



FRIEDRICH-LOEFFLER-INSTITUT

seit 1910

FLI

Bundesforschungsinstitut für Tiergesundheit
Federal Research Institute for Animal Health

Die Maul- und Klauenseuche

Eine ständige Bedrohung auch für Deutschland

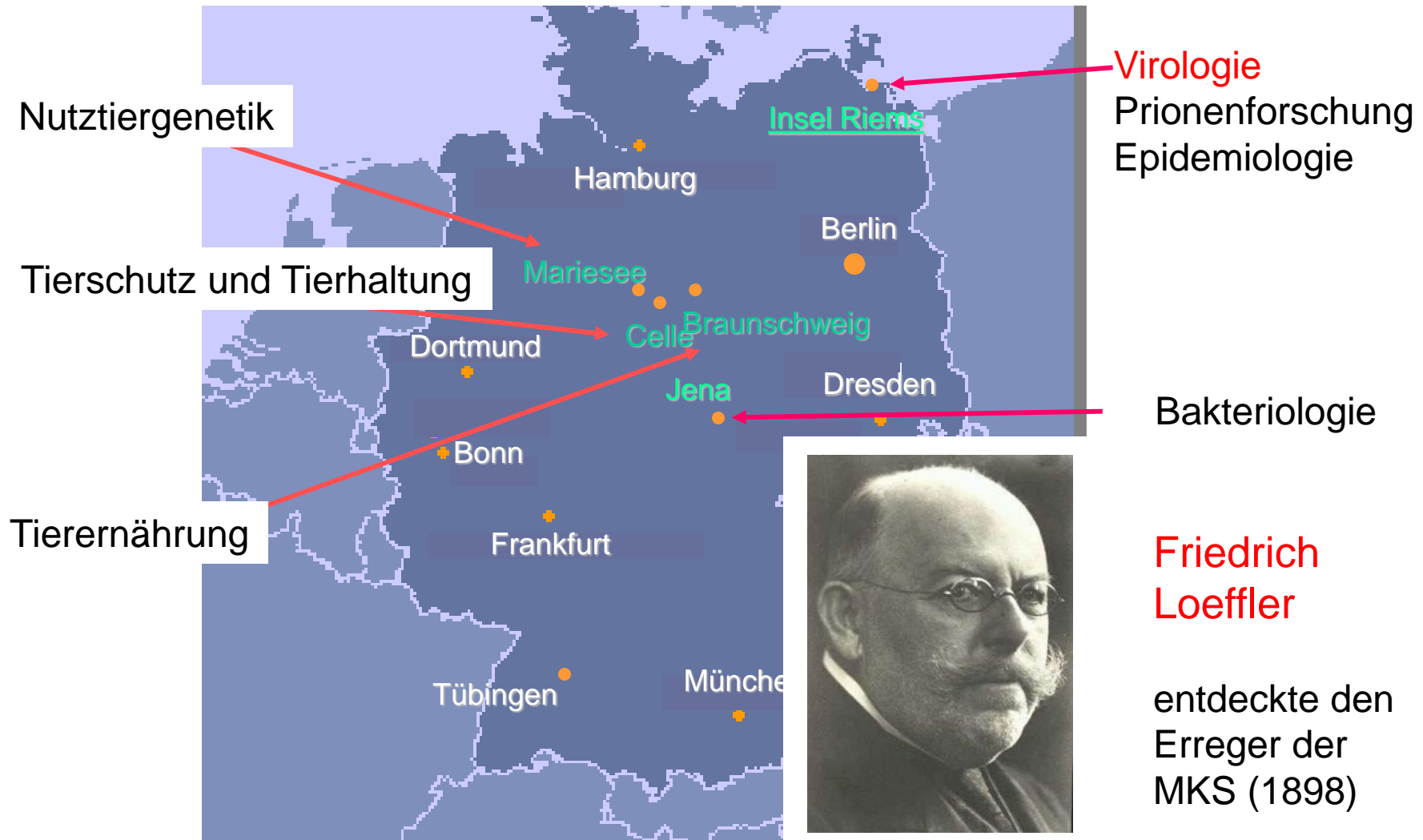
Bernd Haas, NRL für MKS, IVD, FLI Riems

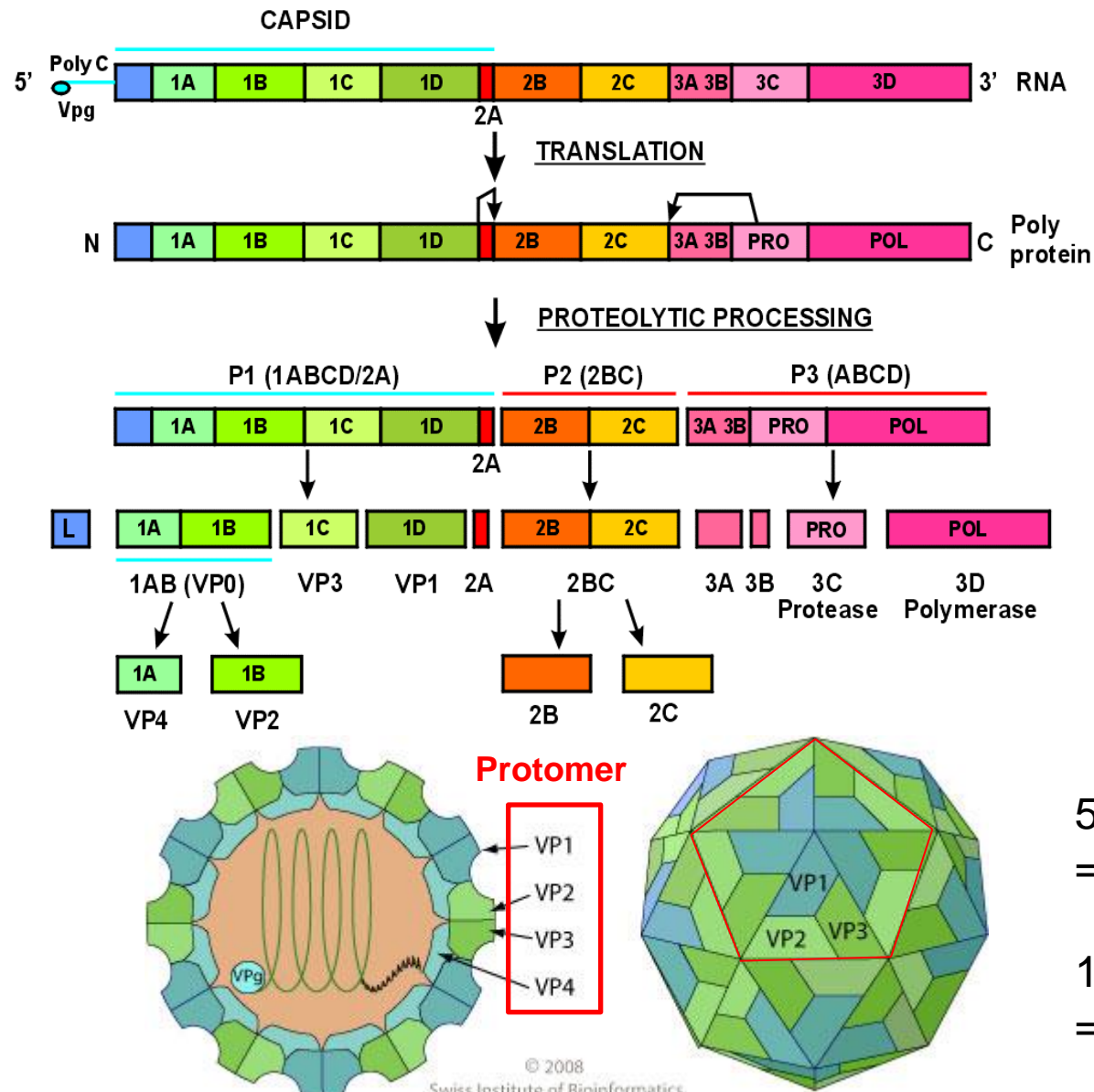






Friedrich-Loeffler-Institut Bundesforschungsinstitut für Tiergesundheit





5 Protomers
= 1 Pentamer

12 Pentamers
= Capsid



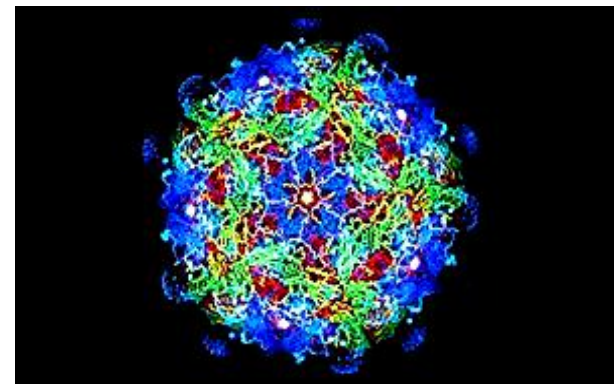
Foot-and-mouth disease virus (FMDV)

Family Picornaviridae

Genus Aphthovirus

7 Serotypes – 46 Topotypes - Hundreds of sub-lineages and strains

Serotype	Topotypes (15 – 20% nucleotide difference)
O	11
A	3
C	3
Asia	1
SAT 1	9
SAT 2	14
SAT 3	5



25nm, non-enveloped, icosahedral



Besonderheiten der MKS:

Geringe Mindestinfektionsdosis

Virus wird in hohen Dosen ausgeschieden

Virus stabil in der Umwelt (aber säurelabil)

Viele Virusstämme, geringer Kreuzschutz

Subklinische Fälle + Viruspersistenz

Viele Spezies empfänglich



Duration of FMD Excretion in Cattle

Blood	5 days	1 - 2 weeks
Semen	10 days	
Urine	7 days	
Milk	5 days	
Saliva	11 days	
Aerosol	5 days	
Nasal Discharge	7 days	
Faeces	5 days	
Probang samples	month to years	



„Carrier Animals“

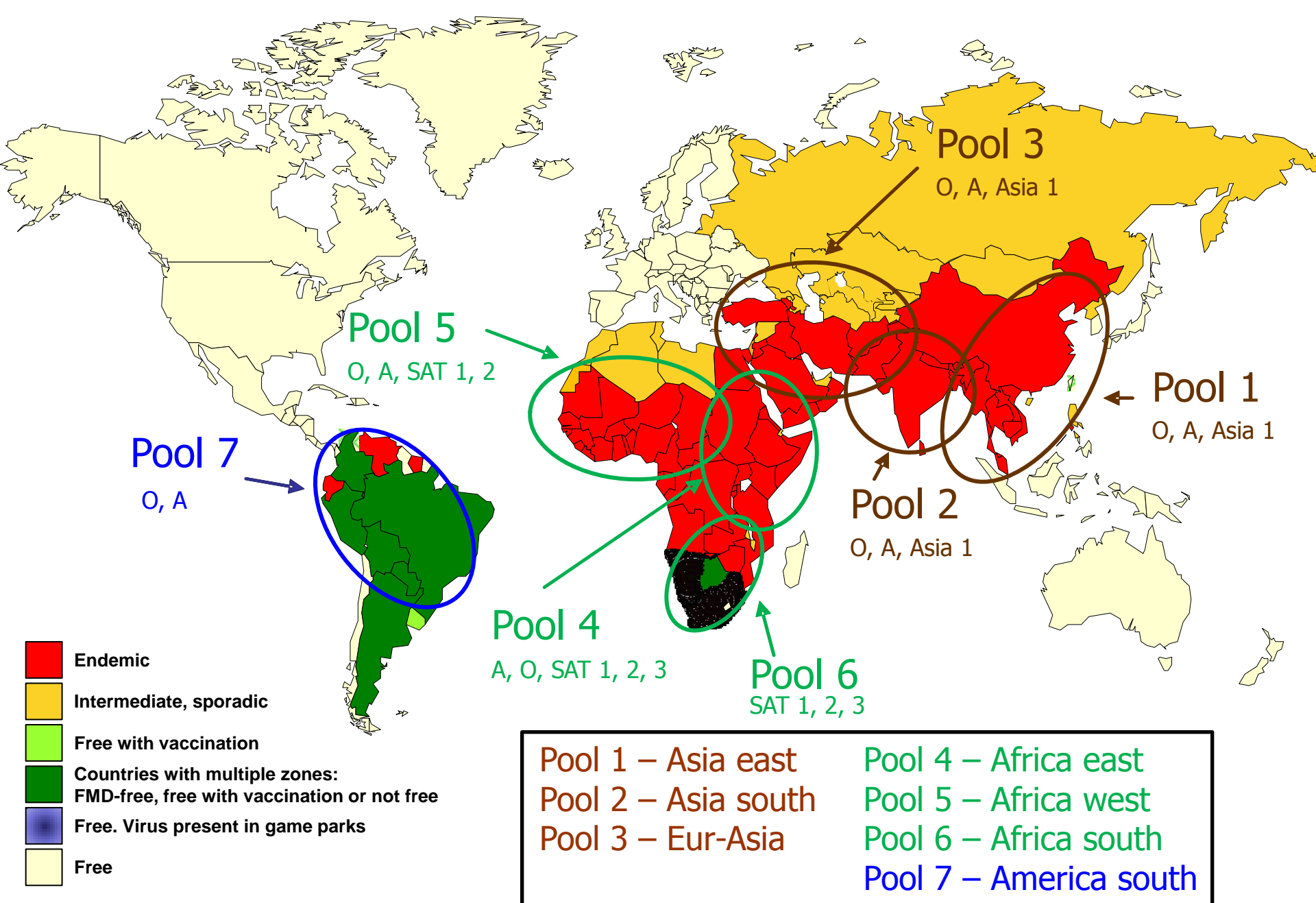
**Probangprobe \geq 28 Tage positiv
Nachweismöglichkeit intermittierend**

