AHEAD IN
ANIMAL HEALTH

Teaming up for animal health

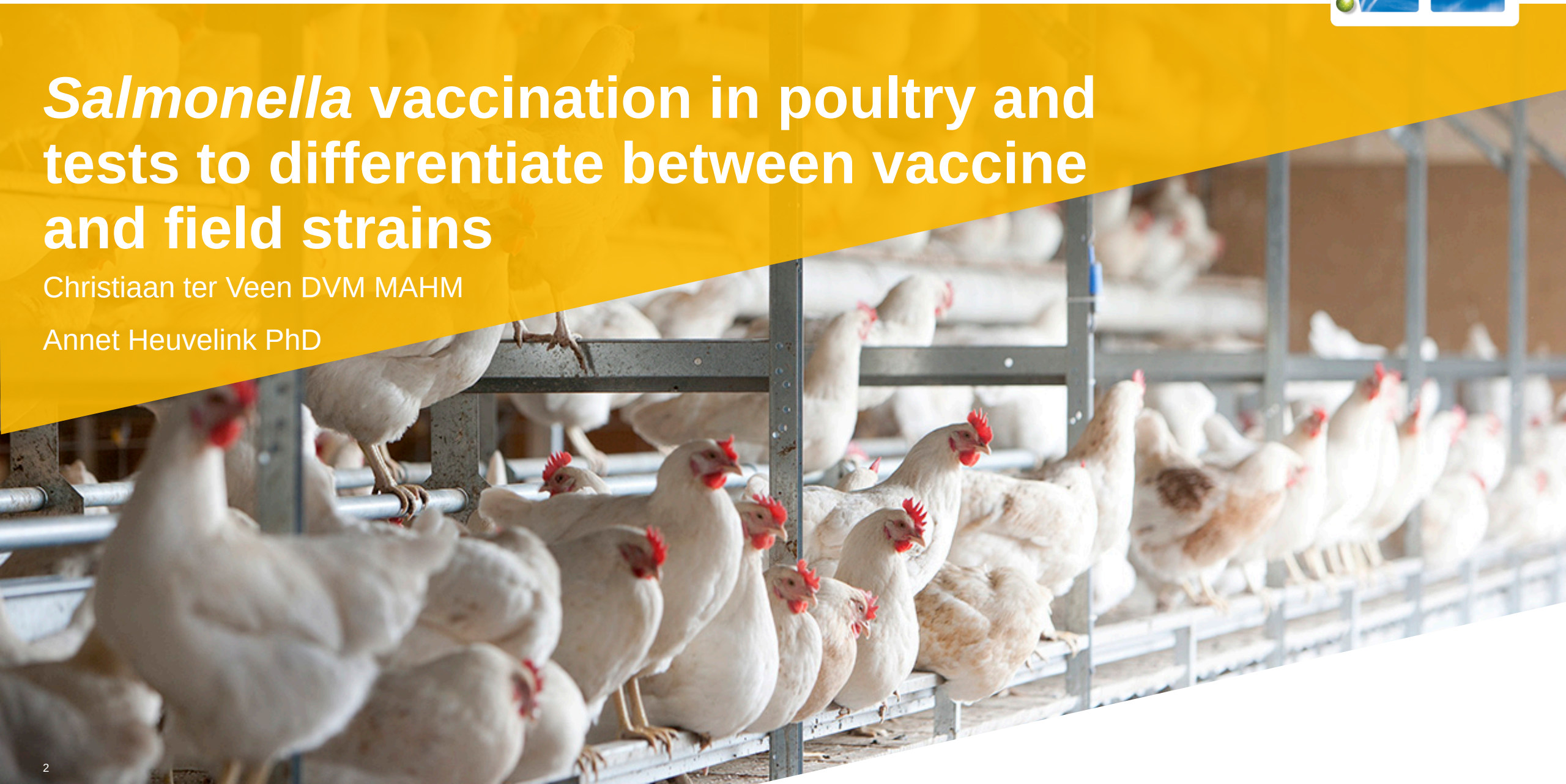
In the interest of animals, their owners and society at large

