BUNDESINSTITUT FÜR RISIKOBEWERTUNG

Bundesinstitut für Risikobewertung

# Emerging pathogens – foodborne viruses

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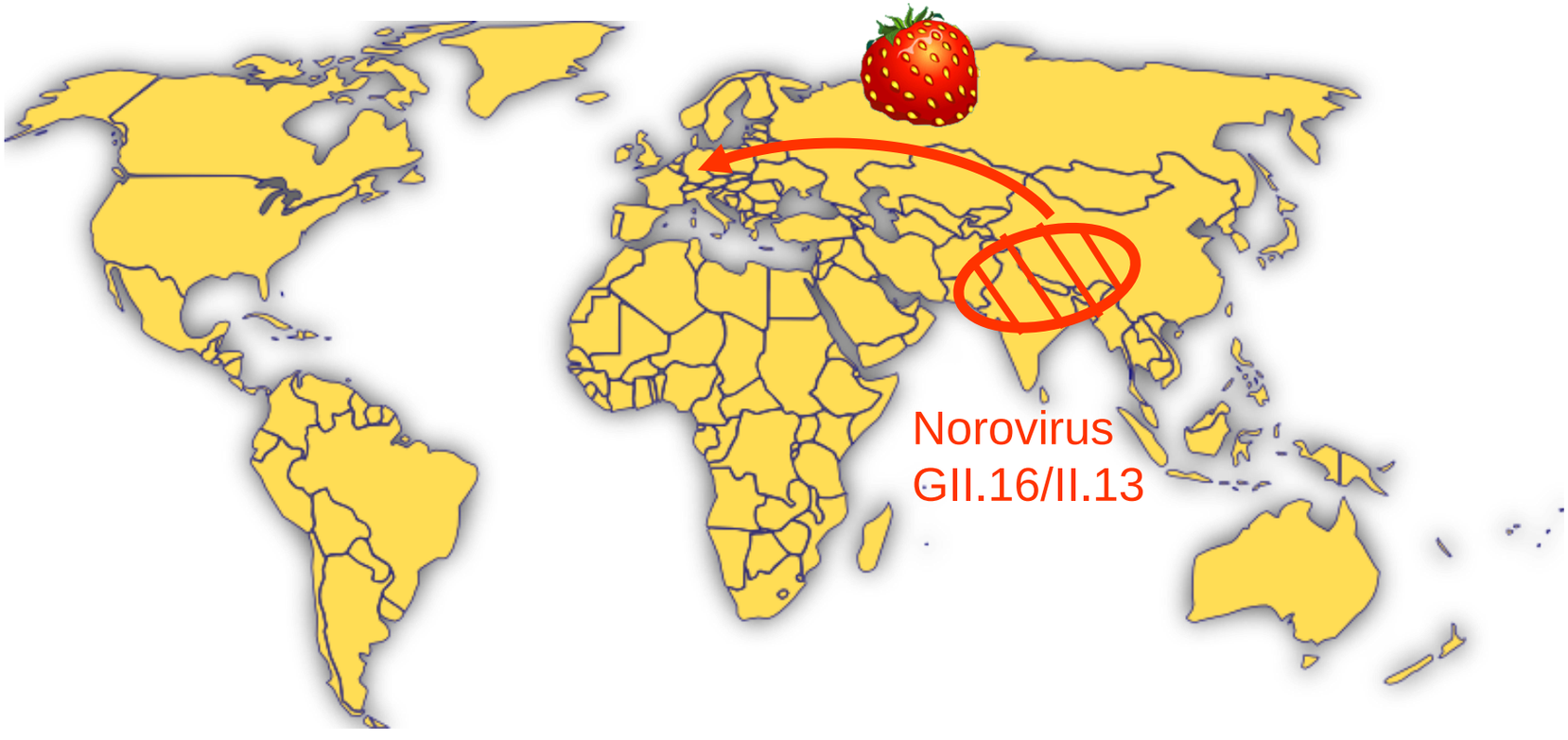
# Norovirus gastroenteritis outbreak in Germany 2012



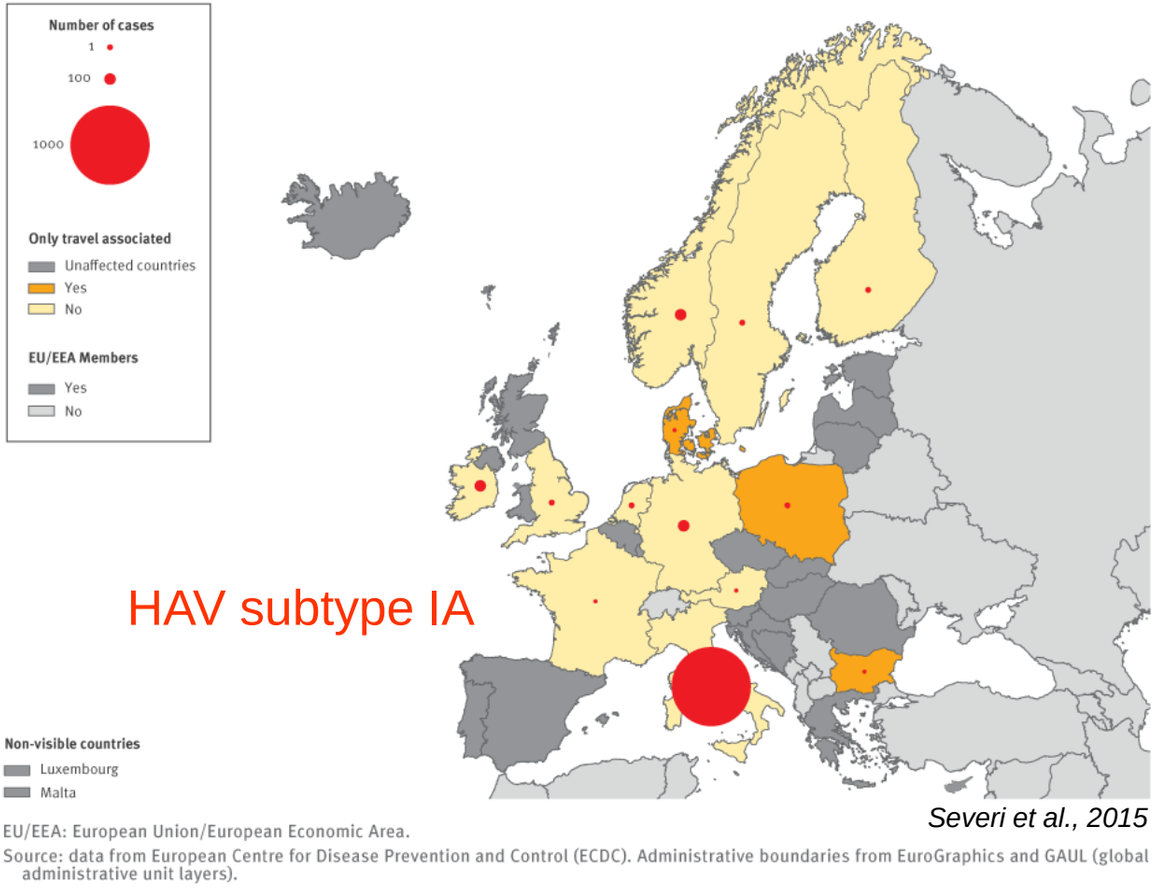

October/November 2012:

→**10.974 diseased children** after consumption of imported frozen strawberries

# Outbreak of hepatitis A in 13 EU countries 2013/2014

**FIGURE 1**

Hepatitis A cases by reporting country and cases' travel history, European Union/European Economic Area countries, 1 January 2013–31 August 2014 (n = 1,589)




HAV subtype IA

EU/EEA: European Union/European Economic Area.