Afrikan. Büffel 5 Jahre

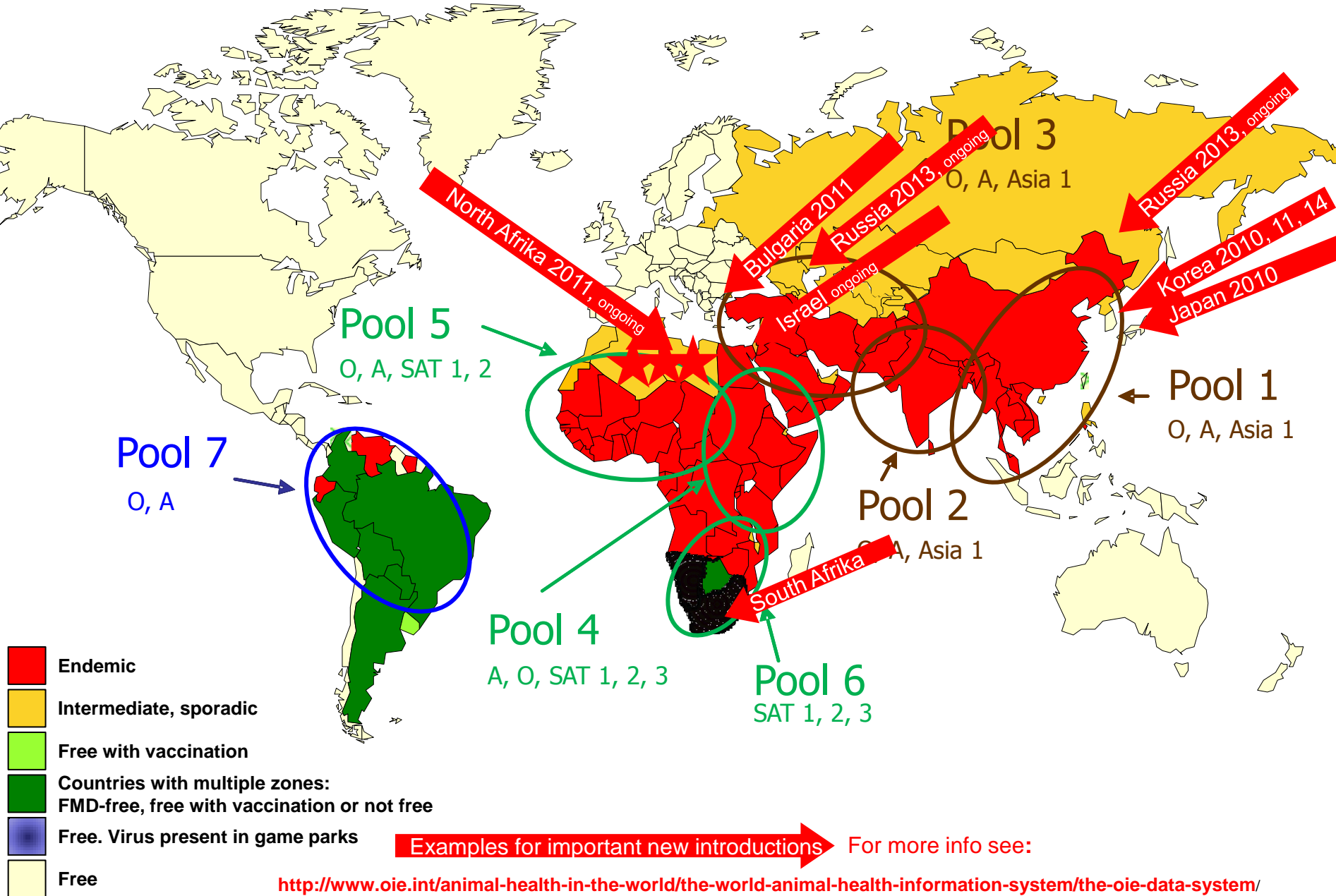
Rind 3,5 Jahre

Kl. Wdk. 9 Monate

Nicht: Schwein



Pool positions are approximate and colours indicate that there are three principal pools, two of which can be subdivided into overlapping areas



Pool positions are approximate and colours indicate that there are three principal pools, two of which can be subdivided into overlapping areas

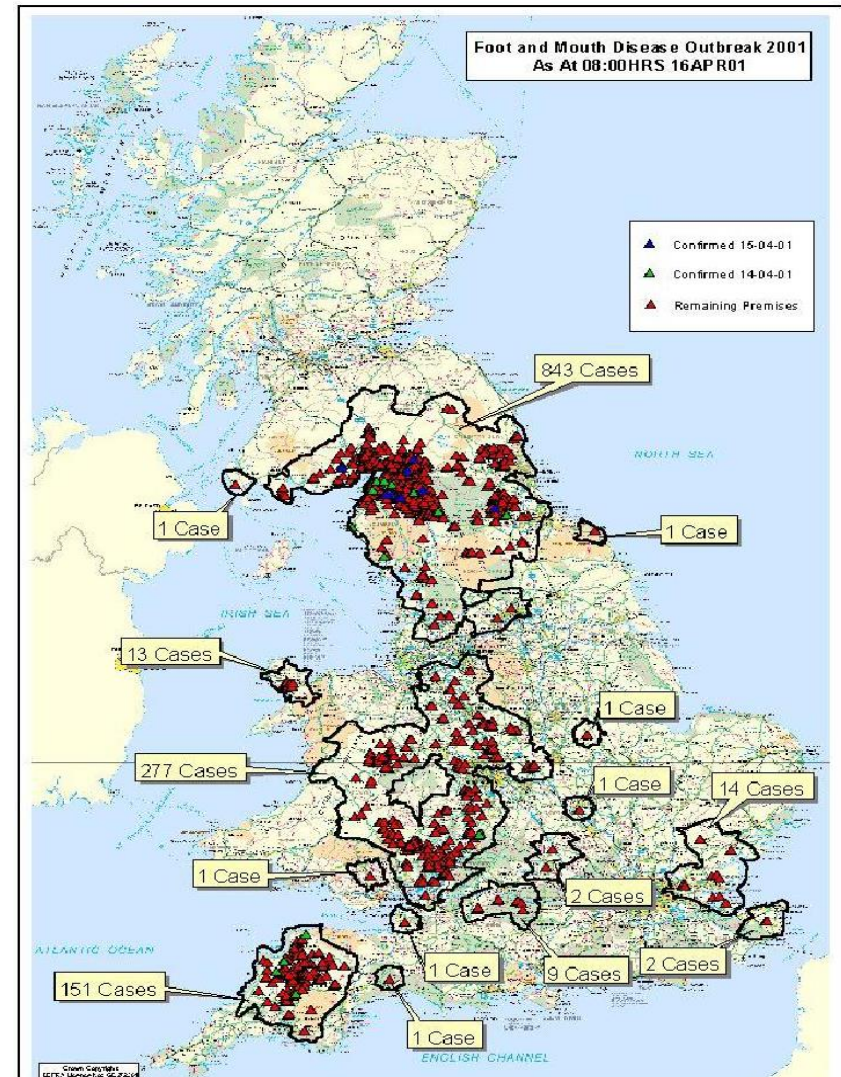
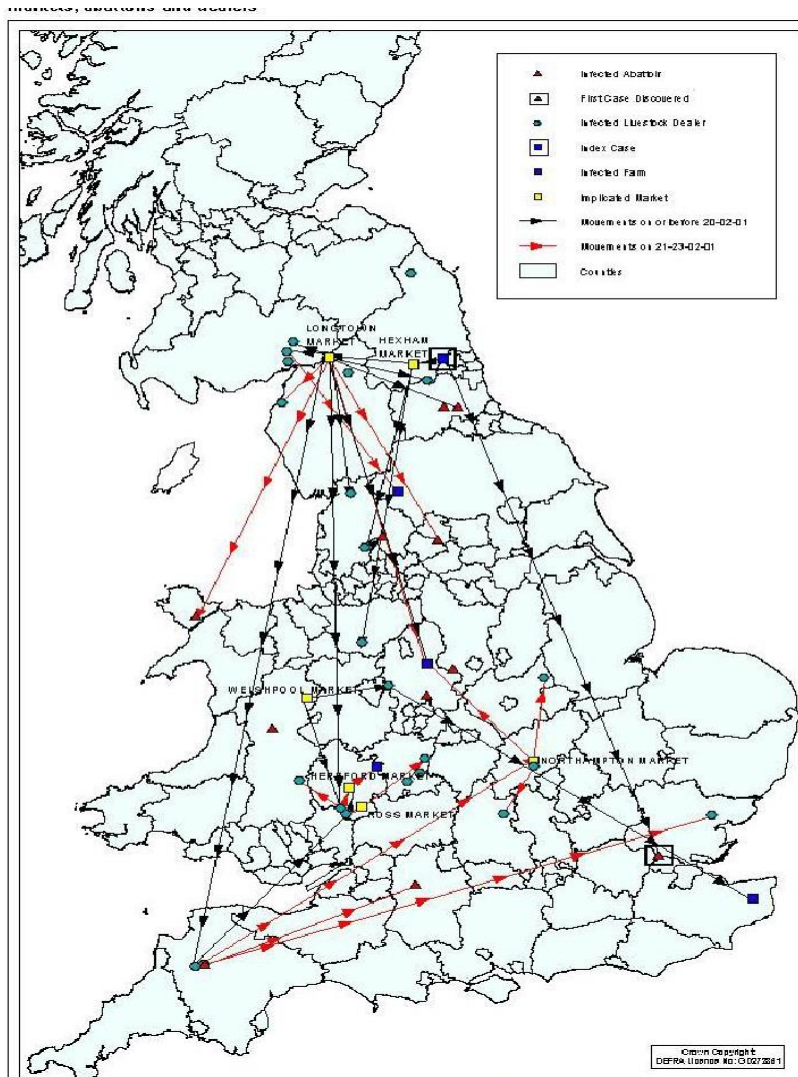


**Table 10. Sources of Primary Outbreaks
of Foot-and-Mouth Disease, Worldwide, 1870-1993**

Source ¹	Percent of Outbreaks ²
Meat, meat products, or garbage	66
Airborne (wind) or migrating birds	22
Livestock importations	6
Contaminated objects or persons	4
Vaccines	3
Wildlife	< 1

¹ known or suspected source

² outbreaks with a reported source (n = 627)