AHEAD IN
ANIMAL HEALTH

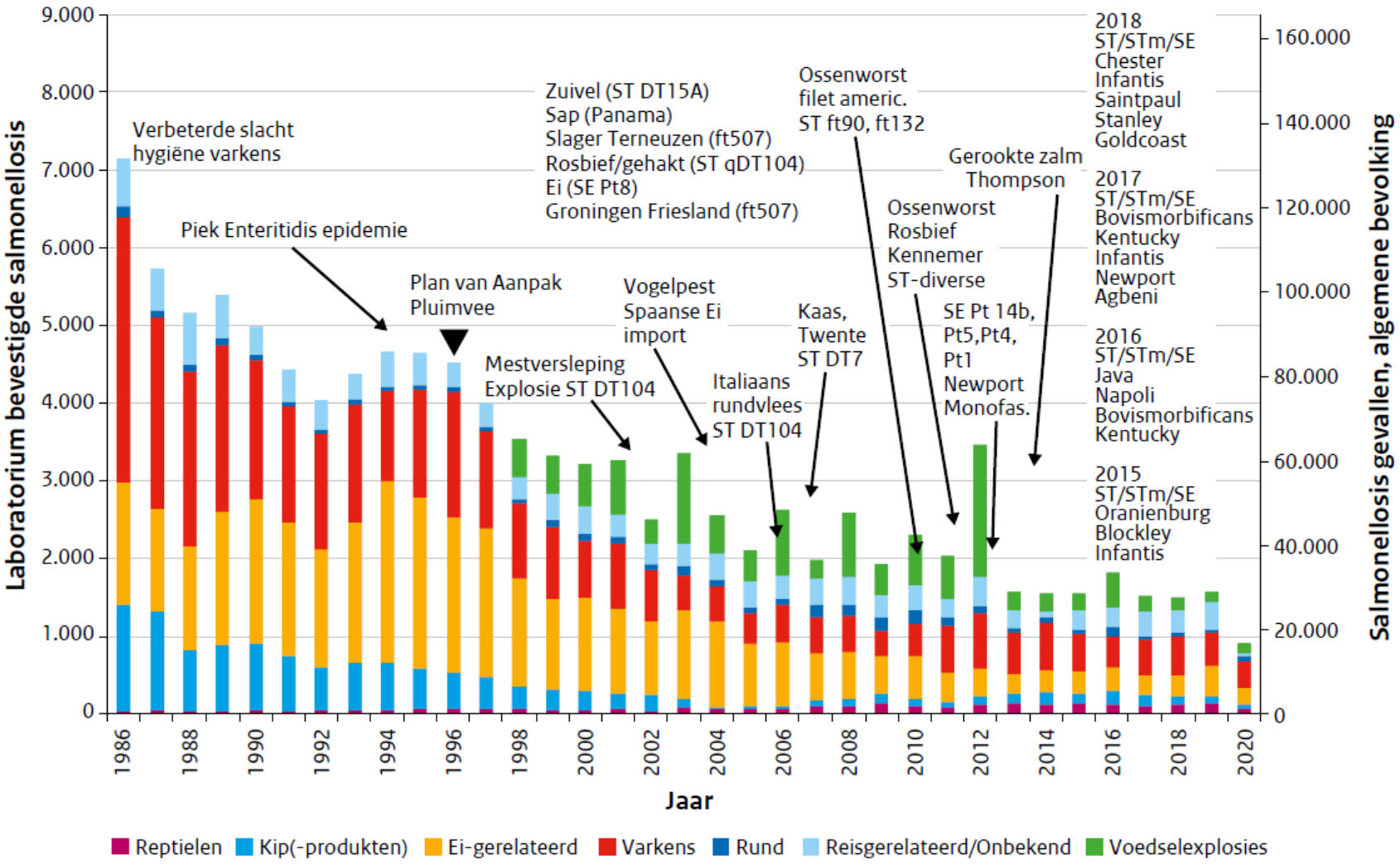
Salmonella vaccination in poultry and tests to differentiate between vaccine and field strains

Christiaan ter Veen DVM MAHM

Annet Heuvelink PhD



Salmonella & poultry



Staat van zoönosen 2020

Salmonella control Dutch poultry

Mainly poultry health problem

S.E. control in breeders

Identification risk factors horizontal transmission poultry

< 1987 | 1987 | 1989 | 1992 | 1995 | 1996

Increase S.E. poultry

Increase S.E. humans

Importance horizontal transmission in spread of S.E. between poultry flocks

Hygiene based control in layers

Salmonella control Dutch poultry

Vaccination against S.G.
Cross protection S.E.