Source: data from European Centre for Disease Prevention and Control (ECDC). Administrative boundaries from EuroGraphics and GAUL (global administrative unit layers).

*Severi et al., 2015*

January 2013 – August 2014:

**→1.589 patients (1.102 hopsitalized)**

after ingestion of frozen berry mix

# Hepatitis E cases in UK in 2017

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News

## 'Brexit virus' caused by EU sausages causes 60,000 Britons to fall ill annually

share 76

Could your full English give you Brexit virus? CREDIT: ABBIE TRAYLER-SMITH

*(The Telegraph, 22.05.2017)*

By Helena Horton

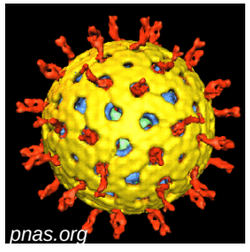
22 MAY 2017 • 9:41AM

Cases of a potentially deadly disease carried in sausages made with EU meat - the so-called 'Brexit virus' - are on the rise.

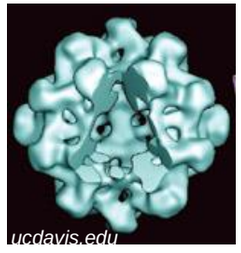
The strain of hepatitis E (HEV) has been linked to pig farms on the Continent after the tropical virus mutated to infect livestock.

Public Health England reported the number of severe cases has almost trebled since 2010, with 1,244 reported in 2016, compared with 368 six

