

COllaborative Management Platform for
detection and Analyses of (Re-) emerging
and foodborne outbreaks in Europe

The merging of fields through NGS applications in emerging infectious diseases

A global platform for the sequence-based rapid identification of pathogens

Prof. Frank M. Aarestrup, coordinator, microbiology (Technical University of Denmark)
Prof. Marion Koopmans, deputy coordinator, virology (Erasmus Medical Center, the Netherlands)



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research and innovation programme under grant agreement No 643476.
research and innovation programme under grant agreement No 643



Infectious diseases in the current era

- Dynamics of common infectious diseases are changing
 - Demographic change, population density, anti vaccine, AMR, etc.
- New diseases emerge frequently
 - Deforestation, population growth, health system inequalities, travel, trade, climate change
- Effects are difficult to predict due to complexity of problems
 - Rapid flexible response
- Public health and clinical response depend on global capacity for disease surveillance
 - Rapid sharing, comparison and analysis of data from multiple sources and using multiple methodologies

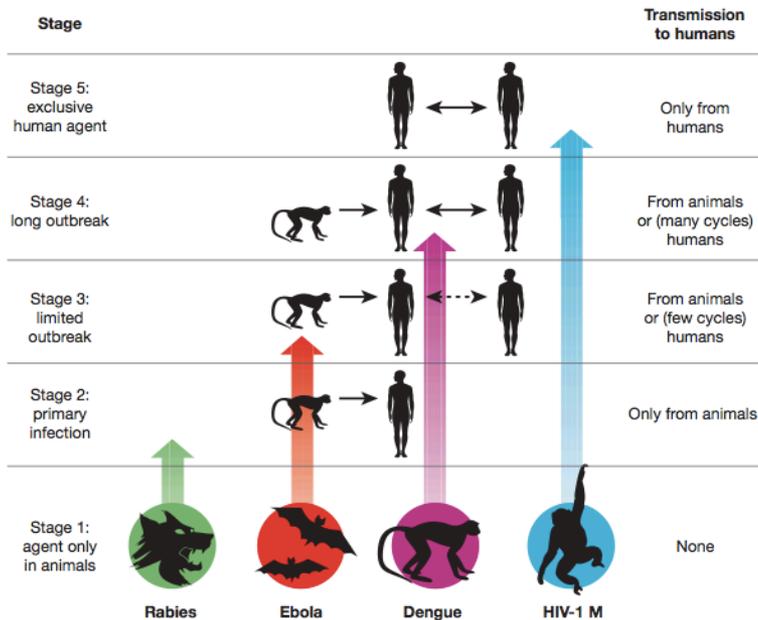
Drivers of EID emergence

- **CHANGE** in demographics of humans and animals (size, age profile, health status)
- **CHANGE** in political landscape (civil unrest, wars, migration)
- **CHANGE** in behavior (sexual behavior, tourism, food habits)
- **CHANGE** in pathogen behavior (new viruses, antimicrobial resistance, evolution)
- **CHANGE** in technology (implants, increased survival, transplantation)
- **CHANGE** in climate
-

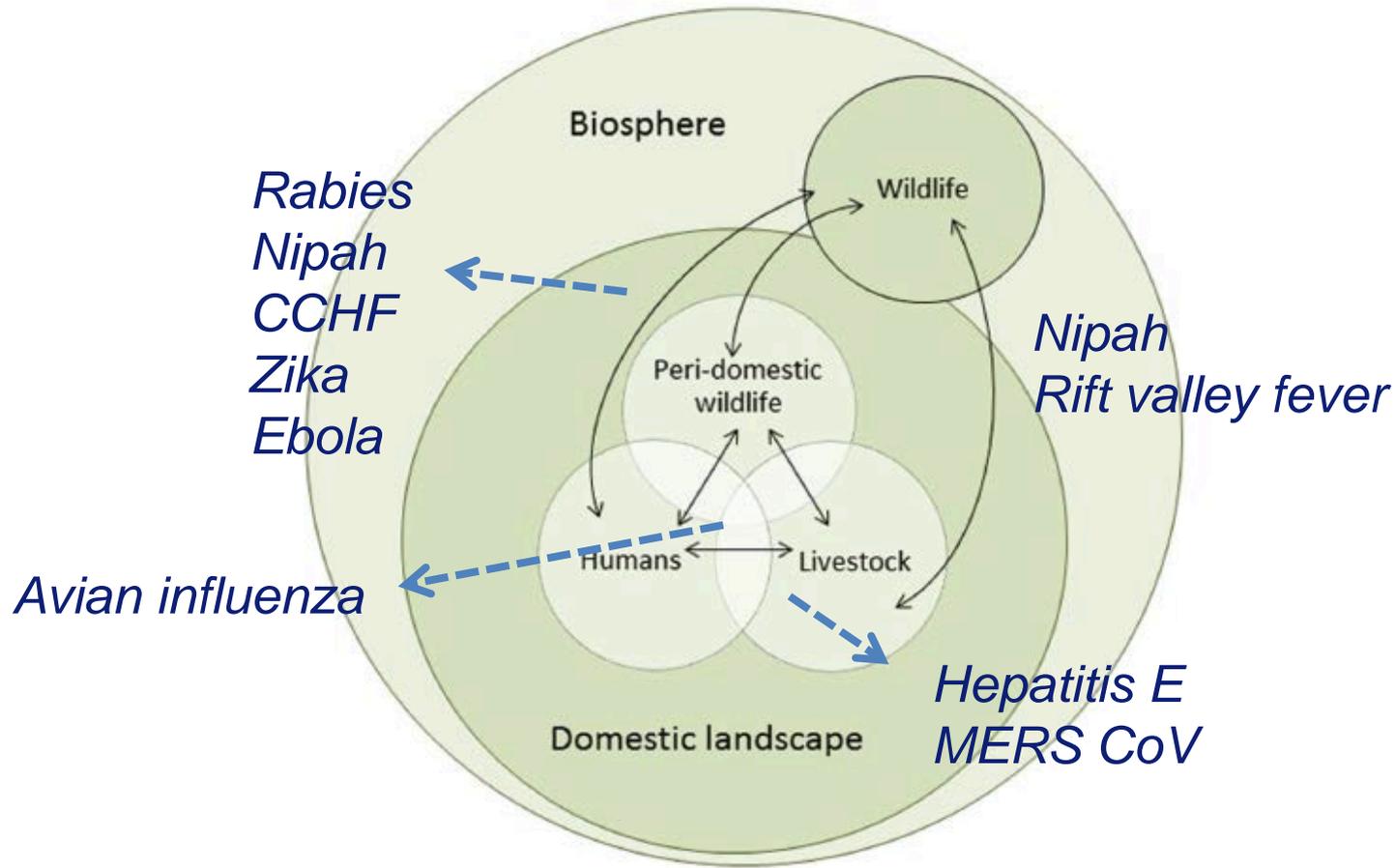
2 main aspects

- Increased likelihood of new pathogens
- Zoonotic diseases!

- Increased ability for transmission

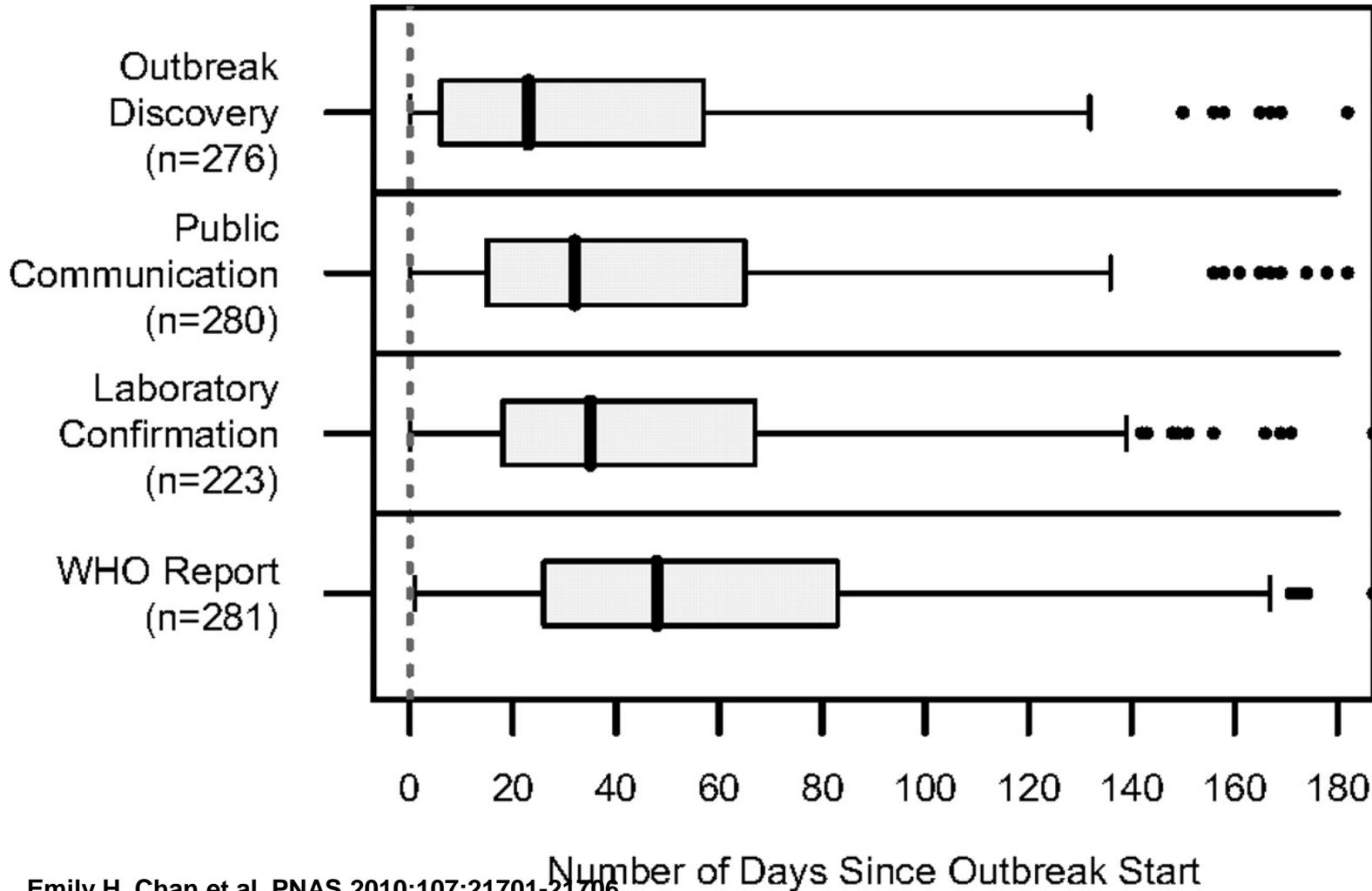


EID require multidisciplinary research



Current performance

late detection, human disease first



Problems in EID detection

- Inequality in health care systems
- Budget cuts in healthcare
- Clinical diagnostic priorities
- Clinician knowledge of zoonoses, emerging infections, exotic infections
- Privacy laws
- Prediction is difficult



Priority research questions EID after disease emergence

Clinic/public health

- How to diagnose/rule out?
- How to track?
- When are hosts infectious?
- Modes of transmission
- Sources of infection
- Role of co-infections
- Risk factors
- Potential non-medical interventions/prevention

Basic

- Comparison with other viruses in family
- Virulence traits
- Evolution after species jump
- Pathogenesis
- Immunity/cross protection/enhancement
- Potential antivirals
- Potential vaccines

Priority research questions EID before disease emergence

Clinic/public health

1. Which discoveries will be relevant?
2. What to prepare for?
3. Who are at risk?
4. How to predict emergence, virulence and spread?
5. Is intervention/prevention needed?

Basic

1. What are drivers of emergence
2. Is this a pathogen?
3. Can health risks be predicted from genomics, structure, in vitro studies?
4. What is the role of cross immunity in emerging viral infections?
5. how can disease emergence be influenced?