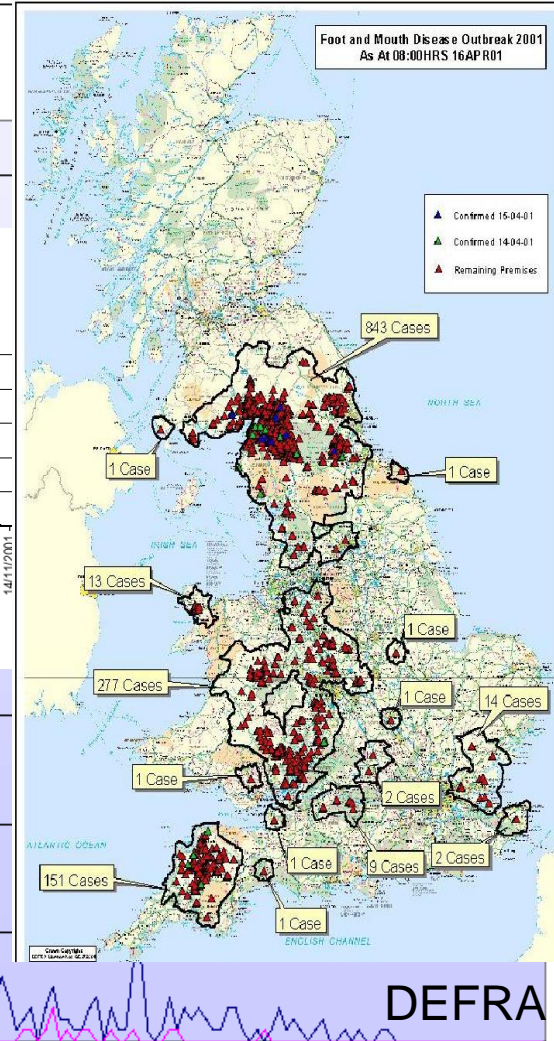
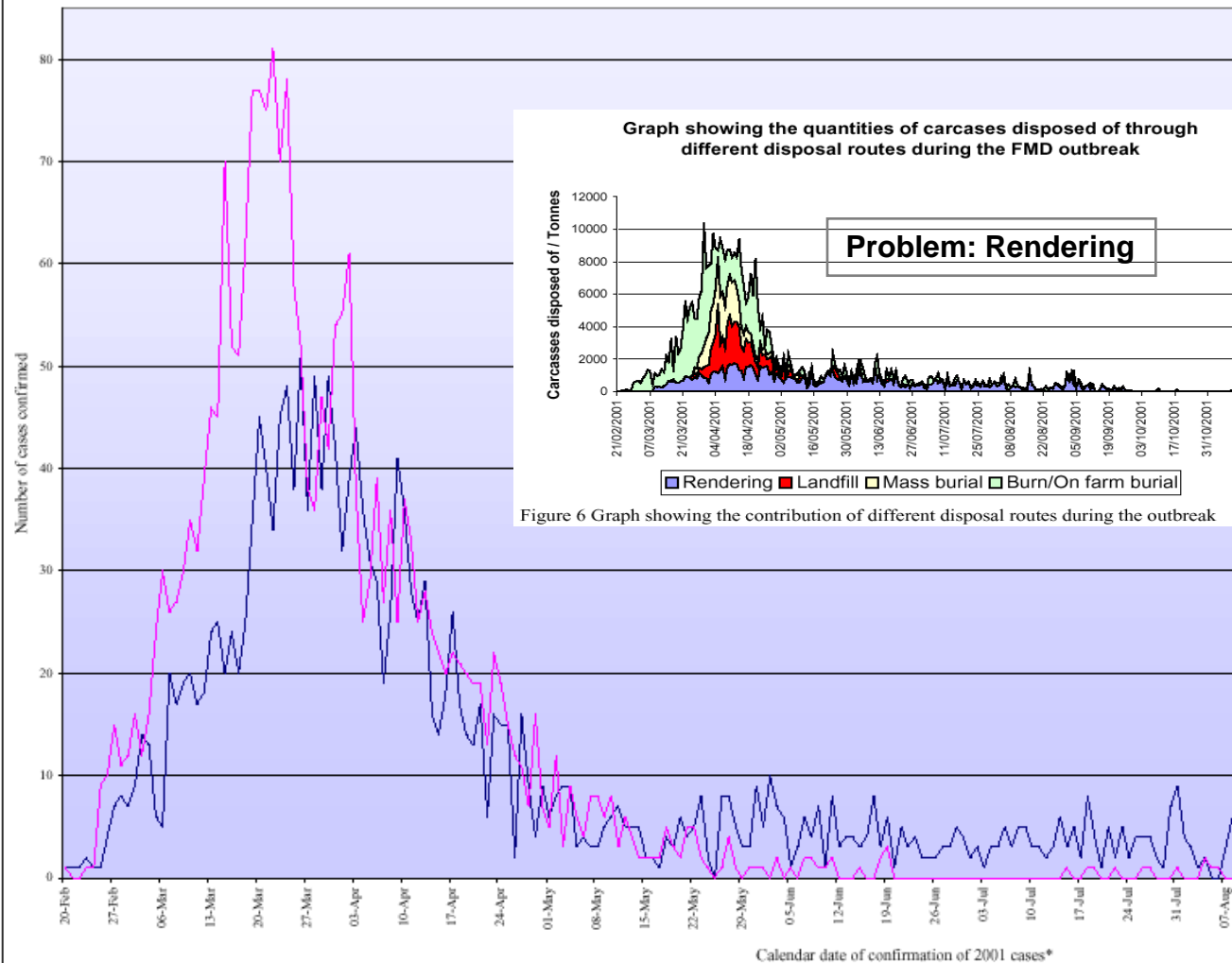
Ringvaccination unmöglich!

DEFRA



FMD in UK, Irland, France, NL 2001

Comparison of daily number of cases confirmed in the 1967/68 and 2001 FMD epidemics in Great Britain





FMD in UK, Irland, France, NL 2001



Holdings Affected

- 2,026 infected premises in GB (UK 2,030)
- 7,139 dangerous contacts (including 3,302 contiguous premises)
- 247 slaughter on suspicion cases

Animals Slaughtered for disease control measures (% of number slaughtered)

- 3,915,849 total animals slaughtered
- 600,600 cattle (15%)
- 3,172,012 sheep (81%)
- 138,731 pigs (3%)
- 2,597 goats (<1%)
- 520 deer (<1%)
- 1,389 other (<1%)

Animals Slaughtered for welfare reasons

- 548,190 under the Light lamb scheme
- 2,000,800 under the Livestock Welfare Disposal Scheme (LWDS)

Source: DEFRA Disease Control System 30 November 2001
Note figures are provisional and subject to change





FMD in Bulgaria 2011

29. Dez. 2010 - **Wildboar** with FMD signs
hunted

04. Jan. 2011 - FMD confirmed

9. Jan. 2011 - Outbreak in Kosti

15. Jan. 2011 - Outbreak in Rezovo

31. Jan. 2011 - Outbreak in Gramatikovo

**19. März 2011 - Outbreak near Kirovo,
Beginning of 2nd series**

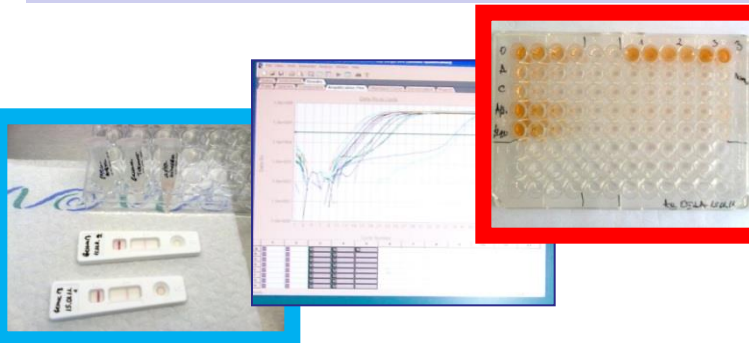
Typing:

*Serotype O, Topotype ME-SA,
PanAsia-2ANT-10*

*Greatest homology with isolates
from Asian part of Turkey*



Trophy boar hunting in Turkey and Bulgaria





FMD in Bulgaria 2011

T. Alexandrov et al. / Veterinary Microbiology 166 (2013) 84–90

85

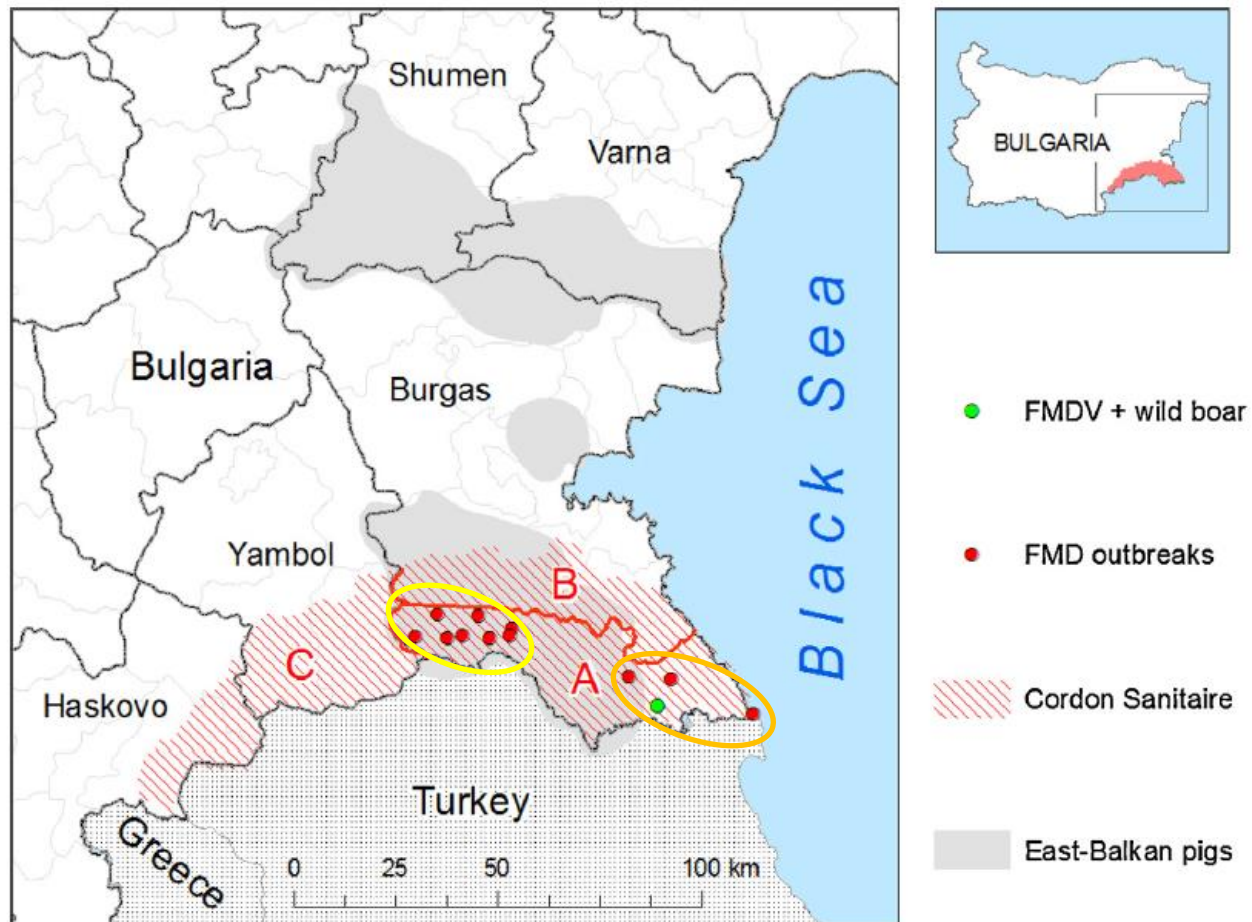
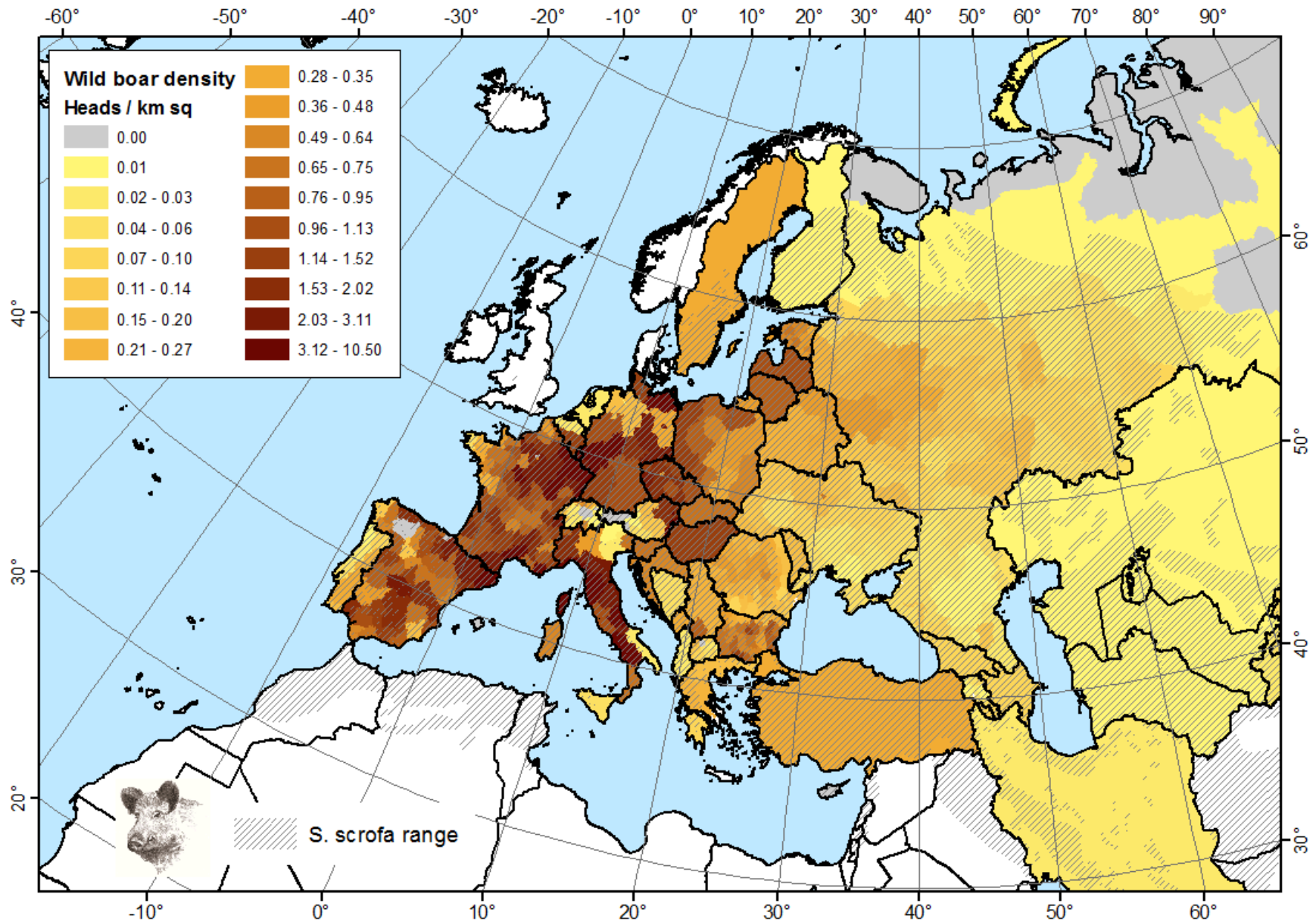


Fig. 1. Locations of the shot FMDV positive wild boar and the 11 outbreaks in domestic livestock within the surveillance area comprising sampling areas A, B and C and the East-Balkan pig herds.