# Important foodborne viruses


**Norovirus** **Rotavirus**

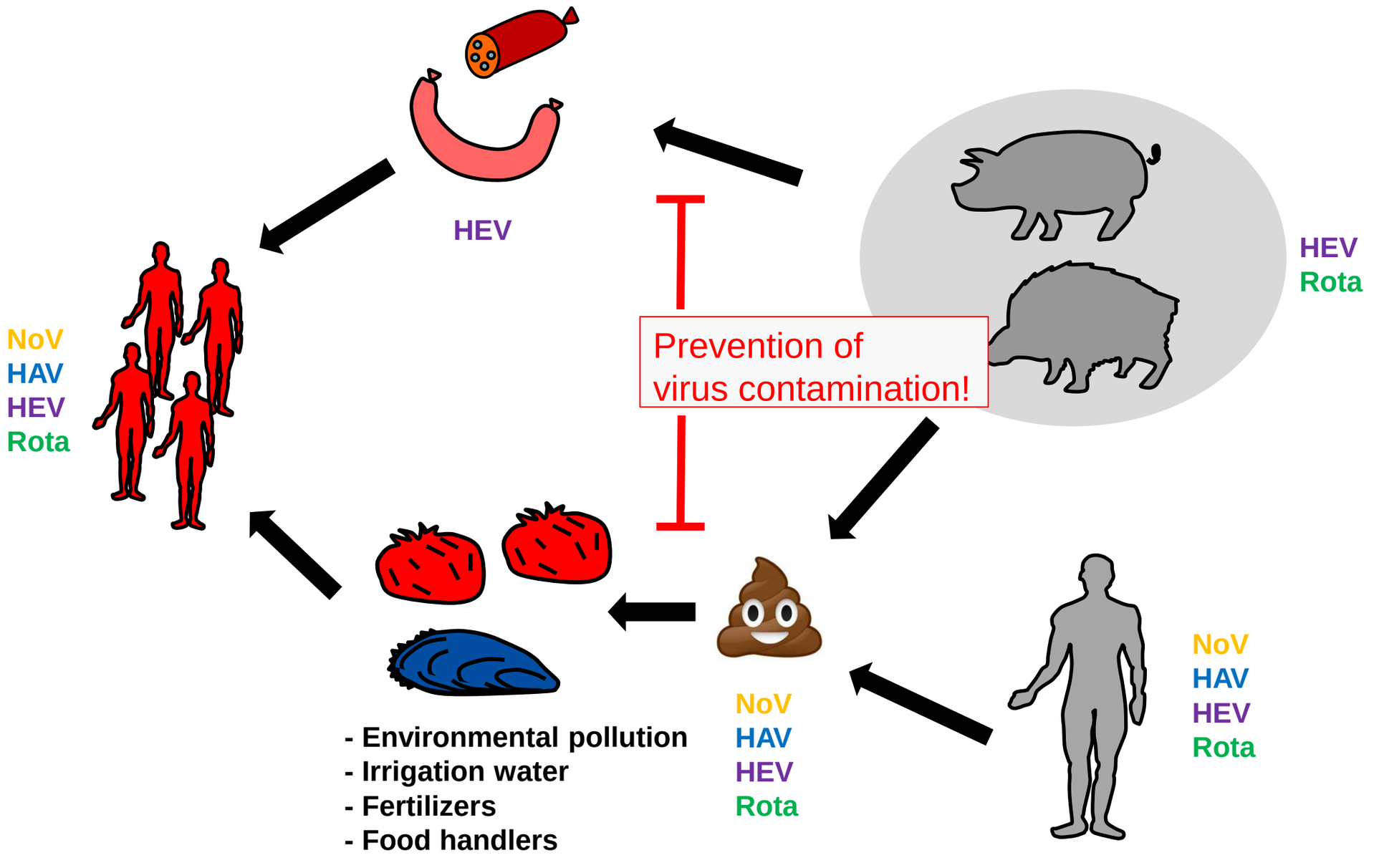




**Hepatitis A-Virus** **Hepatitis E-Virus**

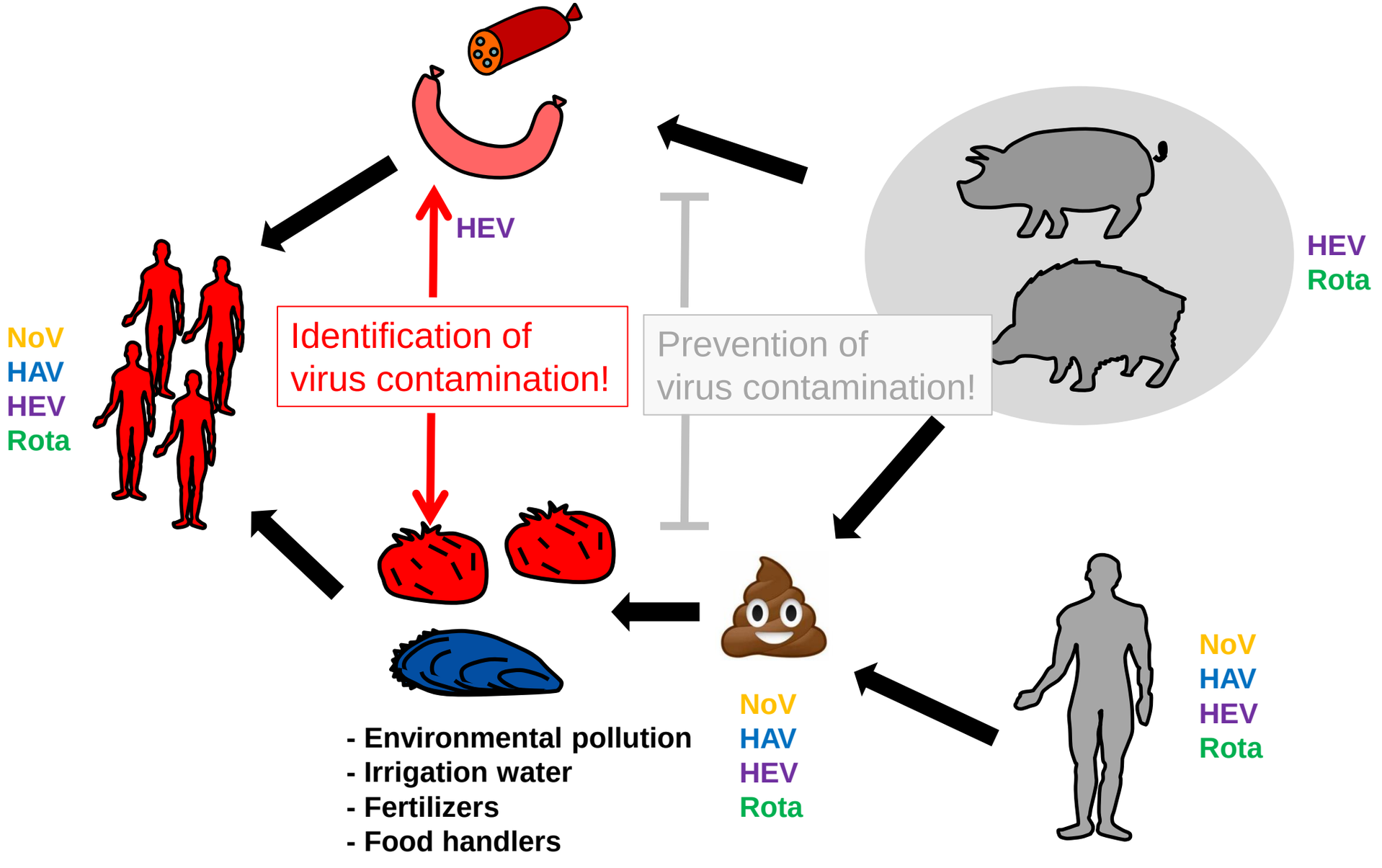

**Vomiting/ Diarrhoa**


**Virus-Hepatitis**

# Transmission of foodborne viruses



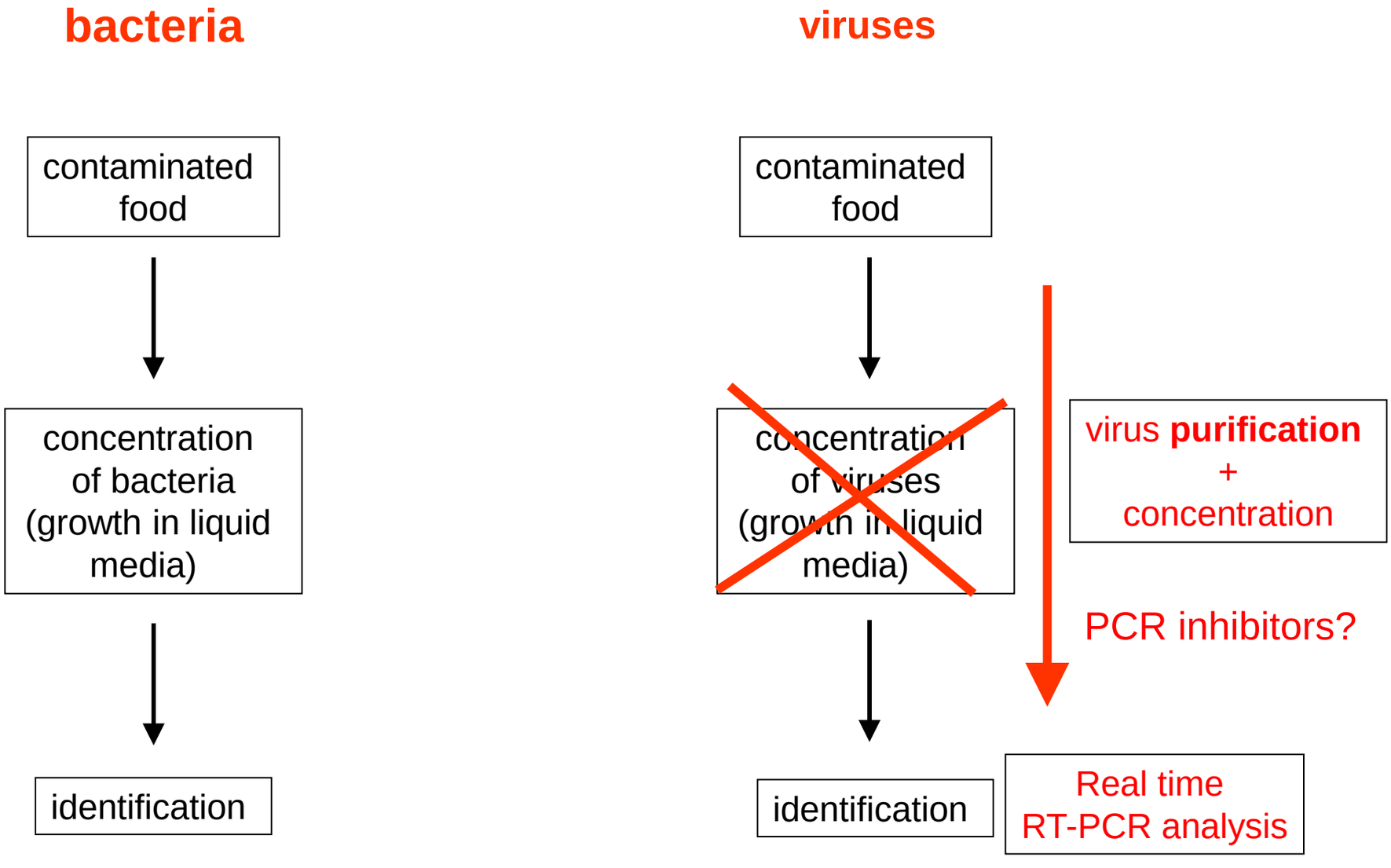
# Transmission of foodborne viruses



# Detection methods

# for viruses in food

# Detection of pathogens in food



# ISO 15216 for soft fruits

berry sample

Tris/Glycin/Beef Extract Buffer and Pectinase

adjust to pH 9.5

homogenize

adjust to pH 9.5

centrifugation 30 min 10.0000 x g

adjust to pH 7.0

**add PEG/NaCl**

stir 1 h at 4°C

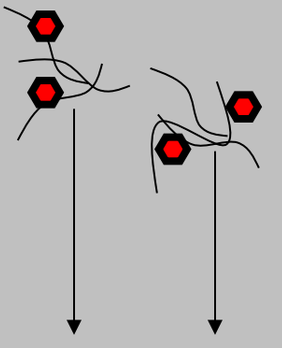
centrifugation 30 min 4°C

wash pellet with Chloroform/Butanol

RNA extraction

real-time RT-PCR

**PEG-Precipitation**



# Method comparison for NoV detection on strawberries

Artificially NoV-contaminated frozen strawberries

| Method | RR mean ± SD (%) | Reference |
|---|---|---|
| **ISO/TS 15216** | **1.71 ± 2.31** | **ISO (2014)** |
| **Ultrafiltration** | 0.98 ± 0.95 | Esseili et al. (2015) |
| **Direct lysis** | 0.52 ± 0.54 | Perrin et al. (2015) |
| **PGM magnetic Beads** | 0.04 ± 0.1 | Tian et al. (2008) |
| **TriReagent** | 0.01 ± 0.03 | Szabo et al. (2015) |

