



Finding novel viruses and their role in disease

Lia van der Hoek



Technique

Novel virus and disease: example



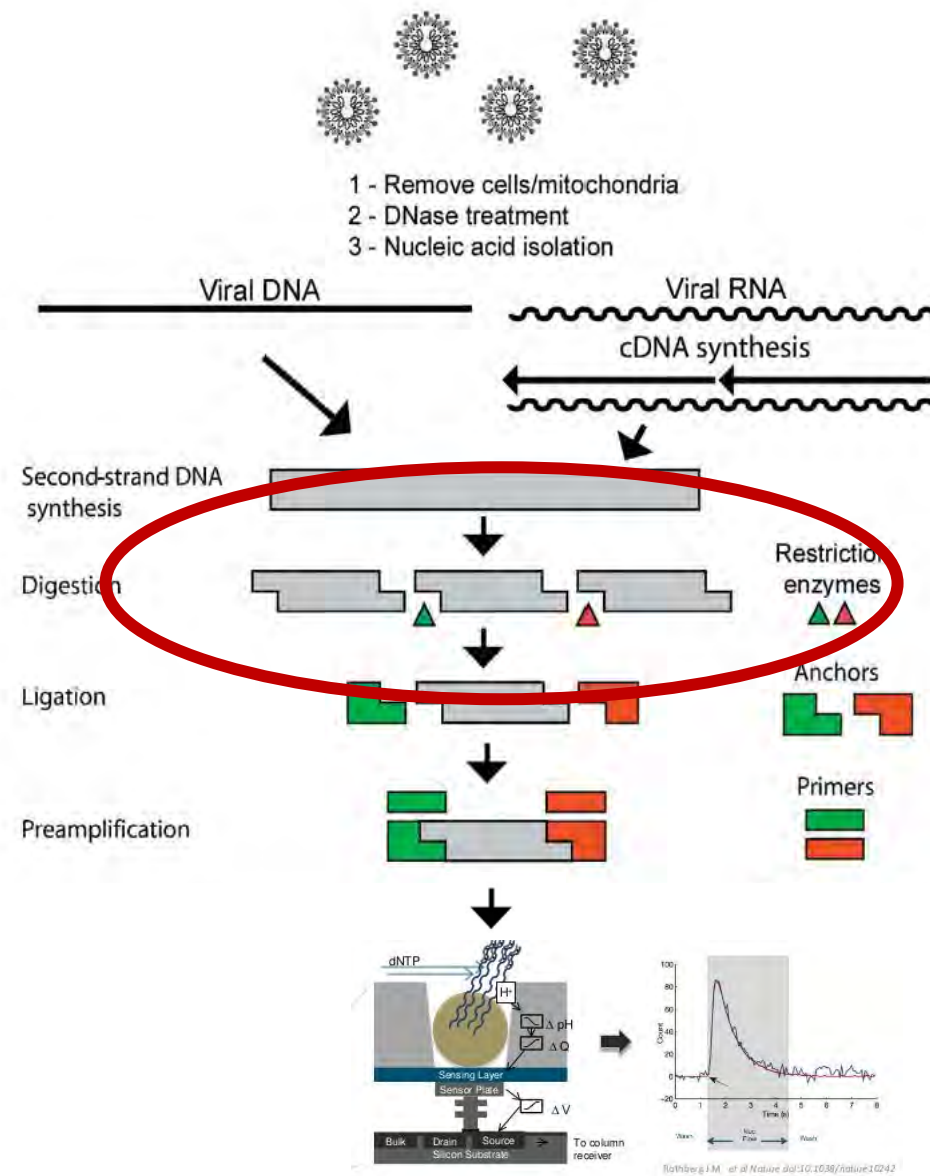
VIDISCA

Virus Discovery cDNA-AFLP

Main characteristics:

Restriction enzyme digestion for fragmentation

VIDISCA scheme

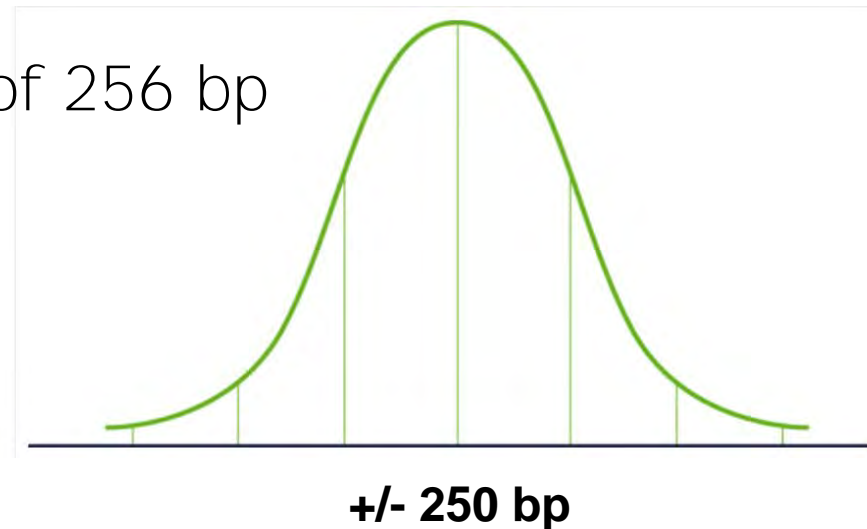


Ion Torrent-S5



MSE1 recognizes a motif of only 4 nucleotides: TTAA

- 1 nt, in every 4 bp
- 2 nt, in every 16 bp
- 3 nt, in every 64 bp
- 4 nt recognition, in every piece of 256 bp





Continued activity of MSE1 digestion during ligation

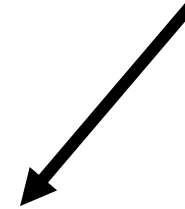
...GCGTACTTAAGCA...
...CGCATGAATTCGT...

...GCGTACTTAAGCA...
...CGCATGAATTCGT...


...GCGTACT PTAAGCA...
...CGCATGAAT_P TCGT...