Non-invasive surveillance

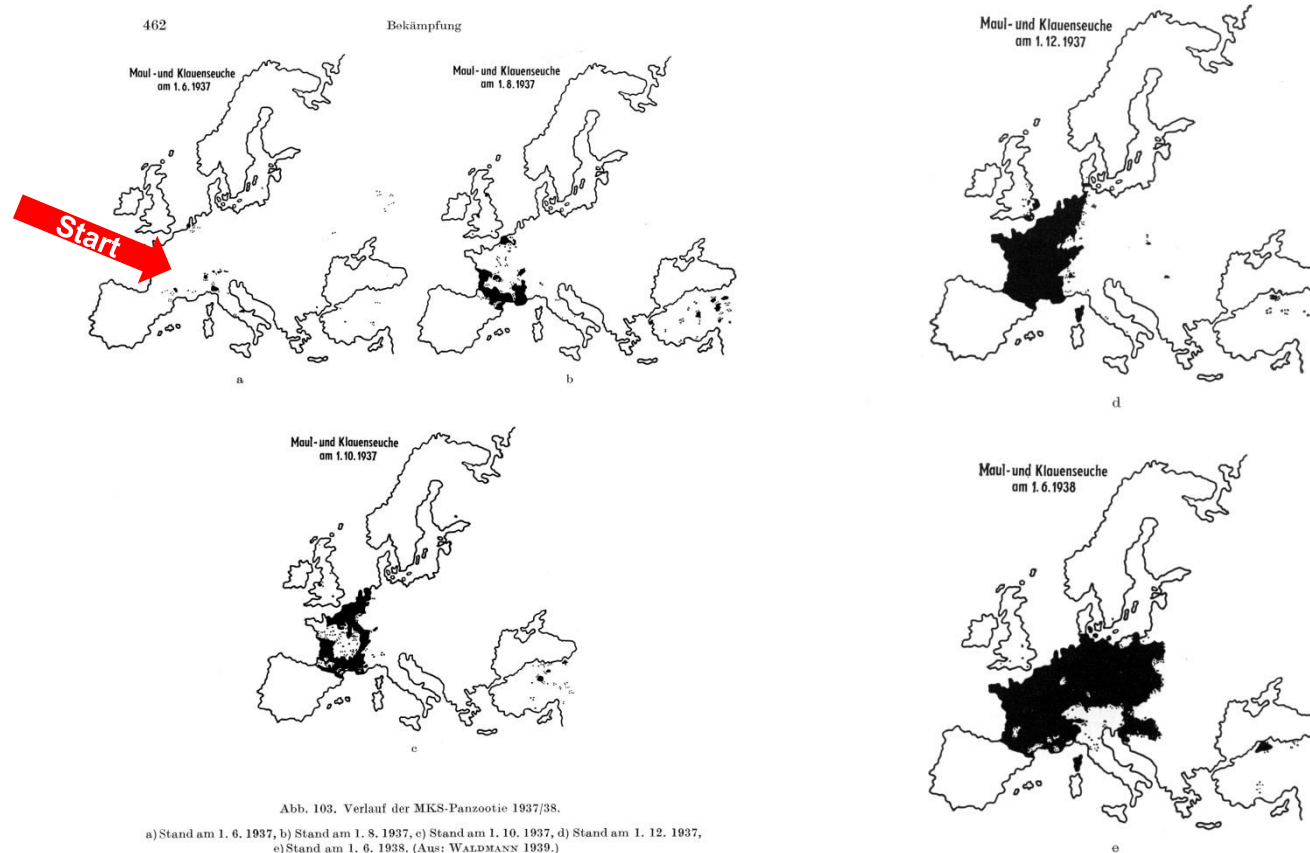
- Aims at detecting virus by PCR; Targets epi-unit (all animals attending a feeding site);
- As frequent as needed



Khomenko, et al, 2013,



Was passiert wenn man die MKS nicht konsequent bekämpft?



Röhrer u. Olechnovitz, 1980

1937 → Nach 1 Jahr: „Land unter“! → 1938

Die MKS hat nichts mit „Massentierhaltung“ zu tun!



Beratung durch den Tierarzt - Was kann der Landwirt tun?

Betriebshygiene!

- Unterbinde Zugang zum Stall durch Betriebsfremde!
- Stelle **betriebseigene** Overalls und Stiefel!
- Reinige *vor* der Desinfektion
 - Schmutz schützt das Virus vor dem Desinfektionsmittel!
- Desinfiziere Oberflächen vollständig
 - Mit Desinfektionsmittel besprühen alleine nützt nichts!
- Beachte die Einwirkungszeit (und Temperatur!)
- Verwende ein geeignetes Desinfektionsmittel (DVG-gelistet, viruzid)





Klinik der MKS

Speicheln

Fieber

Milchrückgang

Jungtiersterben





Tongue of steer with 1-day-old vesicle which ruptured when the tongue was drawn from the mouth.



DEFRA



**Tongue of steer with
3-day-old lesions.**

**Sero-fibrinous exudation
into the lesions has
resulted in a loss of
earlier red raw
appearance and
also sharpness
of margination.**

Early granulation evident.



**4-day-old lesions.
Note progressive
loss of lesion
margination
and extensive
fibrin infilling.**

DEFRA



Steer's tongue with a 10-day-old lesion characterised by loss of papillae, indentation at the site of the lesion and fibrous tissue proliferation.





3 day old vesicle - the epithelium overlying the vesicle is friable and easily stripped off





**2-day-old lesion on the dental pad and upper gum of a sheep.
The margins of the lesion are sharp.**





**2-day-old lesions, inter-digital space, along the coronary bands
Note blanching and swelling**



3-day-old lesions.
Note extensive



DEFRA

**1-day-old vesication of a pig's
snout, gum and lips.**

**4-day-old lesions
Scab formation and healing evident.**



**Unruptured 2-day-old
vesicle on the
supernumerary digit**



**3-day-old
lesions along
the coronary
bands**



Labordiagnostik der MKS



Unterdruck
Doppelte HEPA Filter (Abluft)
Gasdichte Klappen (Zuluft)
Thermische Abwasserbehandlung
(Im Containment)

Chemische Desinfektion oder
Autoklavieren von Ausrüstung und Abfall
Duschen + Quarantäne
Tierkörperbehandlungsanlage
Notstromaggregat



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MKS-Labor



Aufgaben der Labordiagnostik

Bestätigung klinischer Verdachtsfälle

Charakterisierung des isolierten Virus
u.a für die Impfstoffauswahl!

Untersuchungen zur Klärung epidemiologischer
Fragestellungen - „tracing back“ („tracing on“?)

Aufhebungs-, Handelsuntersuchungen



Labordiagnostische Verfahren I

Antigen- Nachweis

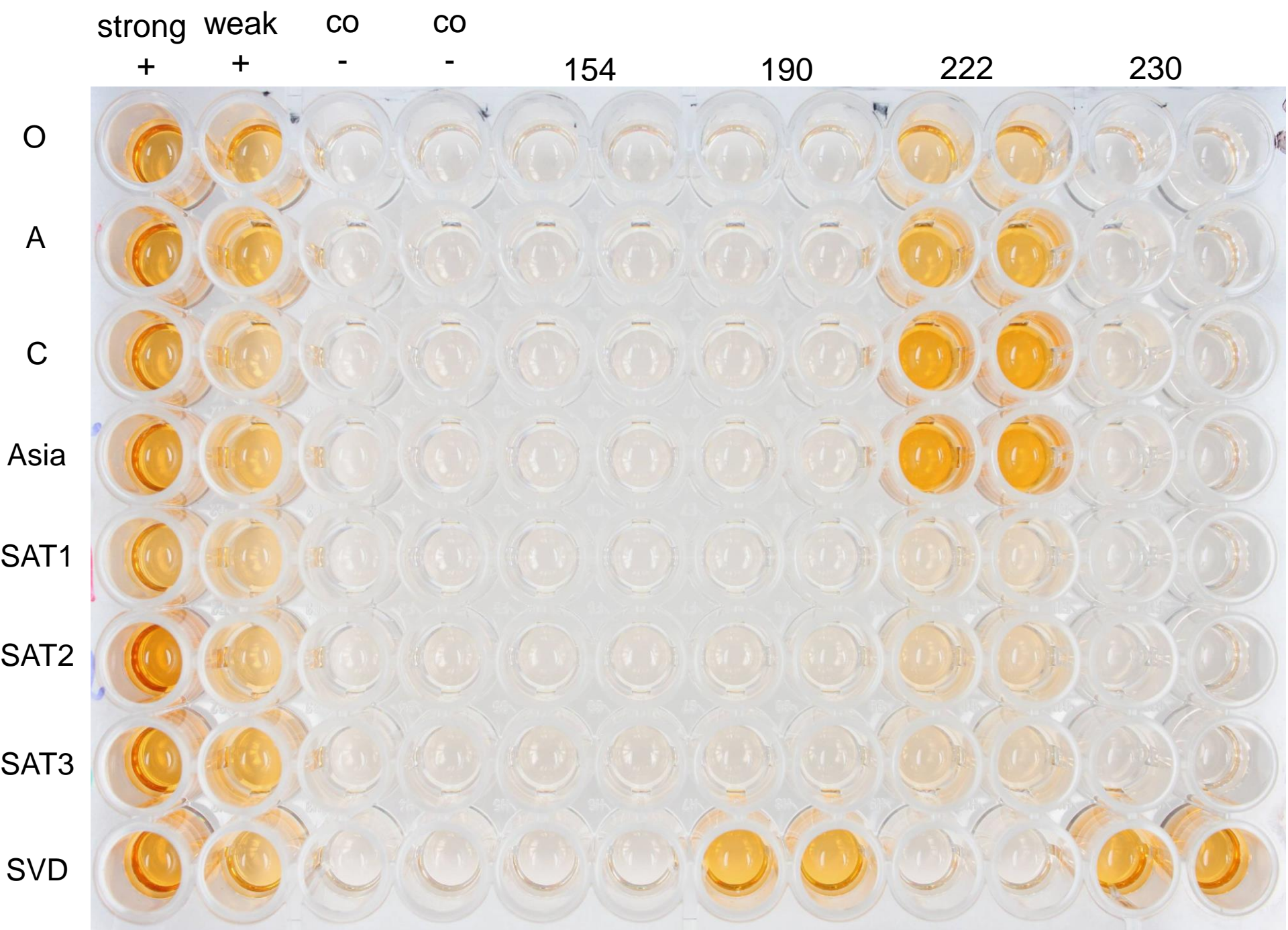
- ELISA, früher: KBR
- Künftig: LFDs

Nukleinsäurenachweis

- RT-qPCR

Virusnachweis und Charakterisierung in Zellkultur (Nur im MKSV – Hochsicherheitslabor FLI Riems!)