***RR*** *Recovery rate* | ***SD*** *Standard deviation*

→ ISO method most reliable
→ still low recovery rate

# ISO 15216 for soft fruits (expanded)

berry sample

↓

Tris/Glycin/Beef Extract Buffer and Pectinase

↓

adjust to pH 9.5

↓

homogenize

↓

adjust to pH 9.5

↓

centrifugation 30 min 10.0000 x g

↓

adjust to pH 7.0

↓

**add PEG/NaCl**

↓

stir 1 h at 4°C

↓

centrifugation 30 min 4°C

↓

wash pellet with Chloroform/Butanol

↓

RNA extraction

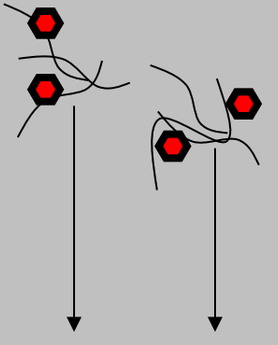
↓

Sephacryl column purification

↓

real-time RT-PCR

**PEG-Precipitation**



# Optimization of the ISO method using Sephacryl columns

| Batch | ISO method<br>NoV RR ± SD (%) | ISO method<br>+ Sephacryl columns<br>NoV RR ± SD (%) |
|---|---|---|
| **3** | 2.83 ± 2.92 | **15.28 ± 9.73** |
| **4** | 0.59 ± 0.49 | **5.60 ± 1.58** |

***NoV*** *Norovirus* | ***RR*** *Recovery rate* | ***SD*** *Standard deviation*

→Improved detection usiung of Sephacryl columns

# Analysis of frozen strawberry samples involved in the NoV outbreak in Germany 2012

| | ISO method NoV RR ± SD (%) | ISO method + Sephacryl columns NoV RR ± SD (%) |
|---|---|---|
| **Positive / samples tested** | 9 / 22 | **20 / 22** |
| **Detection rate in %** | 40.1 | **90.9** |

→ Higher detection rate using Sephacryl columns

# Next Generation Sequencing –

## Analysis of frozen strawberry samples involved in the NoV outbreak in Germany 2012

~ 29 million reads in total

| *Family* | *Species* | SkTax | FamTax | Tax | counts |
|---|---|---|---|---|---|
| *Rosaceae* | *Sanguisorba sitchensis* | 2759 | 3745 | 1037063 | 15346144 |
| *Hydrangeaceae* | *Jamesia americana* | 2759 | 23097 | 152292 | 2486369 |
| *Rosaceae* | *Fragaria vesca* | 2759 | 3745 | 57918 | 2355716 |
| *Saccharomycetaceae* | *Saccharomyces cerevisiae* | 2759 | 4893 | 4932 | 892091 |
| *Rhizopodaceae* | *Rhizopus stolonifer* | 2759 | 1344955 | 4846 | 595163 |

eukaryotes

| *Family* | *Species* | SkTax | FamTax | Tax | counts |
|---|---|---|---|---|---|
| *Erwiniaceae* | *Tatumella citrea* | 2 | 1903409 | 53336 | 3119912 |
| *Enterobacteriaceae* | *Salmonella enterica* | 2 | 543 | 28901 | 221477 |
| *Enterobacteriaceae* | *Kosakonia oryzae* | 2 | 543 | 497725 | 107219 |
| *Rhodanobacteraceae* | *Frateuria aurantia* | 2 | 1775411 | 81475 | 17074 |
| *Rhodanobacteraceae* | *uncultured Frateuria sp* | 2 | 1775411 | 390515 | 10321 |

bacteria

| *Family* | *Species* | SkTax | FamTax | Tax | counts |
|---|---|---|---|---|---|
| *Alphaflexiviridae* | *Strawberry mild yellow edge virus* | 10239 | 675064 | 12187 | 1208 |
| *Caulimoviridae* | *Strawberry vein banding virus* | 10239 | 186534 | 47903 | 292 |
| *Myoviridae* | *Escherichia phage ESCO13* | 10239 | 10662 | 1881104 | 218 |
| *Podoviridae* | *Pantoea virus Limelight* | 10239 | 10744 | 1985729 | 217 |
| *Myoviridae* | *Enterobacteria phage ECGD1* | 10239 | 10662 | 1784948 | 183 |

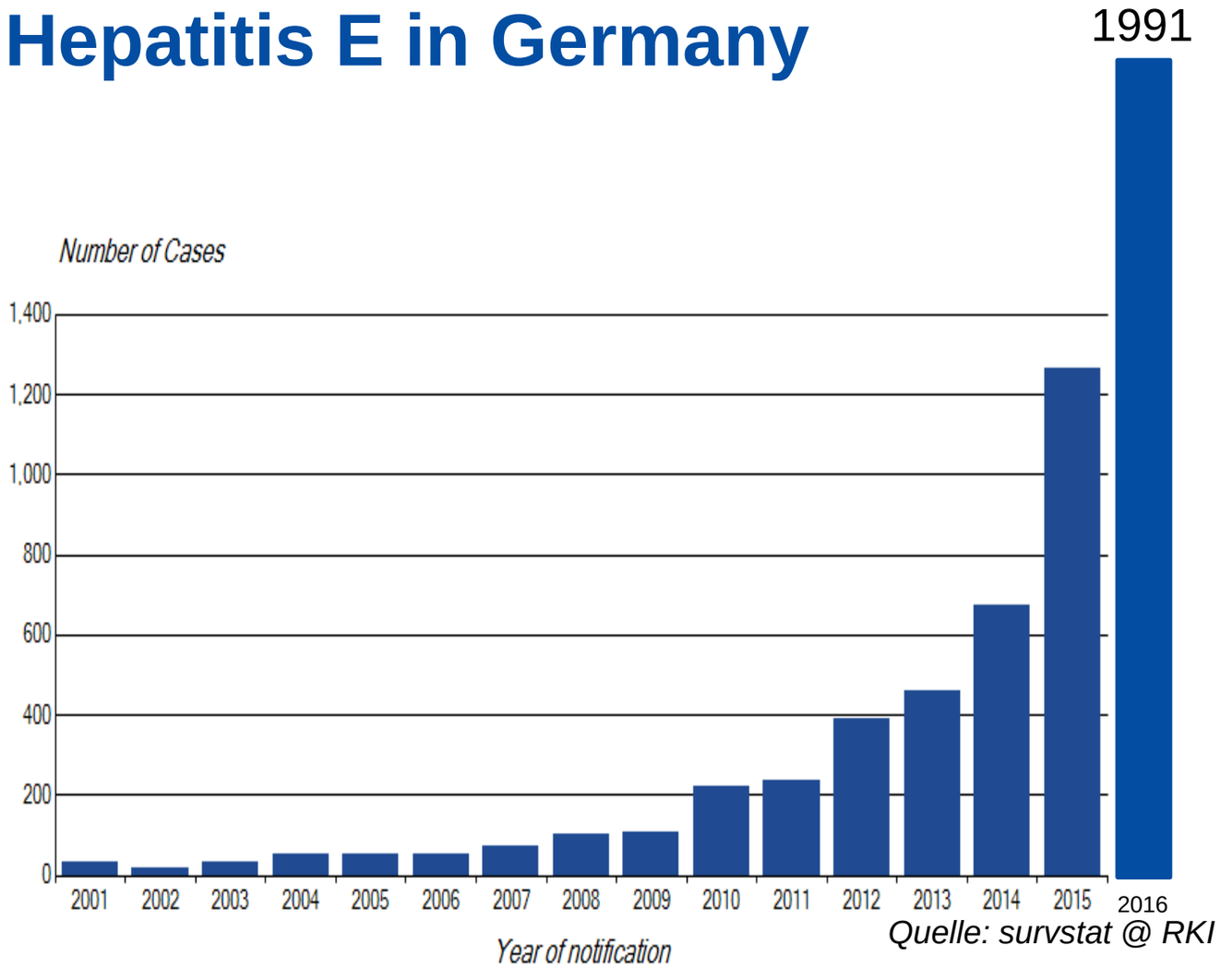
viruses

only 2 human norovirus reads (genotype II.16/II.13 = outbreak strain)

