Use of attenuated live rabies virus, strain SPBN GASGAS as vaccine to protect wildlife and dogs against rabies – are there any risks left?

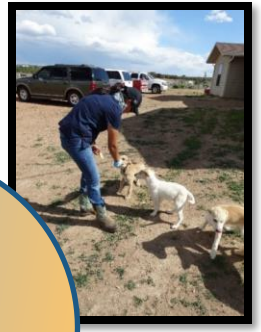
Ad Vos

9th MEACB, 6-7 November 2019

Oral vaccination of wildlife and free-roaming dogs against rabies

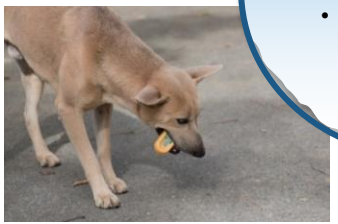
The three pillars:

1. Vaccine
   - Safe for target and non-target species
   - Efficacious

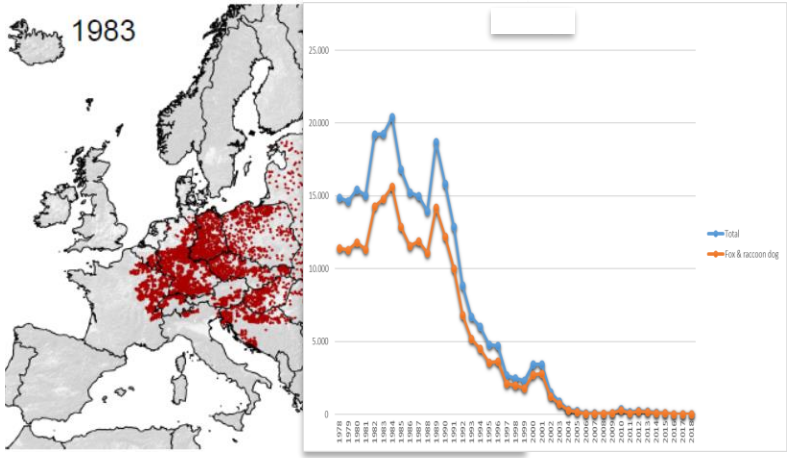


2. Bait
   - highly attractive
   - optimal release of vaccine in oral cavity

3. Distribution system
   - Optimizing bait availability to target species
   - Limit non-target species contact, incl. humans



Oral vaccination of foxes against rabies



A breakthrough in wildlife disease management

Source: Friedrich Loeffler Institute, Insel Riems – Greifswald, Germany

rabies: How big is the problem

EACH YEAR

59,000

PEOPLE DIE FROM RABIES[1]

99%

OF HUMAN CASES ARE CAUSED BY DOGS[1]

122

COUNTRIES WITH ENDEMIC DOG RABIES VIRUS

560

MILLION DOGS

Dog rabies: Parenteral Mass Dog Vaccination

inaccessibility of a large fraction of the dog population (free-roaming dogs)

The tools ‘vaccine‘ and ‘syringe‘ are there **but how to reach the free-roaming dog with parenteral vaccines?**

Oral vaccination of wildlife and free-roaming dogs against rabies

If it works for her, …

why would it not work for him? …

… it does!

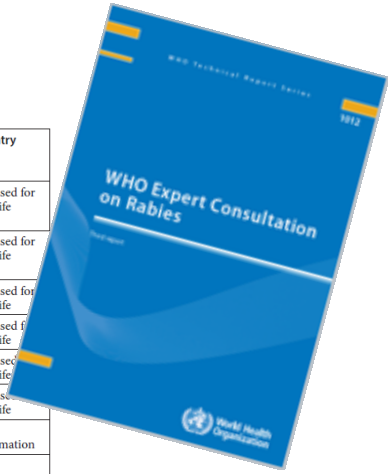
6

Oral vaccination of dogs against rabies

dogs and humans share the same environment, hence safety comes first, since all oral rabies vaccines are based on live replication-competent viruses