Live S.E. vaccine
No growth monitoring

Start EU control programme

1999 | 2000 | 2001 | 2003 | 2010 | 2020

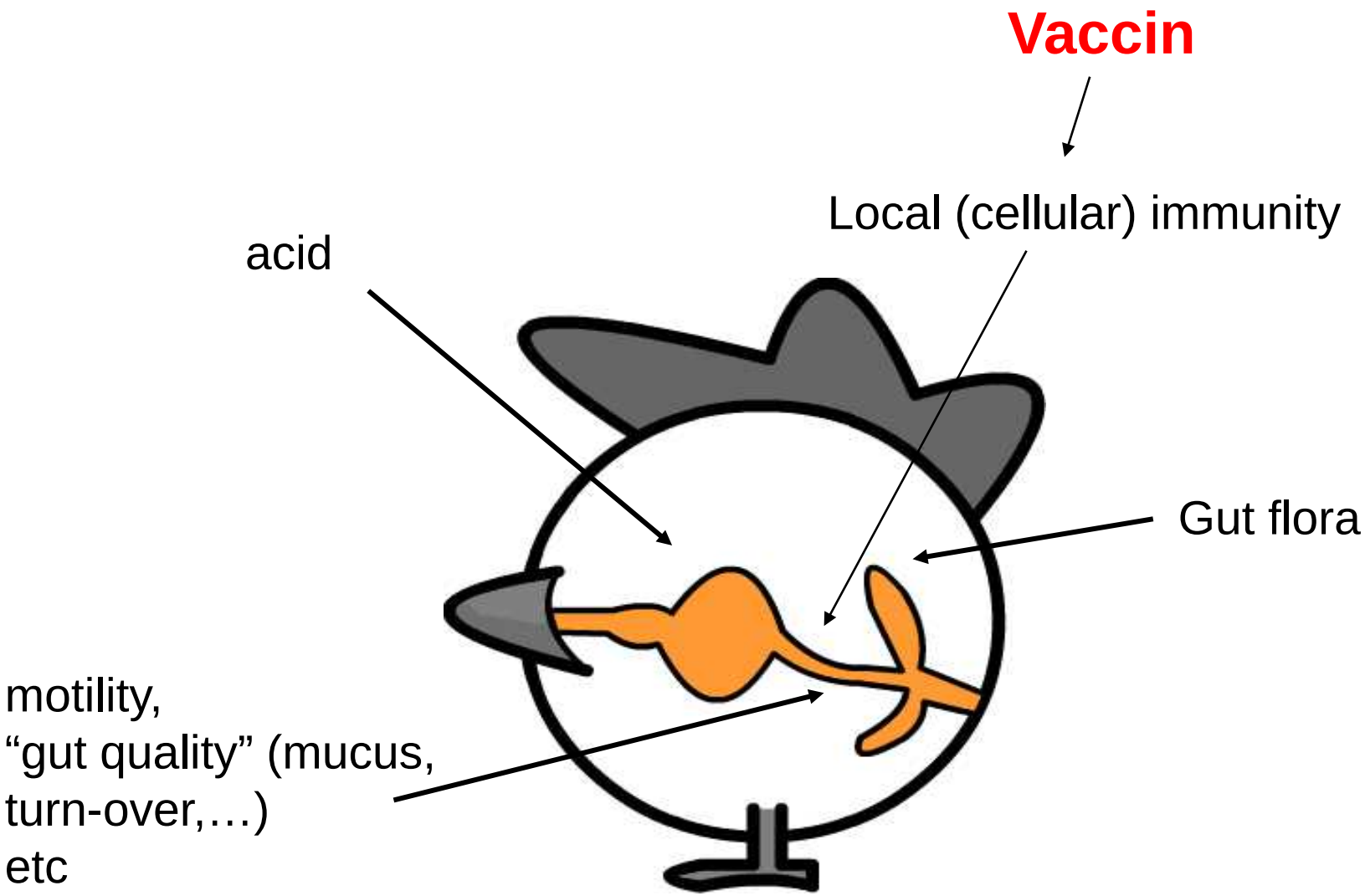
Killed S.E. / S.T. vaccine

Second live S.E. vaccine,
Growth monitoring → DIVA

EU regulation 2160/2003
control of Salmonellosis

Re-sampling of positive flocks only in exceptional cases

Role of vaccination



Killed & Live vaccines

	Live vaccines	Killed vaccines
Application	Drinking water	Individual injection
Colonization	Yes	No
Shedding	Yes	No
Spread between chickens	Yes	No
Immunity	Primarily local	Primarily systemic
Detection during monitoring	Depends on strain	No
Detection during serology	Hardly	Yes