# Hepatitis E Virus

# (HEV)

# Hepatitis E in Germany



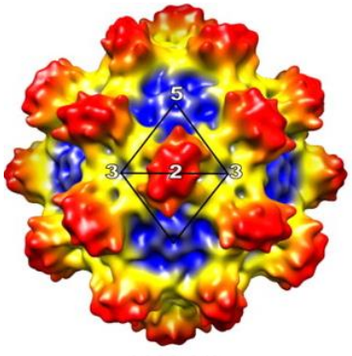

notified Hepatitis E cases in Germany

*Quelle: survstat @ RKI*

- Case/fatality rate <4%: Risk groups are
  - persons with underlaying liver disease
  - (- pregnant women)
  - immunosuppressed people (transplant patients)
- many subclinical cases (16.8 % seroprevalence in Germany)

# Hepatitis E Virus

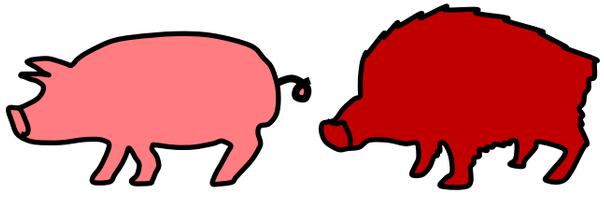
- Genotypes 1 – 4 with simliar disease course, but different transmission modes



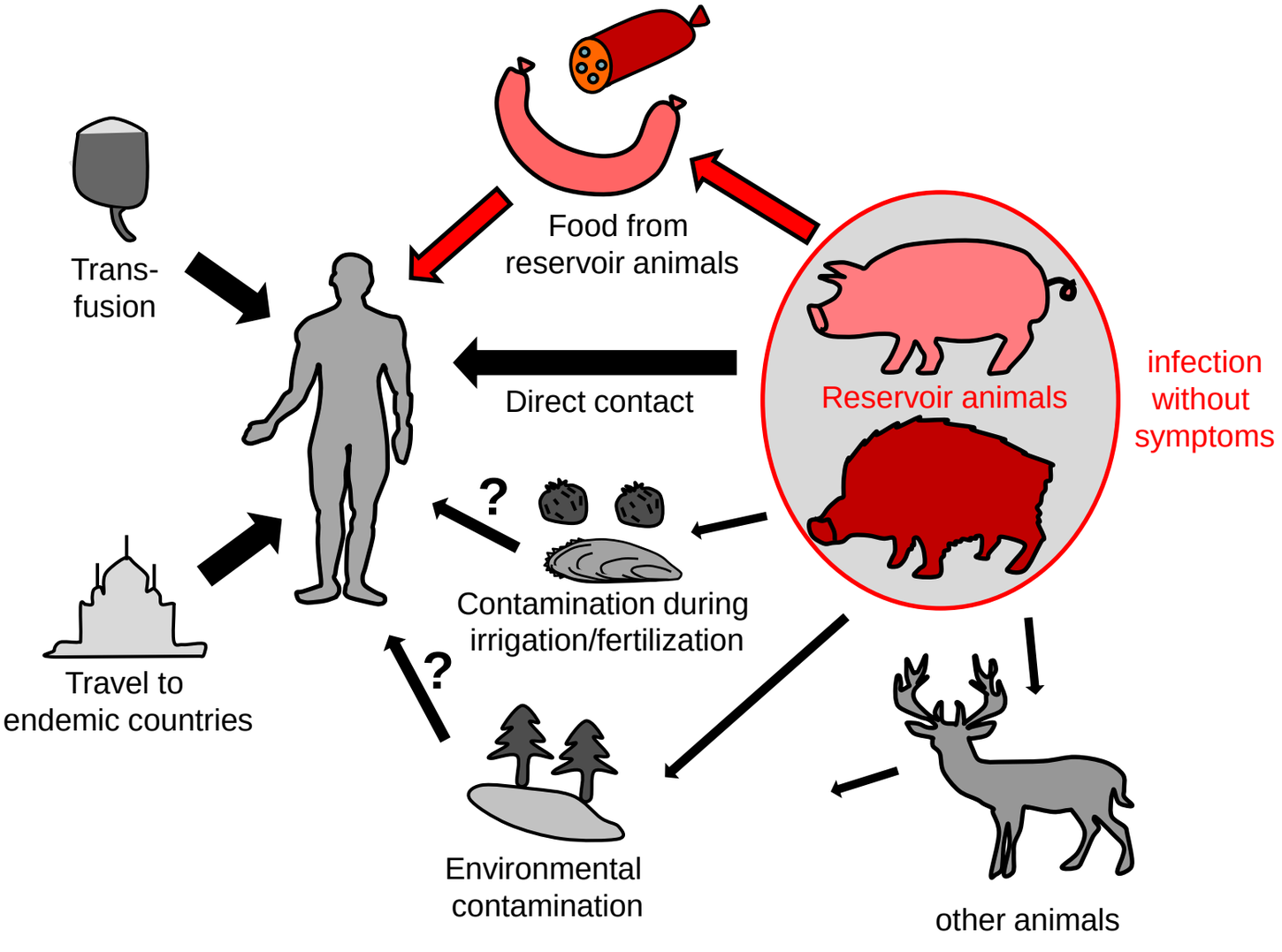
pnas.org

HEV

- Genotype 1: human
- Genotype 2: human
- Genotype 3: human, pig, wild boar, deer
- Genotype 4: human, pig



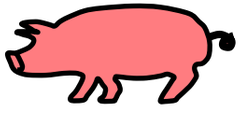
# Transmission pathways of HEV



# HEV in reservoir animals in Germany

**Wild boar:** 

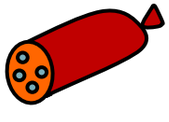
→ 29.9% (*Adlhoch et al., 2009*) to 33.0% (*Denzin er al., 2013)* antibody-positive

**Pig:** 

42.7% *(Dremsek et al., 2013)* to 49.8% *(Bächlein et al., 2010)* antibody-positive