Priority research questions EID during disease emergence

- How to diagnose/rule out?
- Which to track? What are drivers per emergence with other relevant? discoveries with similar cases and how can
- How to predict transmission? When are hosts likely to be infected? Is it used in family
- Modes of transmission? Sources of virulence and of sources of
- What to prepare? Role of
- Who are at risk? Risk factors
- Is intervention needed? Potential interventions



COMPARE: developing an enabling system and tools for collaborative preparedness and outbreak research

- Collaboration between “users” of NGS and bio-informaticians
- Develop validated workflows for top 5 questions in disease preparedness research and outbreak research
- Use datasharing hubs and building from existing infrastructure
- sector-, domain- and pathogen-independent
- Flexible, scalable and open-source based information-sharing platforms

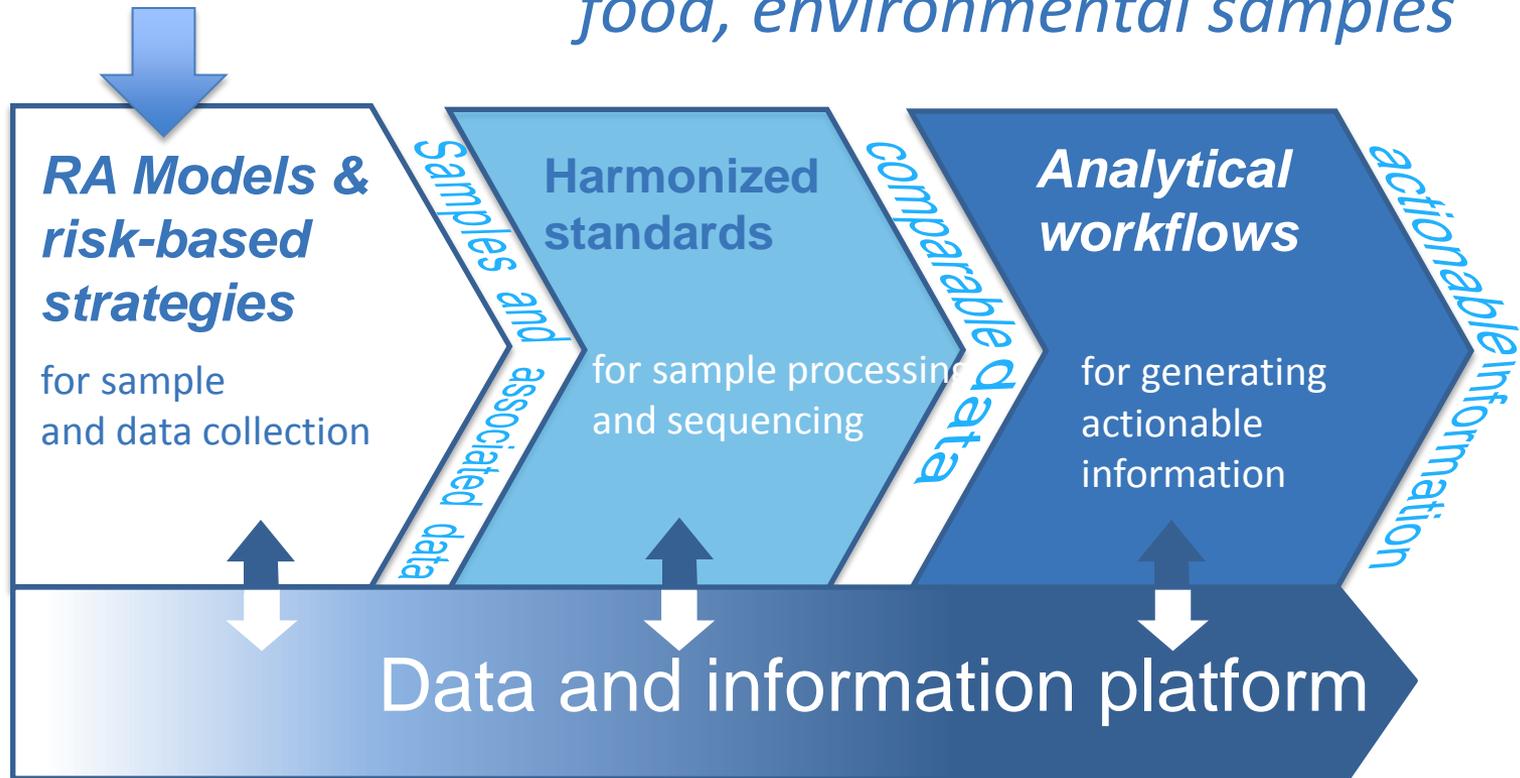
What to sample?

Where?

When?

How?

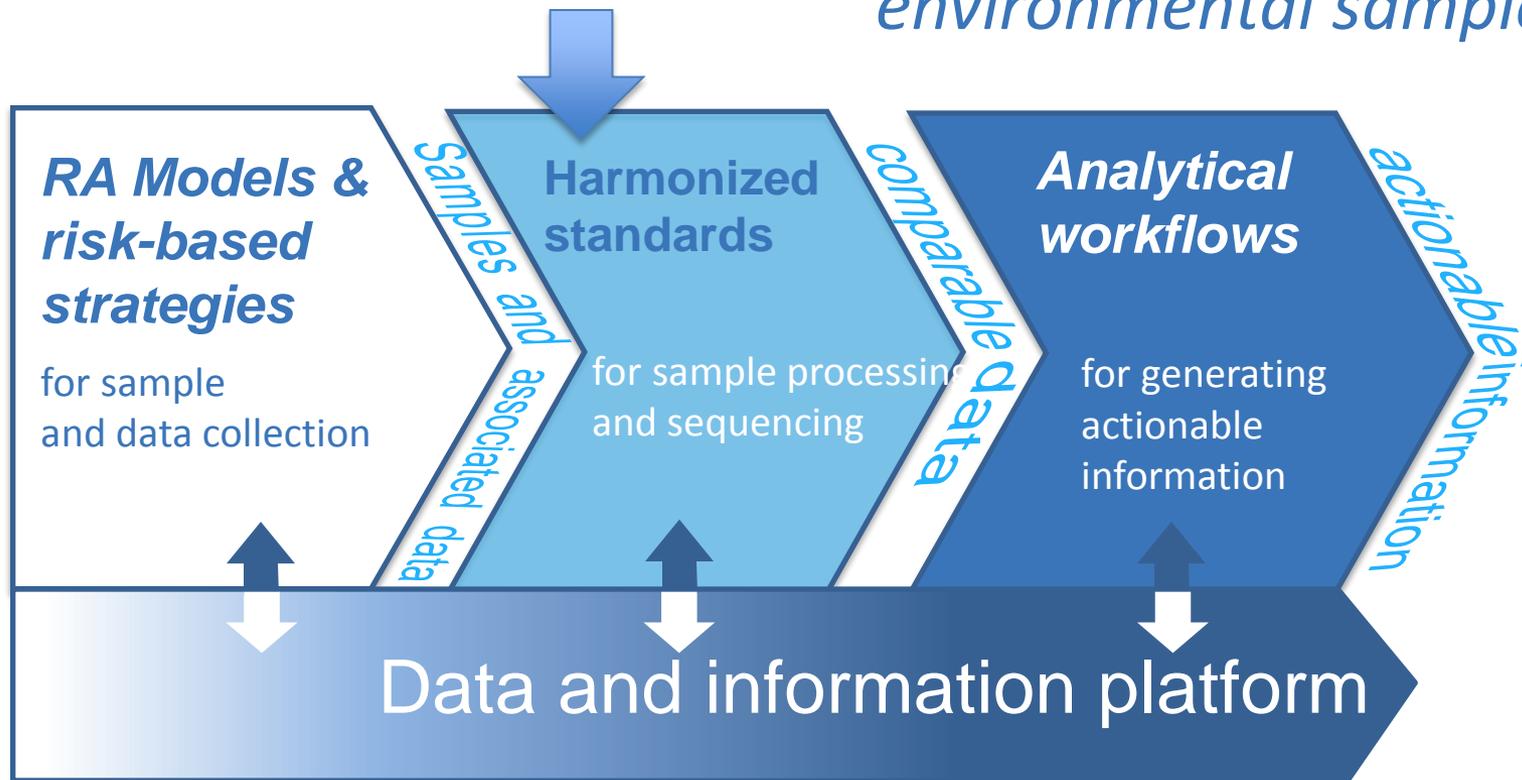
*Humans, livestock, wildlife,
food, environmental samples*





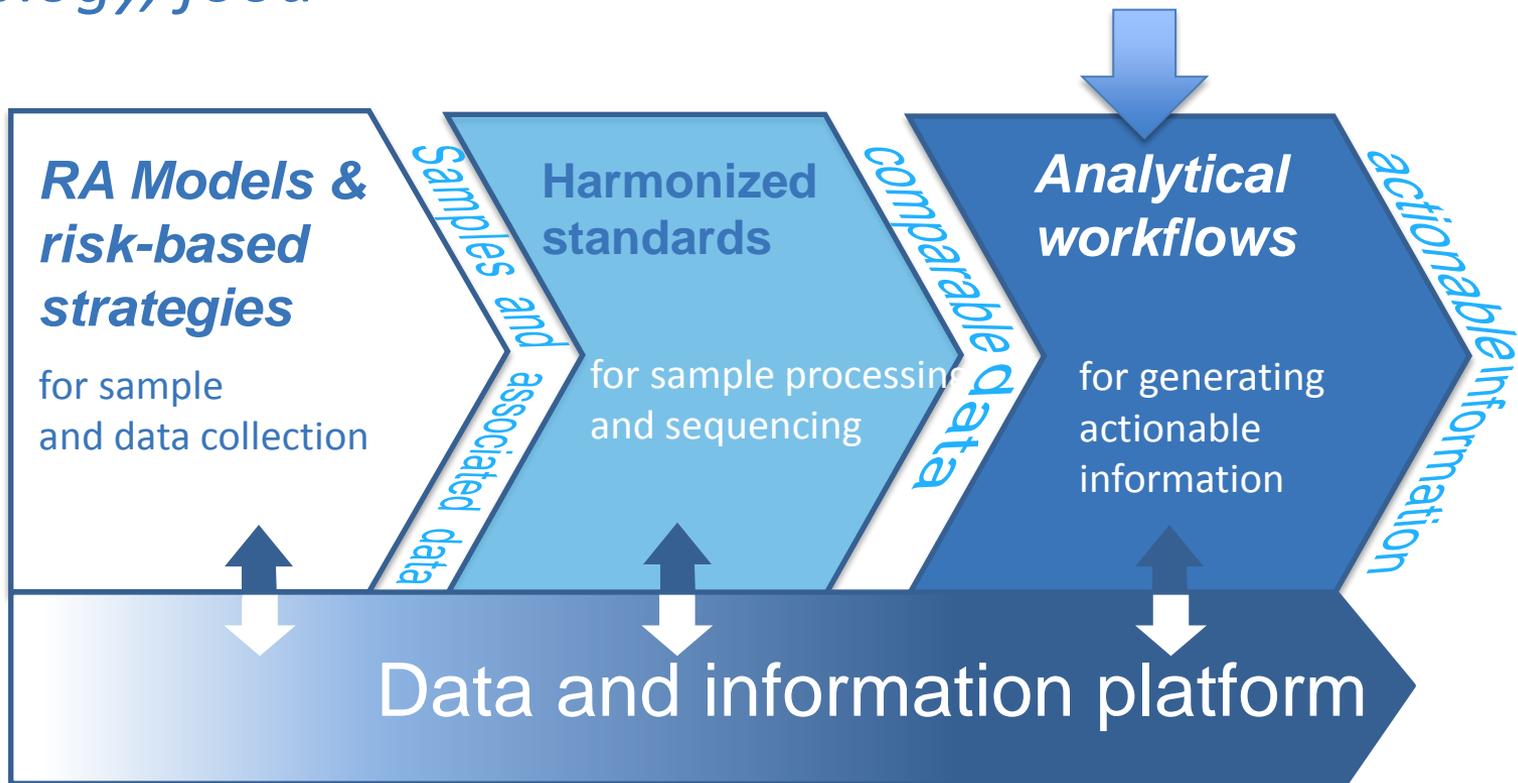
*How to sequence?
Pathogen targeted
Metagenomic, Deep sequencing
Viruses, bacteria, parasites
QUALITY ASSURANCE*

*Clinical, tissue, food,
environmental samples*



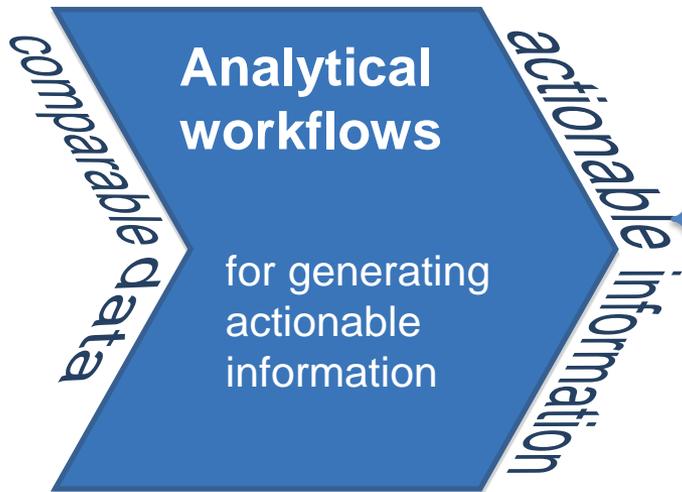
*Clinical, public health, research
Human, veterinary
Ecology, food*