Annex 10. Currently available oral rabies vaccine products

Vaccine strain	Product name or brand name	Formulation	Vial size	Company	Country
SPBN-GASGAS	IDT Biologika	RABV	3rd	Reverse genetics with site-directed mutagenesis	Licensed for wildlife
ERA G333	Prokov	RABV	3rd	Reverse genetics with site-directed mutagenesis	Licensed for wildlife
SAG2*	Virbac	RABV	2nd	Monoclonal selection mutant	Licensed for wildlife
SAD B19	IDT Biologika	RABV	1st	Serial (passaged in vivo/in vitro)	Licensed f wildlife
SAD Bern	Bioveta	RABV	1st	Serial (passaged in vivo/ in vitro)	Licensed wildlife
RB-97	FGBI "ARRAIH"	RABV	1st	Serial (passaged in vivo/ in vitro)	Licens wildlife
VRC-RZ2	No information	RABV	1st	Serial (passaged in vivo/ in vitro)	No information
KMIEV-94	No information	RABV	1st	Serial (passaged in vivo/ in vitro)	No information
V-RG*	Merial	Vaccinia virus		Recombinant, expressing rabies glycoprotein	Licensed for wildlife
AdRG1.3	Artemis Technologies	Adenovirus		Recombinant, expressing rabies glycoprotein	Licensed for wildlife



… Countries that are considering use of ORV of dogs should ensure the **safety** of the viral construct on the target and non-target species ….

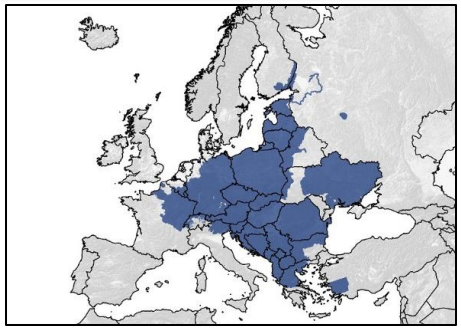
Oral rabies vaccines commercially available (past & present)

1st generation MLV: conventional attenuated vaccine strains through serial in vivo and /or in vitro passaging, e.g. SAD Bern, SAD B19, SAD P5/88

2nd generation MLV: selection of attenuated vaccine virus mutants (monoclonal antibodies);
e.g. SAG1, SAG2, SAD VA1

3rd generation MLV: developed through reverse genetics with site-directed mutagenesis;
e.g. SPBN GASGAS, ERA G333

1st generation GMO: viral vectors expressing the rabies glycoprotein;
e.g. V-RG (vaccinia), HAdV5-RG1.3 (Adenovirus)



Number of vaccine baits distributed in Europe (1978 – 2014)

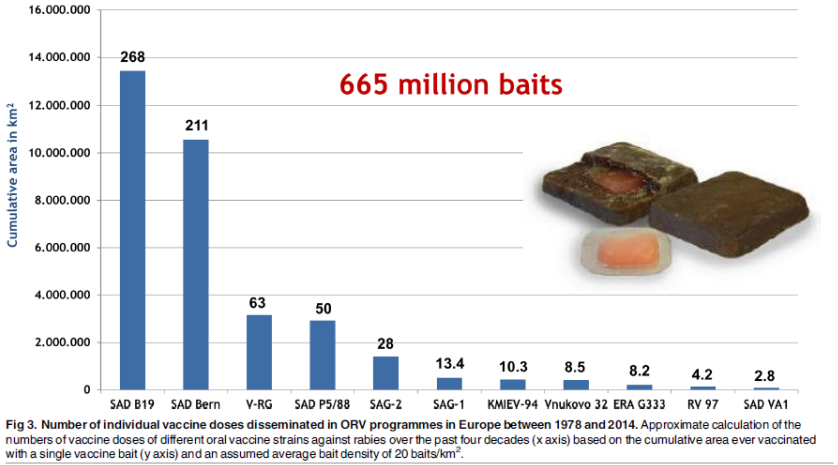


Fig 3. Number of individual vaccine doses disseminated in ORV programmes in Europe between 1978 and 2014. Approximate calculation of the numbers of vaccine doses of different oral vaccine strains against rabies over the past four decades (x axis) based on the cumulative area ever vaccinated with a single vaccine bait (y axis) and an assumed average bait density of 20 baits/km².

Oral vaccination of wildlife and free-roaming dogs against rabies: safety

… vaccine virus associated cases have been reported with 1st generation oral rabies virus vaccines

ERA VACCINE-DERIVED CASES OF RABIES IN WILDLIFE AND DOMESTIC ANIMALS IN ONTARIO, CANADA, 1989–2004

Christine Fehlner-Gardiner,[1] Susan M… Janet Armstrong,[1] Frances Muldoon,[1] Peter Bachmann,[2] and Alexander W…

Journal of Wildlife Diseases, 44(1), 2008, pp. 71–85 © Wildlife Disease Association 2008

