The Importance of Effective Virus Reduction in the Virus Safety of Biologically Derived Medicinal Products

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Overview

- Emerging infectious diseases
  - Defining the problem
  - Factors influencing the emergence of new viruses
  - Examples of emerging viruses
- Paradigms for controlling virus risk in biopharmaceutical products
  - Regulatory guidelines
  - How can we best control the risk?
  - Robust steps for manufacturing biopharmaceutical products

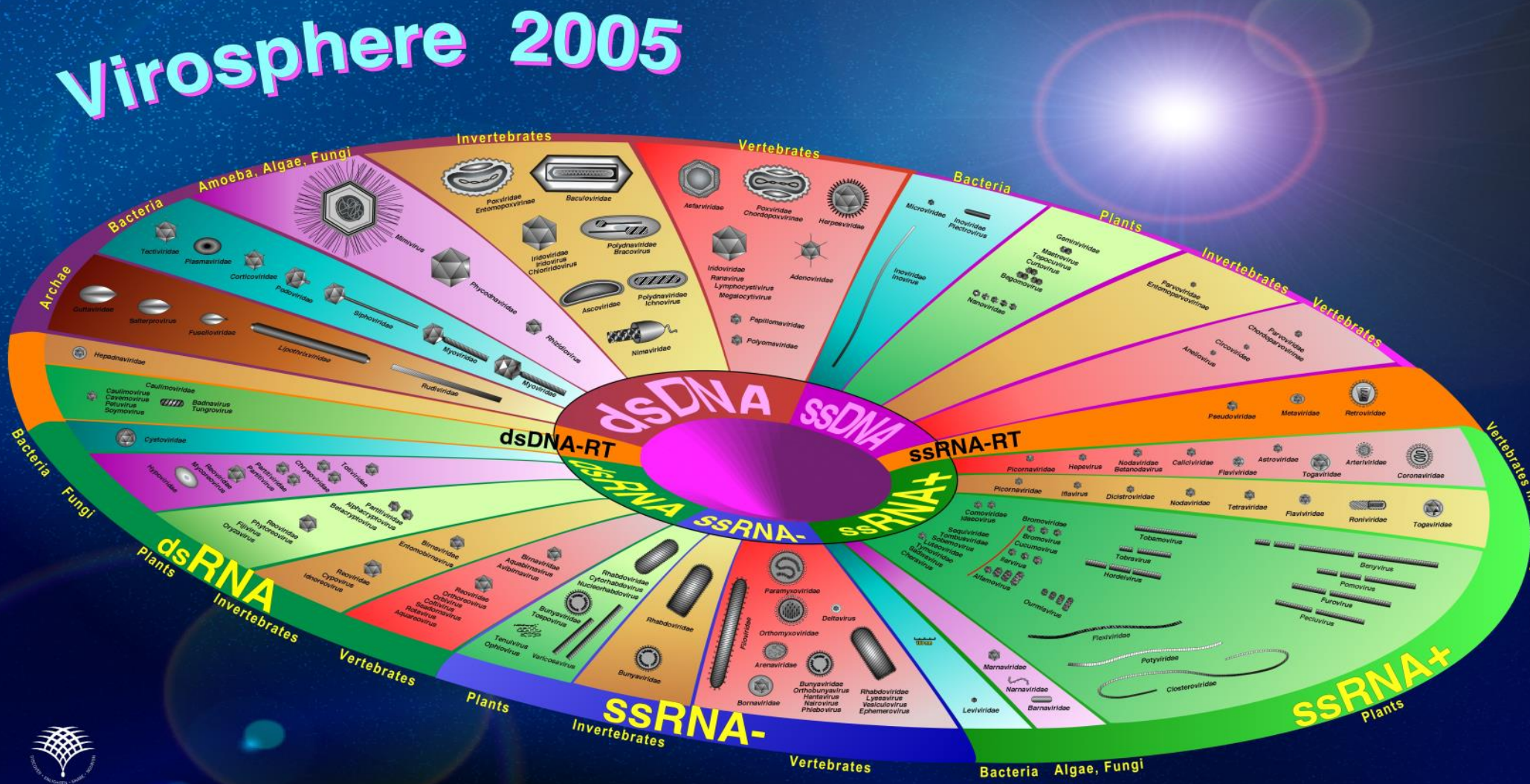
History of Virus Contamination in Biopharmaceutical Products

We have seen a number of virus contamination events that have resulted in the transmission of virus to humans:

- Most severe virus contamination events have occurred with human derived medicinal products, e.g.:
  - Human plasma-derived products
  - Products derived from other human tissues
- Transmission of viruses to humans from animal derived products is more rare
- To date, there are no known virus transmission events that have occurred with recombinant products:
  - But we need to be careful- these products can also be contaminated with viruses (see later)

PDA
Parenteral Drug Association

The Realm of Viruses



DONALD DANFORTH
PLANT SCIENCE CENTER

International Committee on Taxonomy of Viruses

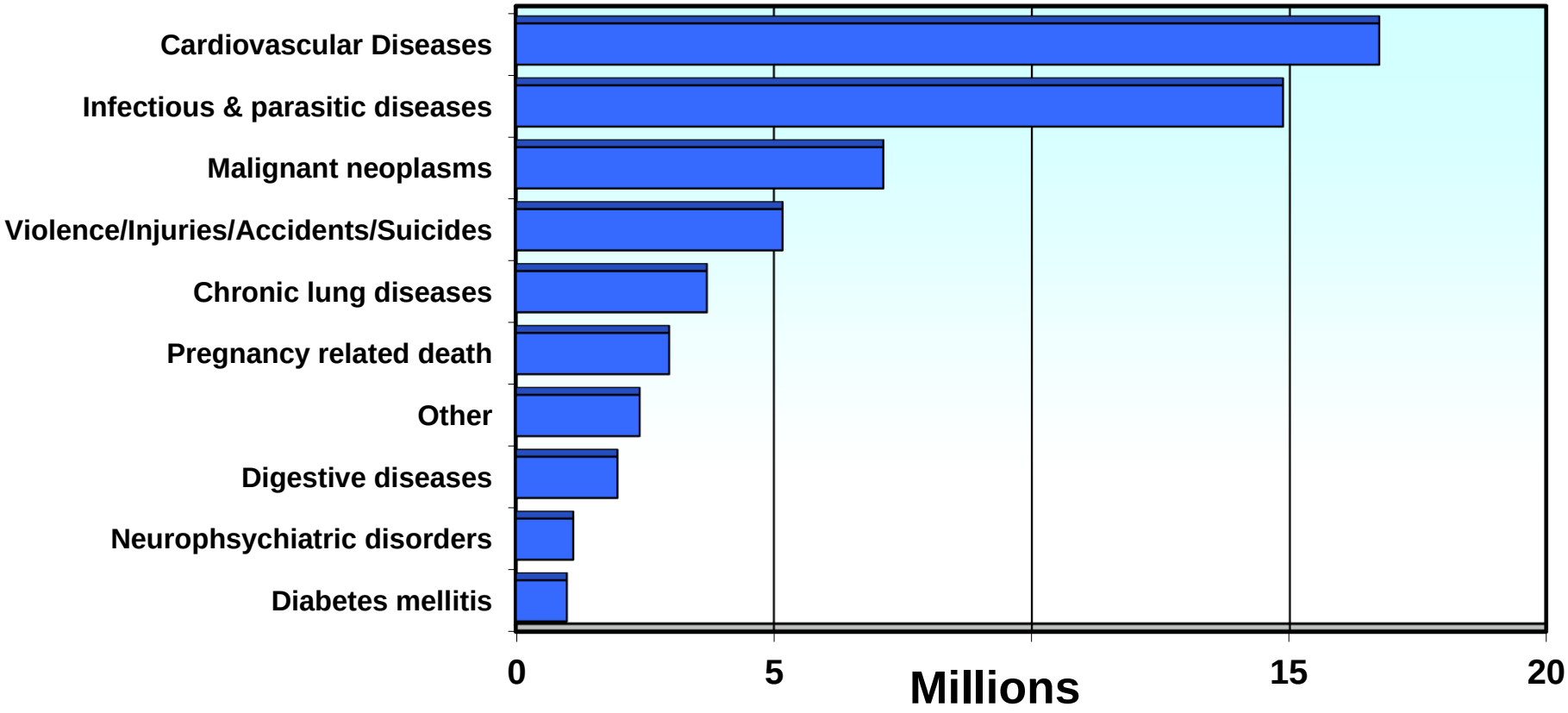
Emerging Viruses

What is an Emerging Virus?