*How to analyze?
What does the analysis mean?
How do I use it ?*



From comparable data to actionable information

Different users need different Analytical workflows



Frontline diagnostics in human and veterinary clinical microbiology

Surbhi Malhortra

WP leader



Menno de Jong **Anne Pohlmann**

WP co-leader



WP co-leader



Detection and analysis of foodborne outbreaks

Eva Møller-Nielsen

WP leader



Tine Hald

WP co-leader



Anne Brissabois

WP co-leader



Detection and analysis of (re-) emerging outbreaks

Ron Fouchier

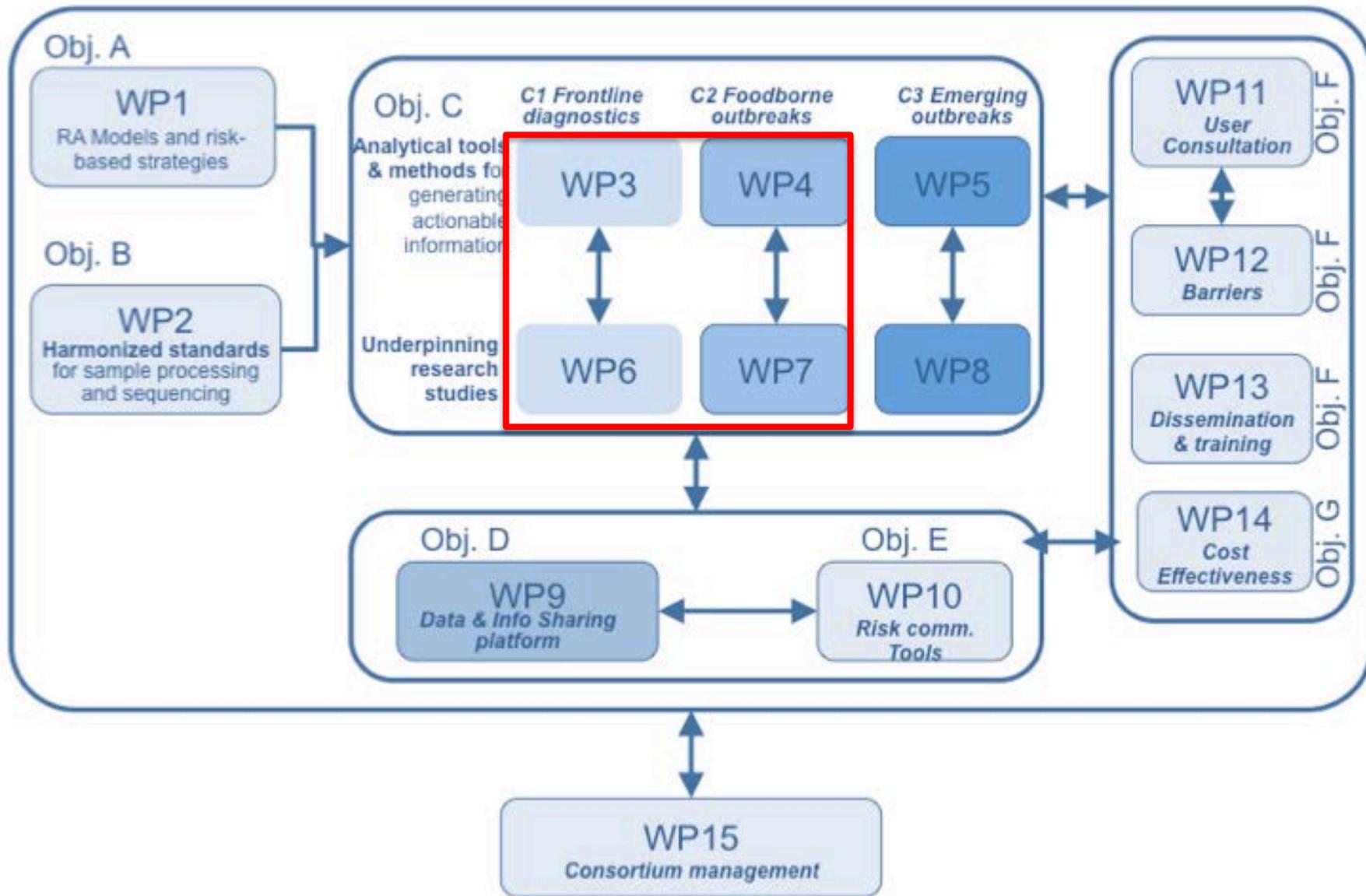
WP leader

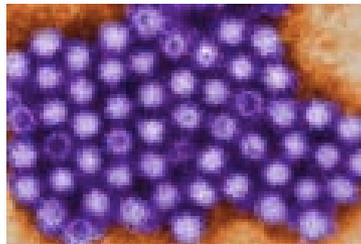


Mark Woolhouse

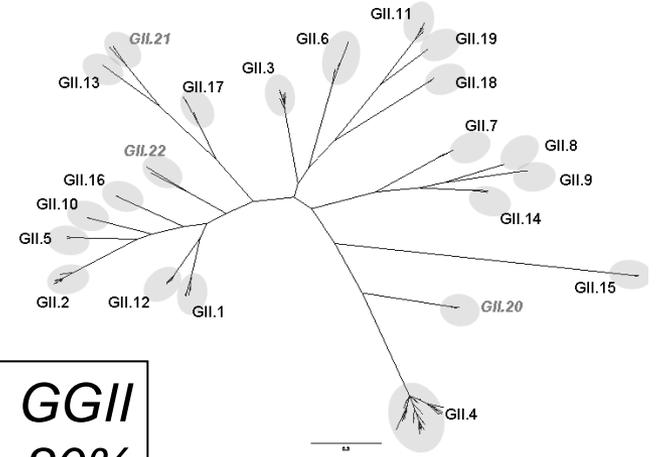
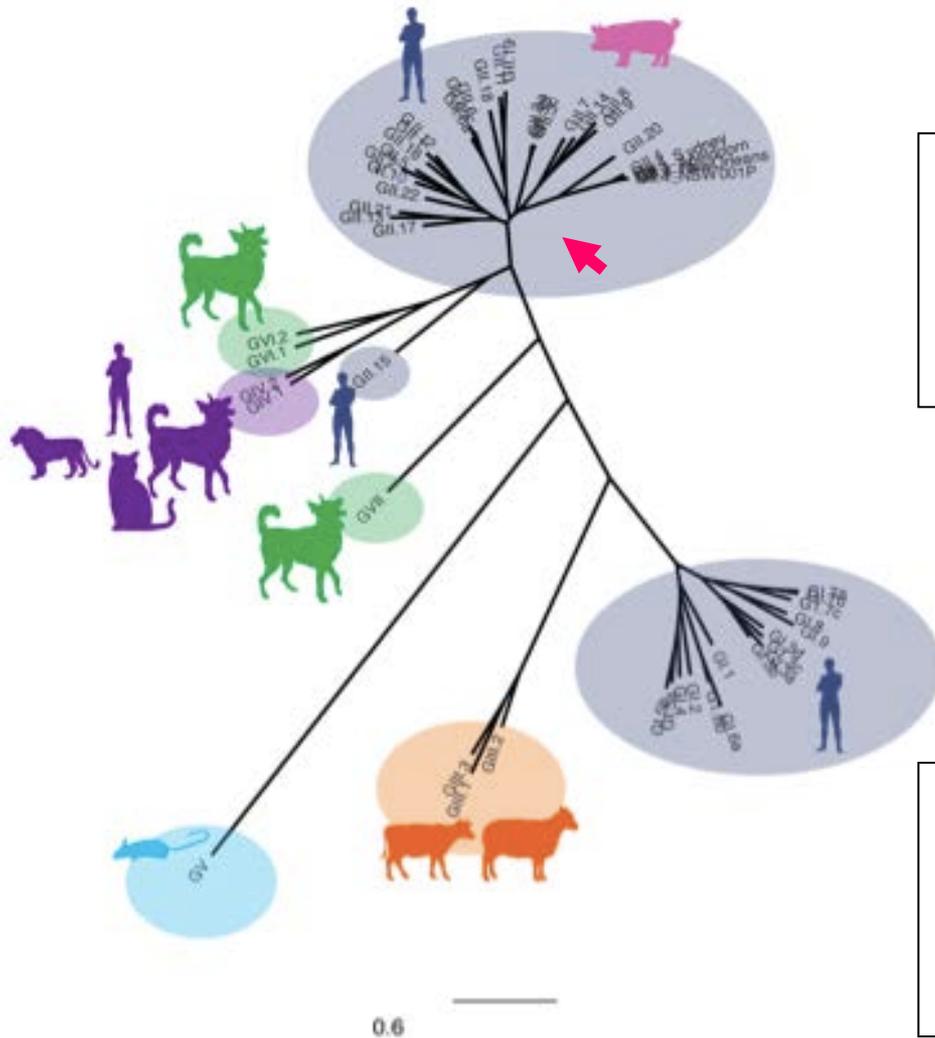
WP co-leader





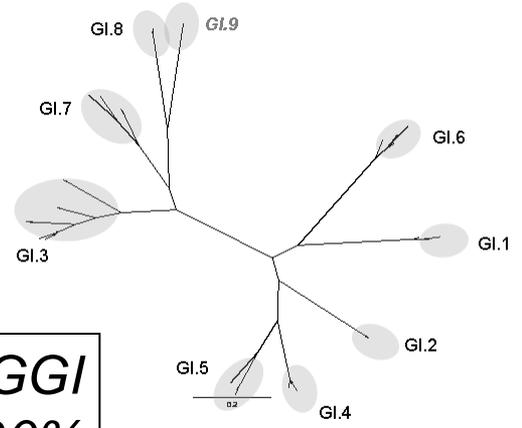


Norovirus



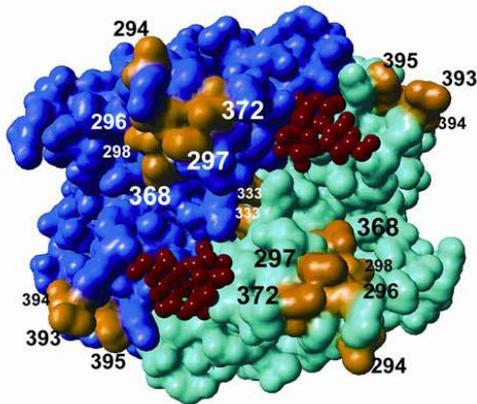
GGII
~80%
Seasonal
HCAI

Dominant Variant
(GII.4)



GGI
~20%
More varied
Food

Noroviruses persist in the population through evolution

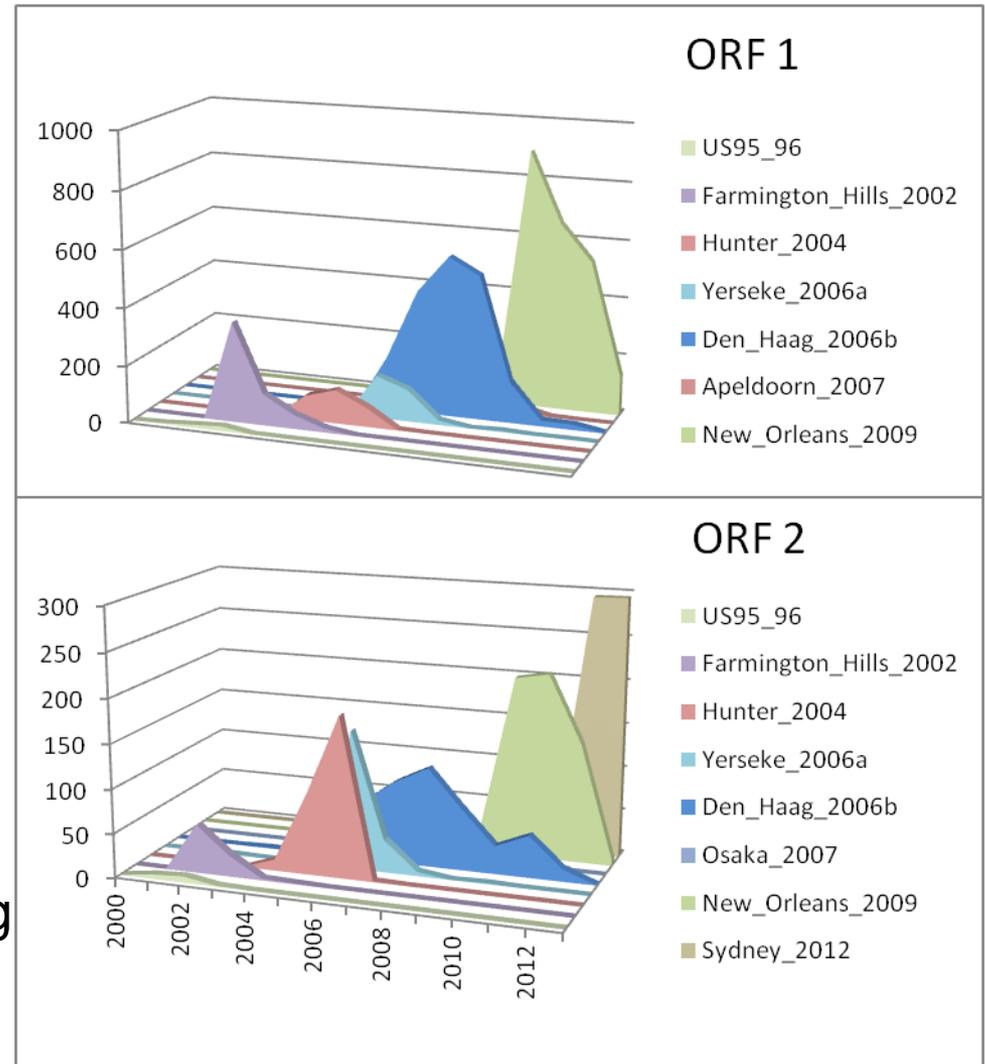


Effects of mutations:

Escape mutants (drift)

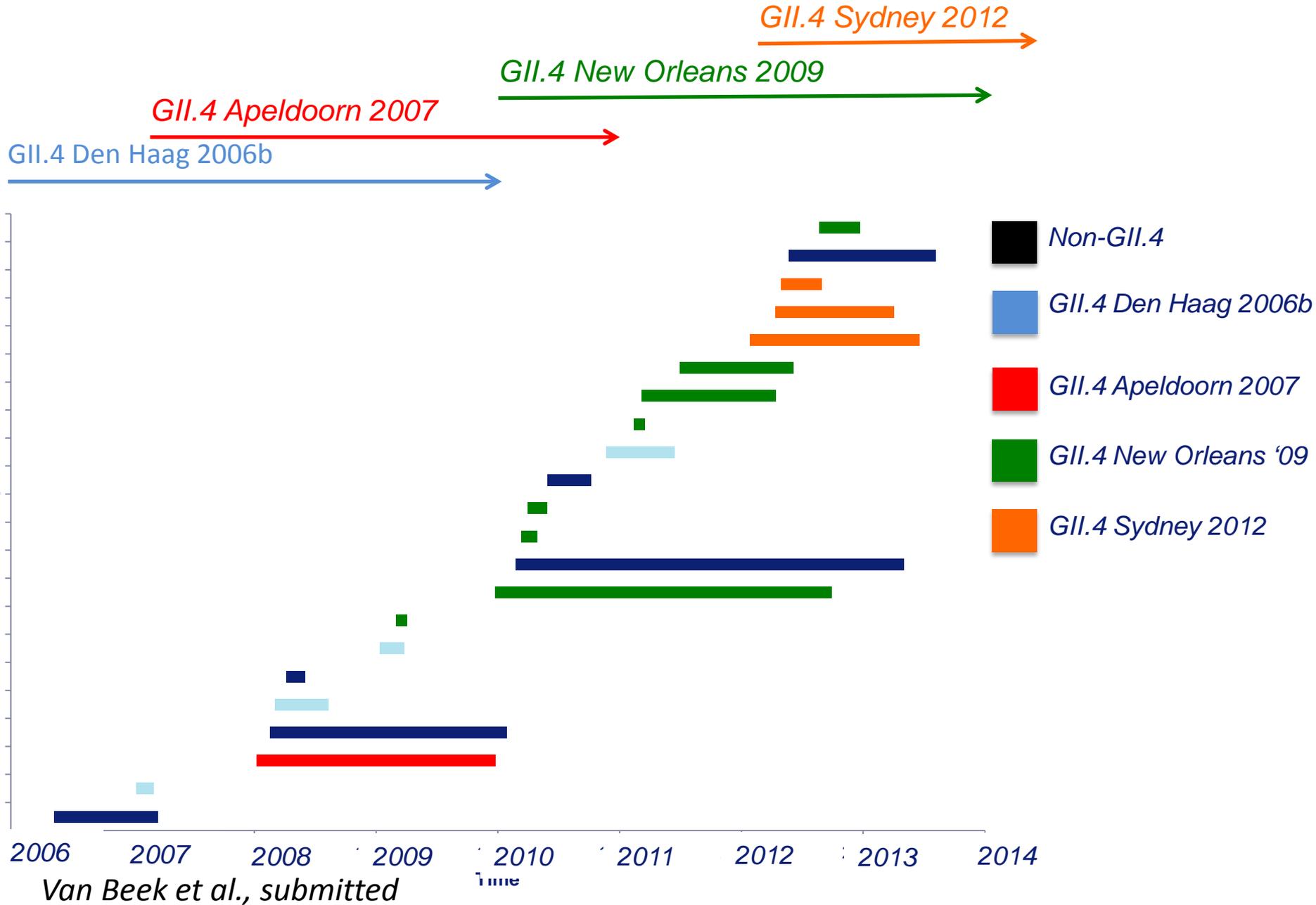
➤ No protective immunity

Differences in host cell binding
> New host range



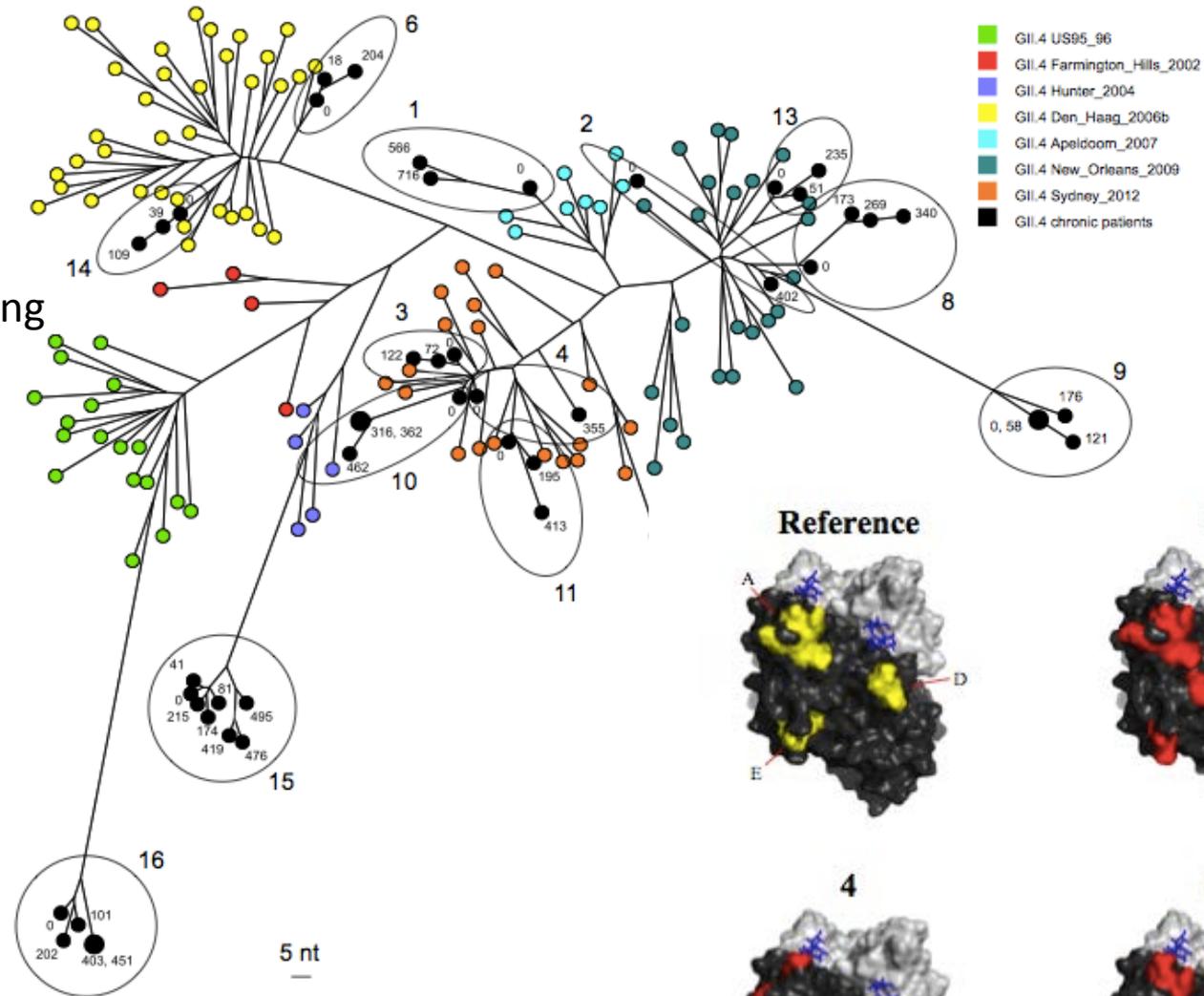
*Siebenga et al., 2008; Allen et al., 2008; Parra et al., 2012; Tan et al., 2003
Lindesmith et al., 2008; Bok et al., 2009; Siebenga et al. 2010*

A newly recognized problem: chronically infected patients



Evolution of drift variants in chronic shedders

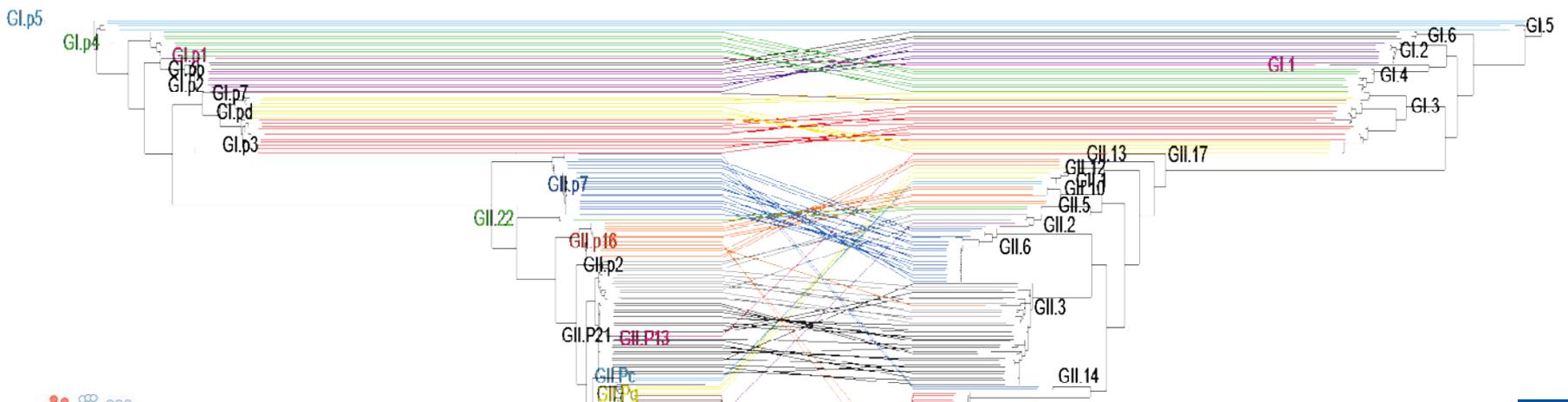
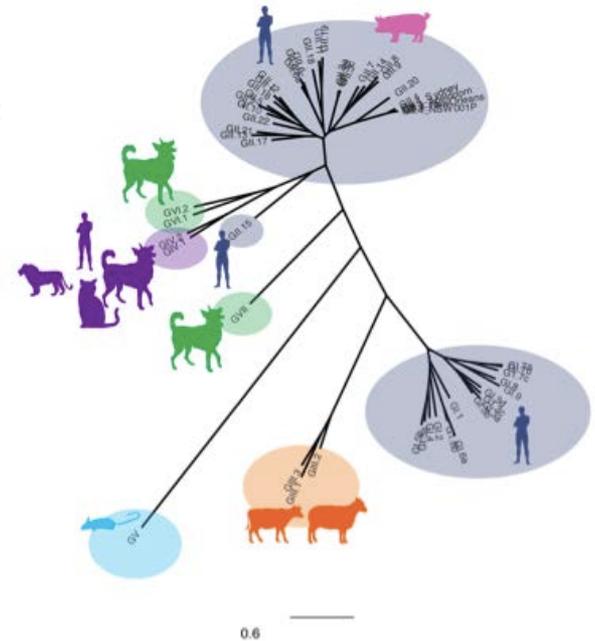
RNA isolation
Random priming
MiSeq paired



Van Beek et al., in preparation

Recombination

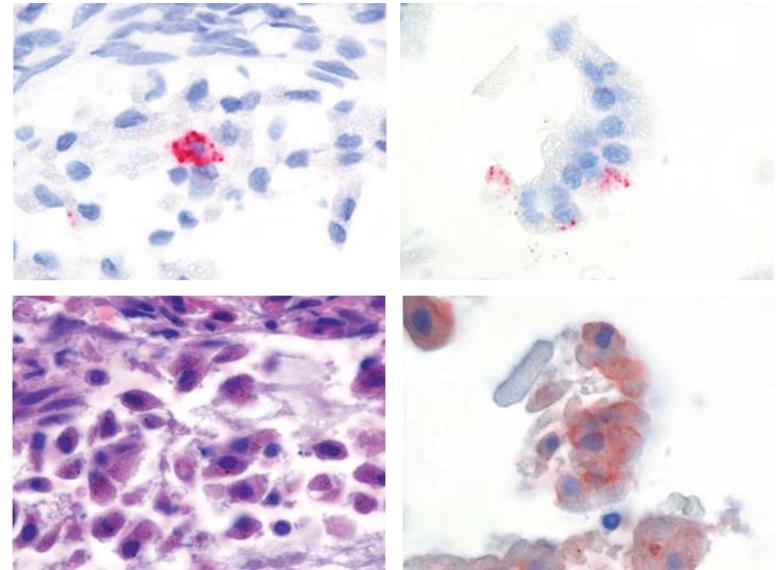
- Infection of a single cell with more than one strain : progeny virus with mixed genome
- More likely when humans are exposed to multiple viruses
 - Eg foodborne outbreaks
 - Daycare centres
 - Immunocompromised persons
- Potential for introduction of novel viruses into the population (mixed genomes)



Marine mammals as a reservoir for norovirus?