Arch Virol (2009) 154:1081–1091
DOI 10.1007/s00705-009-0408-7

ORIGINAL ARTICLE

Analysis of vaccine-virus-associated rabies cases in red foxes (*Vulpes vulpes*) after oral rabies vaccination campaigns in Germany and Austria

Thomas Müller · H.-J. Bärz… · C. M… · C. Bunzenthal… · Geue… · Revill… · T. C…

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

ELSEVIER

Vaccine-induced rabies case in a cow (*Bos taurus*): Molecular characterisation of vaccine strain in brain tissue

DOI: 10.7589/2013-… Vlad Vuta [a,b], Evelyne Picard-Meyer [c], Emmanuelle Robardet [c], Gheorghe Barboi [a], Razvan Motiu [a], Florica Barbuceanu [a,b], Constantin Vlagioiu [b], Florence Cliquet [c,*]

Vaccine-induced Rabies in a Red Fox (*Vulpes vulpes*): Isolatio… Vaccine Virus in Brain Tissue and Salivary Glands

Peter Hostnik,[1] Evelyne Picard-Meyer,[2] Danijela Rihtarič,[1] Ivan Toplak,[1] and Florence Cliquet[2,3]

Unit, Veterinary Faculty, Institute of Microbiology and Parasitology, University of Ljubljana, Gerbičeva 6 …

Vaccine 37 (2019) 4758–4765

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

ELSEVIER

In-depth genome analyses of viruses from vaccine-derived rabies cases and corresponding live-attenuated oral rabies vaccines

Florian Pfaff [a], Thomas Müller [b], Conrad M. Freuling [b], Christine Fehlner-Gardiner [c], Susan Nadin-Davis [c], Emmanuelle Robardet [d], Florence Cliquet [d], Vlad Vuta [e], Peter Hostnik [f], Thomas C. Mettenleiter [b], Martin Beer [a], Dirk Höper [a,*]

Safety studies before licensure may not detect such low frequency events caused by residual pathogenicity

Oral vaccination of wildlife and free-roaming dogs against rabies: safety

1st generation vaccines: genetic stability and purity

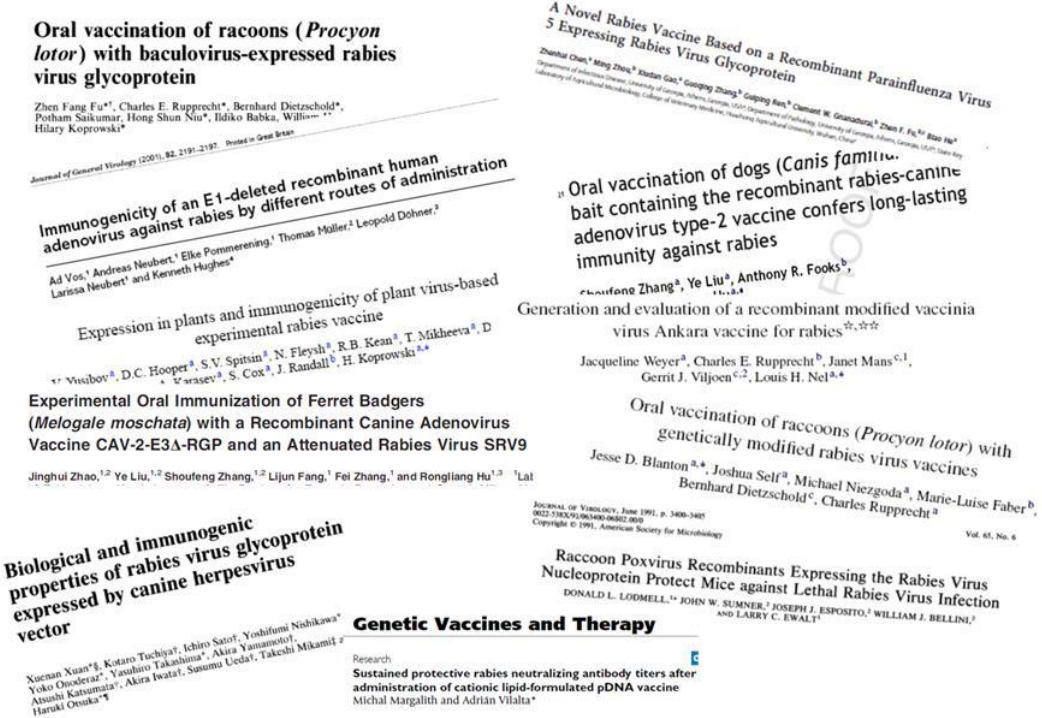
[High definition viral vaccine strain identity and stability testing using full-genome population data--The next generation of vaccine quality control.](#)
Höper D, Freuling CM, Müller T, Hanke D, von Messling V, Duchow K, Beer M, Mettenleiter TC.
Vaccine. 2015 Oct 26;33(43):5829-5837.