An **emergent virus** is a virus that has adapted (changed) and emerged as a new disease or pathogenic variant, with properties that result in increased pathogenicity in an area not normally associated with that virus:

- This includes both new strains that have previously never infected humans, as well as those viruses which have increased in incidence due to other factors (e.g. globalisation, insect vector migration ...)
- Most emergent viruses can be categorised as zoonotic (an animal disease that can be transmitted to humans)
  - i.e. they have one or more animal reservoirs

Estimates of Cause of Death

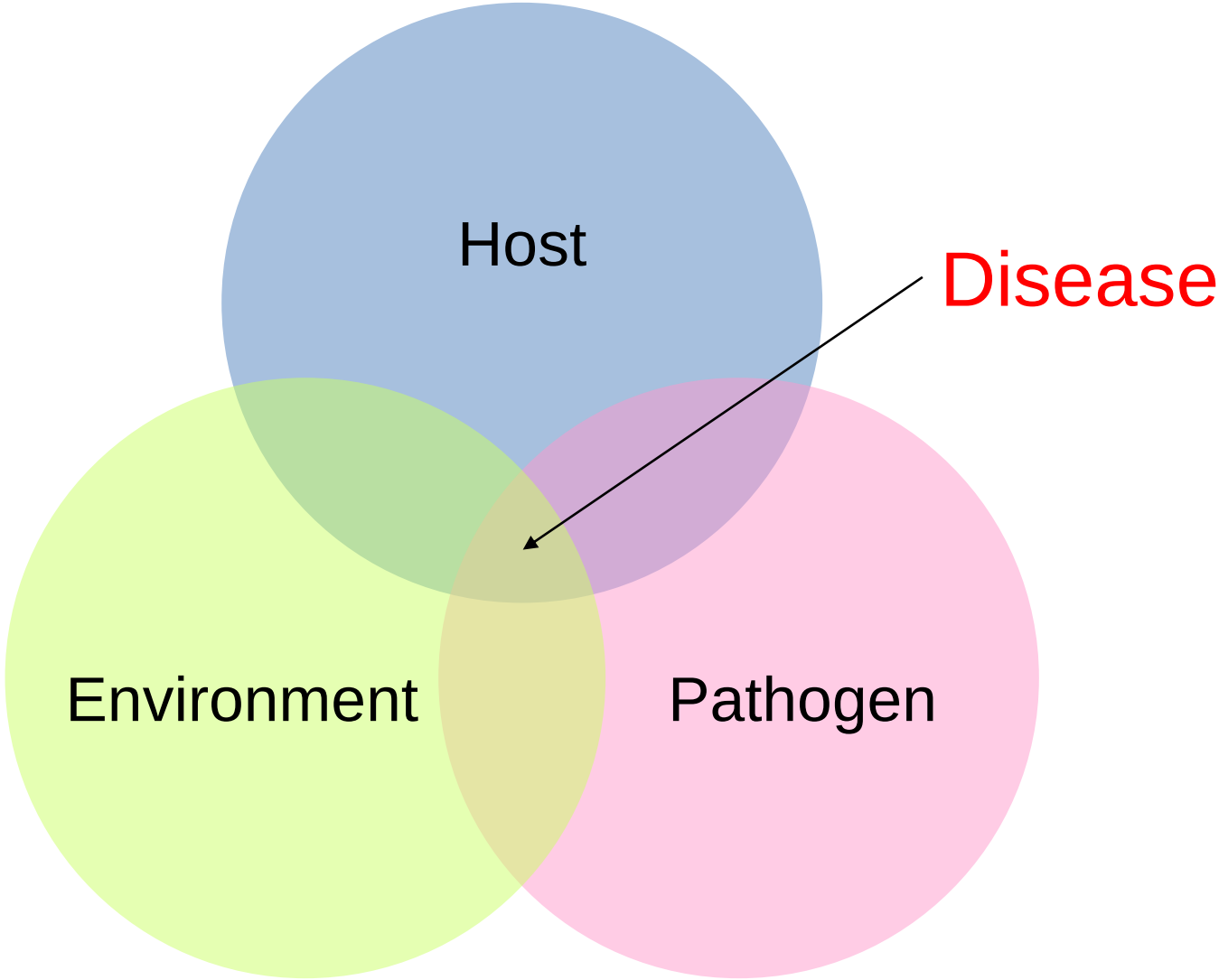


Data: Fauci *et al.*, EID Vol. 11, No. 4, April 2005

ProMed Health Map: Infectious Disease Incidents in One Month: Feb 2016

http://healthmap.org/en

Factors Influencing Emerging Infections: Host-Pathogen-Environment Interactions



Disease Interactions: Host Factors

- Immunological status
  - Immunologically naive population?
  - Herd immunity?
- Poverty
  - Hunger & weakness
- Exposure risks
  - Cultural exposure to high risks (e.g. chicken/human interactions in Asia)
  - Transfusion related transmissions

Disease Interactions: Environmental Factors

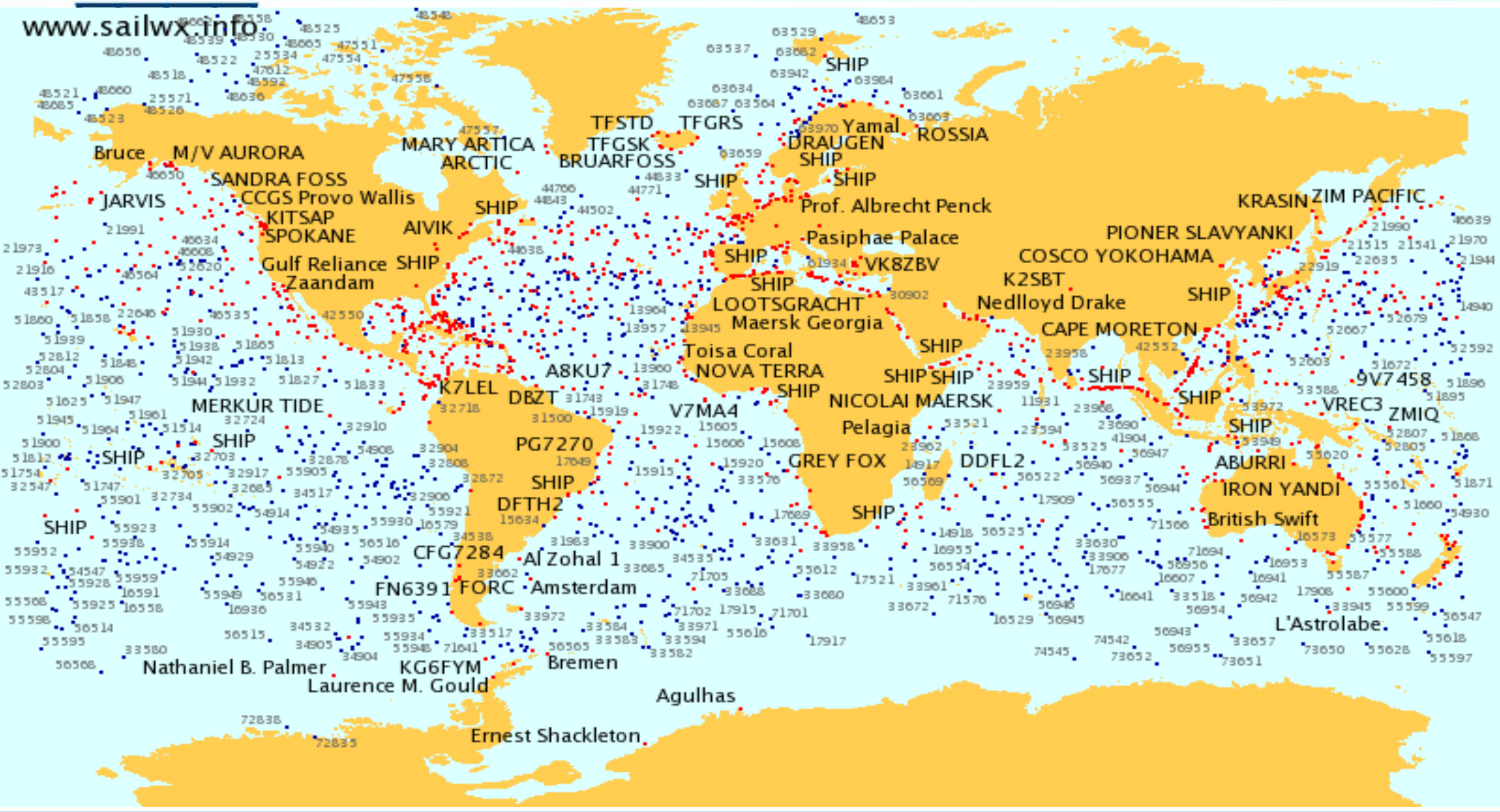
- Zoonoses circulating in the animal population
  - SARS, MERS, Avian influenza, Chikungunya, Hepatitis E
- Society- the way we live?
  - Close interactions with zoonotic pools of virus
  - Poverty- Sanitary conditions
  - Consumption of contaminated meat (i.e. Bushmeat trade)
  - Population density
- Travel and transport
  - Increase travel to exotic lands and the global trade and transport of goods (e.g. MERS arriving in South Korea)
- Climate changes
  - Insect vector population changes / migratory changes