Since 1967, small cetaceans—mainly harbor porpoises—that strand alive along the Dutch, Belgian and German coasts have been rehabilitated at the dolfinarium Harderwijk and subsequently released into the wild.

- Agnostic RNA-seq, 454 sequencing > norovirus (HPNV)
- Production of VLPs (plasmid-based transfections) for serology
- Antibodies in 25% of animals



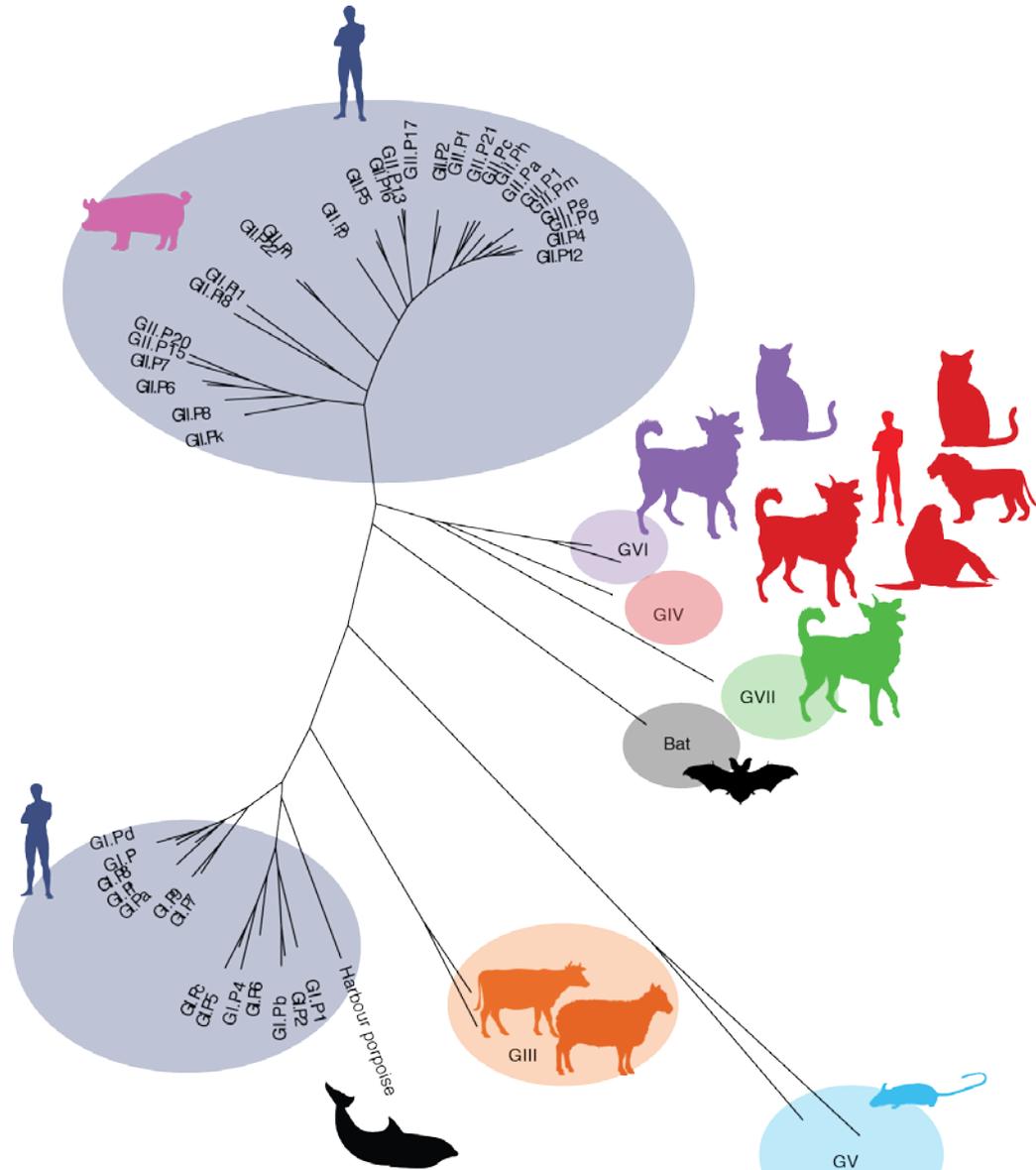
De Graaf et al., in press



This project has received funding from the *European Union's Horizon 2020 research and innovation programme* under grant agreement No 643476.



Maximum likelihood tree -RdRp-



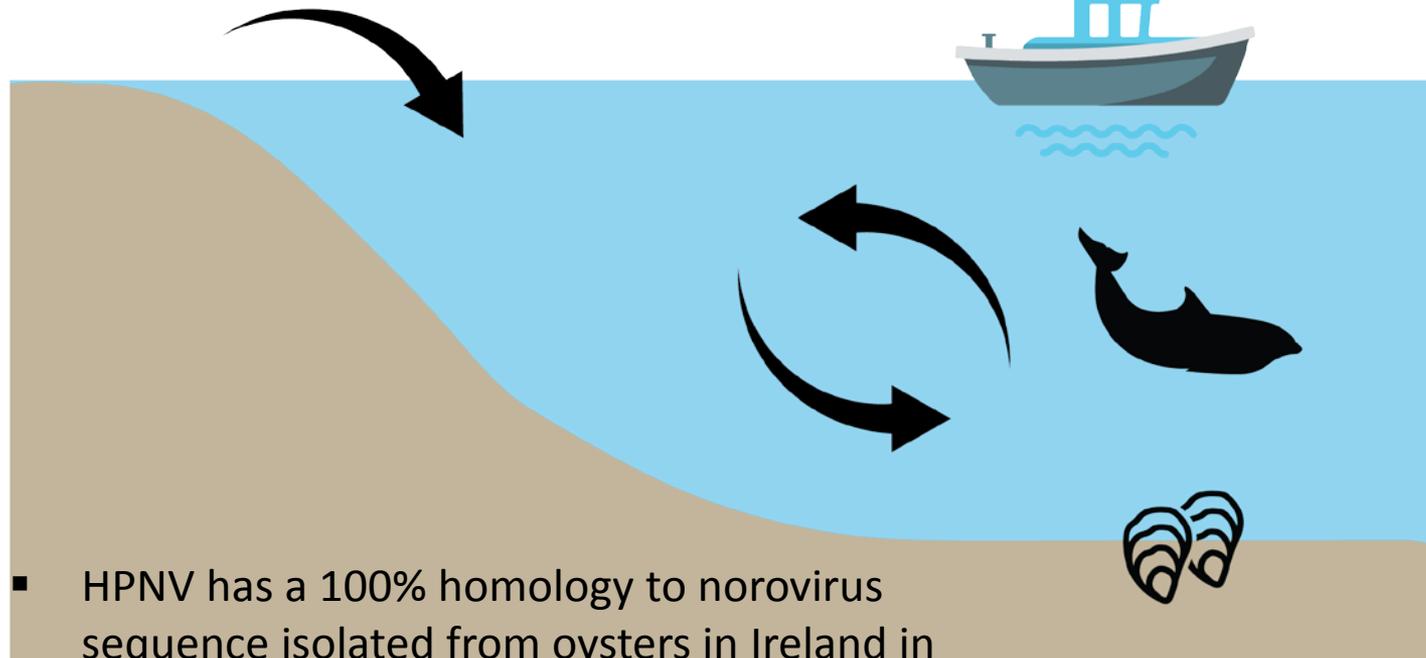
Norovirus gene introduction via sea food?

Agriculture

Sewage treatment

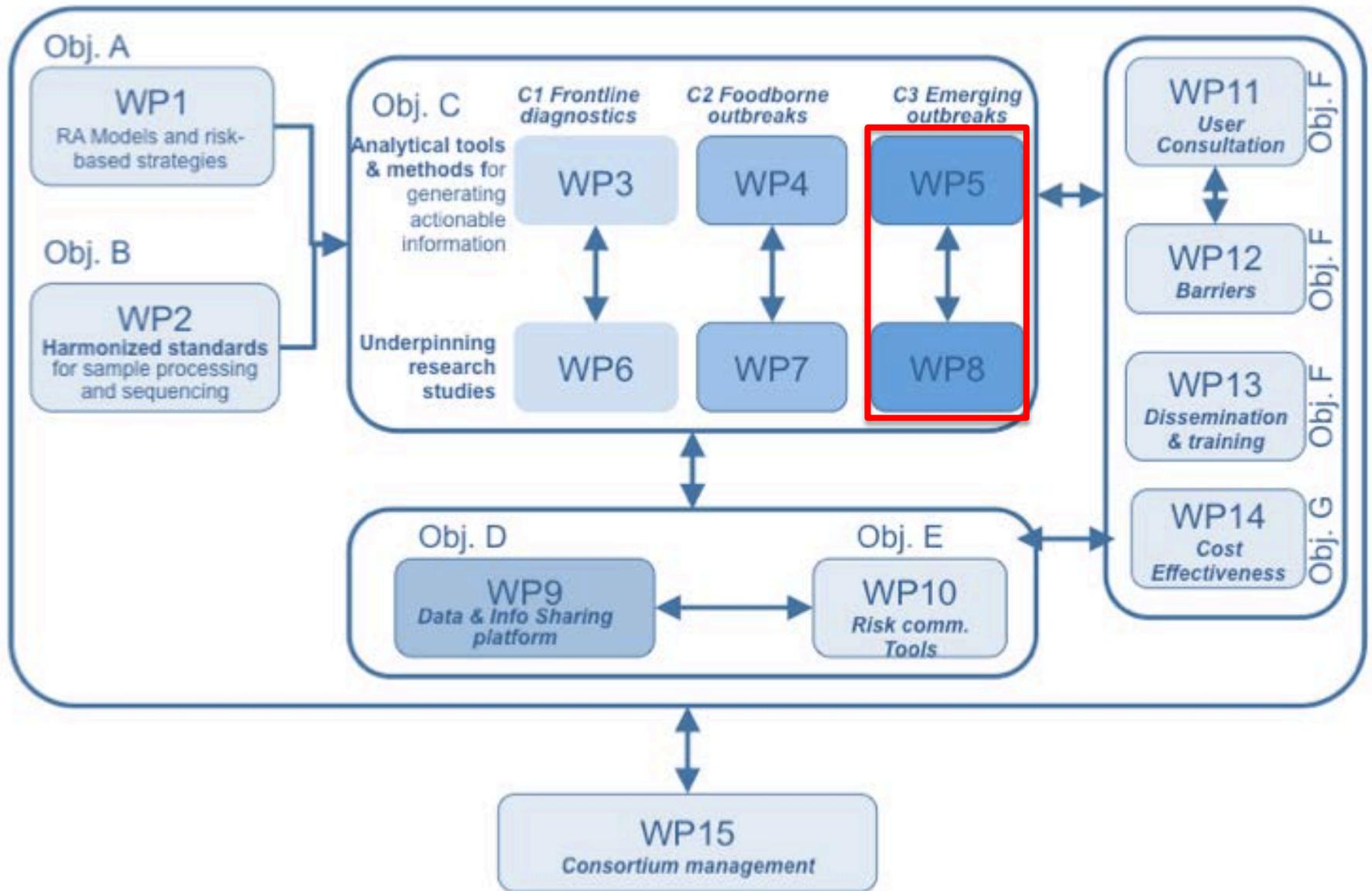
Human & animal noroviruses

Fishery wastes

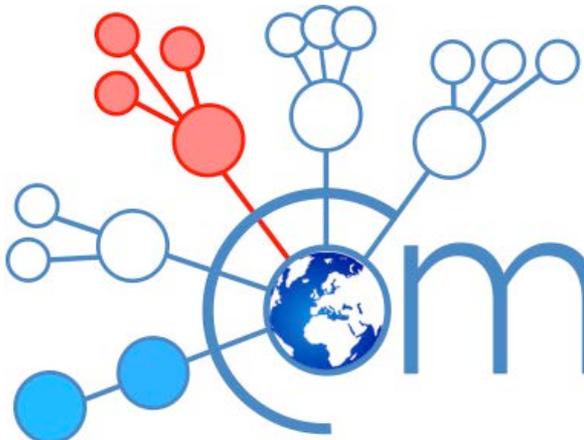


- HPNV has a 100% homology to norovirus sequence isolated from oysters in Ireland in 2012

De Graaf et al., in press



H5N8 pilot project



COllaborative Management Platform for detection and Analyses of (Re-) emerging and foodborne outbreaks in Europe

**"H5N8 AI
인체감염 사례는
전 세계적으로 없어"**



금번 국내에서 발생한 H5N8형 AI는, 전 세계적으로 사람에게 감염을 일으킨 사례가 없습니다.