[In-Depth Characterization of Live Vaccines Used in Europe for Oral Rabies Vaccination of Wildlife.](#)
Cliquet F, Picard-Meyer E, Mojzis M, Dirbakova Z, Muizniece Z, Jaceviciene I, Mutinelli F, Matulova M, Frolichova J, Rychlik I, Celer V.
PLoS One. 2015 Oct 28;10(10):e0141537.

[In-depth genome analyses of viruses from vaccine-derived rabies cases and corresponding live-attenuated oral rabies vaccines.](#)
Pfaff F, Müller T, Freuling CM, Fehlner-Gardiner C, Nadin-Davis S, Robardet E, Cliquet F, Vuta V, Hostnik P, Mettenleiter TC, Beer M, Höper D.
Vaccine. 2018 Feb 10. pii: S0264-410X(18)30156-7.

Oral vaccination against rabies: Vaccine candidates

After 25 years, it is time for a safer highly attenuated vaccine virus construct, but which one?

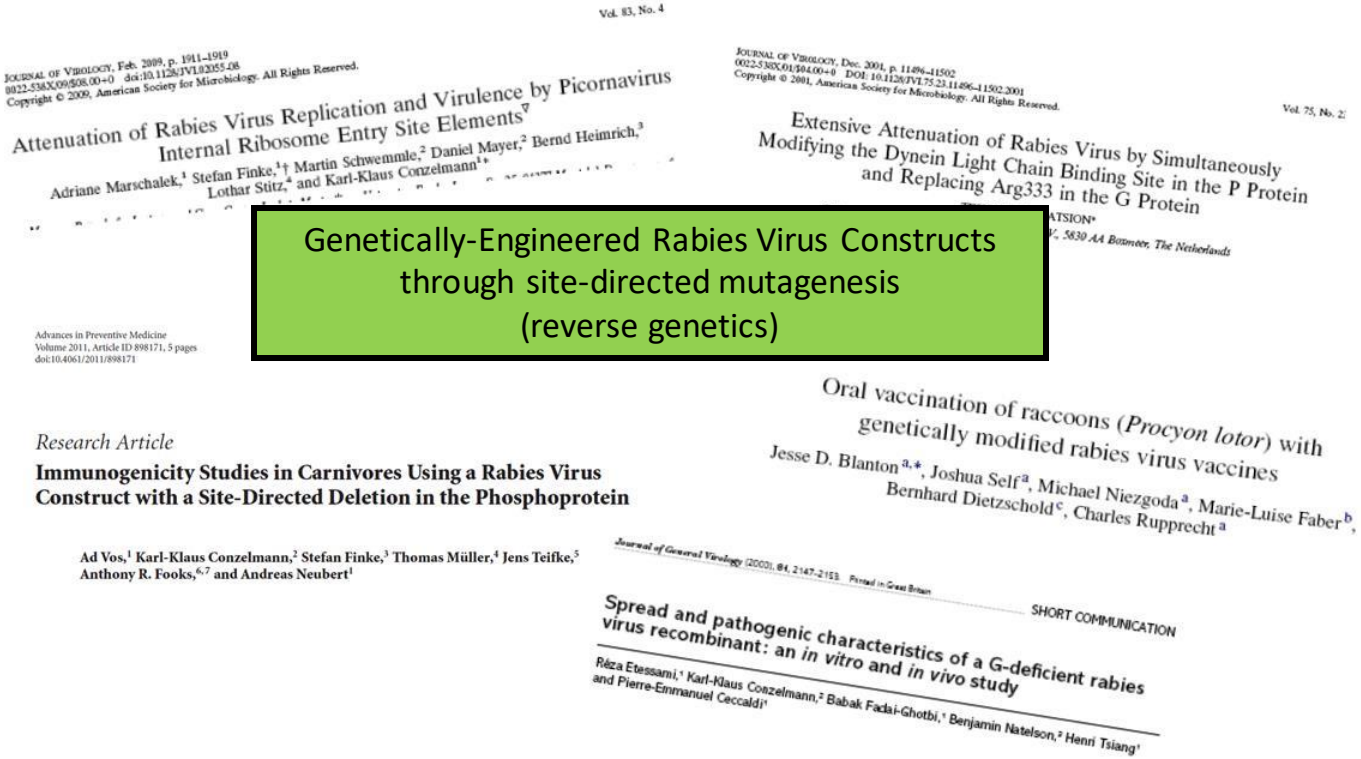


Oral vaccination against rabies: Vaccine candidates

Desired parameters (key attributes) of an ideal oral rabies vaccine:

