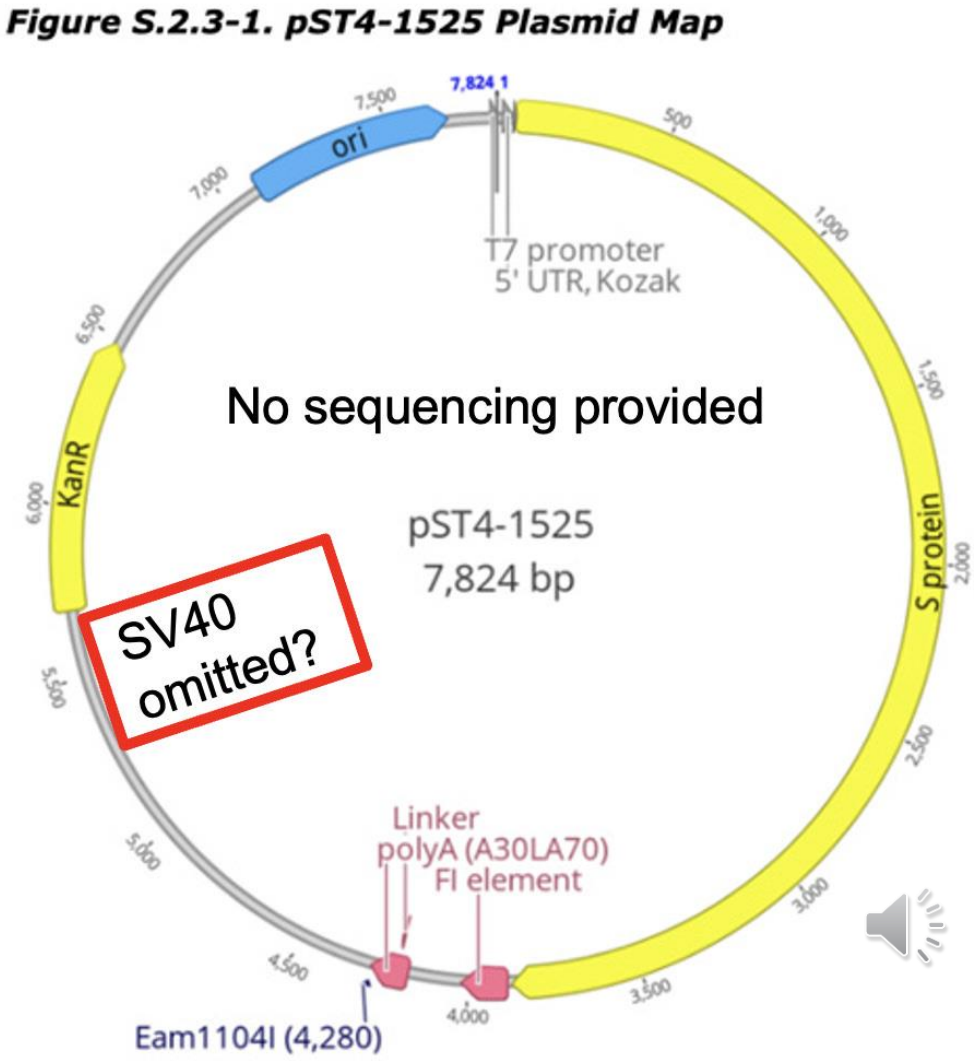
Ease of Adulteration, Contamination and Weaponization

Detection requires high-tech gene sequencing labs, equipment and expertise

Independent Illumina sequencing



What was disclosed to the EMA



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Source: Kevin McKernan, *Preprint DOI [10.31219/osf.io/b9t7m](https://doi.org/10.31219/osf.io/b9t7m)

Persistent Damage to the Gut Microbiome following Messenger RNA SARS-CoV-2 Vaccine

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Abstract
E0141
(S2108)

Introduction

- The human gut microbiome is an essential determinant of human health.
- *Bifidobacterium* decline is associated with inflammatory bowel disease, obesity, neurological disorders, *C. difficile* infection and severe COVID-19 (1-3).
- Long-term effect of messenger RNA vaccines for SARS-CoV-2 on the human gut microbiome is unknown.
- The purpose of this study was to explore longitudinal changes in the Relative Abundance of *Bifidobacterium* after mRNA SARS-CoV-2 vaccination.

Methods

We longitudinally recorded the Relative Abundance of *Bifidobacterium* in four subjects before receiving a mRNA vaccine (Pfizer or Moderna) for SARS-CoV-2, approximately one post-vaccination, as well as 6-9 months post-vaccination. Additional SARS-CoV-2 vaccines were given during that period, totaling 2 to 3 doses. Samples were collected at the time points mentioned. No dietary changes or new medications were introduced throughout the study period. Metagenomic next generation sequencing-based methods were applied to samples obtained from fecal collection. DNA was extracted, and the library prepped, enriched and sequenced on an Illumina Nextseq 550 system. This study was IRB approved.