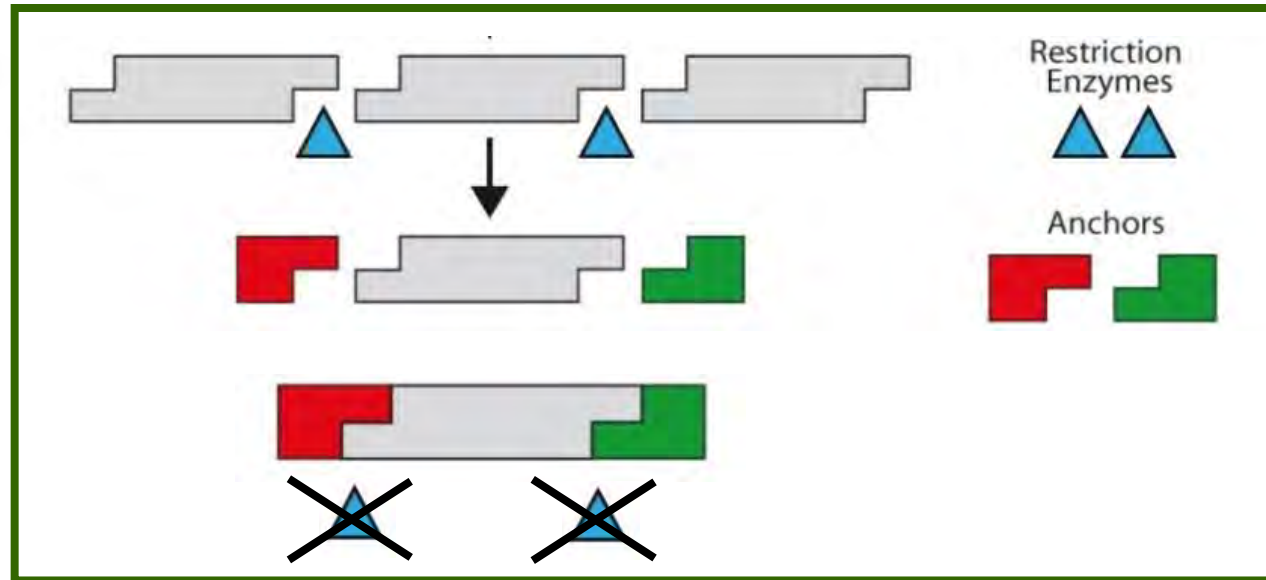
TATCGTTGGGCTAAGTCAAG
AGCAACCCGATTCAGTTC

Anchor



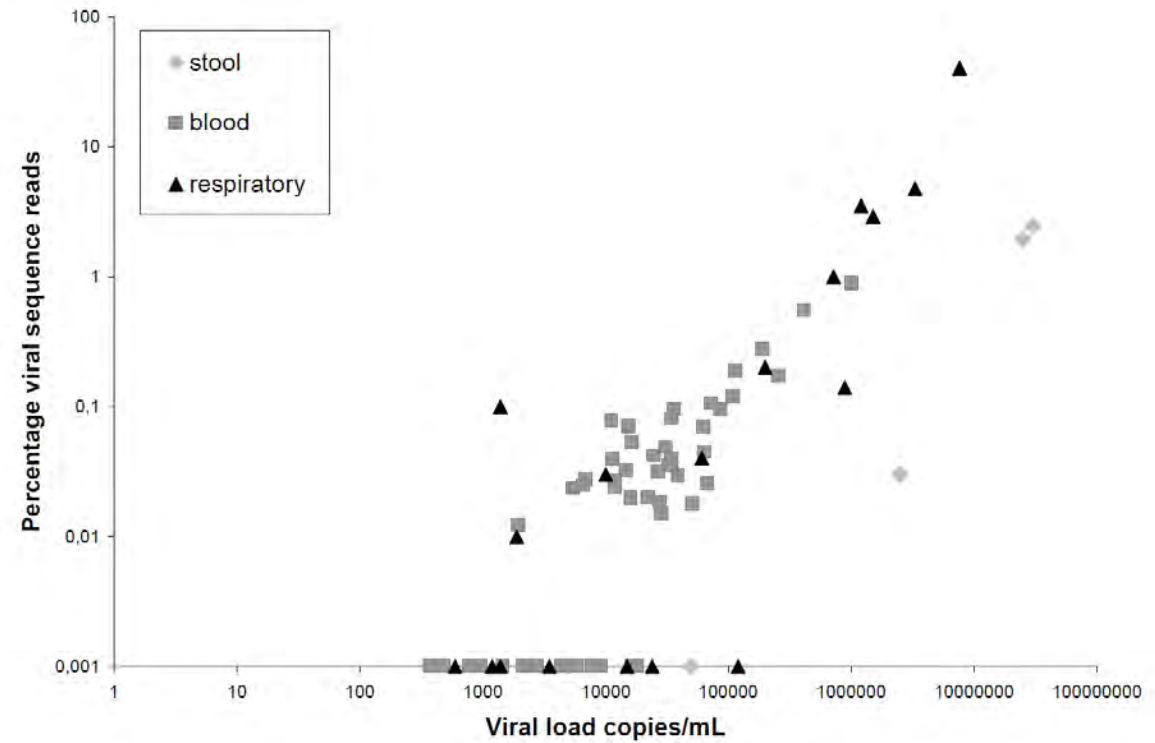
...GCGTACTTATCGTTGGGCTAAGTCAAG
...CGCATGAATAGCAACCCGATTCAGTTC

Loss of restriction site





High sensitivity



- **Concentrations $>10E4$ per mL:** Norovirus, HIV-1, HBV, HCV, PIV1, RSV, InfA, InfB, HCoV-NL63, HCoV-OC43, HRV, Adenovirus, enterovirus: **detected**
- **Only 10.000 – 50.000 sequence reads per sample needed**