- Safety: no adverse reactions in target and non-target species, including humans
- Highly efficacious after a single oral dose
- Rapid onset of protection after administration
- Induces a wide range of appropriate immune responses
- Induces long-lasting immunity
- No interference with pre-existing immunity
- Genetic stability
- Thermo-stability
- Bear genetic markers
- No environmental contamination through active shedding
- Low production costs
- ……

Oral vaccination against rabies: Vaccine candidates



Vol. 83, No. 4

JOURNAL OF VIROLOGY, Feb. 2009, p. 1911–1919
0022-538X/09/$08.00+0 doi:10.1128/JVI.02055-08
Copyright © 2009, American Society for Microbiology. All Rights Reserved.

Attenuation of Rabies Virus Replication and Virulence by Picornavirus Internal Ribosome Entry Site Elements▽

Adriane Marschalek,[1] Stefan Finke,[1]† Martin Schwemmle,[2] Daniel Mayer,[2] Bernd Heimrich,[3] Lothar Stitz,[4] and Karl-Klaus Conzelmann[1]*

JOURNAL OF VIROLOGY, Dec. 2001, p. 11496–11502
0022-538X/01/$04.00+0 DOI: 10.1128/JVI.75.23.11496–11502.2001
Copyright © 2001, American Society for Microbiology. All Rights Reserved.

Vol. 75, No. 2

Extensive Attenuation of Rabies Virus by Simultaneously Modifying the Dynein Light Chain Binding Site in the P Protein and Replacing Arg333 in the G Protein

ATSION*

*, 5830 AA Boxmeer, The Netherlands

Genetically-Engineered Rabies Virus Constructs through site-directed mutagenesis (reverse genetics)

Advances in Preventive Medicine
Volume 2011, Article ID 898171, 5 pages
doi:10.4061/2011/898171

Research Article

Immunogenicity Studies in Carnivores Using a Rabies Virus Construct with a Site-Directed Deletion in the Phosphoprotein

Ad Vos,[1] Karl-Klaus Conzelmann,[2] Stefan Finke,[3] Thomas Müller,[4] Jens Teifke,[5] Anthony R. Fooks,[6,7] and Andreas Neubert[1]

Oral vaccination of raccoons (*Procyon lotor*) with genetically modified rabies virus vaccines

Jesse D. Blanton[a,*], Joshua Self[a], Michael Niezgoda[a], Marie-Luise Faber[b], Bernhard Dietzschold[c], Charles Rupprecht[a]

Journal of General Virology (2000), 81, 2147–2153. Printed in Great Britain

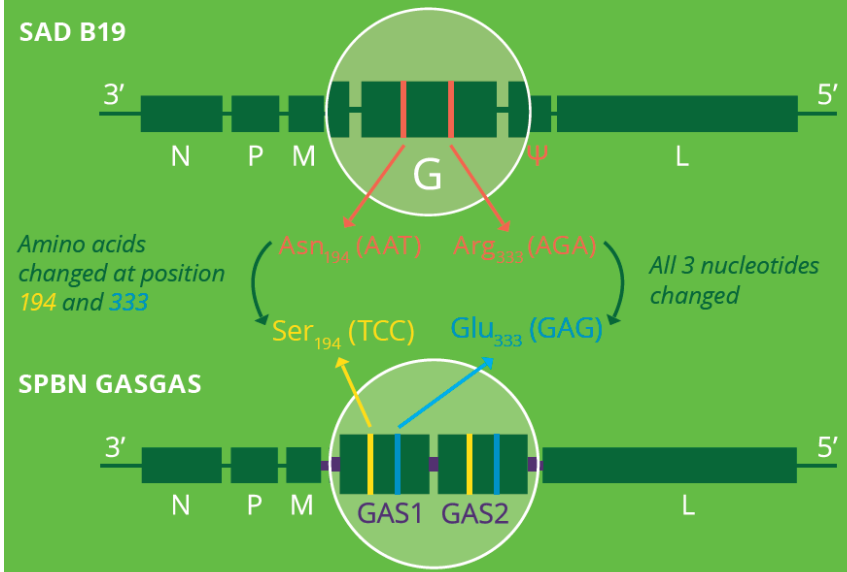
SHORT COMMUNICATION

Spread and pathogenic characteristics of a G-deficient rabies virus recombinant: an *in vitro* and *in vivo* study