- BHK21, ZZ-R127, IB-RS-2

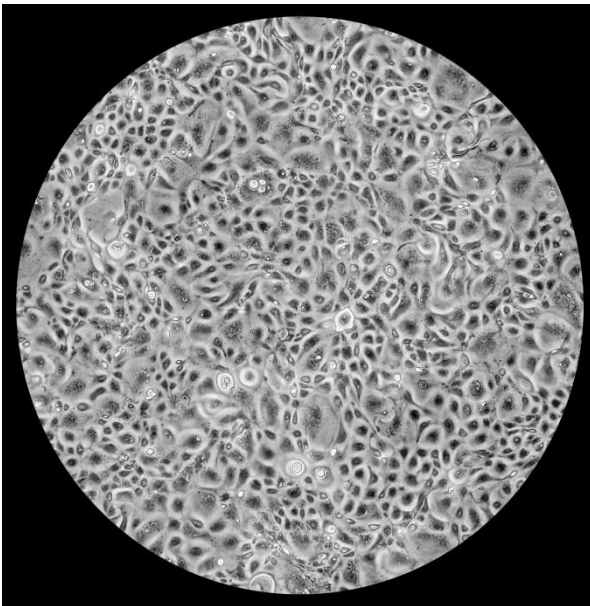


Platte 3

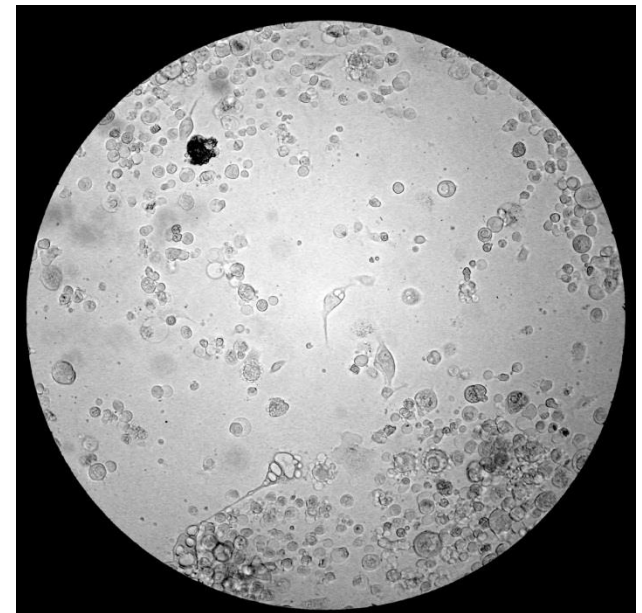


Virus isolation

ZZ R 127 (fetal goat tongue cell)



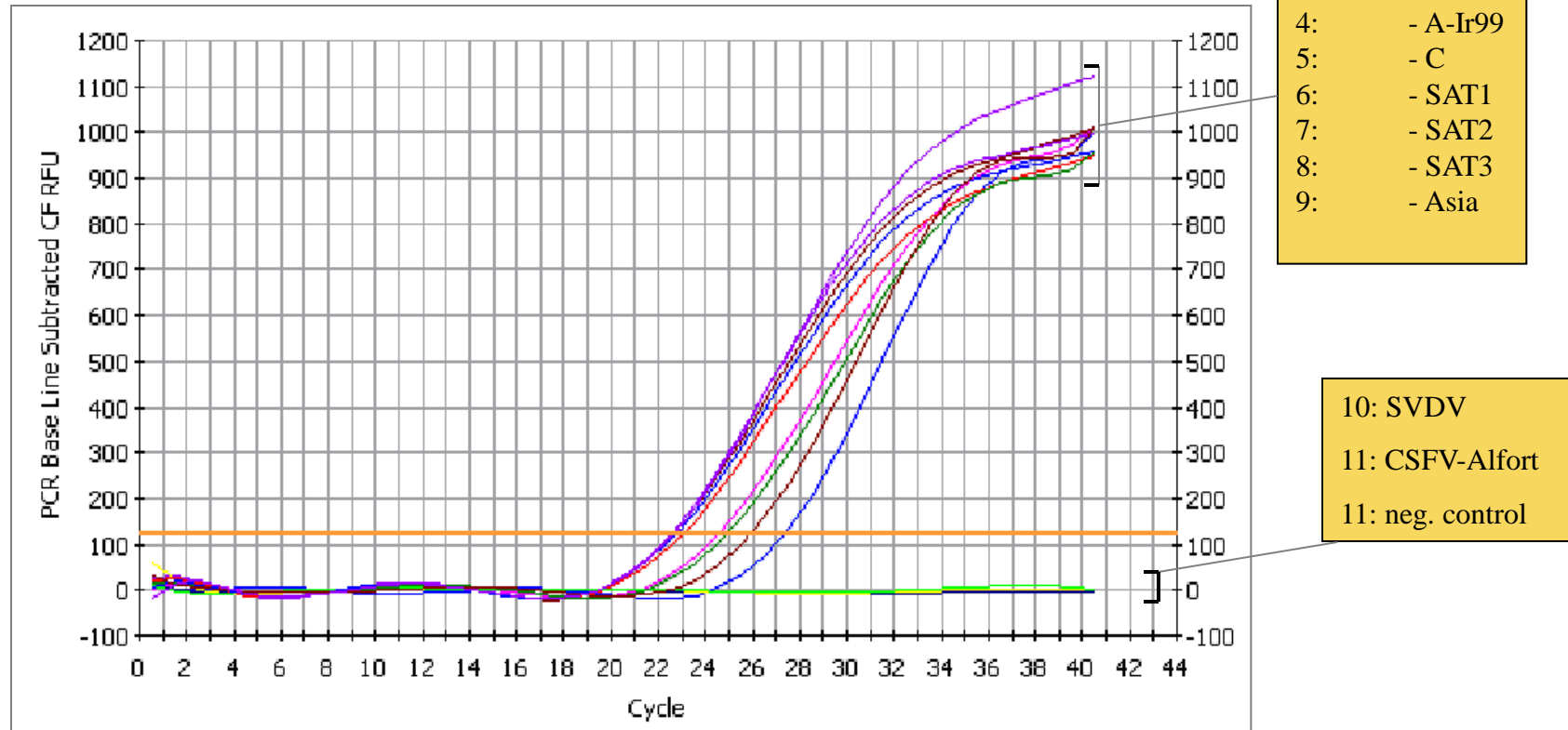
t = 0



t = 24 h

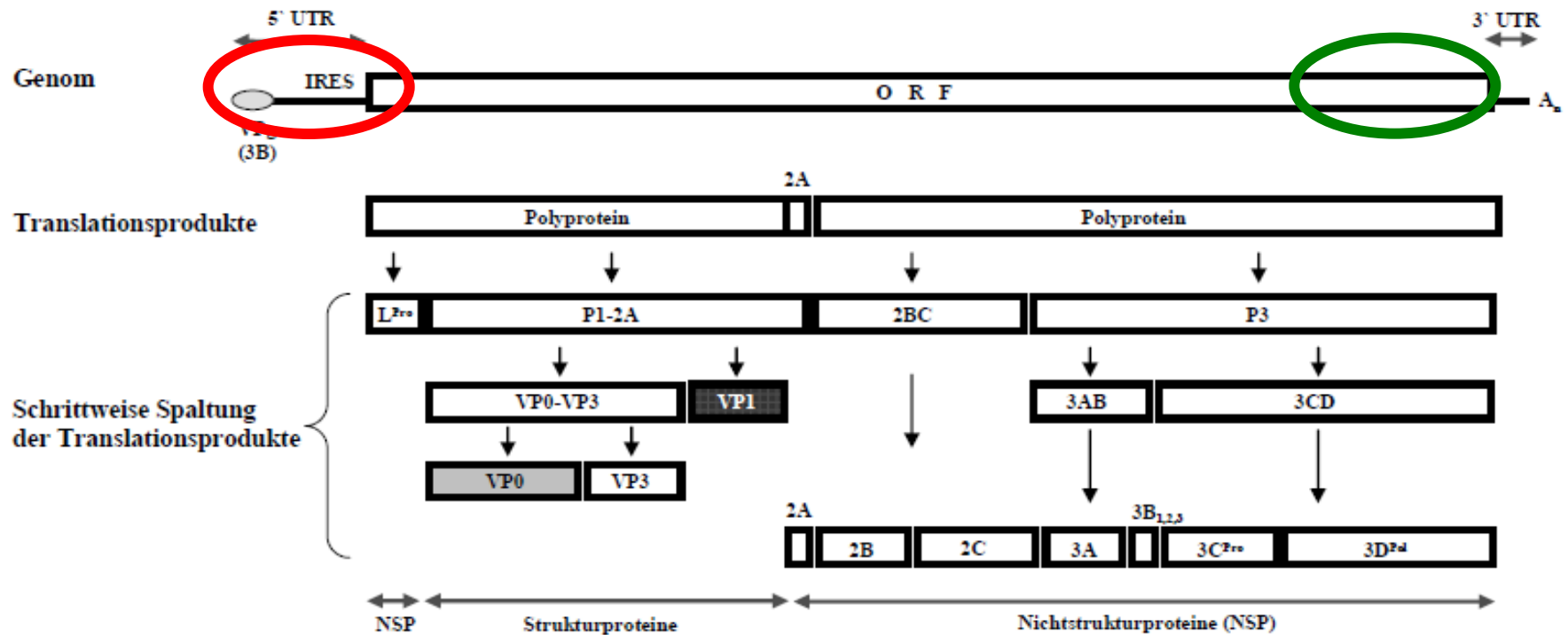


One-tube RT real time PCR





One-tube RT real time PCR





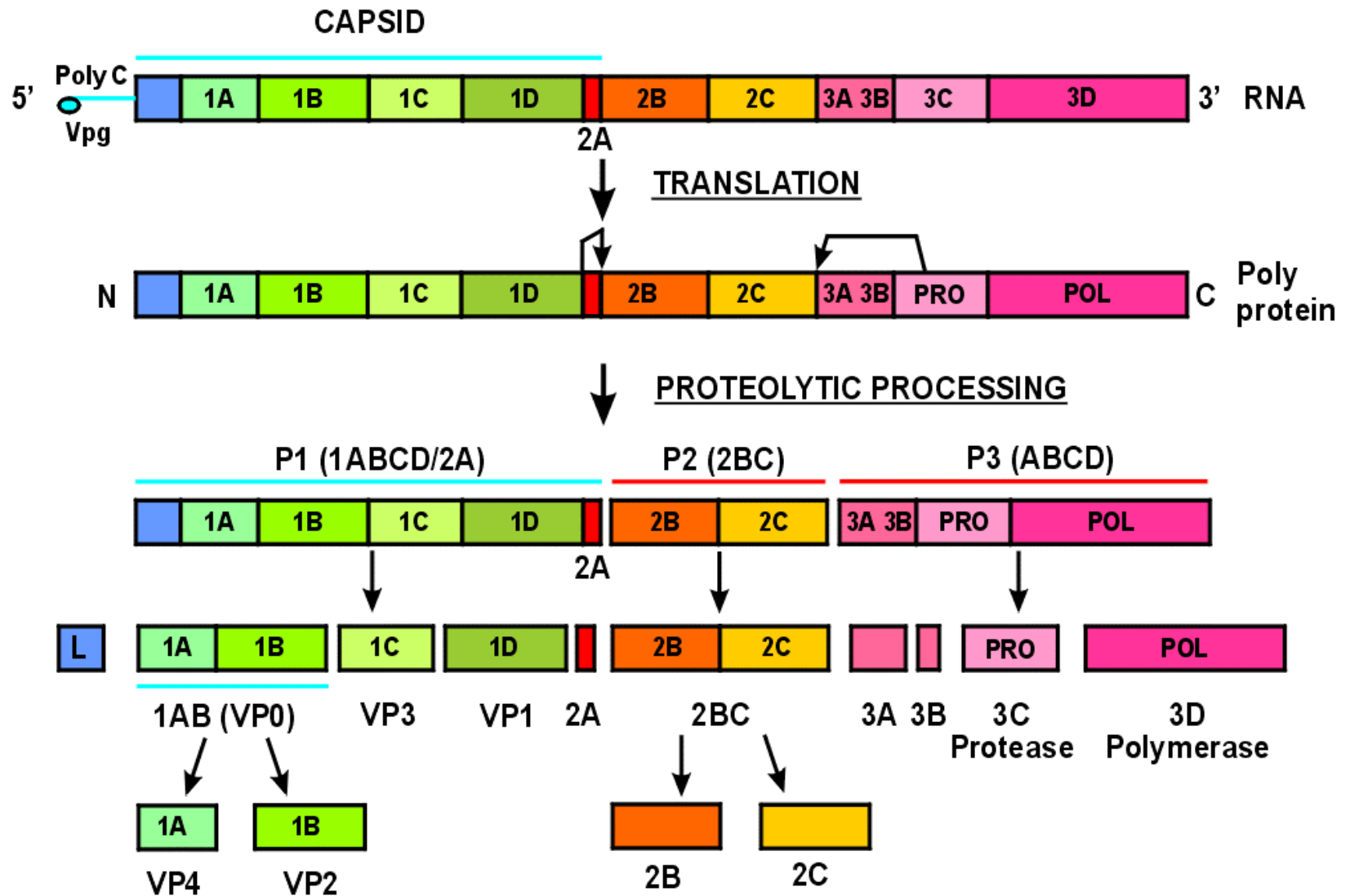
Labordiagnostische Verfahren II

Antikörper gegen Strukturproteine

- Liquid-Phase Blocking ELISA (LPBE)
- Solid Phase Competition ELISA (SPCE)
- Priocheck FMDV type O
- VNT

Antikörper gegen Nicht-Strukturproteine (NSP)

- 3ABC ELISA, EITB
- Commercial NSP ELISA (Priocheck FMDV-NS)