Disease Interactions: Pathogen Factors

- Emergence of immunologically new strains
  - Mutations, co-infection & re-assortment (e.g. genetic shift)- example: Influenza virus
- Increased virulence or pathogenicity
  - Mutations
  - Passage of virus through a new host (e.g. SARS)
- Vector mediated transmission
  - Presence of an appropriate vector insect population

PDA
Parenteral Drug Association

Export-Import: The Global Trade

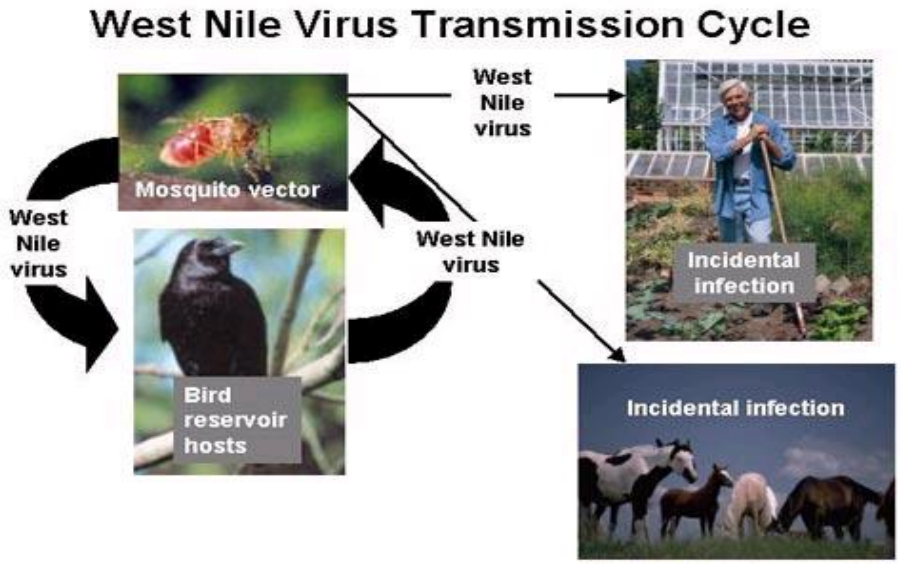


West Nile Virus: Facts

- Virus family *Flaviviridae*
  - Enveloped virus
  - ~40-60 nm
- Transmission cycle involves:
  - Circulation and amplification in birds
  - Transmission via mosquitos
- Disease
  - 80% of infections are asymptomatic
  - 20% develop West Nile Fever:
    - Fever, headache, fatigue
  - A small percentage go on to develop West Nile associated meningitis, encephalitis or poliomyelitis
  - Virus is transmissible via blood products and organ transplants

West Nile Virus Transmission Cycle

- West Nile Virus became a significant threat to the safety of blood products in the USA from 2000 onwards:
  - Transmissions were observed in organ transplant recipients
  - Concerns were raised about the possibility that the virus could also be transmitted through human plasma derived medicinal products