Efficacy of vaccination

TABLE 2: *Results of the challenge studies with strain Salmonella enteritidis 147 N, measured seven days post challenge*

Group	n	Time of Challenge (Week of life)	Challenge strain content (mean ± SD) in log cfu/g	
			Liver	Caeca
1	8	24	1.83 ± 0.33[1]	5.47 ± 0.32[1]
2	8	24	1.36 ± 0.74[1]	5.14 ± 0.72[1]
3	8	24	2.54 ± 0.41	6.28 ± 0.36
1	8	51	1.55 ± 0.37[1]	5.29 ± 0.66[1]
2	8	51	1.40 ± 0.72[1]	4.72 ± 1.15[1]
3	8	51	2.72 ± 0.46	7.33 ± 1.34
1	8	71	1.29 ± 0.64[1]	4.88 ± 1.19[1]
2	8	71	2.04 ± 0.39[1]	5.19 ± 0.64[1]
3	8	71	2.51 ± 0.35	6.62 ± 1.30

Significant difference between the vaccinated group and the control group (Mann Whitney U test, one-tailed test). [1] significance level $p < 0.05$.

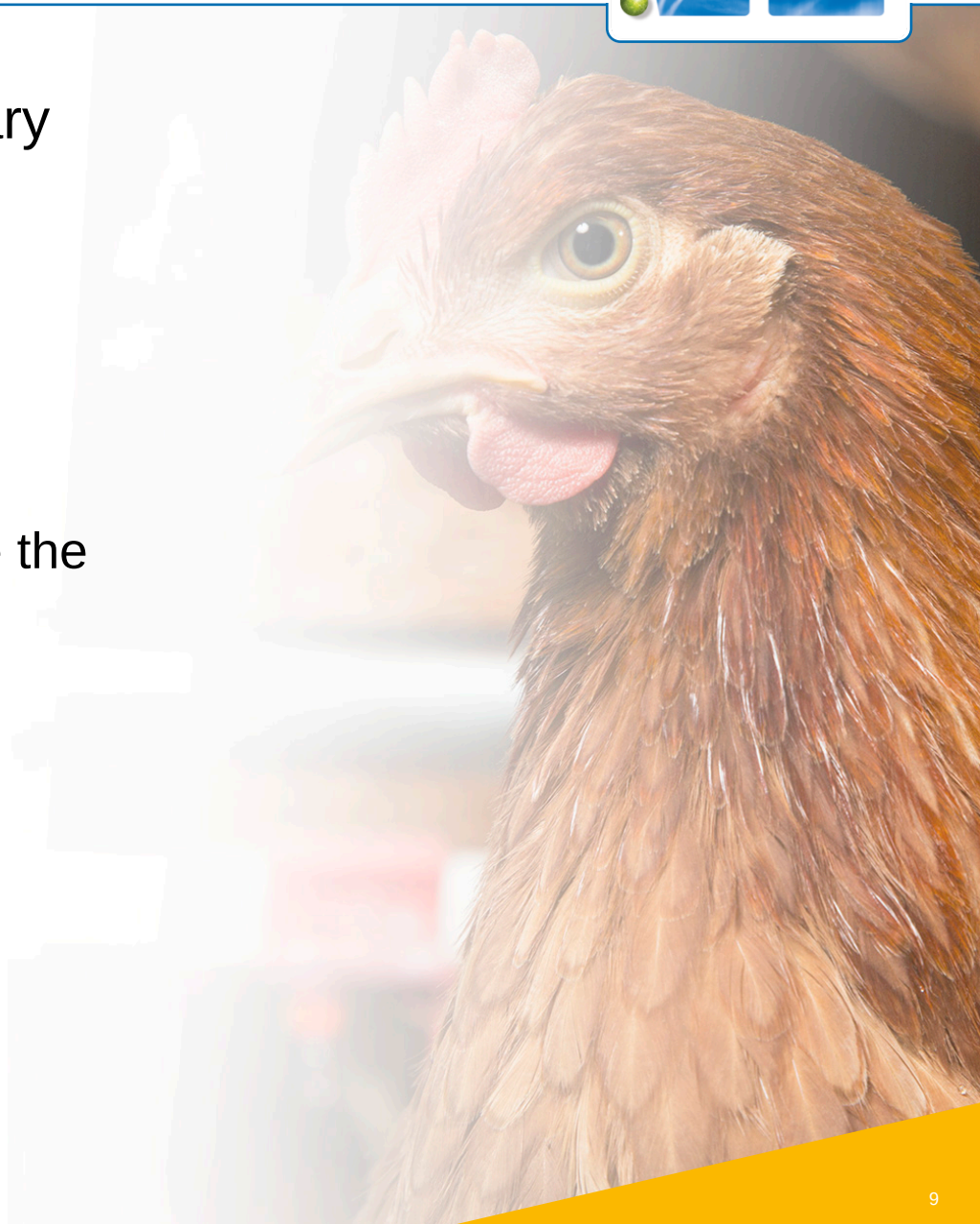
Groups: 1. Live vaccine, 2. Live + killed vaccine, 3. None vaccinated controls