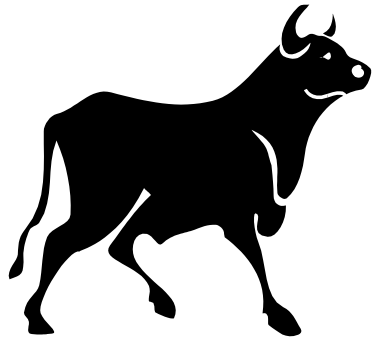




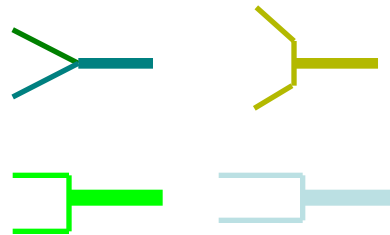
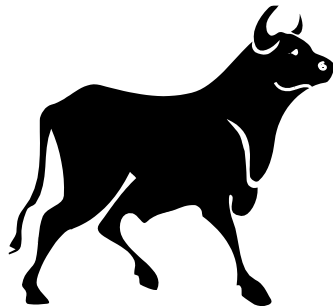
DIVA Konzept für MKS-Impfungen



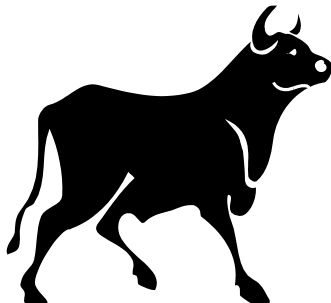
Infiziert



**Geimpft mit
inaktivierter
Vakzine**



Naive





**2001
WRL for FMD
Pirbright, UK**

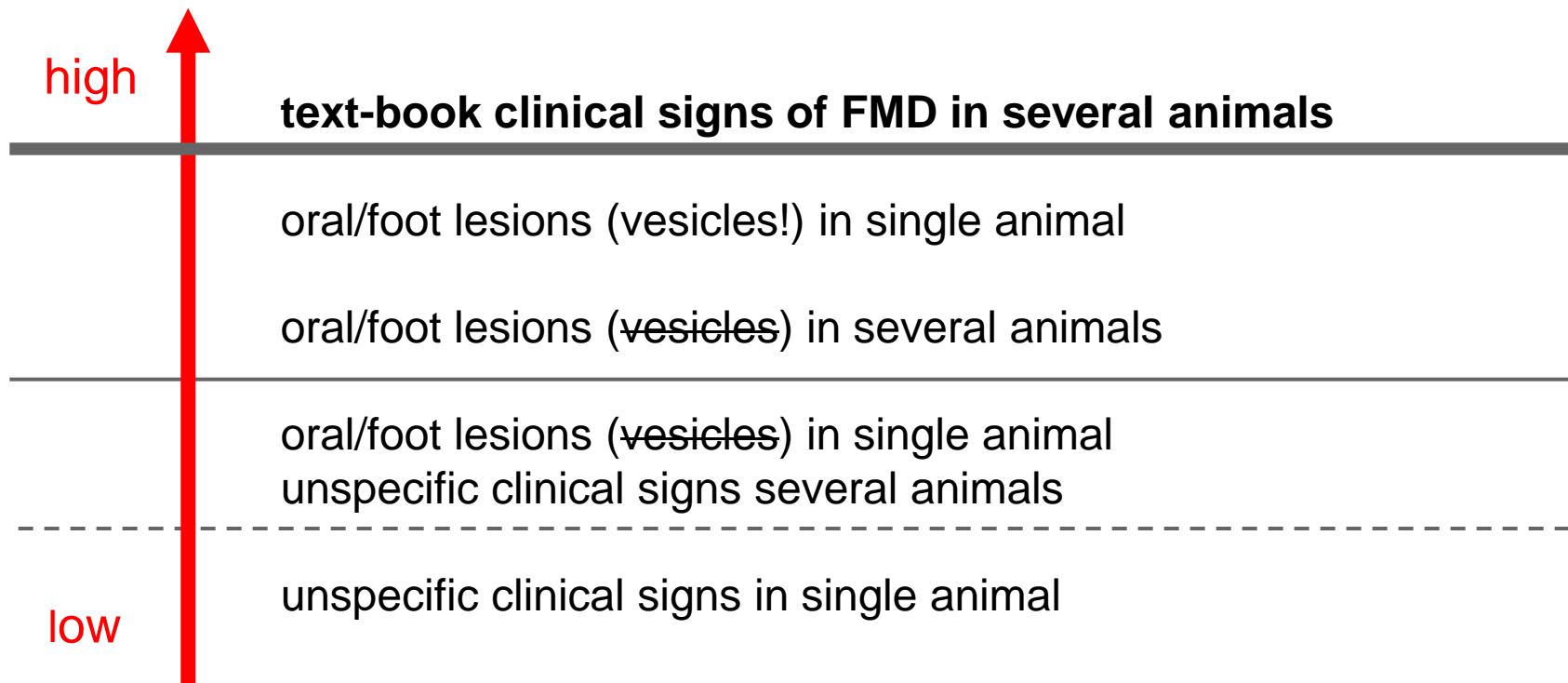
**Zentrales Labor „säuft ab!“
74 Leute, 3 Schichten**

WRLFMD



Threshold for sending samples to lab for FMD testing

What is a suspect case of FMD?





Diagnostic Tests and Containment Standards

Risk of
escape
from lab

high

Large animal
inoculation

Guinea pigs

Cell culture

ELISA

PCR

low

1910

EUFMD MINIMUM STANDARDS

1985/93

2009

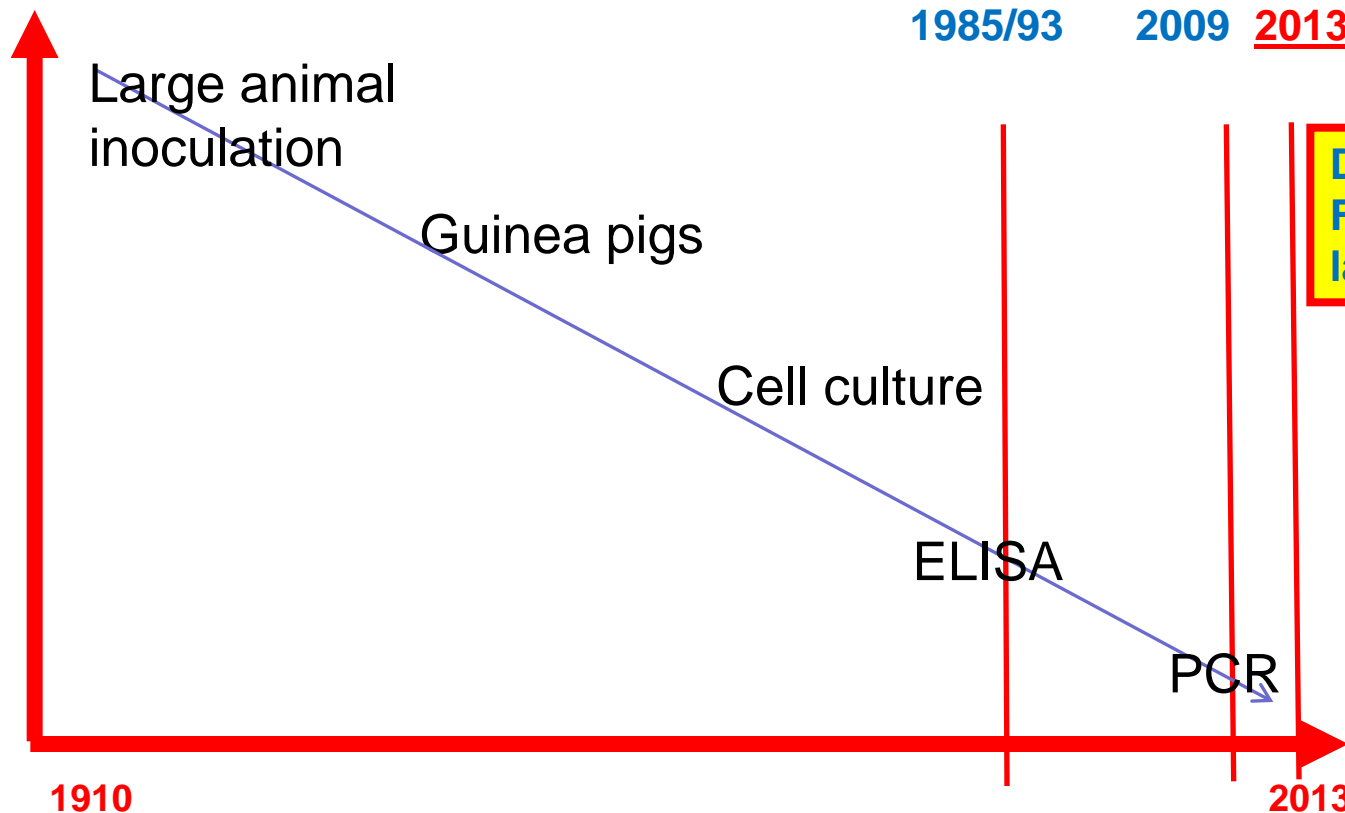
2013

Neu:

Diagnosis of
FMD in regional
labs permitted!

Timeline

2013





MINIMUM BIORISK MANAGEMENT STANDARDS FOR LABORATORIES WORKING WITH FOOT-AND-MOUTH DISEASE VIRUS (2013)

SECTION I.

LABORATORIES WORKING WITH FOOT-AND-MOUTH DISEASE VIRUS
IN VITRO AND IN VIVO

(“MBRM STANDARDS FOR FMDV LABORATORIES”)

(Article 65(d) and Annex XII of Council Directive 2003/85/EC)

SECTION II.

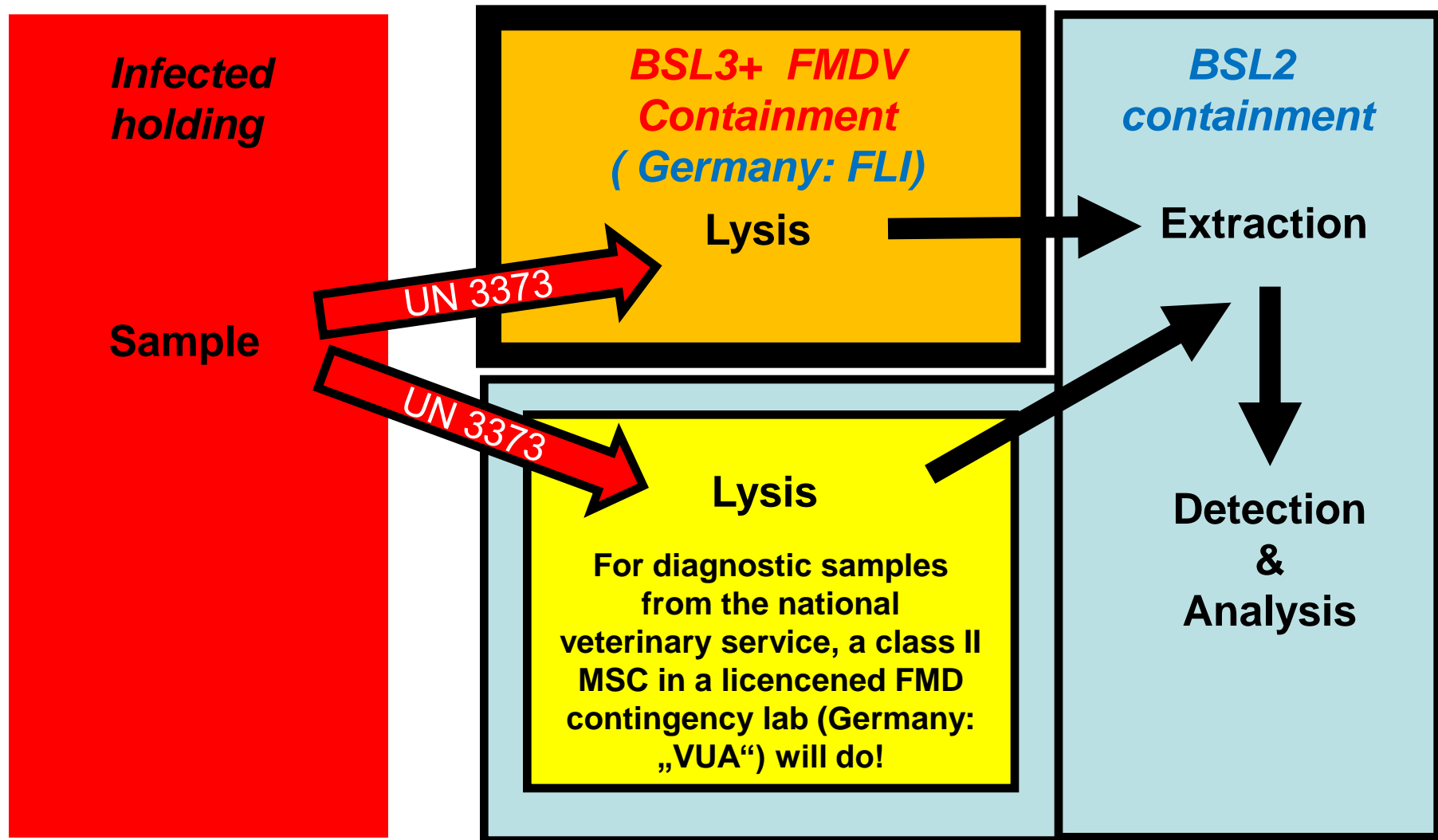
MINIMUM BIORISK MANAGEMENT STANDARDS FOR LABORATORIES
UNDERTAKING DIAGNOSTIC INVESTIGATIONS FOR FMD IN THE FRAMEWORK OF A
NATIONAL CONTINGENCY PLAN