**Pig liver:**

→ 4 % *(Wenzel et al., 2011)* HEV RNA-positive

**Raw and liver sausage:** 

→ 20.0% - 22.0% *(Szabo et al., 2015)* HEV RNA-positive

**Table 1 Summary of reports on detection of HEV RNA in liver, meat and meat products from animals intended for human consumption**

| Animal species | Organ | Geographical area/Country | Detection rate | RNA log copies/g | References |
|---|---|---|---|---|---|
| Pig | Liver | Brazil | 2/118 (2%) | | [115] |
| | | Burkina Faso | 1/157 (1%) | | [116] |
| | | Cameroon | 3/345 (1%) | | [117] |
| | | Canada | 2/19 (10%) | 1.3–1.6 | [90] |
| | | Canada | 25/283 (9%) | 3–6.7 | [118] |
| | | Canada | 9/43 (21%) | 3–7 | [25] |
| | | China | 4/114 (4%) | | [119] |
| | | Czech Republic | 2/40 (5%) | | [26] |
| | | France (Corsica) | 2/24 (8%) | | [55] |
| | | France | 128/3715 (4%) | | [32] |
| | | Germany | 8/200 (4%) | | [120] |
| | | Hong Kong | 7/479 (2%) | | [121] |
| | | India | 2/240 (1%) | | [122] |
| | | Italy | 2/33 (6%) | | [26] |
| | | Japan | 12/243 (4.9%) | | [123] |
| | | Japan | 0/110 (0%) | | [124] |
| | | Japan | 4/390 (1%) | | [125] |
| | | Mexico | 26/127 (20%) | | [126] |
| | | Spain | 1/39 (3%) | | [26] |
| | | Thailand | 3/1090 (1%) | | [127] |
| | | The Netherlands | 4/62 (6%) | | [98] |
| | | United Kingdom | 1/40 (3%) | | [92] |
| | | USA | 14/127 (11%) | | [97] |
| | Meat (muscle) | Canada | 0/599 (0%) | | [118] |
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| | | Thailand | 2/559 (1%) | | [127] |
| | | United Kingdom | 0/40 (0%) | | [92] |
| | Sausages (and other products) containing liver | Canada | 36/76 (47%) | 0.6–2.7 | [90] |
| | | France | 68/394 (17.3%) | 2.2–6.3 | [87] |
| | | France | 22/70 (31%) | 1.6–6.2 | [91] |
| | | Germany | 11/50 (22%) | | [89] |
| | | Italy | 11/68 (16%) | 3.4–5.3 | [88] |

*From:*
*Pavio, …, Johne:*
*Vet. Res. 2017, 48:78*

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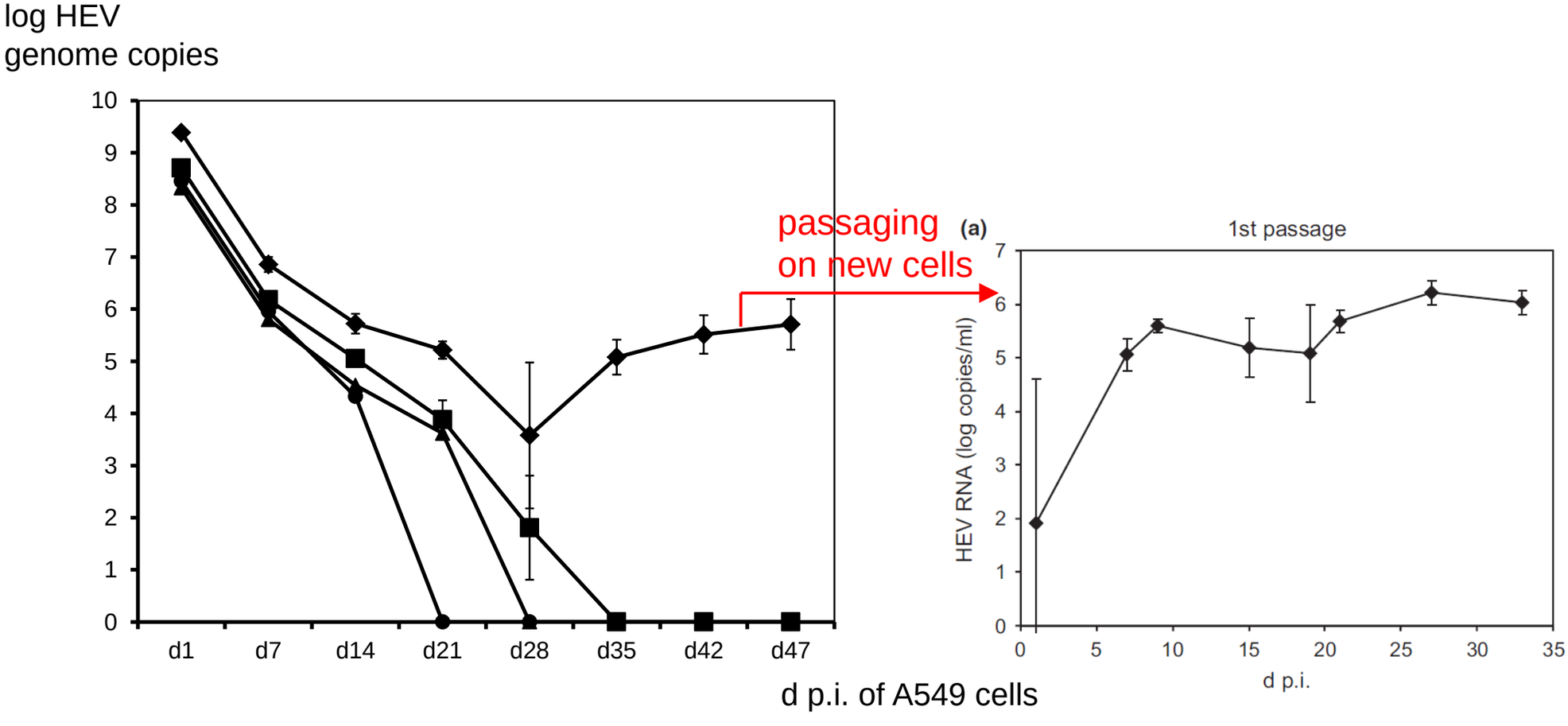
→ Is the virus still infectious in food?