New viruses



Human coronavirus NL63	(Nature Medicine 2004)
Human immunodeficiency virus novel subtype	(Aids res hum. Retrovir. 2007)
Bat parvoviruses Eh-BtPV-1 and Aj-BtPV-1	(PLoS One 2011)
Torque teno mini virus -13	(Virology Journal 2013)
Immunodeficiency associated stool virus	(BMC Infectious disease 2014)
Human gamma papillomavirus 126	(Frontiers in Microbiology 2014)
Husavirus	(Journal of General Virology 2015)
Rhinovirus C54	(Viruses 2015)
Slow loris parvovirus Sl.L-PV-1	(Frontiers in Microbiology 2015)
Scale drop disease virus (SDDV)	(PLoS Pathogens 2015)
Bastrovirus	(J. Mol Evol 2016)
Congenital tremor: porcine pestivirus	(Viruses 2016)
Novel flaviviruses 4x	(Archives Virology 2018)
Ntwetwe virus	(Clin. Inf. Disease 2018)
CRESS viruses (3X) and their hosts	(Nature Communications 2020)
Canine adenovirus	(J. Virol Methods 2012)
Anelloviruses	(Schizophrenia Research 2015)

New viruses



Human coronavirus NL63	(Nature Medicine 2004)
Human immunodeficiency virus novel subtype	(Aids res hum. Retrovir. 2007)
Bat parvoviruses Eh-BtPV-1 and Aj-BtPV-1	(PLoS One 2011)
Torque teno mini virus -13	(Virology Journal 2013)
Immunodeficiency associated stool virus	(BMC Infectious disease 2014)
Human gamma papillomavirus 126	(Frontiers in Microbiology 2014)
Husavirus	(Journal of General Virology 2015)
Rhinovirus C54	(Viruses 2015)
Slow loris parvovirus Sl.L-PV-1	(Frontiers in Microbiology 2015)
Scale drop disease virus (SDDV)	(PLoS Pathogens 2015)
Bastrovirus	(J. Mol Evol 2016)
Porcine pestivirus	(Viruses 2016)
Novel flaviviruses 4x	(Archives Virology 2018)
Ntwetwe virus	(Clin. Inf. Disease 2018)
CRESS viruses (3X) and their hosts	(Nature Communications 2020)
Canine adenovirus	(J. Virol Methods 2012)
Anelloviruses	(Schizophrenia Research 2015)



Congenital tremor type A-II
(CT-type A-II) in piglets



CONGENITAL TREMOR IN PIGLETS AT A FARM IN TANJUNG SEPAT. Michelle Fong Wai Cheng, Ooi Peck Toung, Noordin Mohamed Mustapha, Low Wei Hong, Chow Guo Hao. Malaysian Association of Veterinary Pathology Conference 2012.

Symptoms



- Only symptoms in newborns
- **First described by Kinsley: “Dancing Pigs”**
Veterinary Medicine, 17: 123. 1922
- Tremors of the head and limbs, reduced or absent during sleep
- Tremors diminish within weeks/month
- Not deadly, but difficulty to find a teat to suckle

Infectious?



- More often when gilts (1st time pregnancy) give birth
- Once given birth to an affected litter, the sow rarely gives birth to another litter with disease (protective immunity)




Outbreaks in the Netherlands

- Several pig farms followed
- Farm 1 several outbreaks
 - Early 2012: 48 of the 231 litters affected
 - During the peak: 85% of the litters affected
 - 26% mortality within litters