Results

Subject	Change in Relative Abundance of *Bifidobacterium* (% of pre-vaccine level)	
	1 month post-vaccine	6-9 months post-vaccine
1	38%	15%
2	258%	0%
3	49%	35%
4	90%	60%

Table 1. Change in Relative Abundance of *Bifidobacterium* after SARS-CoV-2 mRNA vaccination.

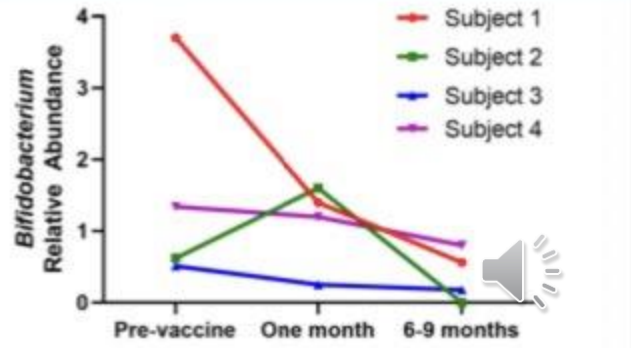

Figure 1. Decline in Relative Abundance of *Bifidobacterium* after SARS-CoV-2 mRNA vaccination.

Discussion

- At 1 month post-vaccination, 3 of 4 subjects experienced a decrease in Relative Abundance of *Bifidobacterium* below pre-vaccination levels.
- At 6-9 months post-vaccination, all subjects experienced a decrease in Relative Abundance of *Bifidobacterium* below pre-vaccination levels.
- No subjects exhibited significant post-vaccine complications.
- The lasting decrease in *Bifidobacterium* levels may contribute to SARS-CoV-2 infection post vaccination.
- Gut dysbiosis after mRNA SARS-CoV-2 vaccination may be a future indication for restoration of *Bifidobacterium* via oral or fecal transplant routes.

References

1. Ruiz L, et al. *Front Microbiol*. 2017;8:2345.
2. Suganya K, Koo BS. *Int J Mol Sci*. 2020;21(20):7551.
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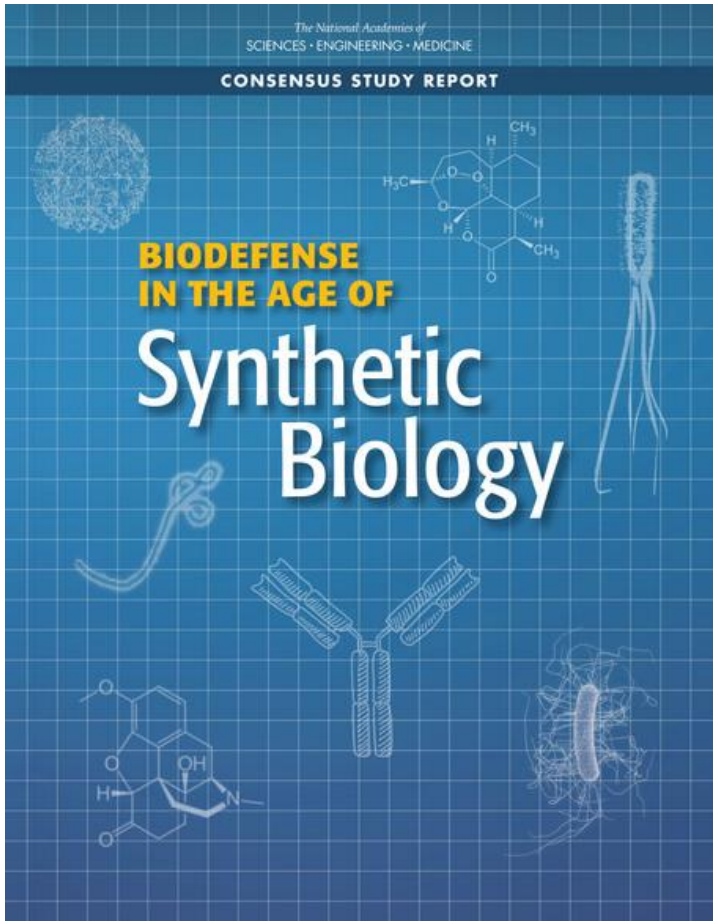

Chapter 6: Assessment of Concerns Related to Bioweapons that Alter the Human Host

"Human health is highly dependent upon the human microbiome—the microorganisms that live on and within us, especially those associated with the gut, oral cavity, nasopharyngeal space, and skin. These populations of microbes are likely far easier to manipulate than the human host itself, making the microbiome a potentially accessible vector for attack".