*From:*
*Pavio, …, Johne:*
*Vet. Res. 2017, 48:78*



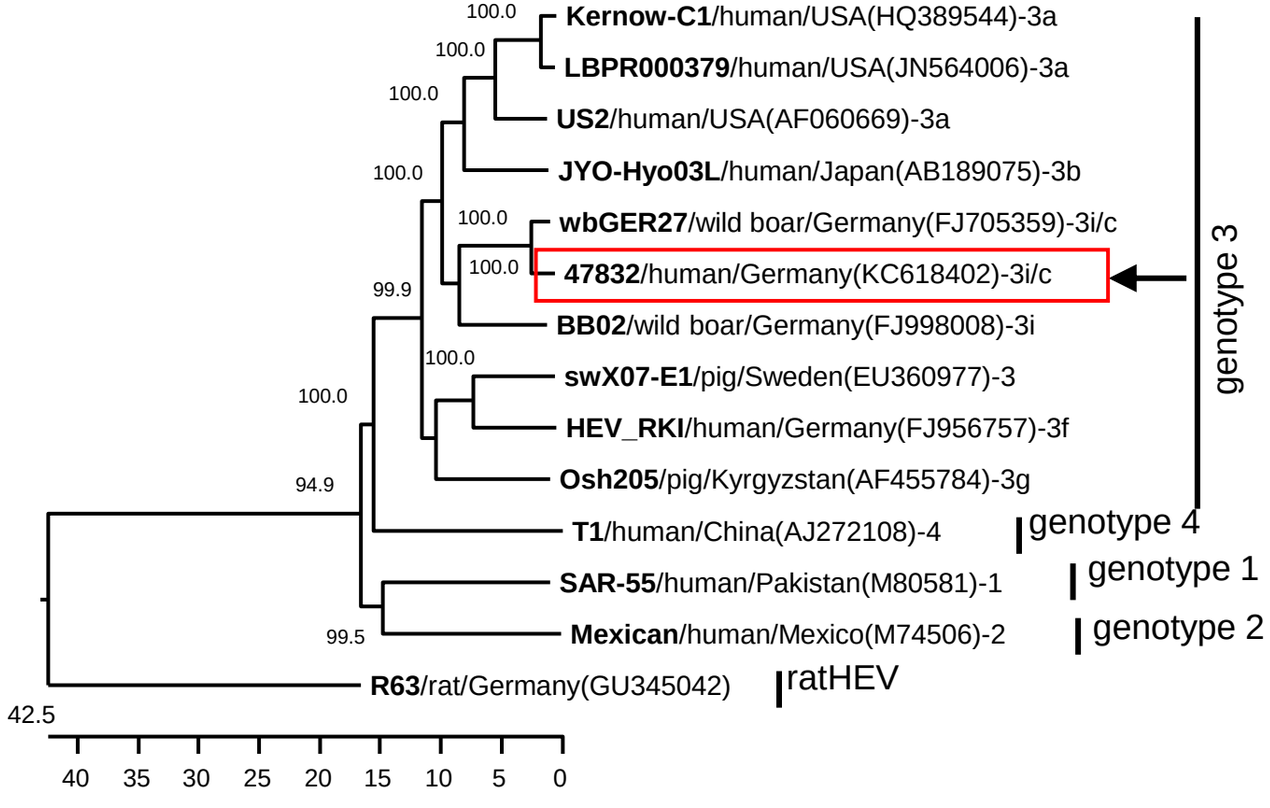
BfR

# HEV cell culture – isolation of strains

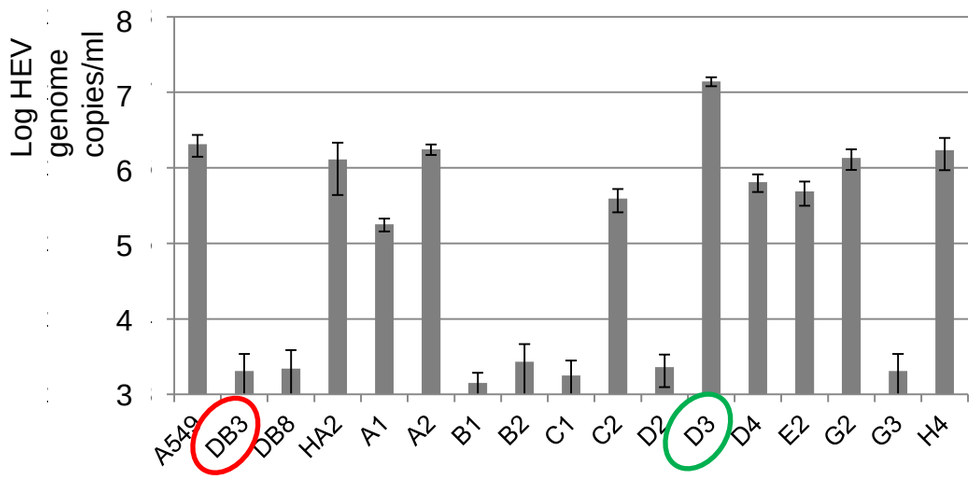


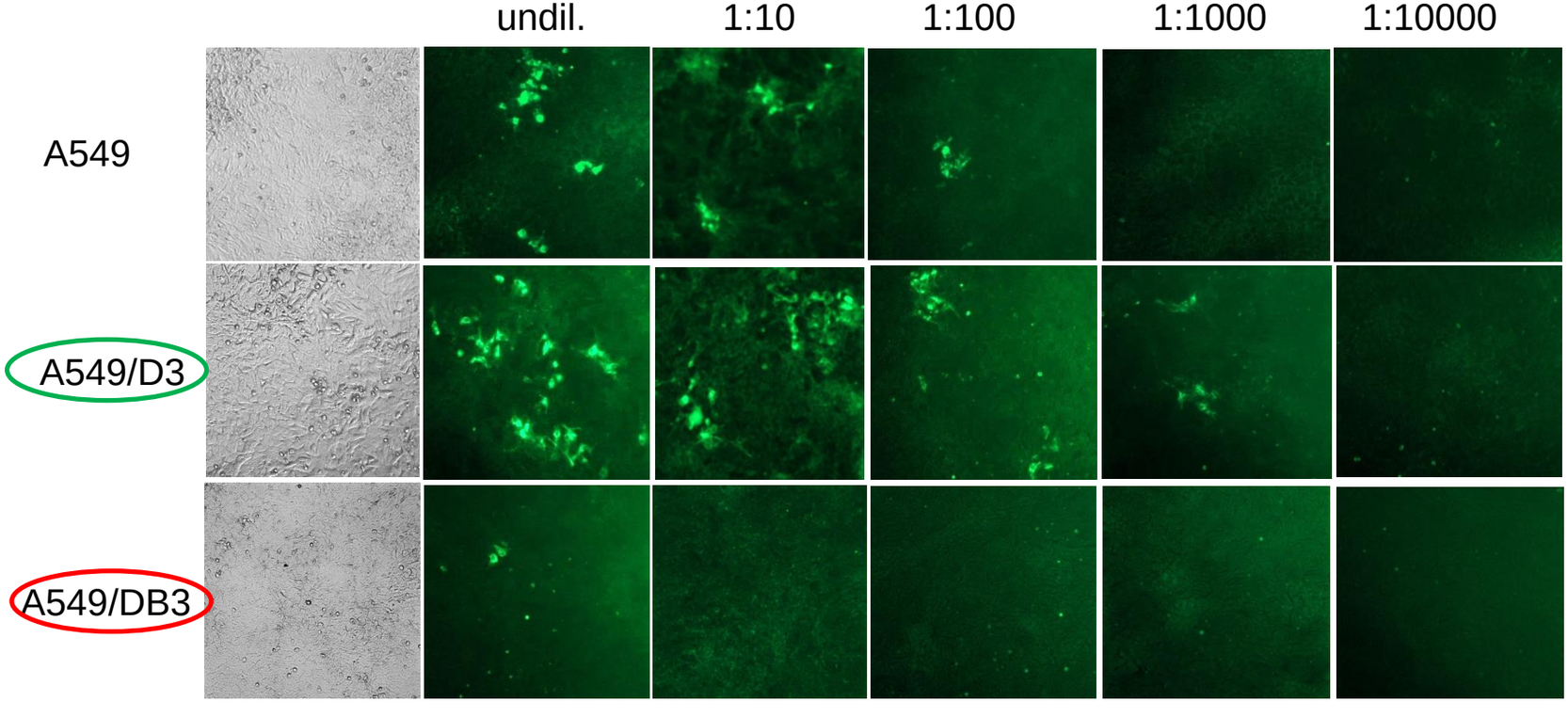
# Genome analysis of the isolated HEV strain 47832

→ genotype 3, related to HEV strains of humans and wild boars from Germany, contains an unusual insertion in ist ORF 1