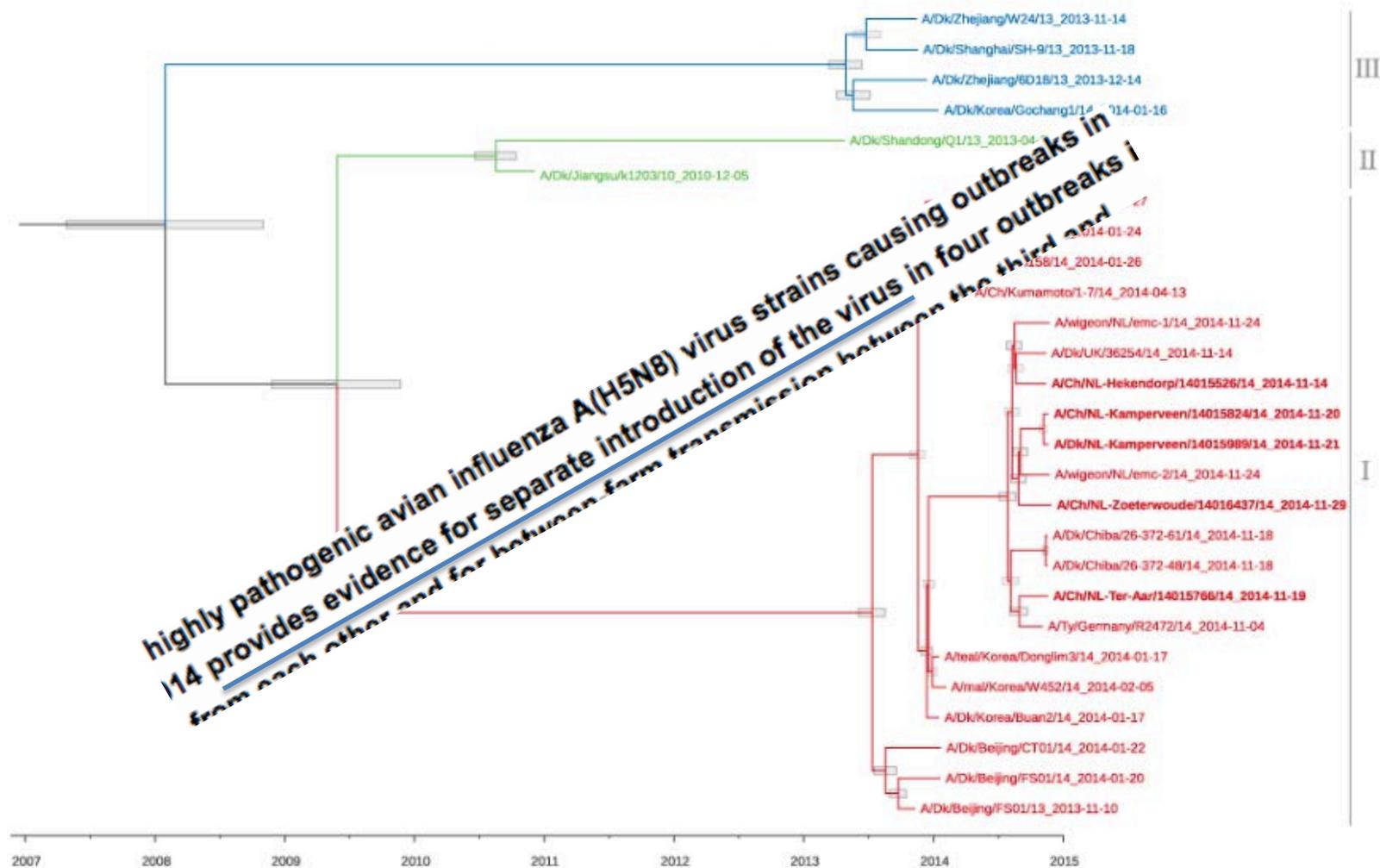
- 1 H5N8형 AI는 1983년 아일랜드에서 칠면조, 2010년 중국에서 오리들 중심으로 발생한 바는 있으나 인체 감염은 없었으며,
- 2 과거 다른 나라에서 발생하여 사람에게 감염을 일으켜 사망에 이르게 한 H5N1, H7N9형과는 다른 혈청형을 갖는 AI입니다.

*우리나라에서 2003년 이후 4차례 발생했던 H5N1형 AI유행에서도 인체감염 사례는 없었습니다



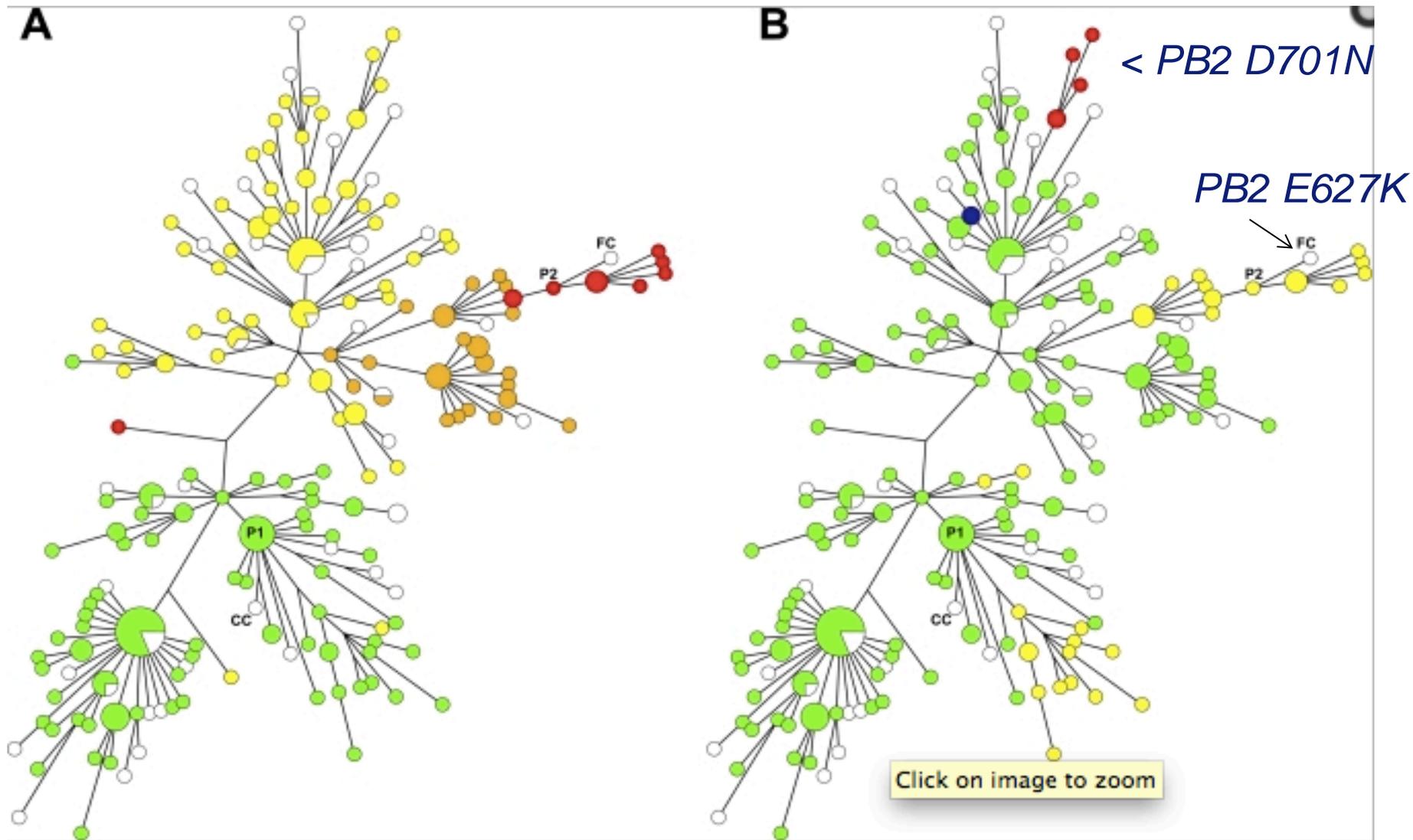
FIGURE 3

Phylogenetic trees derived from complete genome sequences of highly pathogenic avian influenza A(H5N8) viruses



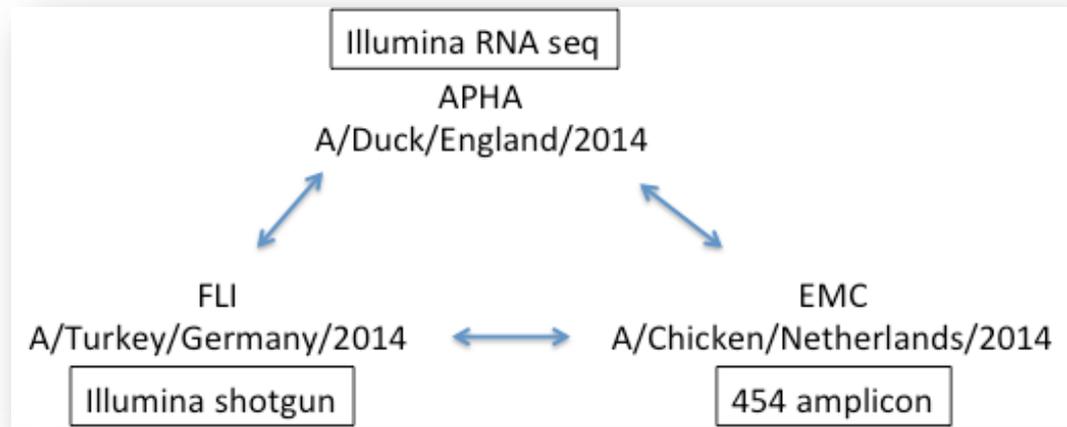
Time-scaled phylogenies (dates shown on the horizontal axis) were inferred using strict-clock Bayesian Markov Chain Monte Carlo analysis including all genome segments. Times of most recent common ancestors with 95% highest posterior density intervals are shown by the horizontal bars at each node. The three distinct evolutionary lineages are indicated in different colours and the symbols I, II and III. Indicated dates are the dates of sampling. A/Ch/NL-Kamperveen was detected at Farm 3 and A/Dk/NL-Kamperveen on Farm 4. Viruses detected at the Dutch farms are shown in bold.





H5N8 pilots

- What is the added value of deep sequence data over consensus sequences (SNPs) (epidemiology)?
- How fast are SNPs generated in various species, how well can we detect them?
- How is this (and conclusions) influenced by platforms, chemistry, workflows?