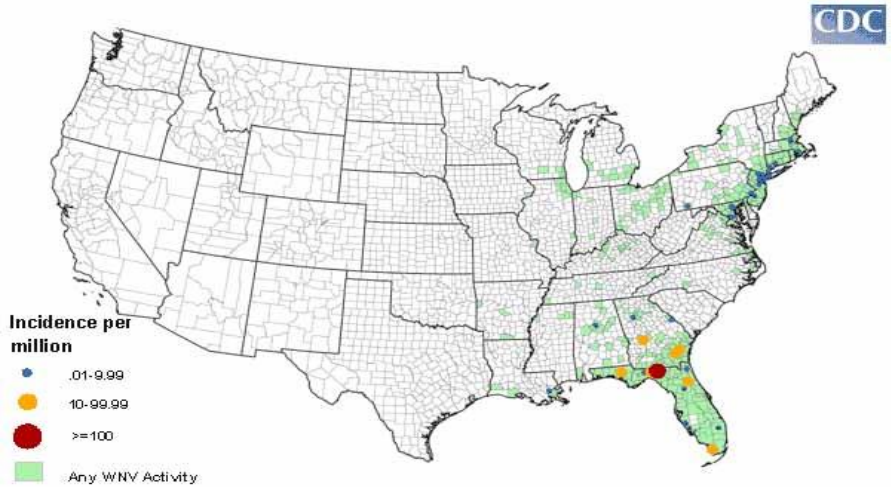


www.cdc.gov

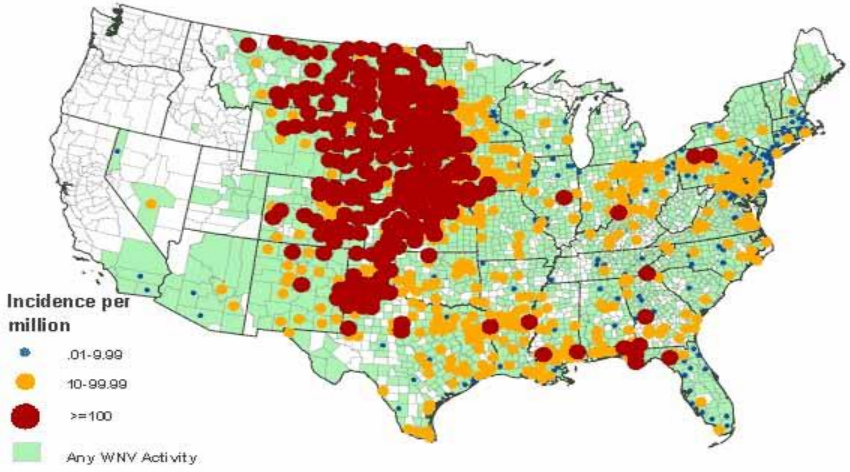
West Nile Virus in US: 1999-2005

1999

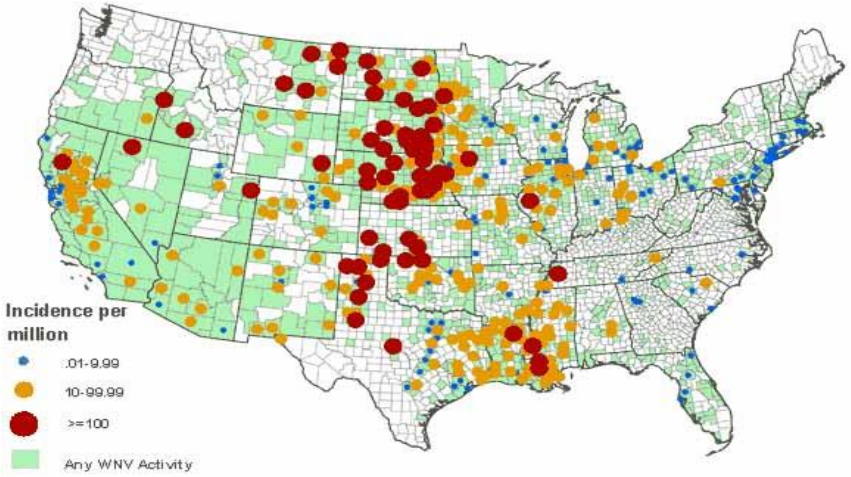
2001



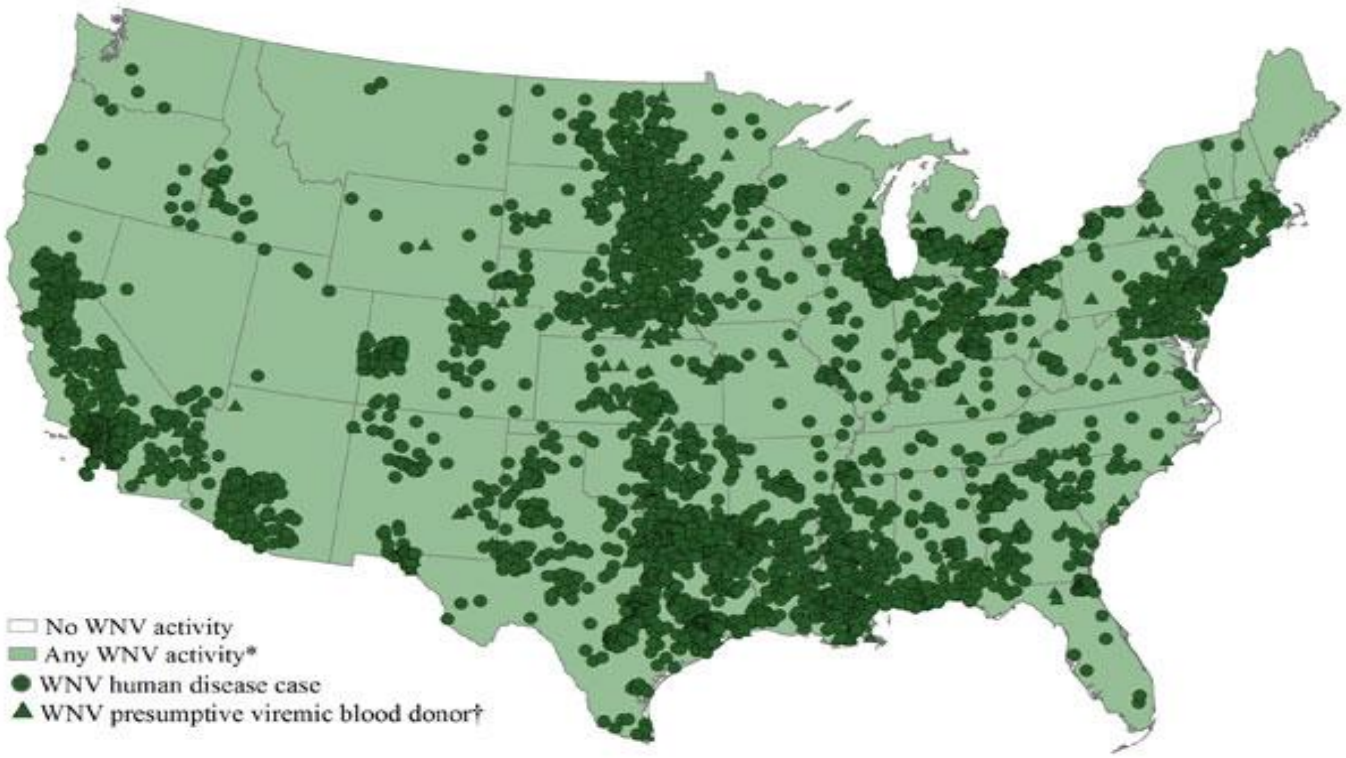
2003



2005



WNV in 2012?



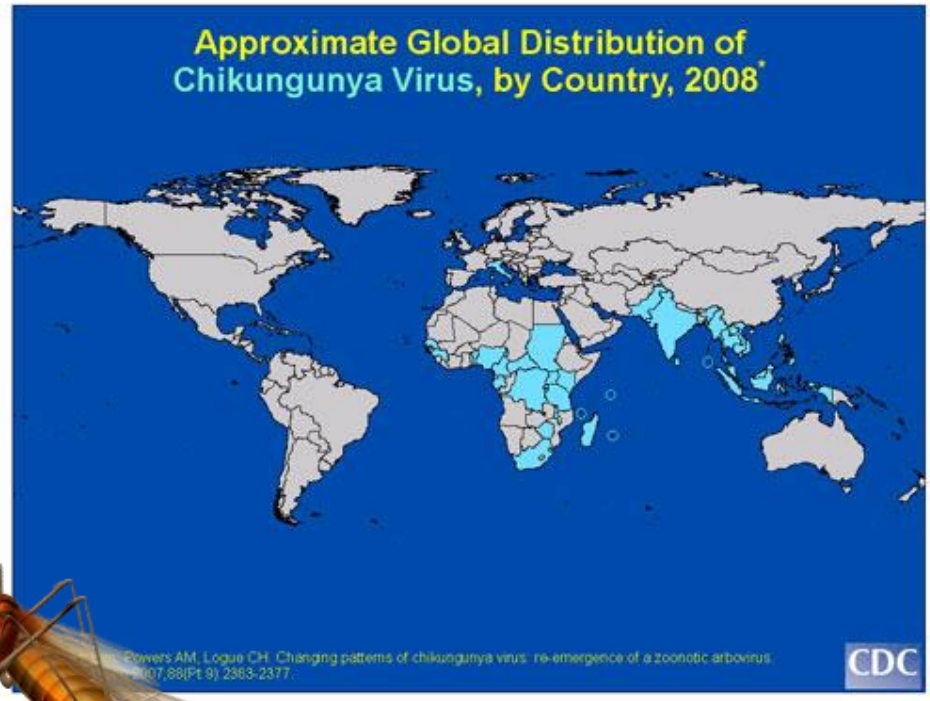
Human disease cases report to CDC					
State	Neuroinvasive disease	Non-neuroinvasive disease	Total cases	Deaths	Presumptive viraemic blood donors †
Totals	2,873	2,801	5,674	286	**703**

† Presumptive viraemic blood donors have a positive screening test which has not necessarily been confirmed

http://www.cdc.gov/ncidod/dvbid/westnile/surv&control/CaseCount12_detailed.htm

Chikungunya

- Alpha virus in the Family *Togaviridae*
  - Enveloped virus (Dengue-like)
- Mosquito transmitted
- Transmissible by blood and organ transplantation
- Recent outbreaks:
  - Kenya (2004)
  - Reunion Island (2005-6)
  - India (2006)
  - Italy (2007)



Outbreak of Chikungunya in Italy- 2007

Index case:

- Infected foreigner from the Indian subcontinent
- Arrived in Italy on June 21 and developed symptoms two days later