Springer *et al.* 2011, Berl Münch Tierärztl Wochenschr 124, 89-93

EU law

- Registered under Regulation (EU) 2019/6 on veterinary medicinal products
  - Details on live *Salmonella* Enteritidis and *Salmonella* Typhimurium vaccines in European Pharmacopoeia

- Monitoring exemption for reporting of vaccine strains:
  - “A [laying] flock shall be considered positive (…) where the presence of relevant *Salmonella* serotypes (other than vaccine strains) has been detected (…) in the flock.”



EU Regulation 200/2010, 217/2011, 200/2012, 1190/2012

Registration of vaccines

1. Characteristics
    1. “The strain has a stable marker or markers to distinguish it from wild-type strains”

2. Safety
    1. General safety (for the chicken)
    2. Excretion, duration of excretion and survival in the environment
    3. Spread of the vaccine strain
    4. Dissimination and survival of the vaccine strain in vaccinated chickens after each vaccination
    5. Increase in virulence

3. Immunogenicity
    1. Immunogenicity
    2. Immunogenicity at the end of the laying period

European Pharmacopoeia 8.0

Results in Summary of product characteristics

4.4 Special warnings for each target species

Bell drinkers are preferred during first days of life, the use of nipple drinkers for one day old chickens can only be recommended if used according to national regulations.

The differentiation between vaccine and field strains is done by means of an antibiogram. In contrast to field strains, vaccine strains are sensitive to erythromycin (recommended concentration 15-30 µg/ml) and resistant to streptomycin and rifampicin (recommended concentration 200 µg/ml).

Results in Summary of product characteristics

4.5 Special precautions for use

Special precautions for use in animals

Not tested in ornamental and pure-bred poultry.

The vaccine strain may spread to susceptible birds in contact with vaccinates. Vaccinated birds shed the vaccine strain until 14 days after the vaccination.

Appropriate veterinary and husbandry measures should be taken to avoid spread of the vaccine strain to susceptible species.

4.11 Withdrawal period(s)

Meat and offal: 21 days after 1st, 2nd and 3rd vaccination.

Meat and offal: 14 days after 4th vaccination.