Réza Etessami,[1] Karl-Klaus Conzelmann,[2] Babak Fadai-Ghotbi,[1] Benjamin Natelson,[2] Henri Tsiang[1] and Pierre-Emmanuel Ceccaldi[1]

Oral vaccination against rabies: the selected construct

Targeted genetic modifications



Rabitec
OUR WORLD RABIES FREE

Modification	Consequence
Arg > Glu @ #333	*Abolish residual pathogenicity**
Asn > Ser @ #194	*Eliminate potential reversion to virulence*
All 3 nucleotides changed at each amino acid position	*Reduce potential back mutation to the original amino acid*
Insertion of a second G-gene	*Enhanced safety measure*

*Residual pathogenicity is defined as mortality in immuno-competetent hosts after i.c. inoculation.

Oral vaccination against rabies: the selected construct

Residual pathogenicity in adult mice and amino acid at position 333 of the RABV glycoprotein

Fuchsoral™

RABITEC™

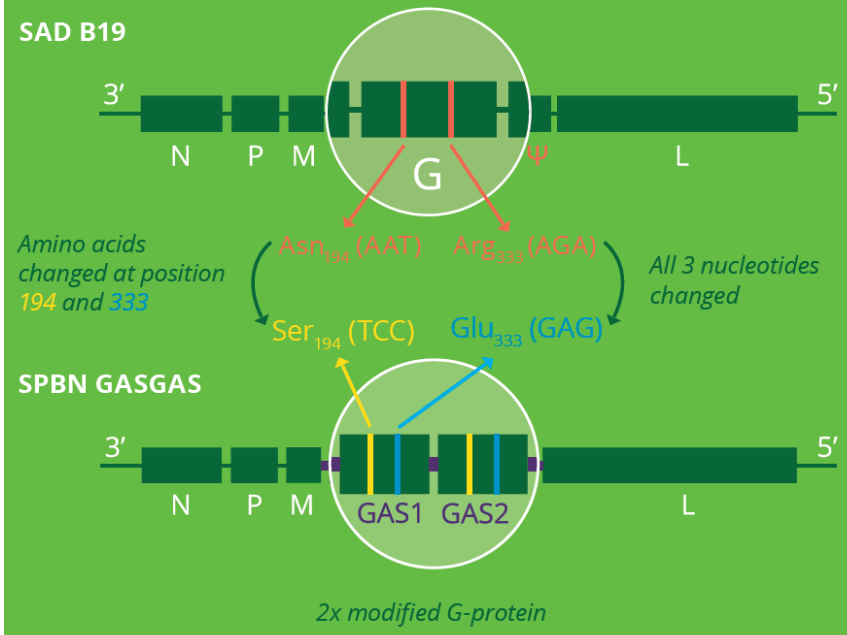
NMRI-mice, 0.03ml, i.c.

Construct	SAD B19	SPBN GASGAS
amino acid (333)	Arginine	Glutamic Acid
nucleotide	AGA	GAG
Dose (FFU/ml)	mortality rate (n/N)	
$10^{7.0}$	5/5	0/5
$10^{5.0}$	5/5	0/5
$10^{3.0}$	5/5	0/5
$10^{1.0}$	4/5	0/5

Rabitec
OUR WORLD RABIES FREE

Oral vaccination against rabies: the selected construct

Targeted genetic modifications



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OUR WORLD RABIES FREE

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Oral rabies vaccines for wildlife and free-roaming dogs: safety

CHAPTER 2.1.17.

RABIES (INFECTION WITH RABIES VIRUS AND OTHER LYSSAVIRUSES)

Updated May 2018

Minimum safety requirements

- **Target species**
  - overdose (incl. shedding)
  - reversion-to-virulence

- **Non-target species**
  - dogs
  - cats
  - rodents
  - immunocompromised hosts (SCID and/or nude mice)
  - humans (risk assessment human safety and likelihood human contacts with vaccine virus)

SPBN GASGAS: safety studies in target and non-target species

Species	Intrinsic safety	Repeated dose	Horizontal trans-mission	Shed-ding	Different routes*	Dissemination
Dog	x	---	x	x	---	x
Fox	x	x	x	x	---	x
Raccoon dog	x	x	x	x	---	x
Small Indian mongoose	x	x	x	x	x	x
Striped skunk	x	---	---	x	---	x
Raccoon	x	---	---	---	---	---
Cat	x	---	x	x	---	---
Domestic pig	x	---	x	x	---	x
Field mouse	x	---	x	---	---	---
House mouse	x	---	x	---	---	---
Guinea pig	x	---	---	---	---	---
Lab mouse	x	---	x	---	---	---
SCID/nude mice	x	---	x	---	x	---

Note: “Different routes” tested in addition to oral administration; e.g. i.c., i.m., s.c..