The peak of the epidemic curve occurred during the third week of August

- By Sept 4, 2007 a total of 197 cases had been reported

Eurosurveillance, Volume 12, Issue 36, 06 September 2007

Chikungunya: The Pivotal Role of Seasonality in Epidemics

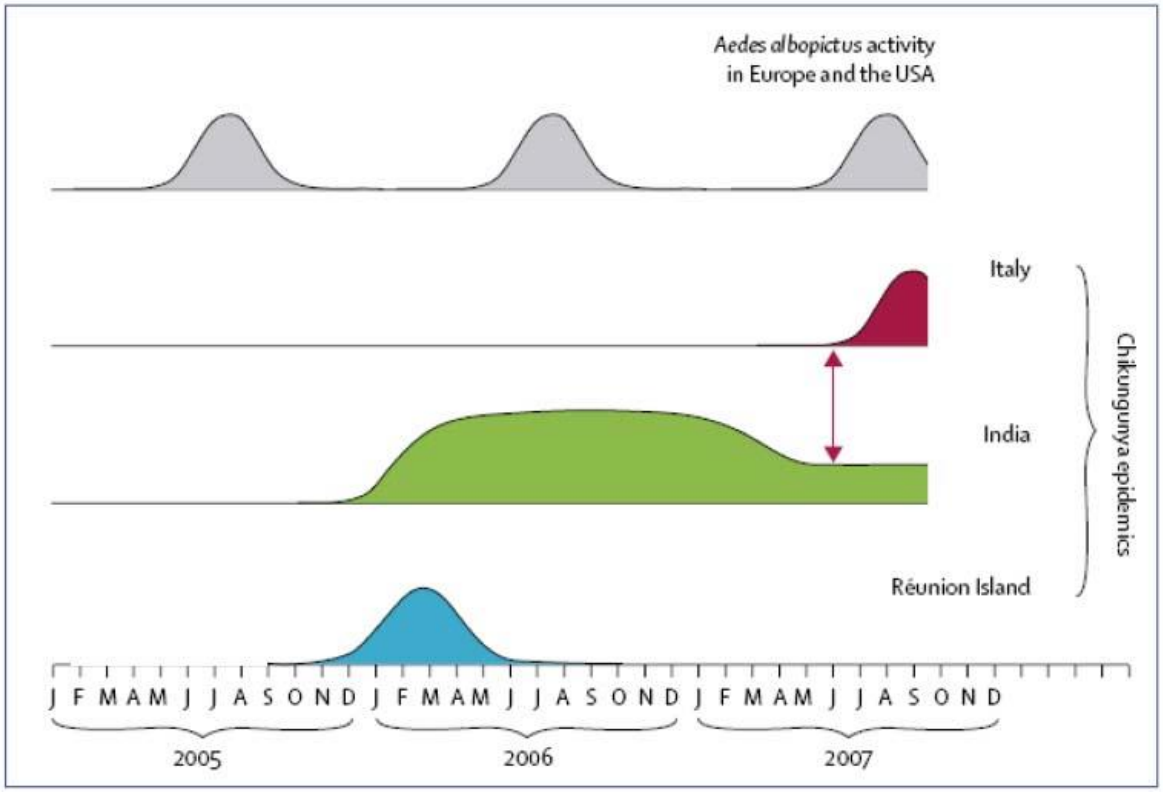
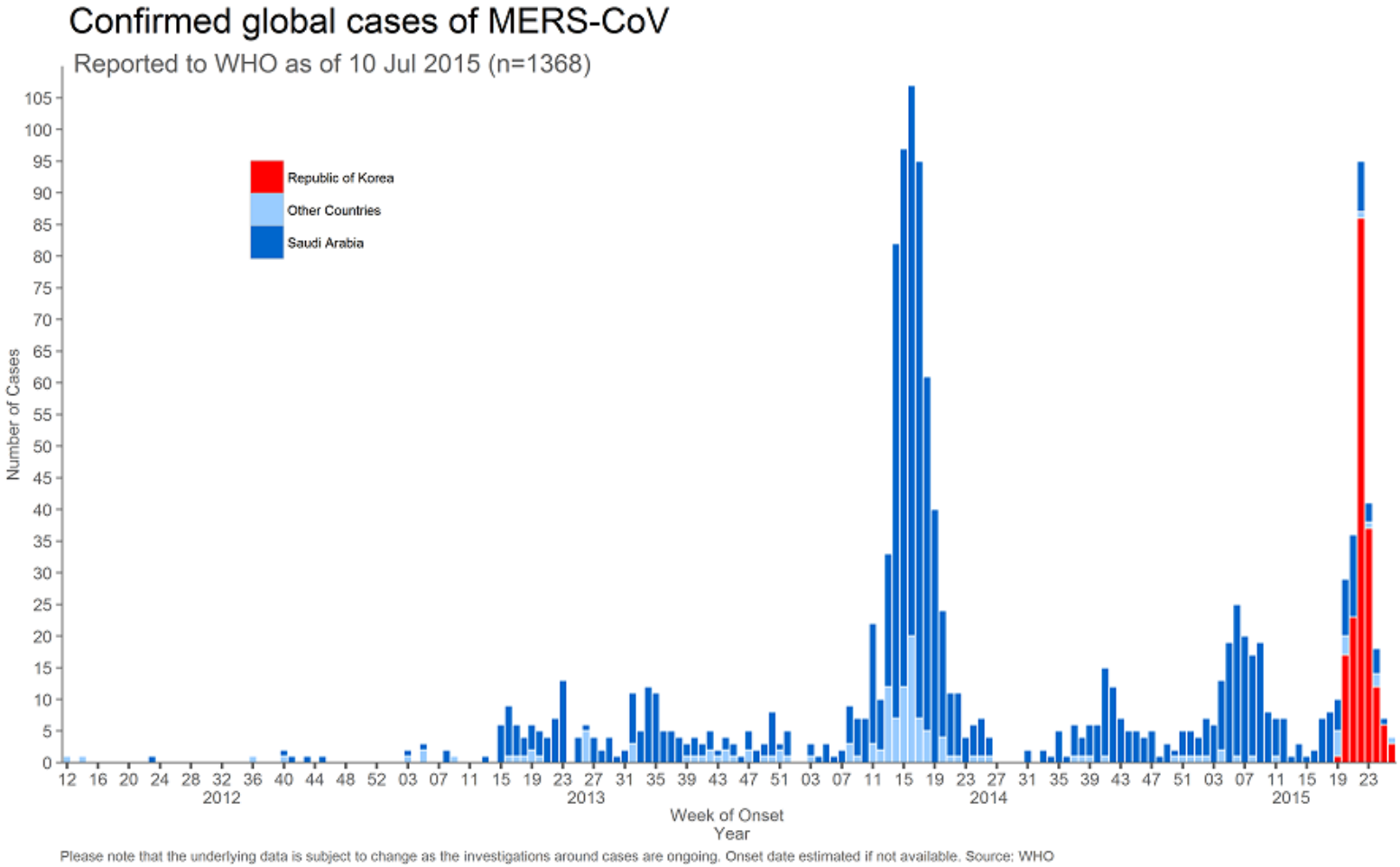


Figure: Synchronisation of *Aedes albopictus* activity in Europe and the USA and of Asian and Indian Oceans outbreaks
The arrow indicates the date of return from India to Italy of the presumed index case of the Italian outbreak.

Data: The Lancet Infectious Diseases, Volume 8, Issue 1, Page 5; R.Charrel

Importing Disease?: MERS in Korea



MERS Virus- Important Facts

- Worldwide 1100+ cases
  - Korea- 186 cases
  - Case fatality rate ~40% (~19% in Korea)
- Intermediate host – dromedary camel (maybe also bats)
  - High prevalence of anti-MERS antibodies in camels
  - Serum from camels applied in mouse model was effective for treatment
    - Zhao J et. Al; J. Virology (in press)

Are MERS Infection Rates Higher than Reported?

A recent publication of the prevalence of antibodies to MERS virus in Saudi Arabia has shown higher than expected levels of antibodies to MERS in camel exposed persons (Mueller *et. al.*; Lancet Inf. Dis.; 2015):

- Shepherds: 2.3%
- Slaughterhouse workers: 3.6%
- At this level of sero-prevalence, this calculates to a total of 44,951 individuals older than 15 years who have been exposed to MERS
  - But the total worldwide case numbers is only around 1,000-2,000
  - Is there a sub-population who can be infected with the virus but are sub-clinically infected?
  - Does this represent a risk for e.g. blood products?

Plasma Derived Products: Examples of Contamination

Historically (80-ies)

- ~10,000 patients (primarily haemophiliacs) infected with HIV
- ~20,000 patients infected with HCV

Recent past

- vCJD, HAV and B19 have been occasionally transmitted by plasma products

Today and in the future

- New emerging viruses may contaminate plasma pools (e.g. WNV, SARS-CoV, MERs, vCJD …….)

Not the focus of today's presentation- but there are lessons we can learn (see later)

Cell Culture Derived Products: Examples of Contamination

- Cell culture derived products
  - Cell line derived:
    - Endogenous Retroviruses
    - Latent Herpesviruses (e.g. EBV)
  - From animal derived components:
    - Porcine parvovirus (PPV)- e.g. trypsin
    - Porcine circovirus (PCV) - e.g. trypsin
    - Bovine viral diarrhoea virus (BVDV)
    - Bovine polyomavirus (BPyV)
    - Equine haemorrhagic disease virus (EHDV)
    - Cache valley virus (CVV)
  - Of as yet non clearly defined aetiology
    - Mice minute virus (MMV)
    - Vesivirus 2117 (probably animal derived)

Virus Contamination Events in Cell Manufactured Biologicals

Virus	Cell	Year	Company	Animal Component Suspected?
EHDV	CHO	1988	Bioferon GmbH	Yes
MMV	CHO	1993	Genentech	??
MMV	CHO	1994	Genentech	??
Reovirus	Human 1° Kidney	1999	Abbott Labs	Yes
Cache Valley virus	CHO	2000	(not publicly available)	Yes
Human Adenovirus	HEK 293	2002	Eli Lilly	??
Cache Valley virus	CHO	2003	(not publicly available)	Yes
MMV	CHO	2008	Amgen	??
MMV	CHO	2009	Merrimack	??
MMV	BHK	2010	Foot & Mouth Disease (Institute of Turkey)	??
Vesivirus 2117	CHO	2003 (1998)	Boehringer Ingelheim	??/Yes
	CHO	2008	Genzyme, Belgium	??/Yes
	CHO	2008	Genzyme, USA	??/Yes
	CHO	2009	Genzyme, USA	??/Yes
PCV-1	Vero	2010	GlaxoSmithKline	Yes
PCV-1/PCV-2	Vero	2010	Merck	Yes

Adapted from Dayue Chen; PDA Virus and TSE Safety Forum, Washington, 2014

Basic Strategies for Controlling Virus Risk

Guidelines on Virus Safety (1)

FDA

Points to Consider in the Characterisation of Cell Lines Used to Produces Biologics (1993).

Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use (1997).

ICH

Q5A: Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin (1997)

Q5D: Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products (1998)

Guidelines on Viral Safety (2)

CPMP

- Note for Guidance on the Production and Quality Control of Animal Immunoglobulins and Immunosera for Human Use (CPMP/BWP/3354/99)
- Note for Guidance on the Use of Bovine Serum in the Manufacture of Human Medicinal Products (CPMP/BWP/1793/02)
- Production and Quality Control of Monoclonal Antibodies (2007; CHMP/BWP/157653/07)
- Revised CPMP Guidelines on Virus Validation Studies (1996; CPMP/BWP/268/95)

Guidelines on Viral Safety (3)

CPMP (continued)

- Position Statement on Creutzfeldt-Jakob Disease and the Safety of Plasma- and Urine-Derived Medicinal Products (EMEA/CPMP/BWP/2379/02 Rev. 1
- Guidelines on the Investigation of Manufacturing Processes for Human Plasma-Derived Medicinal Products with Respect to vCJD Risk
- Guideline on Viral Safety Evaluation of Biotechnological Investigational Medicinal Products (EMEA/CHMP/BWP/398498/2005-corr)

The Safety Tripod (1)

Characterisation of Starting Material

In-Process and Final Product Testing

Virus Validation of the Manufacturing Process

The Safety Tripod (2)

Characterisation of Starting Material

- e.g. Characterised master cell banks
- Selection of raw materials (i.e. serum) for low risk
- Sourcing policies (e.g. location)

In-Process and Final Product Testing

- Infectivity or PCR screening batches

Virus Validation of the Manufacturing Process

- e.g. chromatography, nanofiltration, solvent/detergent

Methods of Virus Detection Included in Guidelines?