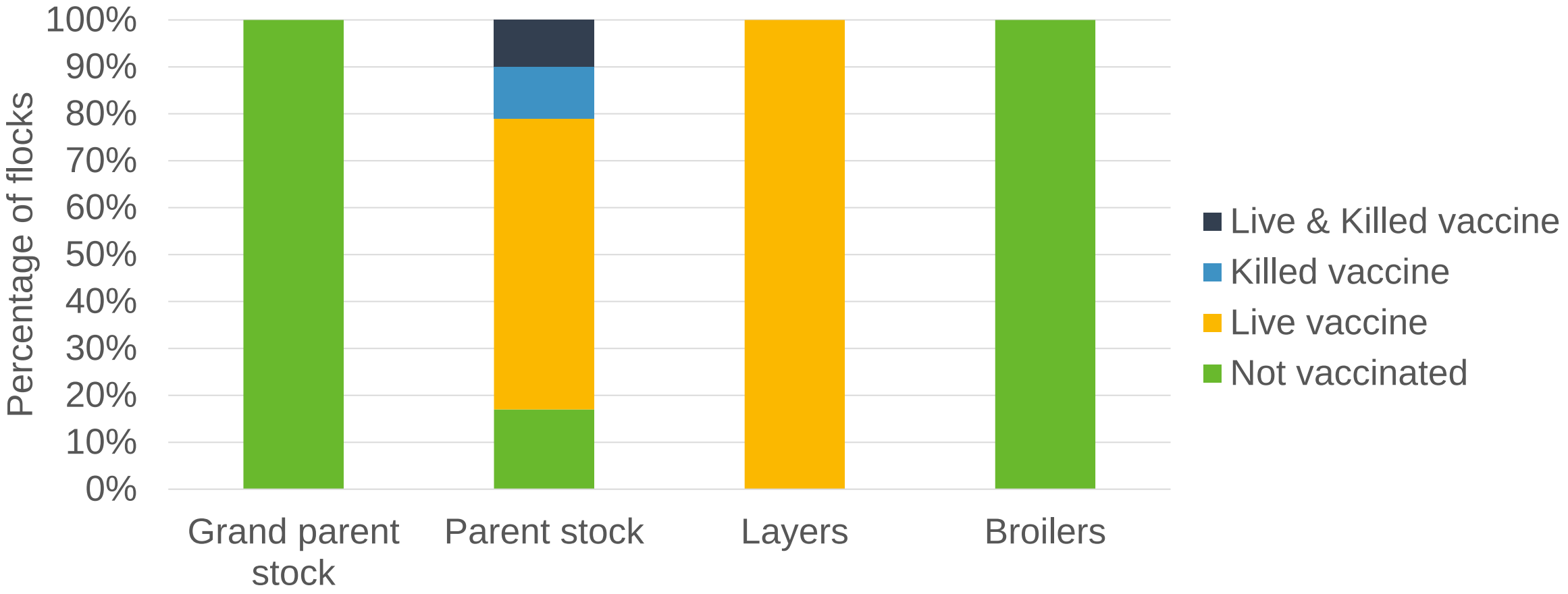
Eggs: zero days after 4th vaccination.

SPC Calier Primun Salmonella

Vaccination schemes

- Vaccination during rearing (all live vaccines)
  - Week 1
  - Week 6-8
  - Week 13-20
- Vaccination during lay (rare)
  - Week 55

Use of vaccines in the Netherlands



Pers. Communication / Ter Veen *et al.* 2022, Avian Pathol 51(1) 19-25

ISO 6579-1:2017 – Primary production stage

Isolation procedure:

1. Pre-enrichment in **BPW**
2. Selective enrichment in
   - **MSRV** agar
     - Optional: MKTTn broth
3. Plating out on
   - **XLD** agar
   - 2nd selective agar
4. Confirmation and serotyping

ISO 6579-1:2017 – Primary production stage

Isolation procedure:

1. Pre-enrichment in **BPW**
2. Selective enrichment in
   - **MSRV** agar
     - Optional: MKTTn broth
3. Plating out on
   - **XLD** agar
   - 2nd selective agar
4. Confirmation and serotyping

Growth of vaccine strains:

- Yes
- Only motile *Salmonella* strains
- Strain dependent

ISO 6579-1:2017 – Primary production stage

Culture result:

Salmonella Enteritidis (SE) / *Salmonella* Typhimurium (ST)

Live vaccine used?

➡ **Differentiating Infected from Vaccinated Animals (DIVA) test**

DIVA tests

- Antimicrobiological susceptibility testing (AST)

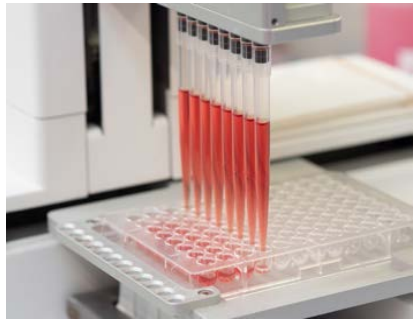
- Molecular biological tests:
  - Real-time PCR
  - Restriction Fragment Length Polymorphism (RFLP)
  - Pulsed-Field Gel Electrophoresis (PFGE)