Addtionally, reversion to virulence (genetic stability) through serial *in vivo* and *in vitro* passaging

SPBN GASGAS: Human safety – risk assessment

direct exposures to vaccine (bait)

Simplified Pathway to S.A.E. (serious adverse event)

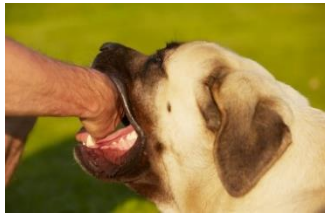
no

yes

S.A.E

yes

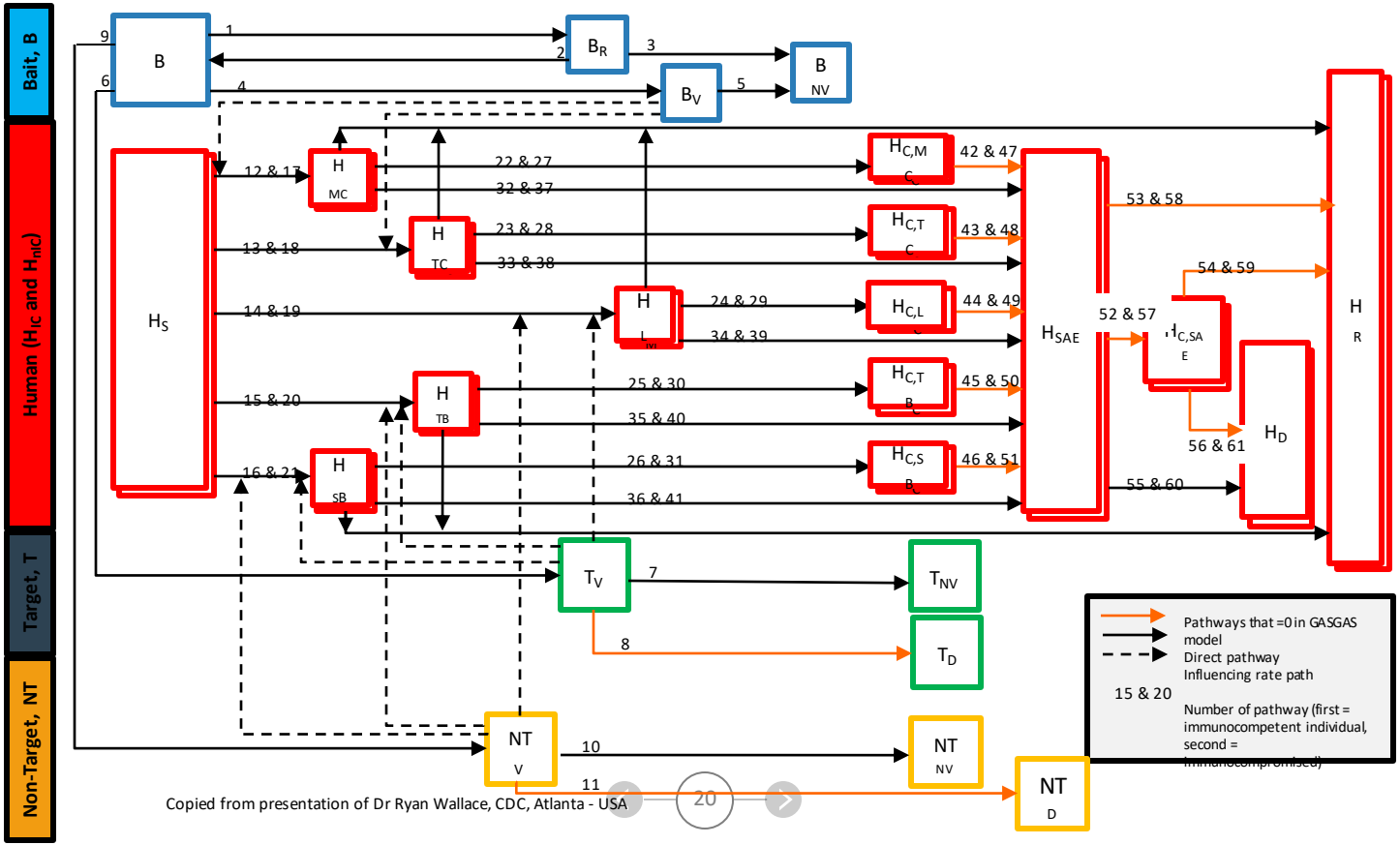
no



indirect exposures to vaccine through recently vaccinated animals

SPBN GASGAS: Human safety – risk assessment

Markov Chain Model: Decision Tree



Copied from presentation of Dr Ryan Wallace, CDC, Atlanta - USA

Markov Chain Model: Decision Tree

Some of the parameters incorporated to determine the path of the vaccine and possible consequences:

- Probability of a vaccine bait left unconsumed in the environment
- Period that vaccine virus remains viable in baits/blisters in the environment
- Period that viable vaccine virus is present in oral cavity after bait consumption
- Probability that a person is bitten or licked by a vaccinated target / non-target species
- Probability that a person is bitten or licked resulting in mucosal contact, transdermal contact, severly bitten, etc.
- Proportion of immune-compromised and immune-competent persons
- Probability of inducing rabies in target and non-target species (vaccine-associated case)
- Duration of active environmental shedding of animal consuming a vaccine bait
- Probability of seeking medical care / advice after potential exposure
- Daily bite rate by target and non-target species
- Bait-uptake by target and non-target species

Outcome depends on vaccine characteristics (safety, stability, etc.), distribution system, target species, location

SPBN GASGAS: Human safety – risk assessment

Results: Markov Chain Model

- **>1000 iterations of 400,000 ORV bait campaign**

vaccine	SAD B19	SAD B19	SPBN GASGAS
target	fox	dog	dog
total exposures per campaign	3.31	21.5	19.37
human death	0.21%	0.60%	0%
mucosal contact	31.65%	4.67%	2.32%
transdermal contact	34.06%	5.04%	2.22%
transdermal bite	33.45%	81.64%	95.14%
severe bite	0%	0%	0%