- *In vivo* tests
  - Antibody production tests
  - Animal safety tests
- *In vitro* tests
  - Cell culture based
  - Molecular biological methods
    - e.g. PCR, Reverse Transciptase assays (e.g. FPERT)
- Electron microscopy
- Immunoassays

Virus Inactivation Steps

KINETICS

Blood Products

- Solvent detergent
- Lyophilisation/Dry Heat
- Pasteurisation
- Methylen Blue/Light
- Beta Propriolacton/UV
- Octanoic Acid

Recombinant Products

- Low pH Treatment
- Microwave
- UV Inactivation

Other

- Gamma Irradiation
- Formaldehyde
- Guanidine Hydrochloride
- Oxidative Treatment H_2O_2
- Organic solvents

Virus Removal Steps

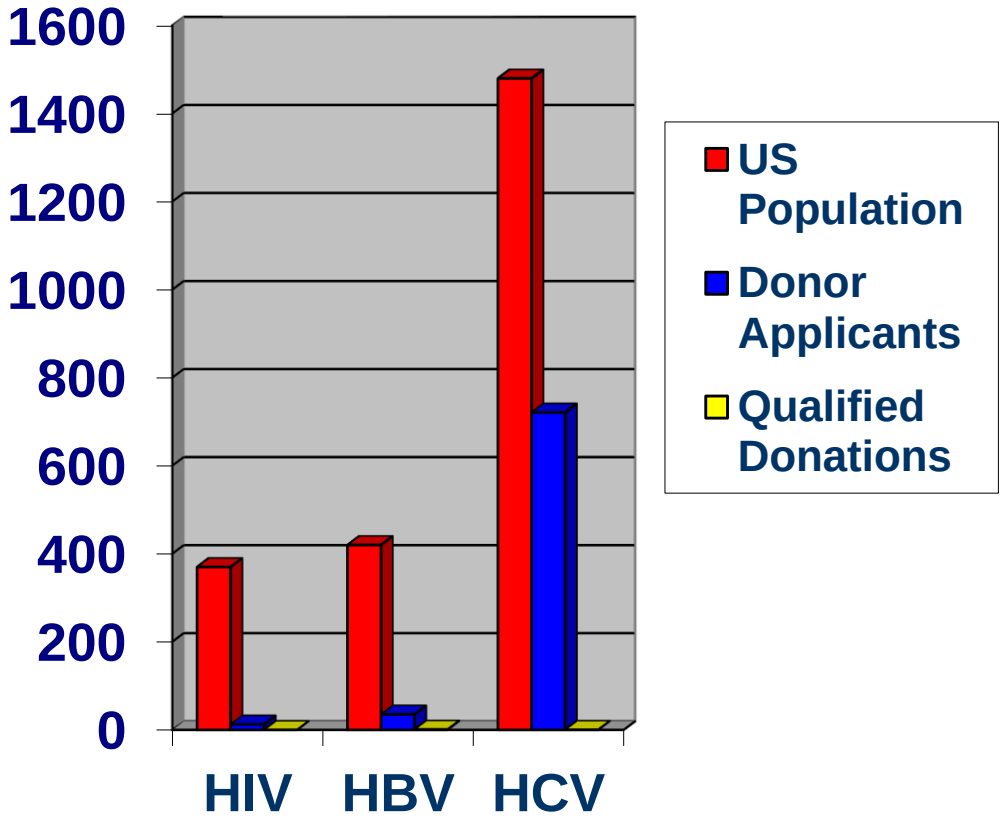
- Chromatographic steps
  - Anion Exchange Column (*binding, non-binding)*
  - Cation Exchange Column *(binding , non-binding)*
  - Affinity Chromatography
  - Hydrophobic Interaction
  - Size exclusion Chromatography
- Virus Removal Filtration
- Membrane Absorber Filter
- Precipitation/Filtration

The Changing Paradigm

Over the years, regulators have shifted more and more focus onto the manufacturing process and the potential for the inactivation or removal of viruses:

The reason?- A realisation that viral clearance affords a significantly greater level of risk reduction than can be achieved through sourcing or testing

Human Plasma: The Contribution of Sourcing and Testing to Risk Reduction



Data from Waytes *et. al.* Dev Biol Stand 2000;102:37-51

- Donor sourcing can provide a significant reduction in viral marker rates and therefore risk
- However, even with improved donor sourcing, transmissions have occurred without additional measures to control risk.
- Reduction of risk:
  - Donor selection: ~1-2 $\log_{10}$
  - Donor testing: ~1-2 $\log_{10}$

How to Effectively Reduce the Risk: Sourcing, Testing & Virus Clearance

Sourcing risk reduction

- Based on plasma experience maximum: **~2 logs**
  - Waytes et. al. *Dev Biol Stand* 2000;102:37-51

Testing risk reduction

- Based on limits of sensitivity, a virus load in the order of **2-3 logs** could still be present

Virus inactivation or removal

- e.g. **>5 logs** inactivation where 1 robust step is present
- e.g. **>10 logs** inactivation where 2 robust steps are present

→ In comparison to sourcing & testing, the level of risk reduction through virus clearance is significantly greater!

Lessons Learned from Virus Inactivation & Removal with Plasma Products

- The input load should be carefully controlled through e.g. PCR testing
  - Insufficient alone however to eliminate transmissions of viruses like Hepatitis A/B/C or Parvovirus B19
- Inactivation procedures in the manufacturing process must be robust:
  - Complete inactivation or removal to below the limit of detection is desired- "***robustness***" is a key parameter
  - e.g. To stop the transmission of HCV in blood products, a "robust" inactivation (i.e. more than 6-7 logs inactivation through S/D treatment) was necessary

Example Retrovirus Load Risk Assessment

Retrovirus particle count in the bulk harvest of a biopharmaceutical is normally determined by transmission electron microscopy (TEM)

Not related to infectious titre

Represents a ‘worst case’ retroviral load

Can be related to clearance and reduction values obtained in virus validation studies

Example of a classical virus removal/mitigation strategy:

Enveloped viruses: at least 2 orthogonal steps-

Overall LRV > 10 log

Non-enveloped viruses: at least one step-

Overall LRV > 5- 6 log

Example Risk Calculation from ICH Q5A

APPENDIX 5

Calculation of Estimated Particles per Dose

This is applicable to those viruses for which an estimate of starting numbers can be made, such as endogenous retroviruses.

Example:

I. Assumptions

Measured or estimated concentration of virus in cell culture harvest = 10^6/ml

Calculated viral clearance factor = $>10^{15}$

Volume of culture harvest needed to make a dose of product = 1 litre (10^3ml)

II. Calculation of Estimated Particles/Dose

$$\frac{(10^6 \text{ virus units/ml}) \times (10^3 \text{ml/dose})}{\text{Clearance factor} > 10^{15}}$$

$$= \frac{10^9 \text{ particles/dose}}{\text{Clearance factor} > 10^{15}}$$

$$= < 10^{-6} \text{ particles/dose2}$$

Therefore, less than one particle per million doses would be expected.