DIVA tests - AST

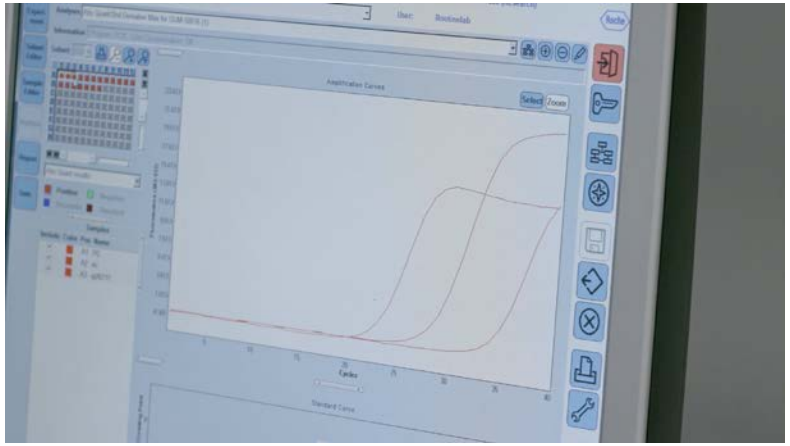
AST procedure:

1. Inoculum preparation (0.5 McFarland)
2. Broth inoculation (Mueller-Hinton broth)
3. Broth microdilution
4. Sealing and incubation (o/n)
5. Results (growth yes/no)

(MIC=Minimum Inhibitory Concentration)



DIVA tests – real-time PCR



Real-time PCR procedure:

1. DNA extraction
2. Extracted DNA + PCR reaction mixture
3. Start PCR run

	Temp (°C)	Time	Step
Polymerase activation	95	10 min	
PCR (35 cycli)	95	15 sec	Denaturation
	60	1 min	Annealing / elongation

4. Results (Ct-value)

DIVA tests - examples

ELANCO

- **AviPro SALMONELLA VAC E**
  - SE strain Sm24/Rifl2/Ssq
  - Susceptible for erythromycin (15 - 30 µg/ml)
  - Resistant to streptomycin (200 µg/ml) and rifampicin (200 µg/ml)

- **AviPro SALMONELLA VAC T**
  - ST strain Nal 2/Rif 9/Rtt
  - Susceptible for erythromycin (15 - 30 µg/ml)
  - Resistant to nalidixic acid (20 µg/ml) and rifampicin (200 µg/ml)

- **AviPro SALMONELLA VAC DUO**
  - SE strain Sm24/Rifl2/Ssq & ST strain Nal 2/Rif 9/Rtt