Vectors of biological attack discussed:

- Delivery of harmful cargo via microbiome (RNA and plasmid DNA or viral vectors) via injections or horizontal transfer (shedding)
- Enhancement of the attack via other pathways – animal vaccines, food: "domestic animals could be used as carriers for engineered agents transmitted via the microbiome".

Contributor(s): National Academies of Sciences, Engineering, and Medicine; Division on Earth and Life Studies; Board on Chemical Sciences and Technology; Board on Life Sciences; Committee on Strategies for Identifying and Addressing Potential Biodefense Vulnerabilities Posed by Synthetic Biology

mRNA-Technology is seen as gold standard for the future

World Health Organization

SEVENTH MEETING OF THE INTERGOVERNMENTAL NEGOTIATING BODY TO DRAFT AND NEGOTIATE A WHO CONVENTION, AGREEMENT OR OTHER INTERNATIONAL INSTRUMENT ON PANDEMIC PREVENTION, PREPAREDNESS AND RESPONSE
Provisional agenda item x

A/INB/7/x
October 2023

DRAFT

Negotiating Text of the WHO convention, agreement or other international instrument on pandemic prevention, preparedness and response (WHO Pandemic Agreement)

Advanced unedited version - 16 October 2023

- $$$$ for WHO Biodefense
- Required collection of DNA samples from countries
- Identification of most toxic agents and sharing with WHO
- Mandatory RNA/DNA injections for “new pathogens” manufactured in 100 days (no safety!)

The mRNA vaccine technology transfer hub

Quelle:
https://www.who.int/initiatives/the-mrna-vaccine-technology-transfer-hub

United States already subject to WHO decision when to announce a PHEIC

15 US states v HHS Petition for Rulemaking – was filed 1/18/2023, dismissed, not being appealed

- "…Oklahoma, Alabama, Arizona, Arkansas, Florida, Georgia, Indiana, Louisiana, Mississippi, Missouri, Montana, Nebraska, South Carolina, Texas, and Utah […] petition the U.S. Department of Health and Human Services (HHS) to amend its definition of "public health emergency" in 42 C.F.R. § 70.1. See 5 U.S.C. § 553(e).
- The Rule exceeds the agency's authority and infringes on U.S. and State sovereignty by unlawfully delegating to the World Health Organization (WHO) the authority to invoke health emergency powers solely based on decisions of the WHO.
- HHS admitted that the declaration by the WHO or notification to t
  Emergency of International Concern is a "way for HHS/CDC to d
  precommunicable stage of a quarantinable communicable dis
  public health emergency if transmitted to other individuals." Id. at
  disclaiming any need to use definitions (3), (4), and (5) [definitions made by WHO] of public health emergency, HHS proceeded to finalize a rule containing those definitions."

Declaration of "pandemic" based on theoretical/modeled potential without need to show any actual mass illness/deaths or economic impact

Questions we should all be asking:

- Is the “emergency” real or only/largely based on PCR and computer models?
- Is there hard evidence or real illness? real economic impact?
- Why the need for total genetic surveillance?
- Why are cell/nucleus/gene transfectants being pushed as the solution for respiratory illness?
- What are the long-term effects of genetic agents on animal microbiome, health and nutritional quality of animal products?
- What are the effects of shedding synthetic DNA/RNA and their byproducts into the food products or environment (other species, or humans that work with transfected animals or transfectants)?

Appendix

Table 1. Overview of licensed fish vaccines that have been used in global aquaculture.