Different platforms different data: Coverage

SAMEA3493153 (BritishDuck) Segment 4 HA

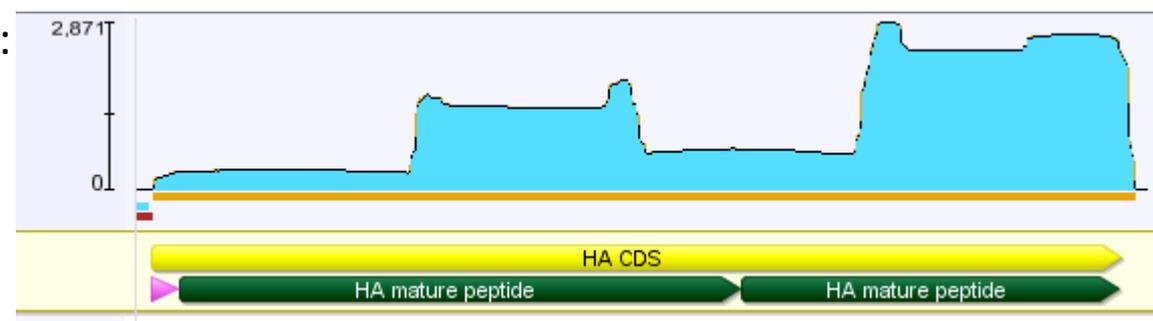
APHA
Illumina RNA seq
Newbler Mapping

Mean coverage:
4,626



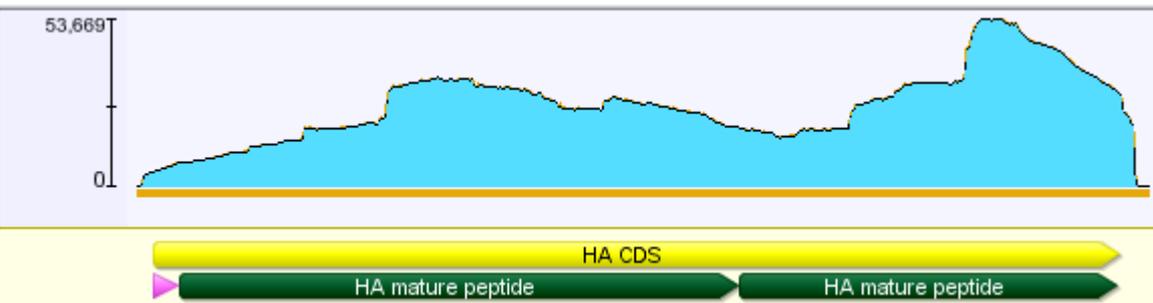
EMC 454
PCR amplification
Newbler Mapping

Mean coverage:
1,292



FLI
Illumina shot gun
Newbler Mapping

Mean coverage:
25,572

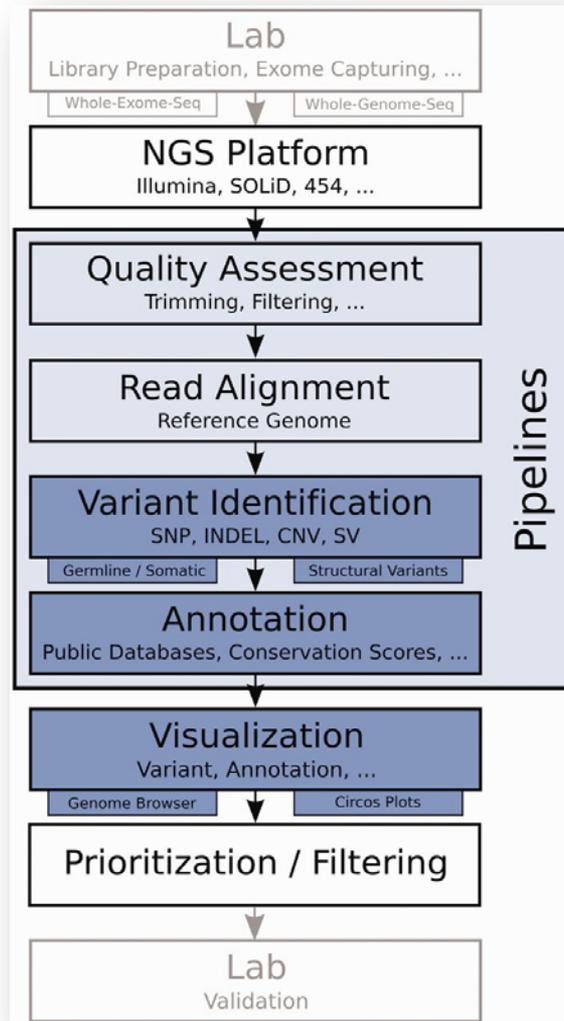


Different data different conclusions?

Table 33. The number of consensus nucleotide mutations and low frequency variants compared to the reference sequence per genome segment.

Genome segment	England		Germany		Netherlands	
	Consensus	Low frequency	Consensus	Low frequency	Consensus	Low frequency
1	9	1	4	2	2	3
2	6	0	5	0	1	1
3	8	0	6	1	1	0
4	5	1	7	2	0	0
5	5	0	3	0	0	1
6	5	1	2	0	0	0
7	1	0	0	0	0	0
8	1	0	0	0	0	0

A survey of tools for variant analysis of next-generation genome sequencing data



11 packages

17 packages

29 packages

74 packages

40 packages

13 pipeline packages

How to choose?

Is it important to choose?

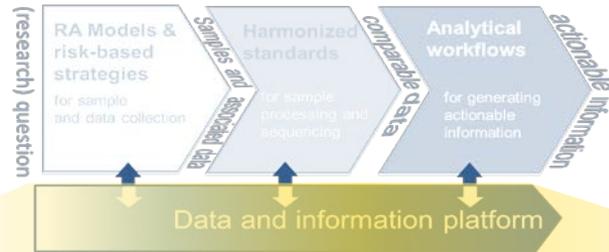
Who compares?

How to keep up to date?

What can be automated?