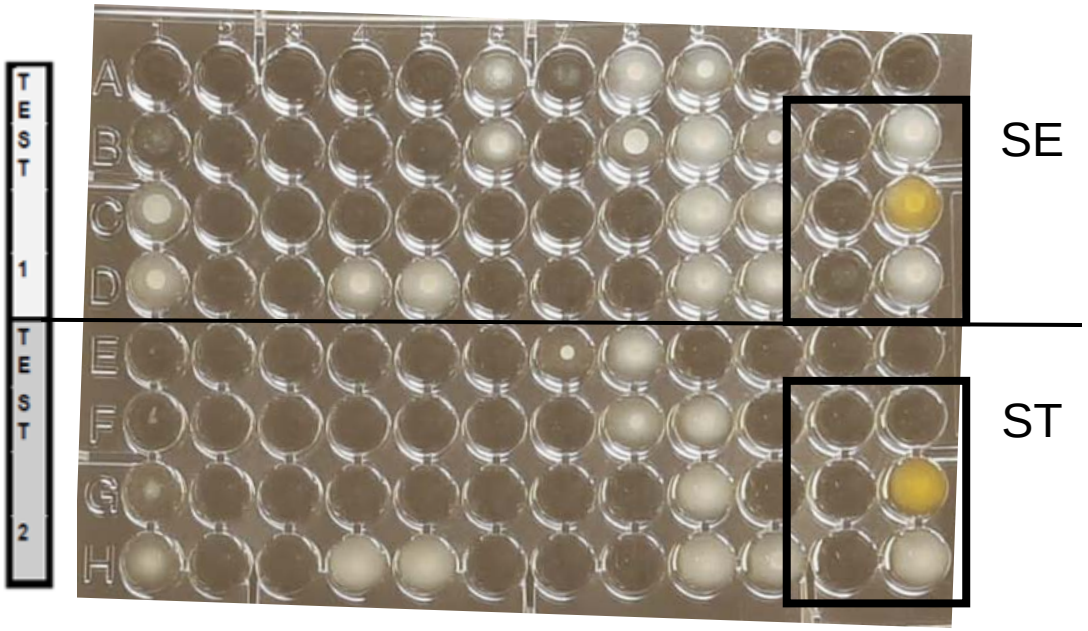
DIVA tests - examples

AviPro® PLATE

DIFFERENTIATION

Elanco

TEST		1	2	3	4	5	6	7	8	9	10	11	12
1	A	PEN 2	AMX 16	ENR 2	CET 2	TET 8	TILM 16	LIS 8/32	OXA 2	ERY 4	DOX 2	DOX 4	DOX 8
	B	PEN 0.5	AMX 8	ENR 1	CPP 4	TET 4	TILM 8	T/S 2/38	OXA 0,25	ERY 1	TIA 16	ERD 30	STRE 200
	C	PEN 0.25	AMX 4	ENR 0.5	CTX 1	TET 2	NEO 16	T/S 1/19	COL 4	ERY 0.5	TIA 8	ERD 20	RAM 50
	D	PEN 0.125	AMX 2	ENR 0.25	TLS 0.5	TLS 1.0	NEO 8	T/S 0.5/9.5	COL 2	ERY 0.25	LIN 4	ERD 10	Growth Control
2	E	PEN 2	AMX 16	ENR 2	CET 2	TET 8	TILM 16	LIS 8/32	OXA 2	ERY 4	DOX 2	DOX 4	DOX 8
	F	PEN 0.5	AMX 8	ENR 1	CPP 4	TET 4	TILM 8	T/S 2/38	OXA 0,25	ERY 1	TIA 16	ERD 30	STRE 200
	G	PEN 0.25	AMX 4	ENR 0.5	CTX 1	TET 2	NEO 16	T/S 1/19	COL 4	ERY 0.5	TIA 8	ERD 20	RAM 50
	H	PEN 0.125	AMX 2	ENR 0.25	TLS 0.5	TLS 1.0	NEO 8	T/S 0.5/9.5	COL 2	ERY 0.25	LIN 4	ERD 10	Growth Control



Growth of AviPro vaccine strains:

Antibiotic	SE	ST
ERD 20 µg/ml	No	No
RAM 50 µg/ml	Yes	Yes
STRE 200 µg/ml	Yes	No

AviPro® plate instruction manual

DIVA tests - examples

CEVAC

- **CEVAC Salmovac 440**
  - SE strain 441/014
    - Kylt ® SE DIVA 1
    - Kylt ® SE DIVA 2
    - Kylt ® ST DIVA 1

- SE field strain:
  - positive FAM-curve (signal serovar Enteritidis) (Ct < 30)
  - positive TXR-curve (signal field strain) (Ct < 30)
- SE vaccine strain 441/014:
  - positive FAM-curve (signal serovar Enteritidis) (Ct < 30)
  - positive Cy5-curve (signal vaccine strain) (Ct < 30)

Vaccines used

Vaccine	Type	Serotype	Detected	DIVA
Calier primun salmonella	Live	SE	No experience	AST
CEVAC Salmovac 440	Live	SE	Common	AST, DIVA PCR available
ELANCO Salmonella Vac E	Live	SE	Very rare	AST, DIVA PCR available
ELANCO Salmonella Vac T	Live	ST	Very rare	AST
ELANCO Salmonella Vac Duo	Live	SE ST	Very rare	AST, SE DIVA PCR available
Merial Gallimune SE + ST	Killed	SE ST	No	Not applicable
Merial Gallivac SE	Live	SE	Common	AST
Nobilis Salenvac	Killed	SE	No	Not applicable
Nobilis Salenvac T	Killed	SE ST	No	Not applicable
Nobilis Salenvac ETC	Killed	SE ST SI	No	Not applicable
Nobilis SE Live	Live	SE	Unlikely	AST
Zoosaloral H	Live	ST	No experience	AST, DIVA PCR available
…				

Copyright and intellectual property rights

All rights reserved

All material provided and/or used by Royal GD/GD Academy is protected by copyright. All intellectual property with regard to the material belongs to Royal GD/GD Academy and is rightfully owned by Royal GD/GD Academy. These materials, such as, but not limited to, text, trademarks, illustrations, photos, graphics, files, designs, patents etc., may not be printed, copied, reproduced, republished, downloaded, posted, displayed, modified, reused, or transmitted in any way, except for the user's own personal non-commercial use. Permission for any other type of use must be obtained upfront in writing from Royal GD / GD Academy via E-mail: gdacademy@gdanimalhealth.com.

AHEAD IN
ANIMAL HEALTH

Thank you for your attention!

Salmonella vaccination in Poultry

Christiaan ter Veen DVM MAHM

Annet Heuvelink PhD