Examples of Robust Process Steps in Biopharmaceutical Manufacturing Processes

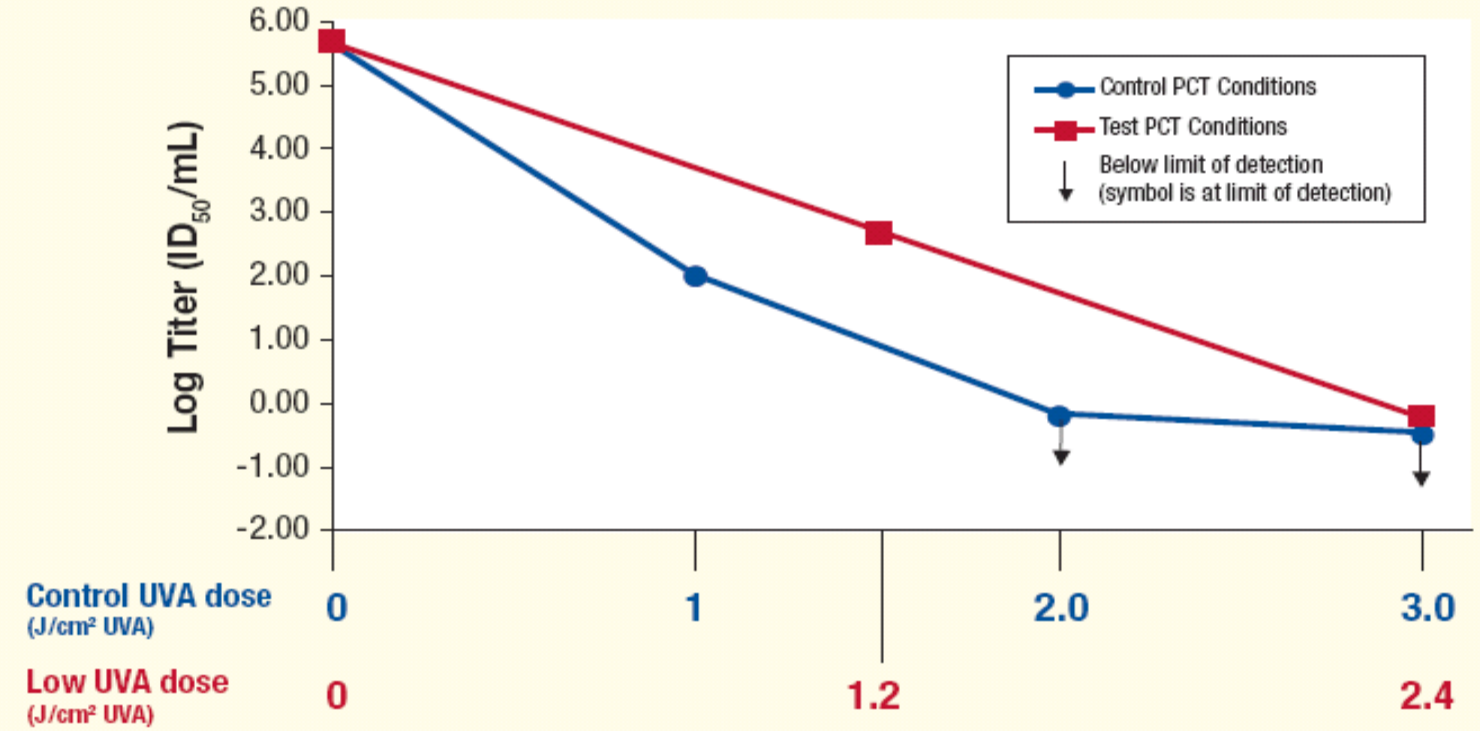
- Removal steps:
  - Virus filtration
    - Viruses larger than the pore size can be effectively removed
  - Ethanol precipitation (not robust for all virus types)
- Inactivation steps
  - Solvent detergent (enveloped viruses only)
  - Heat treatment (e.g. Pasteurisation at 60°C):
    - Some virus types might not be completely inactivated
  - Low pH (enveloped viruses only)
  - Solvent inactivation (primarily enveloped viruses)

Robustness: The Critical Factor for an Effective Virus Clearance Step

- Robustness:
  - The robustness of any given step is defined as sensitivity of the step to process variables:
    - For virus filtration, various factors can potentially impact on the robustness, including pressure, volume to filter area ratio and the matrix being filtered
    - For inactivation steps, the key measure of robustness is the kinetics of inactivation
      - i.e. Complete inactivation within no more than 20-50% of the total inactivation time

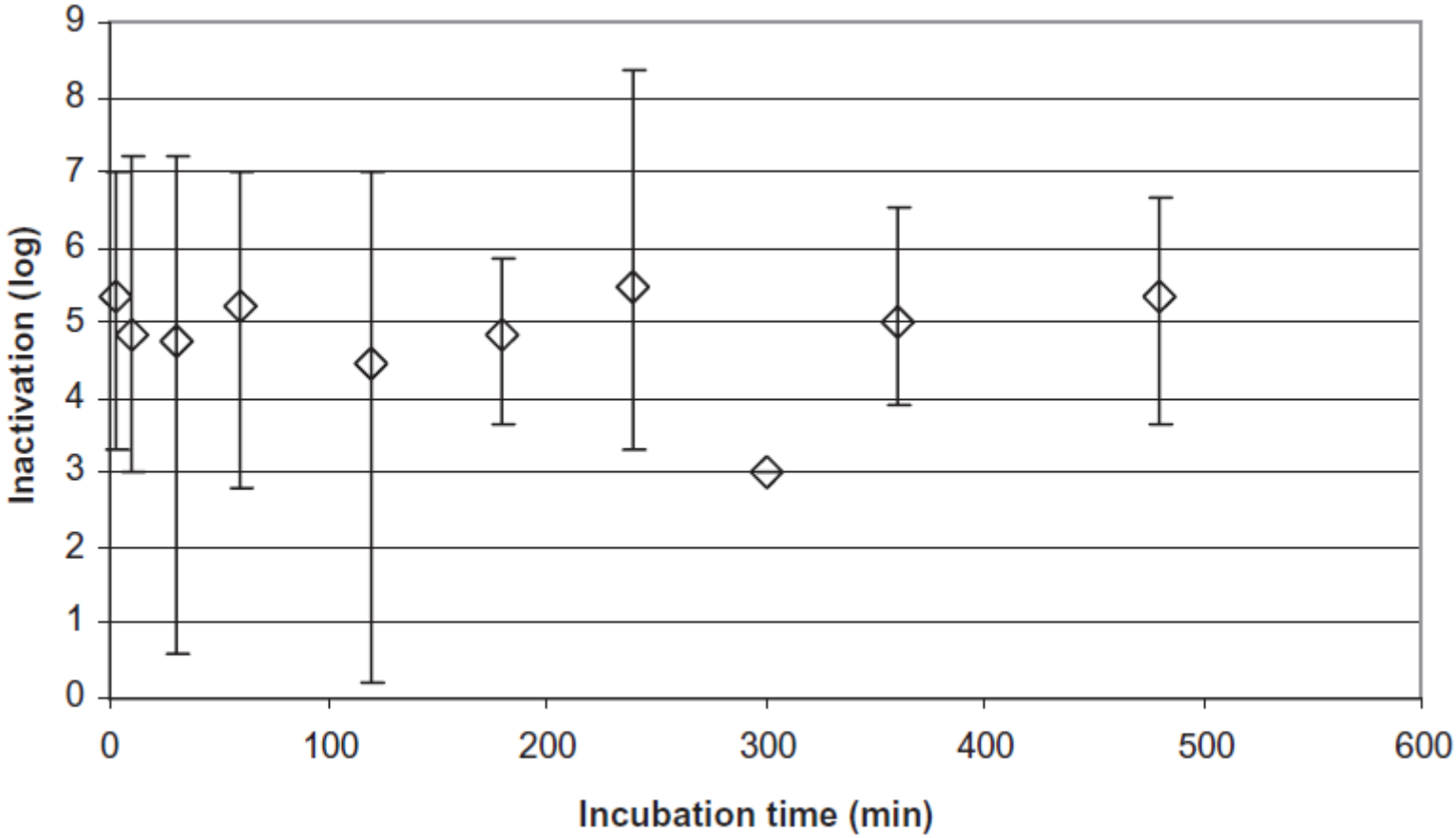
Amotosalen+UVA Light: Kinetics of Inactivation