VIDISCA-NGS

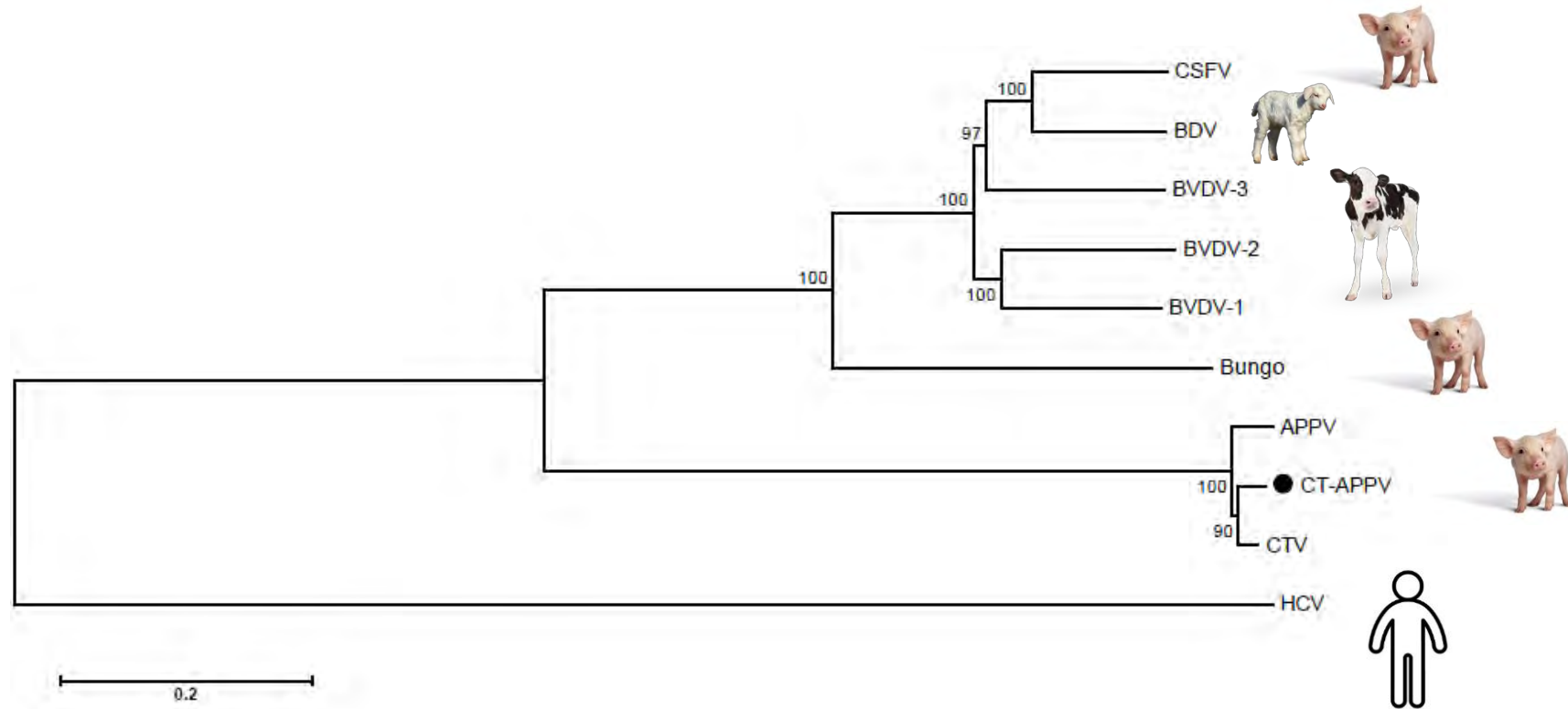


- serum of piglets with congenital tremor type A-II
 - In total 27.000 sequences, 30 sequence reads showed identity with pestiviruses
 - Full genome: novel pestivirus, 7800 nt, ssRNA
- 
- Design of a universal real time RT-PCR for screening
 - Several types of the virus

Disease	Farm	nr of samples	Date	Nr of samples CT-APPV PCR pos (strain number)	Type
CT-A-II*	1	6	15 March 2012	6 (CT-APPV-1)**	A
CT-A-II	1	5	05 April 2012	5 (CT-APPV-1)	A
CT-A-II	1	4	28 January 2013	4 (CT-APPV-1)	A
CT-A-II	1	3	05 March 2013	3 (CT-APPV-1)	A
CT-A-II	1	4	31 January 2014	4 (CT-APPV-1)	A
CT-A-II	1	3	12 February 2014	3 (CT-APPV-1)	A
CT-A-II	2	8	14 August 2013	8 (CT-APPV-2)**	C
CT-A-II	3	8	11 October 2013	8 (CT-APPV-3)	B
CT-A-II	4	5	31 May 2013	5 (CT-APPV-5)	B
CT-A-II	5	10	04 December 2013	10 (CT-APPV-6)	B
CT-A-II	6	15	08 January 2014	15 (CT-APPV-7)	C
CT-A-II	6	4	24 January 2014	4 (CT-APPV-7)	C
CT-A-II	7	4	06 March 2014	4 (CT-APPV-8)**	B
CT-A-II	8	4	12 February 2014	4 (CT-APPV-9)	C
CT-A-II	10	4	10 March 2016	4 (CT-APPV-11)	B
CT-A-II	11	12	25 February 2016	12 (CT-APPV-12)	A
CONTROLS	9	1	05 March 2013	0	
CONTROLS	9	6	18 December 2014	0	
No Symptoms	1	15	20 July 2012	1 (CT-APPV-1)	A
No Symptoms	3	8	11 October 2013	5 (CT-APPV-4)	C
No Symptoms	1	36	08 April 2013	0	

* CT-A-II = congenital tremor type A-II; ** strains of which the sequence is used as reference in type analysis.