# Selection of cells for HEV strain 47832

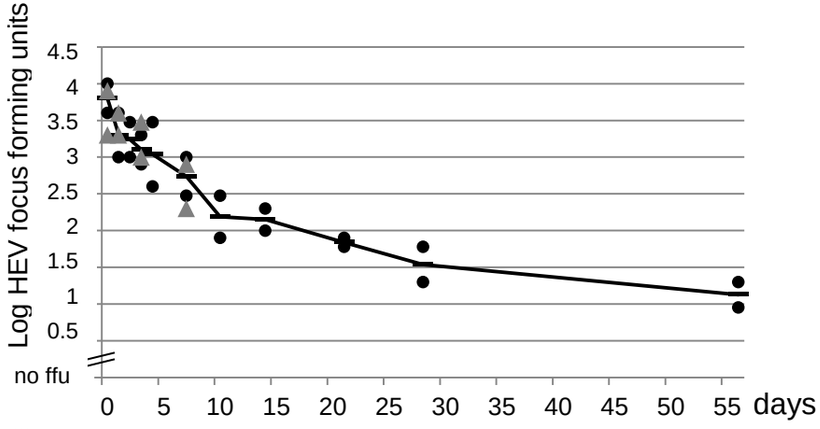


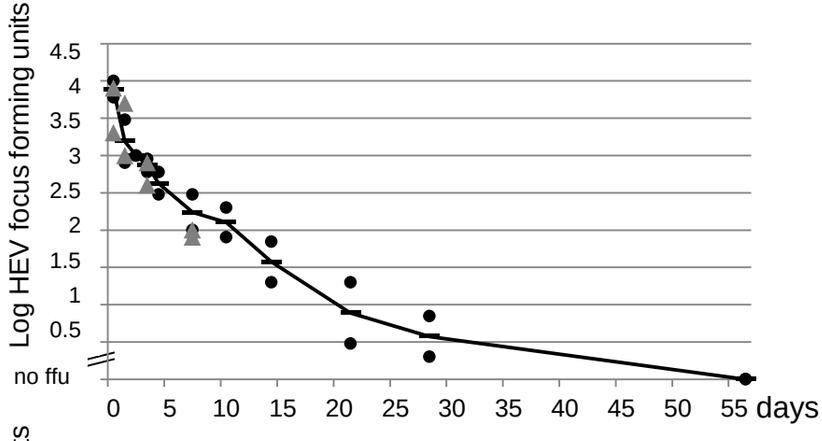

*(Schemmerer et al., 2016)*

# Testing of long-term stability of HEV

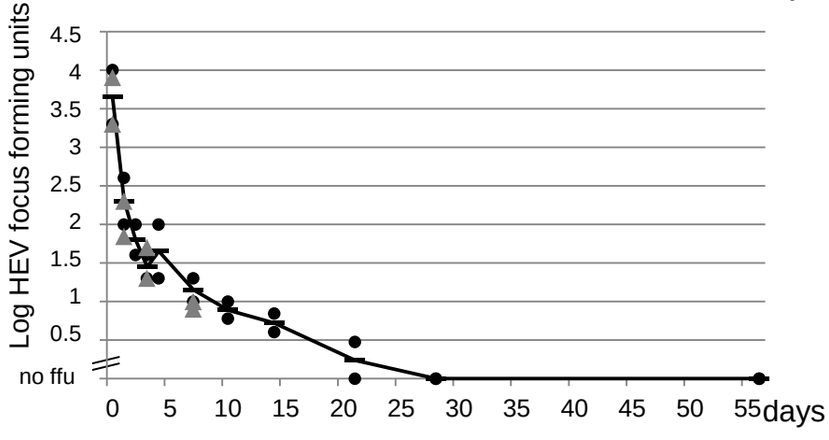
4°C




room temp.

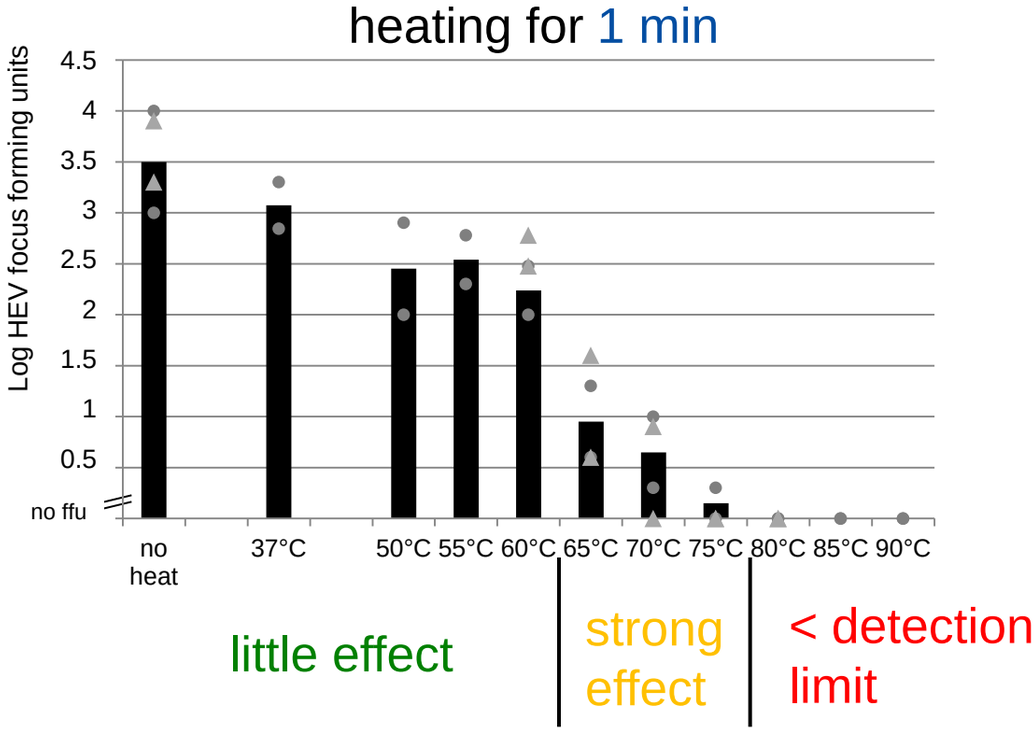


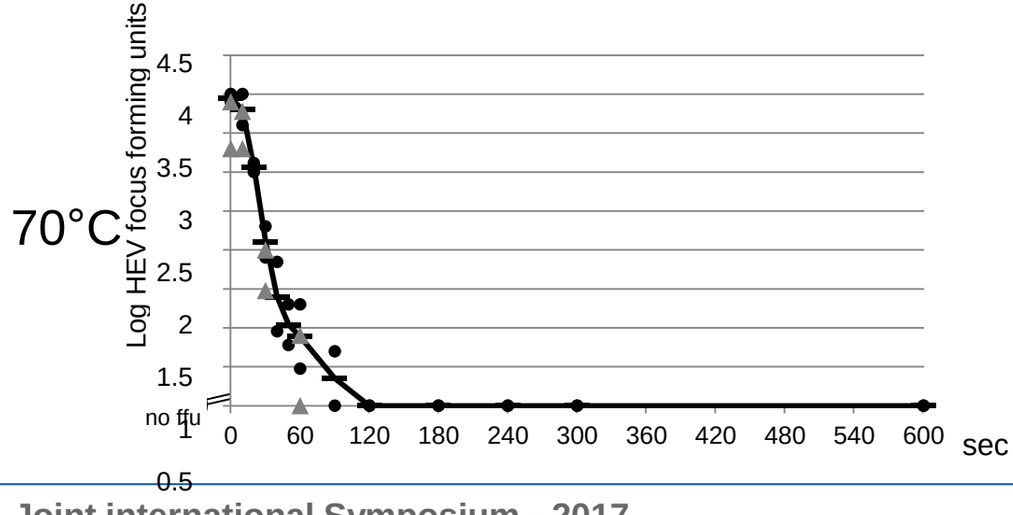

37°C




*(Johne et al., 2016)*

# Testing of short-term heating




*(Johne et al., 2016)*

# Summary

- Foodborne viruses have caused large disease outbreaks during the last years.

- Norovirus and HAV are mainly transmitted by contamination of food with human excretions.

- Detection of viruses in food is complicated by the absence of cell culture techniques and the presence of PCR inhibitors

- Hepatitis E poses an increasing problem in industrialized countries

- Pigs and wild boars are the main reservoirs for HEV

- Infectivity of HEV in meat products remains mostly unknown

- A novel cell culture model can be used for estimation of HEV inactivation under specific physico/chemical conditions

# Acknowledgements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