(“MBRM STANDARDS FOR FMD CONTINGENCY LABORATORIES”)

(point 13 of Annex XV to Council Directive 2003/85/EC)

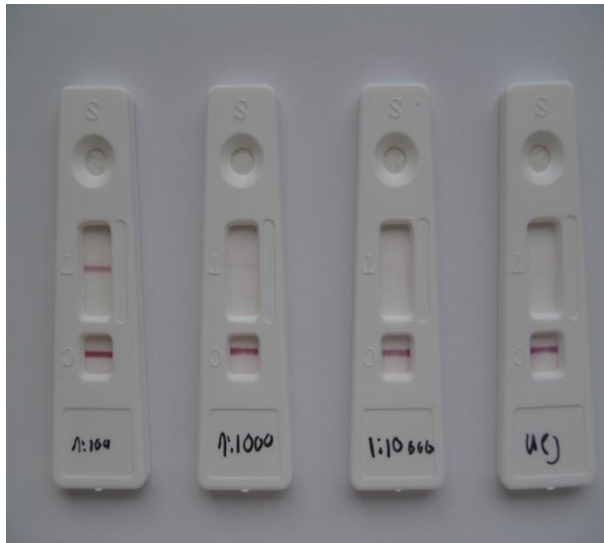


FMDV Nucleic Acid Detection by Real Time PCR





LFDs für Amtstierärzte als „pen-side test“



Der LFD ist bei **Aphthen**material dem Ag-ELISA ebenbürtig, aber schneller und ohne Laborausbildung im Stall einsetzbar

Er gehört in die Hand des entsprechend fortgebildeten Amtstierarztes und soll dessen klinische Einschätzung unterstützen. Er spart insbesondere bei Sekundärausbrüchen Zeit und gibt eine höhere Sicherheit für Tötungsentscheidungen.

Er kann aber **nicht** die Labordiagnostik ersetzen. Für ~~Sekrete und Blut~~ ist der LFD nicht geeignet! Die PCR ist etwa $\geq 1000x$ sensitiver als der LFD.



Impfung gegen die MKS

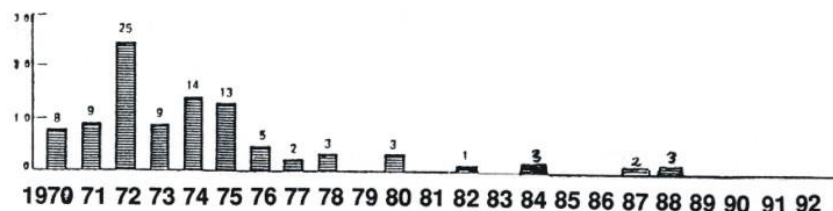
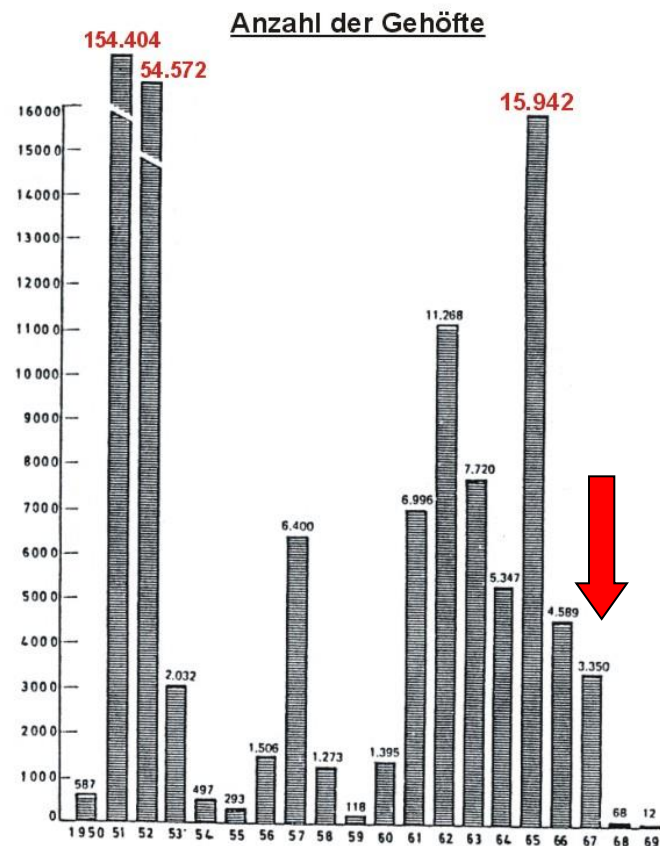
Ab Mitte 1960er Jahre:
Tilgung der endemischen
Stämme durch jährliche
Flächenimpfung der
Rinder

Unterstützt durch
„Stamping out“ und
Verbringungsverbote

Ab 1970: nur noch Einzelfälle,
peinlicherweise z.T. durch
Impfstoff(herstellung)

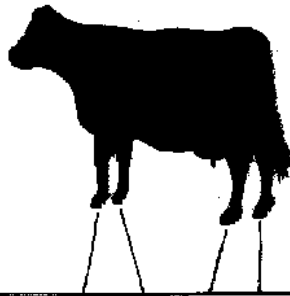
1991: Ende der jährlichen
Impfung in der EU,
Neu: Vakzinebanken,

MKS-Ausbrüche (alte Bundesländer)





Wirksamkeitsprüfung von MKS-Impfstoffen



Tier	Dosis	VL	VR	HL	HR	
1	1/ 1	✓	✓	✓	✓	P
2	1/ 1	✓	✓	✓	✓	P
3	1/ 1	✓	✓	✓	✓	P
4	1/ 1	✓	✓	✓	✓	P
5	1/ 1	✓	✓	✓	✓	P
6	1/ 4	✓	✓	✓	✓	P
7	1/ 4	✓	✓	✓	✓	P
8	1/ 4	✓	✓	✓	✓	P
9	1/ 4	✓	✓	✓	✓	P
10	1/ 4	+	✓	✓	✓	F
11	1/16	✓	✓	✓	✓	P
12	1/16	+	+	+	+	F
13	1/16	+	+	✓	+	F
14	1/16	+	+	+	+	F
15	1/16	+	+	+	+	F
16	Kontr.	+	+	+	+	F
17	Kontr.	+	+	+	+	F

5/5 (rows 1-5)
4/5 (rows 6-9)
1/5 (rows 10-15)