lick	0.12%	0.28%	0.31%
bite from rabid animal	0.51%	7.77%	0%
human death*	0,18	3,35	0

* - human deaths per 10,000,000 baits distributed

SCIENTIFIC REPORTS

Environmental distribution of certain modified live-virus vaccines with a high safety profile presents a low-risk, high-reward to control zoonotic diseases

Jennifer R. Head[1,2], Ad Vos[3], Jesse Blanton[4], Thomas Müller[5], Richard Chipman[6], Emily G. Pieracci[4], Julie Cleaton[4] & Ryan Wallace[4]

Oral rabies vaccines for wildlife and free-roaming dogs: safety

Conclusion:

CHAPTER 2.1.17.

RABIES (INFECTION WITH RABIES VIRUS AND OTHER LYSSAVIRUSES)

Updated May 2018

From ZKBS-website:

Aktualisierung der Allgemeinen Stellungnahme zur Einstufung rekombinanter Rabiesviren

….. in der Stellungnahme die **Herabstufung des Rabiesvirus-Impfstammes SPBN GASGAS** ergänzt. Das attenuierte, rekombinante Rabiesvirus SPBN GASGAS weist ein gutes Sicherheitsprofil auf und wurde Ende 2017 durch die *European Medicines Agency* (EMA) als Impfstoff für wildlebende Füchse in Europa zugelassen. Unter der Voraussetzung, dass die im Rahmen der Zulassung festgelegten Herstellungsbedingungen eingehalten werden (Anzahl der Passagen, verwendete Zellkulturen für die Virusvermehrung), wird der Rabiesvirus-Impfstamm SPBN GASGAS als gentechnisch veränderter Organismus in die **Risikogruppe 1** herabgestuft.

Rabitec (SPBN GASGAS) meets the latest safety requirements as specified in OIE – Terrestrial Manual, incl. human safety

Oral rabies vaccines for wildlife and free-roaming dogs: unresolved issues

Licensing procedure in developing countries:
- no claim for dogs in central EU Marketing Authorization (EMA)

Genetically Engineered vs. Genetically Modified Organism
- Although it is considered a GMO, it does not contain a foreign gene but many countries do not allow GMOs to be used


Rabies in Endangered Ethiopian Wolves


Safely delivering
a rabies
free world.
Safety engineered, by design.
Rabitec
OUR WORLD RABIES FREE

Thank You


Delivering a
world free of
dog-mediated
rabies.