Phylogeny



Is APPV causing the disease?



Is there association with disease?

- Clinical samples (serum) piglets with disease: n= 99 samples.
- Clinical samples (serum) of controls: n=66

% positive of sick animals: 100%

% positive of healthy controls: 9%

Is APPV causing the disease?



Can we reproduce the symptoms with experimental infection?

Test *In vivo*:

- Three pregnant gilts intramuscular inoculated with serum (0.22 micron filtrated), day 32 of gestation
- All three infected by the virus at 10 days post inoculation
 - Gilt 49: 5.8×10^2 RNA copies/mL serum
 - Gilt 50: 1.4×10^4 RNA copies/mL serum
 - Gilt 51: 2.3×10^4 RNA copies/mL serum

Is APPV causing the disease?



Can we reproduce the symptoms with experimental infection?

Test *In vivo*:

- Three pregnant gilts intramuscular inoculated with serum (0.22 micron filtrated), day 32 of gestation
- All three infected by the virus at 10 days post inoculation
 - Gilt 49: 5.8×10^2 RNA copies/mL serum
 - Gilt 50: 1.4×10^4 RNA copies/mL serum
 - Gilt 51: 2.3×10^4 RNA copies/mL serum



Litters born from infected gilts

- Gilt 49, 13 piglets, no symptoms, all negative for the virus
- Gilt 50, 13 piglets, 11 with symptoms, 11 positive for the virus
- Gilt 51, 15 piglets, 13 with symptoms, all positive for the virus



Litters born from infected gilts

- Gilt 49, 13 piglets, no symptoms, all negative for the virus
- Gilt 50, 13 piglets, 11 positive for the virus: 11 with symptoms
- Gilt 51, 15 piglets, all positive for the virus: 13 with symptoms

Conclusions



- Infection during pregnancy followed by trans-placental transmission of the virus is the most likely cause of the disease
 - Ad de Groof et al Viruses 2016 Oct 4;8(10):271. doi: 10.3390/v8100271.
- Could diagnostics be useful to make and/or keep a farm virus-free?

Conclusions



- Infection during pregnancy followed by trans-placental transmission of the virus is the most likely cause of the disease
- Could diagnostics be useful to make and/or keep a farm virus-free?



viruses



Article

Atypical Porcine Pestivirus Circulation and Molecular Evolution within an Affected Swine Herd

Alba Folgueiras-González ^{1,2} , Robin van den Braak ¹ , Bartjan Simmelink ¹, Martin Deijs ²,
Lia van der Hoek ²  and Ad de Groof ^{1,*} 

Viruses **2020**, *12*, 1080; doi:10.3390/v12101080

Conclusions



- Infection during pregnancy followed by trans-placental transmission of the virus is the most likely cause of the disease
- Could diagnostics be useful to make and/or keep a farm virus-free?
- Is vaccination the solution to free a farm from APPV?

HONOURS



This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 721367



Cormac Kinsella

Nadine van Kleef

Arthur W. Edridge

Joanna Kaczorowska

Nathalie van den Berkel

Martin Deijs

Maarten F. Jebbink

Michelle Klein

Margreet Bakker

Lia van der Hoek



Collaboration

- Amsterdam UMC (location AMC):

Martin Deijs, Laura van Os-Galdos, Lia van der Hoek

- MSD-Animal Health:

Alba Folgueiras-González, Robin van den Braak, Bartjan Simmelink, Lars Guelen, Lotte van Grinsven, Wannes Vogels, Carmen Derks, Toine Cruijssen, Victor Geurts, Mieke Vrijenhoek, Janneke Suijskens, Peter van Doorn, Leo van Leengoed, Paul Vermeij, Carla Schrier, Ad de Groof