EP – Challenge Test

3 groups of 5 cattle

1 Dose

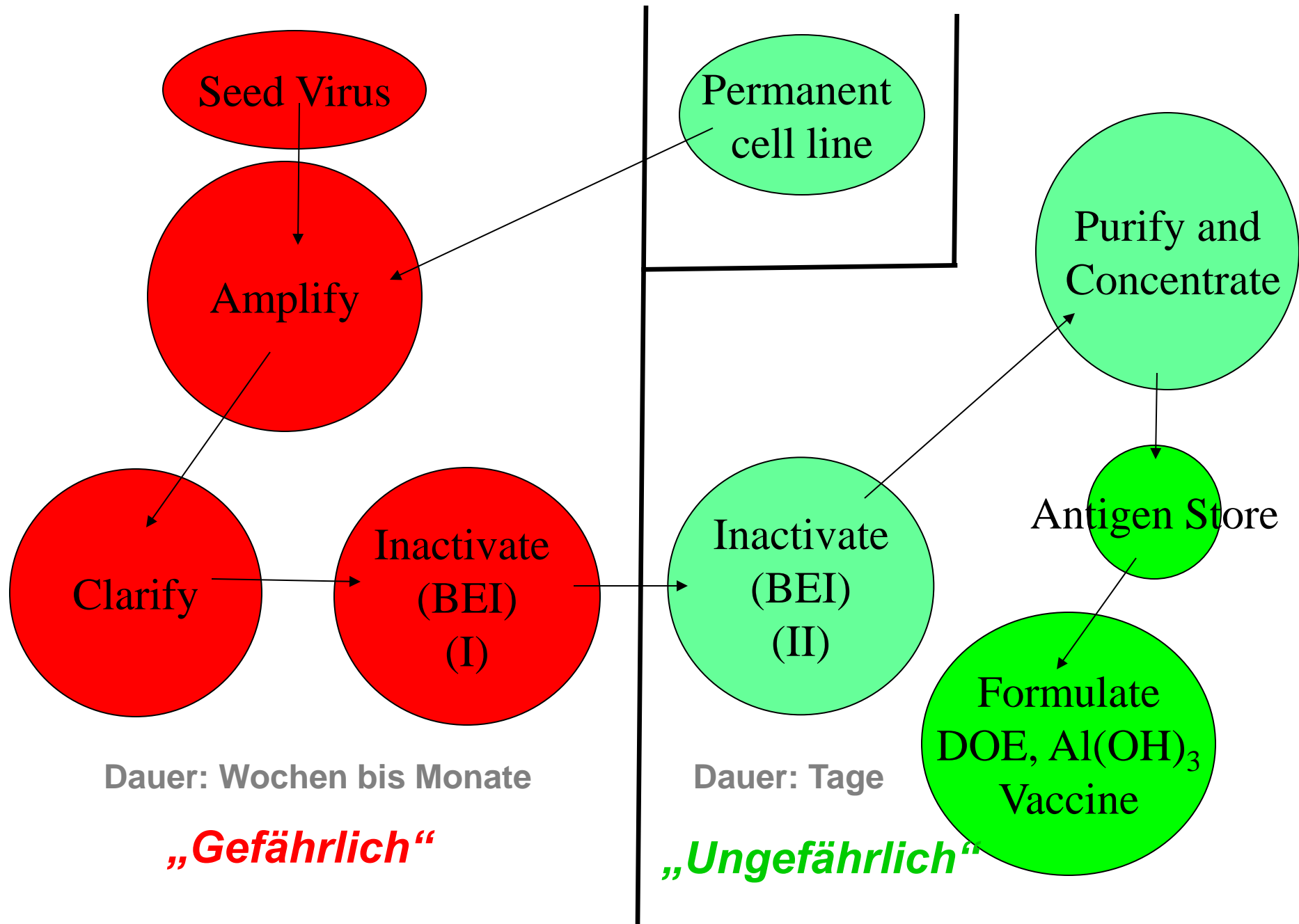
1/4 Dose

1/16 Dose

2 Control animals

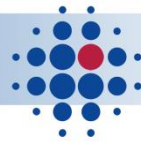
Intradermolingual
infection 21 d.p.i

Schema der MKS Impfstoffherstellung



Problem der limitierten Vakzinestämme

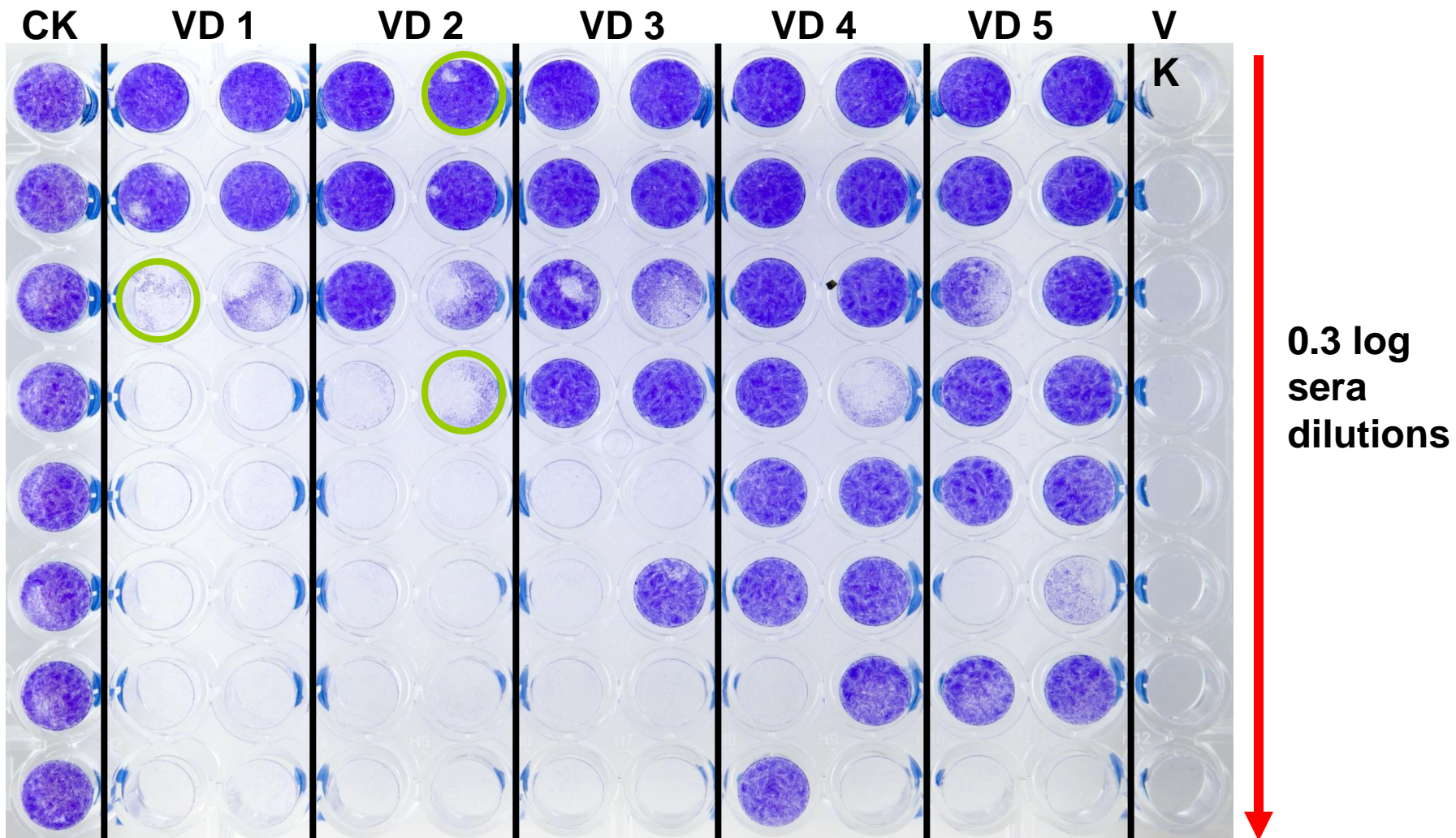
WRL for FMD (Pirbright): Vaccine bank recommendations	Vaccine strain (for each category are not listed in order of importance)
High Priority	<ul style="list-style-type: none"> O Manisa O PanAsia-2 (or equivalent) O BFS or Campos A24 Cruzeiro Asia 1 Shamir A Iran-05 (or A TUR 06) A22 Iraq SAT 2 Saudi Arabia (or equivalent i.e. SAT 2 Eritrea)
Medium Priority	<ul style="list-style-type: none"> A Eritrea SAT 2 Zimbabwe SAT 1 South Africa A Malaysia 97 (or Thai equivalent such as A/NPT/TAI/86) A Argentina 2001 O Taiwan 97 (pig-adapted strain or Philippine equivalent)
Low priority	<ul style="list-style-type: none"> A Iran '96 A Iran '99 A Iran 87 or A Saudi Arabia 23/86 (or equivalent) A15 Bangkok related strain A87 Argentina related strain C Noville SAT 2 Kenya SAT 1 Kenya SAT 3 Zimbabwe A Kenya



Virus Vaccine	A 22 Irak	A 24 Cruzeiro	A Iran 96	A Iran 99
A 22 Irak	≥ 32 PD 50	2,64 PD 50	6,06 PD 50	3,84 PD 50
A 24 Cruzeiro	n.d.	13,93 PD 50	n.d.	n.d.
A Iran 96	2,00 PD 50 8,00 PD 50	n.d.	≥ 32 PD 50	10,56 PD 50
A Iran 99	13,93 PD 50	n.d.	18,38 PD 50	≥ 32 PD 50

A22 vaccine – A Egypt 06: 10,56 PD50

Two dimensional virus neutralisation test - neutralisation





FMDV Strains in Vaccine Banks 2013

Stamm (codiert)	EU	D	Hessen
1	2,5	1,75	0,1
2		1,75	
3	2		
4	5	1,75	0,1
5	4	1,75	
6	2		0,1
7	2,5	1,75	0,1
8	4	1,75	0,1
9	2		0,1
10	2,5		
11	2,5		
12	5	1,75	0,1
13	2		0,1
14	2	1,75	0,1
15	2		0,1
16	2		

Problem of
limited
vaccine doses

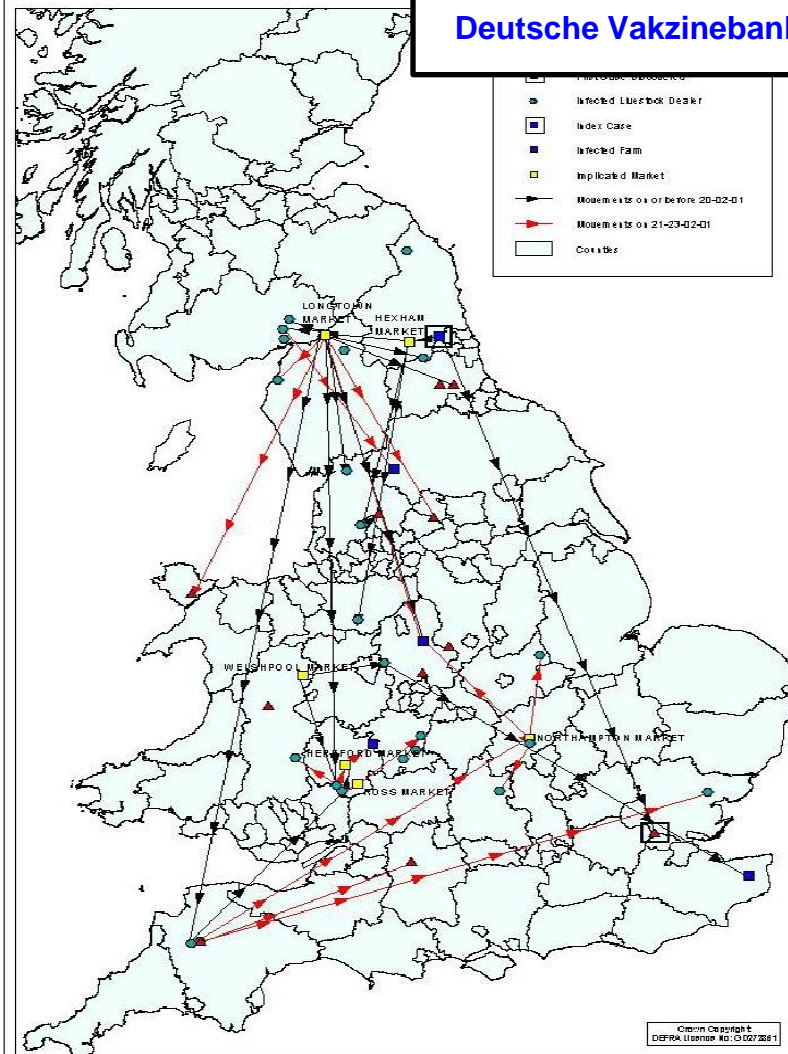
Problem of
limited
vaccine strains

**Strains
confidential!**

European Producers: Merial and MSD/Intervet

Problem der limitierten Vakzinedosen

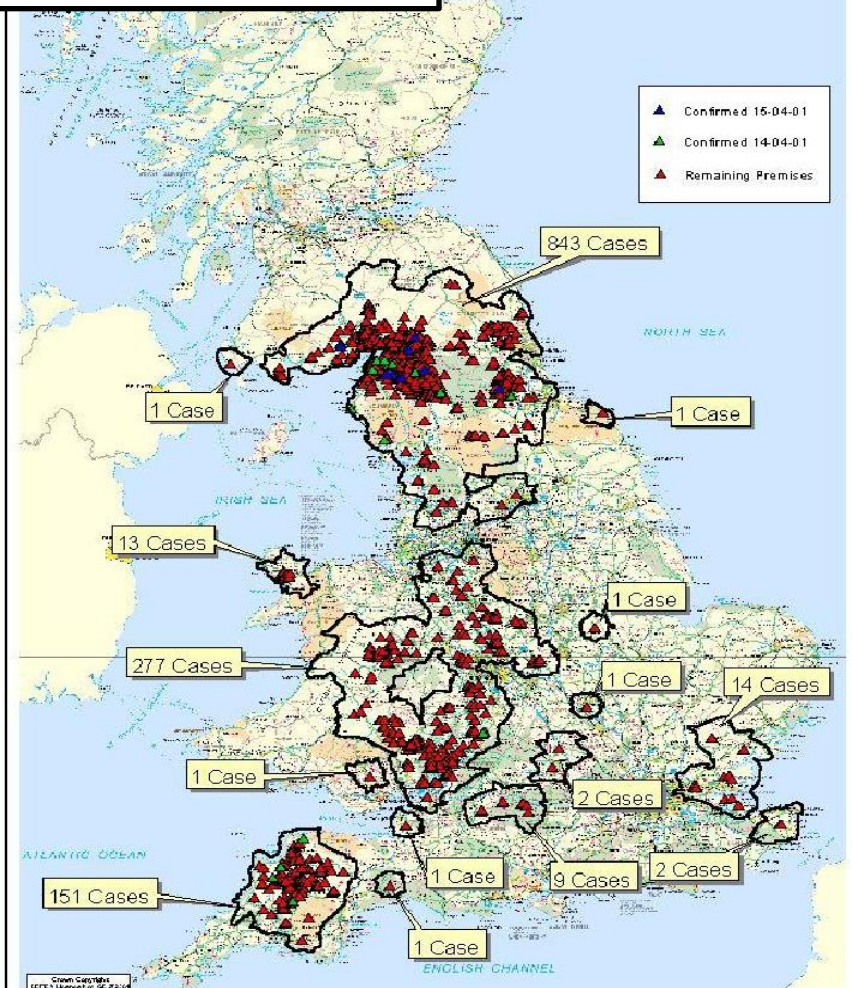
Select Committee - 31 Oct 2001
Movement of FMD infected animals before 23rd F
markets, abattoirs and dealers

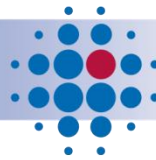


EU-Vakzinebank: max. 5 Mio Dosen pro Stamm

Deutsche Vakzinebank: 1,75 Mio Dosen pro Stamm

Foot and Mouth Disease Outbreak 2001
As At 08:00HRS 16APR01





Internationales Recht

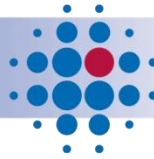
EU: Directive 2003/85

Vaccinate-to-live policy option

Followed by early recovery of OIE status of FMD-free without vaccination

Need post-vaccination serosurveillance to demonstrate absence of infection

- From 30 days after vaccination
- **Either all animals (Impfgebiet) or “5% prevalence with 95% confidence”(Umgebung)**
- **Regional labs using commercial NSP tests do the screening (EUFMD)**



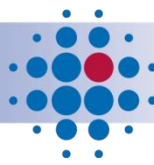
Internationales Recht

OIE: Recovery of free status in a free country or zone without vaccination

3 months after stamping out and serological surveillance (App. 3.8.7)

3 months after slaughter of all vaccinated where stamping out, emergency vaccination and serological surveillance (App. 3.6.7)

6 months after the last case/vaccination, where stamping out, emergency vaccination not followed by the slaughtering of all vaccinated. Serological surveillance according to App. 3.8.7 should demonstrate absence of infection (NSP antibodies) in the remaining vaccinated population.



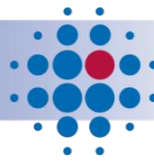
Ansätze für neuartige MKS-Vakzinen

Was leisten MKS-Vakzinen?

- *Verhinderung/Reduktion klinischer Krankheitszeichen*
- *Verringerung der Virusausscheidung*
- *Verhinderung/Reduktion der Ausbreitung*

Was leisten MKS-Vakzinen nicht?

- *Erzeugung einer sterilen Immunität*
- *Verhinderung des Carrierstatus*
- *Abdeckung mehrerer Serotypen*
- *Erzeugung einer lebenslangen Immunität*
- *Stabilität bei RT*



Ansätze für neuartige MKS-Vakzinen

Gentechnisch veränderte MKSV Lebendvakzinen

Gentechnisch verbesserte MKSV Totvakzinen

Empty Capsid Vakzinen

Protein-Vakzinen

Peptid-Vakzinen

DNS-Vakzinen

Rekombinante Vektorvakzinen

**Bisher nicht
reif für
Feldeinsatz!**

**Bedingte Ausnahme:
Ad5-FMDV Vaccine in USA**



Fazit Impfung:

Wenn morgen die MKS ausbricht, muss sie, sofern eine Impfung möglich und sinnvoll erscheint, mit konventionellen Impfstoffen bekämpft werden.

Die dazu nötigen Antigene müssen aus den fl. Stickstofftanks der europäischen Hersteller Merial und MSD/Intervet kommen.

Mehr als ca. 2 – 5 (10 ?) Mio Dosen pro Stamm sind kurzfristig nicht verfügbar.

Die deutsche Vakzinebank enthält 8 Stämme, die EU Bank 16 Stämme.

Dies schränkt die sinnvollen Optionen für den Einsatz der Impfung stark ein.

Problem der
limitierten
Vakzinedosen

Problem der
limitierten
Vakzinestämme