WP9 Information sharing platform



Guy Cochrane

WP leader



Ole Lund

WP co-leader

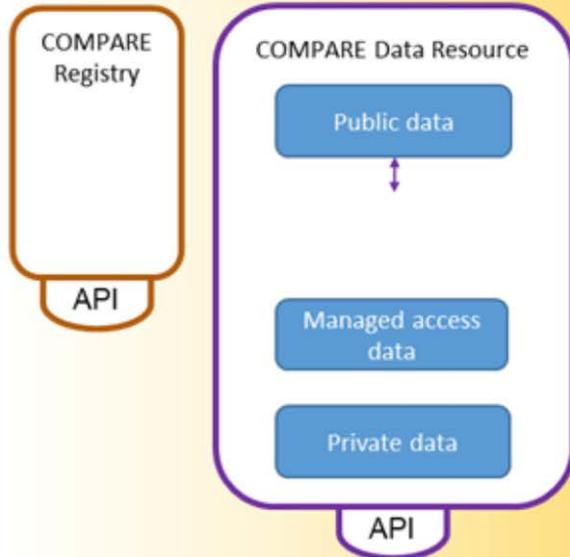


Istvan Csabai

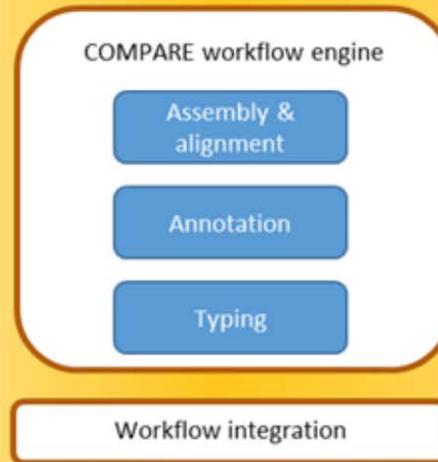
WP co-leader



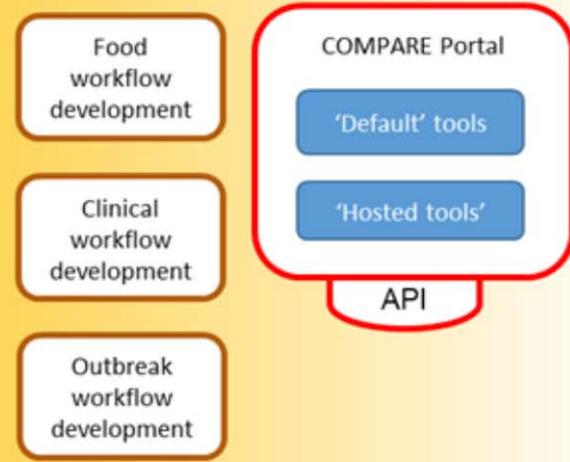
Sources



Processes



Portals and environments



Building on the EU ESFRI Elixir, EMBL and DTU infrastructures



Core capacity is broadly applicable

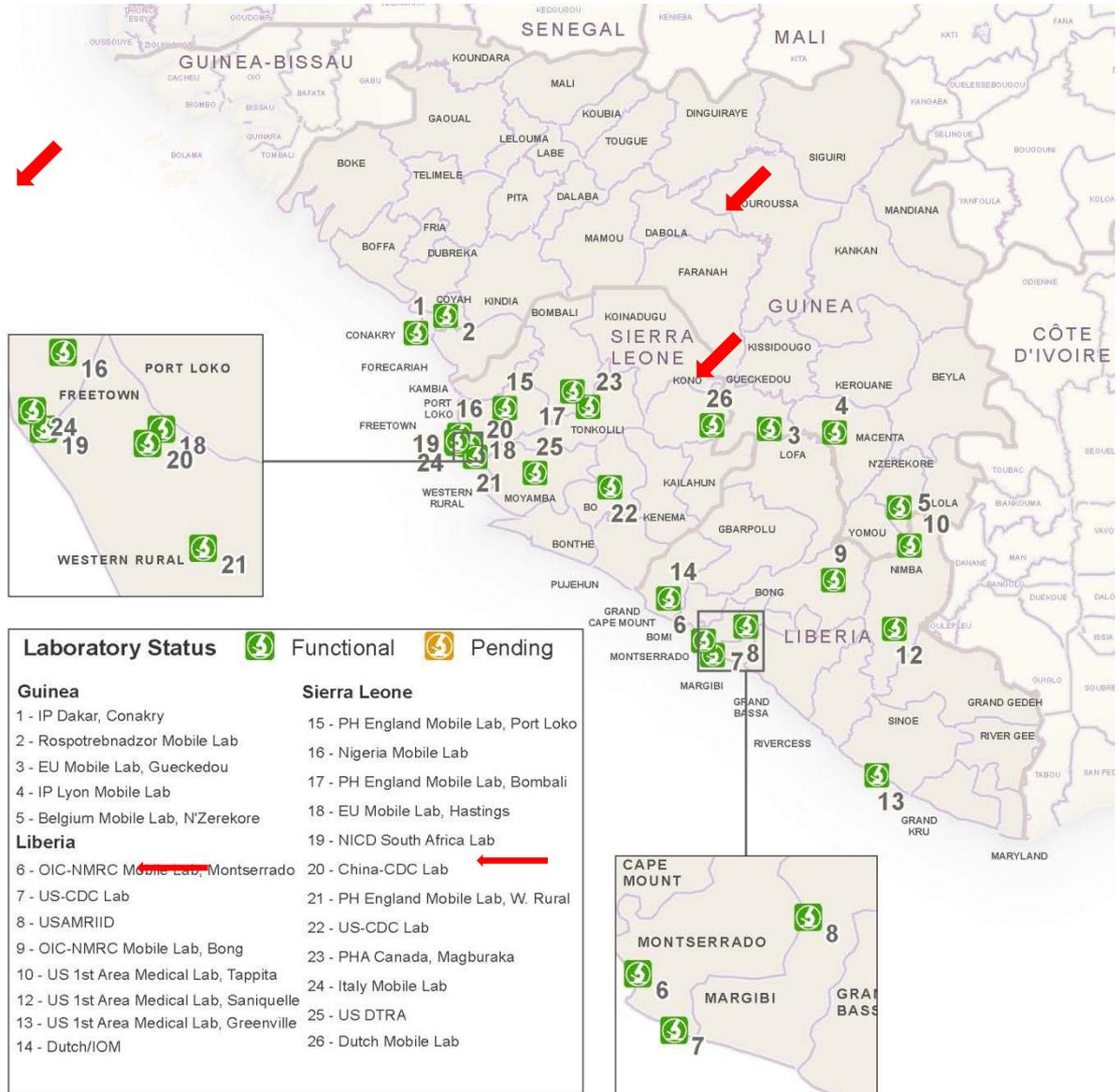
Kono, SL



Freetown, SL



Sinje, Liberia



Mateneh Ebola Treatment Centre Makeni Sierra Leone



1 of 6 DfID-funded treatment centres

Part of network of diagnostic labs set up by EML, DML, PHE, many other groups

Ian Goodfellow
Univ of Cambridge
Sequencing
Tent

Diagnostic
Lab

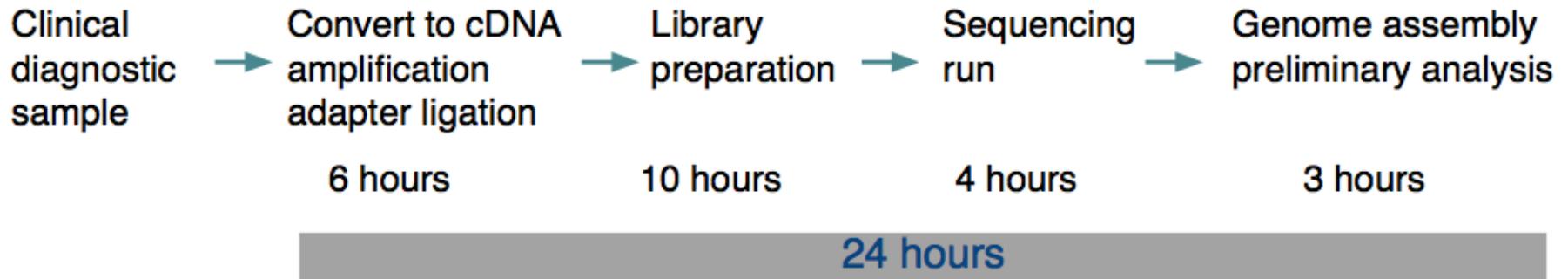


Sequencing Tent

Ian Goodfellow



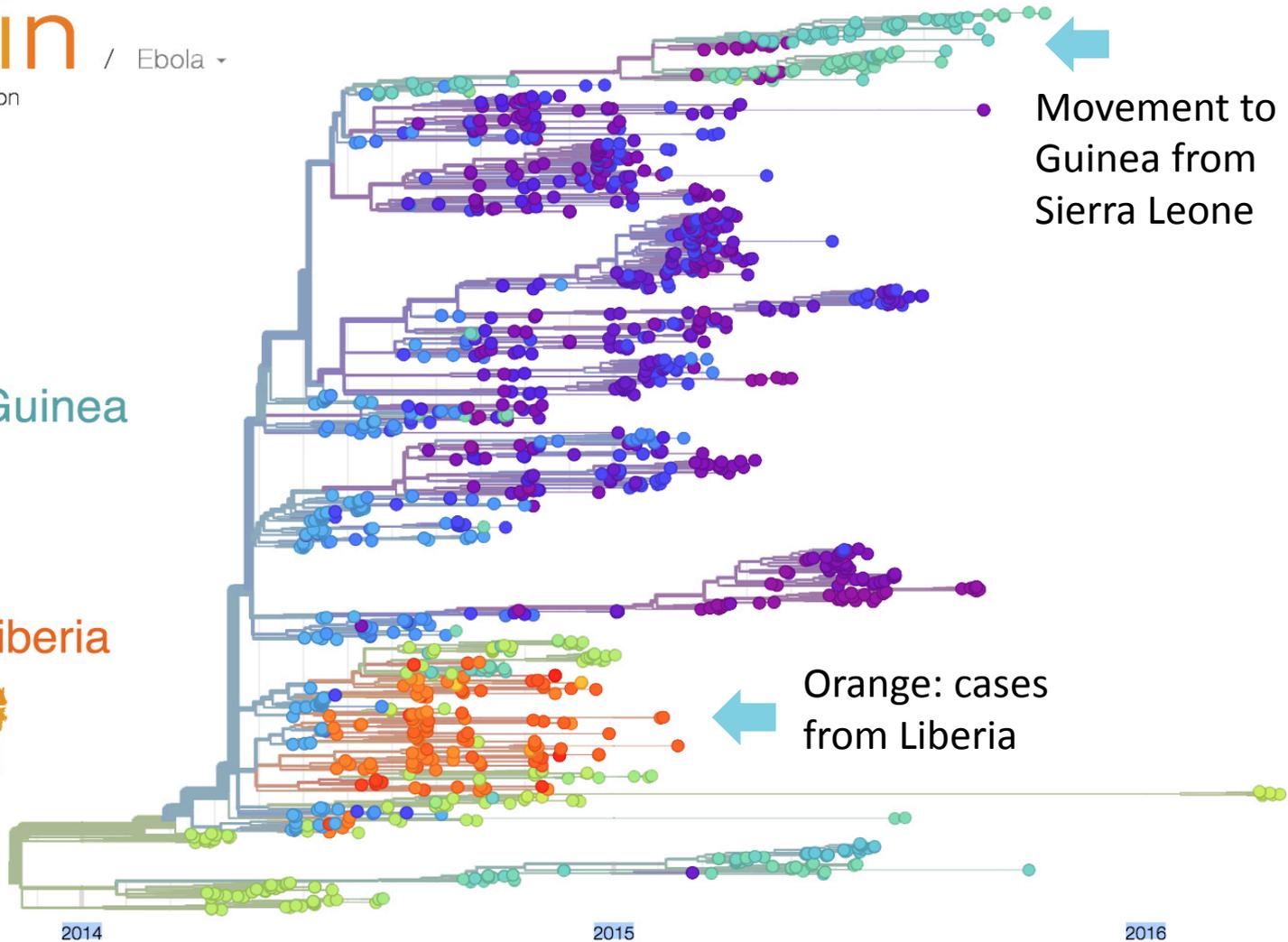
My Phan



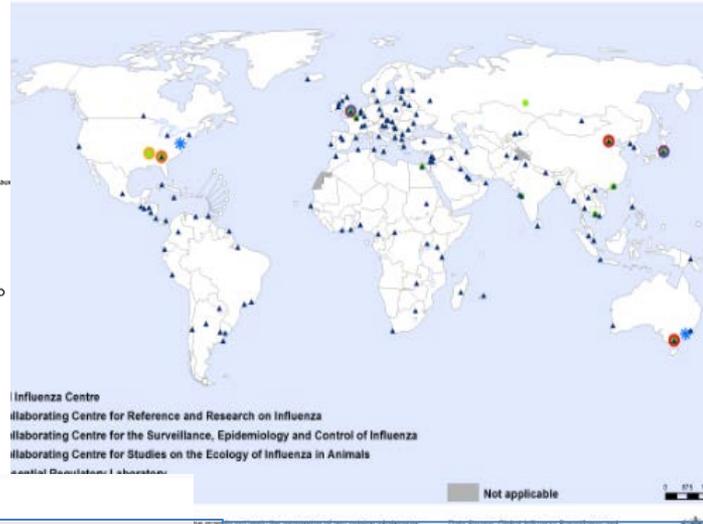
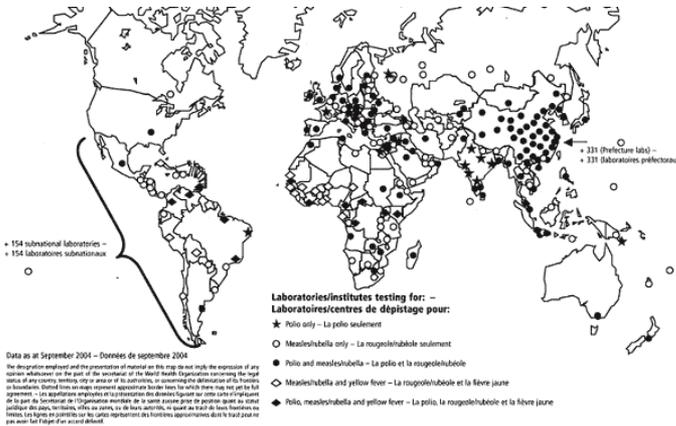
Rapid NGS of all Ebola cases to track source of new infections

nextstrain / Ebola ▾
Real-time tracking of Ebola virus evolution

Richard Neher
Trevor Bedford



We could do things on the fly if capitalizing on existing networks



**OIO, FAO, WHO
networks**
Polio, Measles,
Flu, TB, HIV,
VHF, malaria,
Dengue,
Salmonella, AMR,
Zika

.....

PERSPECTIVES

Laboratory support during and after the Ebola virus endgame: towards a sustained laboratory infrastructure

I Goodfellow¹, C Reusken², M Koopmans (m.koopmans@erasmusmc.nl)^{2,3}

1. University of Cambridge, Department Virology, Cambridge, United Kingdom
2. Erasmus MC, Department Viroscience, Rotterdam, the Netherlands
3. Centre for Infectious Disease Control, Bilthoven, The Netherlands

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Conclusions

- The merging of public health and research activities calls for new models for research collaboration during EID outbreaks
- COMPARE seeks to develop tools, infrastructure, and datasharing hubs to support this increasing
- Bottleneck at level of bioinformatics, therefore key focus
- Open source and access solutions, user defined
- In the coming years, we will be seeking partners for pilot projects

Norovirus:

Miranda de Graaf
David Nieuwenhuijse
Janko van Beek
Rogier Bodewes
Annemiek Vandereijk
Matt Cotten

Influenza

Saskia Smits
Ron Fouchier
Martin Beer
Ian Brown
Thijs Kuiken

Ebola

Saskia Smits
Susan Pas
Chantal Reusken
Bart Haagmans
Ian Goodfellow
Matt Cotten

And many others



Frank Aarestrup, Ole Lund, Technical University of Denmark (DTU)
Marion Koopmans, Ron Fouchier, Thijs Kuiken, Matt Cotton, Erasmus Medical Center (ErasmusMC)
Eva Moller Nielssen, Tine Hald, Statens Serum Institut (SSI)
Martin Beer, Anne Pohlmann, Dirk Hoper, Friedrich-Loeffler-Institute (FLI)
Anne Brisabois, Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES)
Klaus Stark, Andres Nitsche, Robert Koch-Institut (RKI)
Guy Cochrane, European Molecular Biology Laboratory (EMBL)
Simone Caccio, Instituto Superiore di Sanita (ISS)
Annelies Kroneman, George Haringhuizen, Rijksinstituut voor Volksgezondheid en Milieu (RIVM)
Amie Atkin, Emma Snary, Andrew Breed, Ian Brown, Animal Health and Veterinary Laboratories Agency (AHVLA)
Marc Woolhouse, Andrew Rambaut, University of Edinburgh (UEDIN)
Christian Drosten, Universitäts Klinikum Bonn (UK-Bonn)
Menno de Jong, Academic Medical Center (AMC)
Surbhi Malhotra, Universiteit Antwerpen (UA)
Ab Osterhaus, Artemis Wildlife Health BV (Artemis)
Derek Smith, Terry Jones, University of Cambridge (UCAM)
Wolfgang Baumgaertner, Ab Osterhaus, Tierärztliche Hochschule Hannover (TIHO)
Christian Cortazar, Universidad Castilla de la Mancha (UCLM)
Hubert Endtz, Fondation Mérieux (FMER)
Ana Papa, Aristotle University Thessaloniki (AUTH)
Soizick LeGuyader, L'Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER)
Pieter van Baal, Erasmus Universiteit Rotterdam (EUR)
Martyn Kirk, Australian National University (ANU)
Istvan Csabai, Magyar Tudományos Akademia Wigner Fizikai kutatokozpont (Wigner RCP)
Frank Alleweldt, Civic Consulting Alleweldt & Kara Gbr (CIVIC)
Emilio Mordini, Responsible Technology (RT)
University of Bologna (UNIBO)
Ulbrecht Nubel, Leibniz-Institut DSMZ GmbH (DSMZ)
Paul Kellam, David Aanensen, Wellcome Trust Sanger Institute (WTSI)



Satellite Symposium « Emerging Zoonoses »

Erasmus MC / Institut Pasteur

Saturday 22nd October 2016

13h15 – 14h45 Hall A . Floor 1



Introduction and outline of PREDEMICS and ANTIGONE projects	<i>Sylvie van der Werf</i> <i>Thijs Kuiken</i>	Institut Pasteur Erasmus MC
Zoonotic Hepatitis E virus infections in Europe	<i>Helene Norder</i>	Goeteborgs Universitet
The newly emerged reassortant viruses of the influenza H5 A/Goose/Guandong/1/96 lineage	<i>Mathilde Richard</i>	Erasmus MC
The drivers of Ebola spatial spread in West Africa	<i>Philippe Lemey</i>	Rega Institute, KU Leuven
Pathogenesis of MERS in human patients	<i>Christian Drosten</i>	University of Bonn
General Discussion and Summary	<i>Sylvie van der Werf</i> <i>Thijs Kuiken</i>	Institut Pasteur Erasmus MC



ELIXIR

A distributed infrastructure for life-science information



The European Bioinformatics Institute

EMBL-EBI

[Other EMBL locations >](#)

The home for big data in biology

At EMBL-EBI, we use bioinformatics — the science of storing, sharing and analysing biological data — to help people everywhere understand how living systems work, and what makes them change.



EMBL (June 2013)



UK (Sept 2013)



Sweden (Sept 2013)



Switzerland (Oct 2013)



Czech Republic (Nov 2013)



Estonia (Dec 2013)



Norway (Jan 2014)



Netherlands (Feb 2014)



Denmark (Mar 2014)



Israel (May 2014)



Portugal (July 2014)



Finland (Sept 2014)



France (Oct 2015 as Provisional Member)



Spain (Oct 2015 as Provisional Member)



Belgium (Nov 2015)



Italy (Jan 2016)



Slovenia (Feb 2016)



Luxembourg (July 2016)



Ireland (July 2016)



Germany (August 2016)