Disease	Pathogen	Major Fish Host	Vaccine Type	Antigens/Targets	Delivery Methods	Country/Region *	Further Information
Viral Diseases							
Infectious hematopoietic necrosis	IHNV *Rhabdovirus*	Salmonids	DNA	G Glycoprotein	IM	Canada	https://www.dfo-mpo.gc.ca/aquaculture/rp-pr/acrdp-pcrda/projects-projets/P-07-04-010-eng.html
Infectious pancreatic necrosis	IPNV *Birnavirus*	Salmonids, sea bass, sea bream, turbot, Pacific cod	Inactivated	Inactivated IPNV	IP	Norway, Chile, UK	www.pharmaq.no
			Subunit	VP2 and VP3 Capsid Proteins	Oral	Canada, USA	www.aquavac-vaccines.com
			Subunit	VP2 Proteins	IP	Canada, Chile, Norway	http://www.msd-animal-health.no/
Infectious salmon anemia	ISAV *Orthomyxovirus*	Atlantic salmon	Inactivated	Inactivated ISAV	IP	Norway, Chile, Ireland, Finland, Canada	www.pharmaq.no
Pancreatic disease virus	SAV *alphaviruses*	Salmonids	Inactivated	Inactivated SAV	IP	Norway, Chile, UK	https://www.merck-animal-health.co
Spring viremia of carp virus	SVCV *Rhabdovirus*	Carp	Subunit	G Glycoprotein	IP	Belgium	[22]
			Inactivated	Inactivated SVCV	IP	Czech Republic	[23]
Koi herpesvirus disease	KHV *Herpesvirus*	Carp	Attenuated	Attenuated KHV	IMM or IP	Israel	[22]
Infectious spleen and kidney necrosis	ISKNV *Iridovirus*	Asian seabass, grouper, Japanese yellowtail	Inactivated	Inactivated ISKNV	IP	Singapore	https://www.aquavac-vaccines.com/
Bacterial diseases							
Enteric redmouth disease (ERM)	*Yersinia ruckeri*	Salmonids	Inactivated	Inactivated *Y. ruckeri*	IMM or oral	USA, Canada, Europe	http://www.msd-animal-health.ie/products_ni_vet/aquavac-erm-oral/overview.aspx; https://www.msd-animal-health-hub.co.uk
Vibriosis	*Vibrio anguillarum; Vibrio ordalii; Vibrio salmonicida*	Salmonids, ayu, grouper, sea bass, sea bream, yellowtail, cod, halibut	Inactivated	Inactivated *Vibriosis* spp.	IP or IMM	USA, Canada, Japan, Europe, Australia	https://www.merck-animal-health.com/species/aquaculture/trout.aspx;
Furunculosis	*Aeromonas salmonicida* subsp. *salmonicida*	Salmonids	Inactivated	Inactivated *A. salmonicida* spp.	IP or IMM	USA, Canada, Chile, Europe, Australia	https://www.msd-animal-health-me.com/species/aqua.aspx
Bacterial kidney disease (BKD)	*Renibacterium salmoninarum*	Salmonids	Avirulent live culture	*Arthrobacter davidanieli*	IP	Canada, Chile, USA	[24]
Enteric septicemia of catfish (ESC)	*Edwarsiella ictaluri*	Catfish	Inactivated	Inactivated *E. ictaluri*	IP	Vietnam	https://www.pharmaq.no/

Table 1. *Cont.*

Disease	Pathogen	Major Fish Host	Vaccine Type	Antigens/Targets	Delivery Methods	Country/Region *	Further Information
Columnaris disease	*Flavobacterium columnaris*	All freshwater finfish species, bream, bass, turbot, salmon	Attenuated	Attenuated *F. columnare*	IMM	USA	[25]
Pasteurellosis	*Pasteurela piscicida*	Sea bass, sea bream, sole	Inactivated	Inactivated *P. pscicida*	IMM	USA, Europe, Taiwan, Japan	ALPHA JECT 2000
Lactococciosis	*Lactococcus garviae*	Rainbow trout, amberjack, yellowtail	Inactivated	Inactivated *L. garviae*	IP	Spain	https://www.hipra.com/
Streptococcus infections	*Streptococcus* spp.	Tilapia, yellow tail, rainbow trout, ayu, sea bass, sea bream	Inactivated	Inactivated *S. agalactiae* (biotype 1)	IP	Taiwan Province of China, Japan, Brazil, Indonesia	https://www.aquavac-vaccines.com/products/aquavac-strep-sa1/
				Inactivated *S. agalactiae* (biotype 2)	IP		https://www.aquavac-vaccines.com/products/aquavac-strep-sa/
				Inactivated *S. iniae*	IP or IMM		https://www.aquavac-vaccines.com/products/aquavac-strep-si/
Salmonid rickettsial septicemia	*Piscirickettsia salmonis*	Salmonids	Inactivated	Inactivated *P. salmonis*	IP	Chile	Evensen, 2016; https://www.pharmaq.no/products/injectable/
Motile *Aeromonas* septicemia (MAS)	*Aeromonas* spp.	Striped catfish	Inactivated	*A. hydrophila* (serotype A and B)	IP	Vietnam	https://www.pharmaq.no/; ALPHAJECT Panga 2
Wound Disease	*Moritella viscosa*	Salmonids	Inactivated	Inactivated *M. viscosa*	IP	Norway, UK, Ireland, Iceland	https://www.pharmaq.no
Tenacibaculosis	*Tenacibaculum maritimum*	Turbot	Inactivated	Inactivated *T. maritimum*	IP	Spain	https://www.hipra.com/

IHNV: Infectious hematopoietic necrosis virus; IPNV: Infectious pancreatic necrosis virus; ISAV: Infectious salmon anemia virus; SVCV: Spring viremia of carp virus; KHV: Koi herpesvirus; ISKNV: Infectious spleen and kidney necrosis virus; IM: Intramuscular injection; IP: Intraperitoneal injection; IMM: Immersion; * denotes country or region where the vaccine is licensed and sold.