Figure 4: Inactivation of DHBV Under Control and Test Treatment Conditions



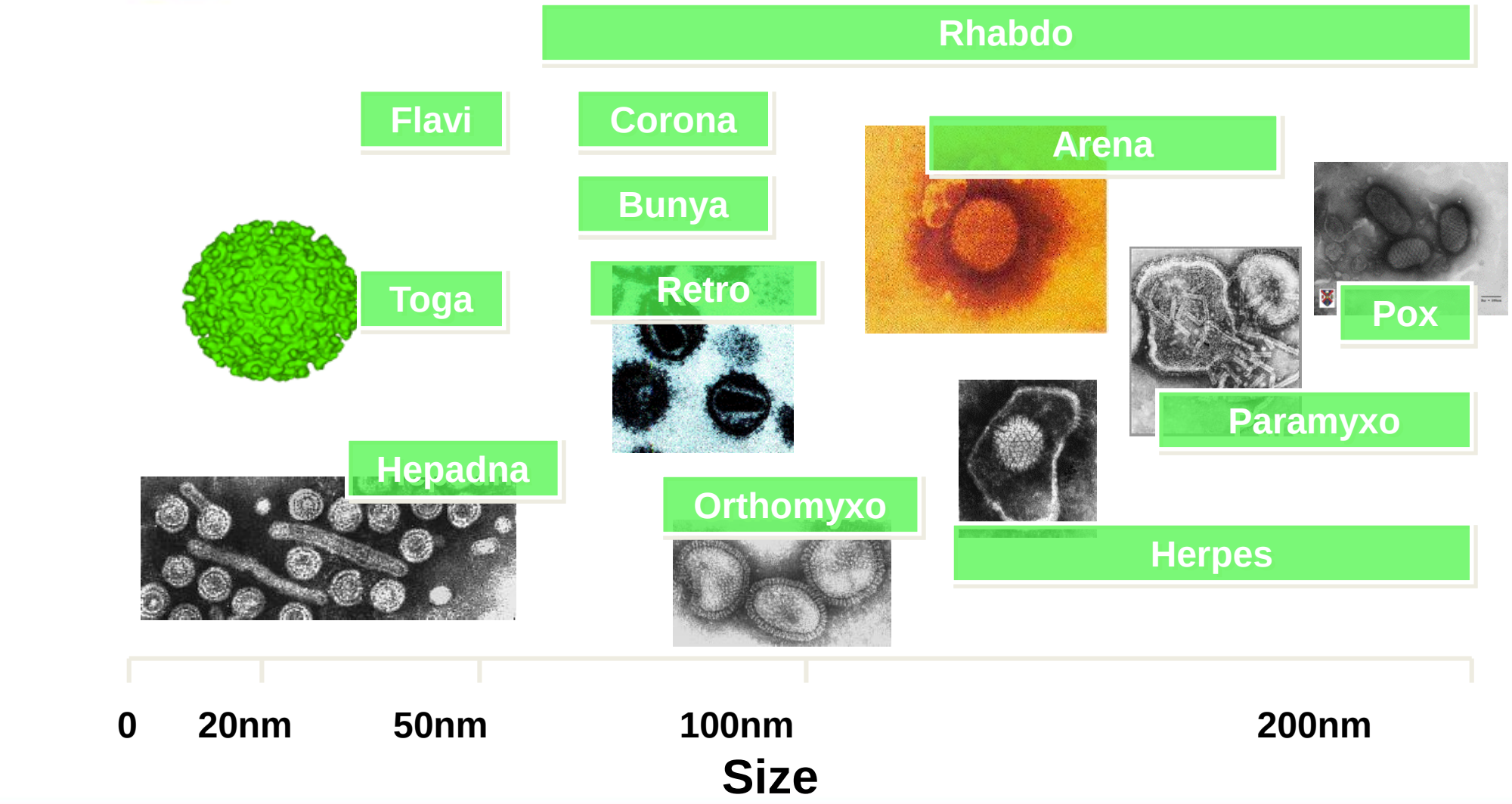
Data: Cerus- Presented at the XVII International Society of Blood Transfusion (ISBT) Regional Congress, Madrid, Spain; June 23 – 27, 2007

S/D Treatment: Kinetics of Inactivation

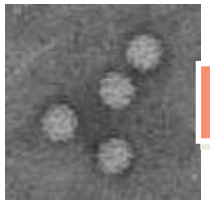


Data from Dichtelmueller et. al. 2009 (Robustness of solvent/detergent treatment of plasma derivatives: a data collection from Plasma Protein Therapeutics Association member companies). Number of experiments: n=128

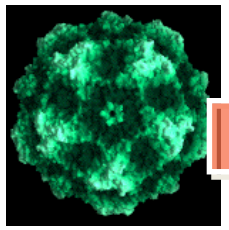
Virus Structure: Enveloped Viruses



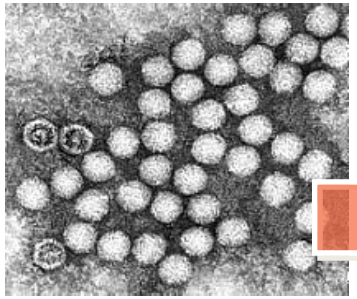
Virus Structure: Unenveloped Viruses



Circo

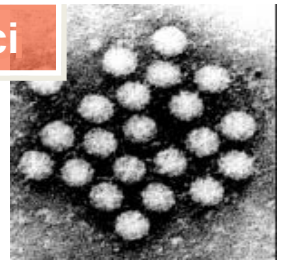


Parvo

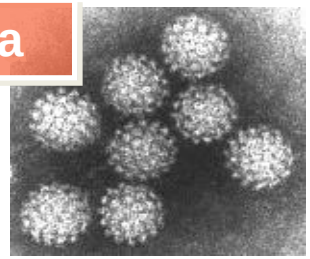


Picorna

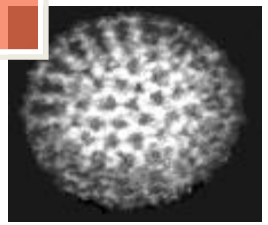
Calici



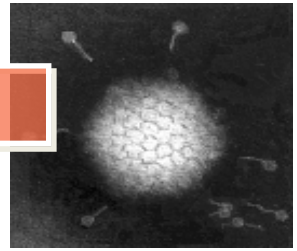
Papova



Reo



Adeno



0 10nm 20nm 50nm 75nm 100nm

Size

Emerging Viruses: Proven or Potential Transfusion Transmitted by Blood Products?

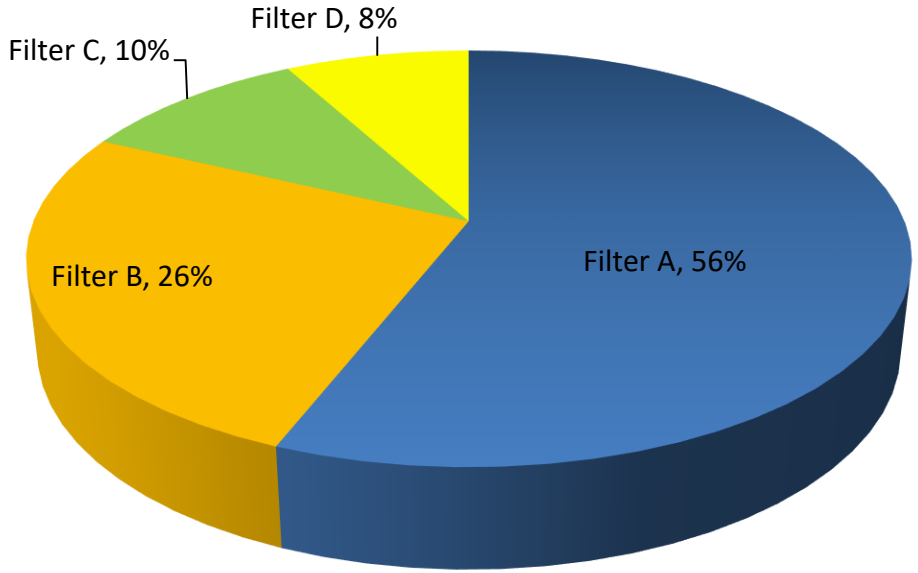
Virus	Family	Characteristics
HIV	*Retroviridae*	Enveloped; RNA
HBV	*Hepadnaviridae*	Enveloped; DNA
HCV & WNV	*Flaviviridae*	Enveloped; RNA
CMV & EBV	*Herpesviridae*	Enveloped; DNA
HTLV-I/II	*Retroviridae*	Enveloped; RNA
SARS/MERS	*Coronaviridae*	Enveloped; RNA
WNV	*Flaviviridae*	Enveloped; RNA
Influenza A	*Orthomyxoviridae*	Enveloped; RNA
HAV	*Picornaviridae*	Unenveloped; RNA
Parvovirus B19	*Parvoviridae*	Unenveloped; DNA
HEV	*Hepeviridae*	Unenveloped; RNA

Viruses Found as Contaminants of Recombinant Products?

Virus	Family	Characteristics
Endogenous Retrovirus	*Retroviridae*	Enveloped; RNA
EBV (Endogenous)	*Herpesviridae*	Enveloped; DNA
BVDV	*Flaviviridae*	Enveloped; RNA
Bovine polyomavirus	*Polyomaviridae*	Unenveloped; DNA
MMV	*Parvoviridae*	Unenveloped; DNA
Vesivirus 2117	*Caliciviridae*	Unenveloped; RNA
PPV	*Parvoviridae*	Unenveloped; DNA
PCV	*Circoviridae*	Unenveloped; DNA
EHDV	*Reoviridae*	Unenveloped; RNA

Virus Filtration- A Robust Step for Enveloped & Non-Enveloped Viruses

Virus Filters Tested



Virus Filter	Average RF* from all studies
Filter A	5.4
Filter B	5.9
Filter C	5.3
Filter D	3.5

i.e. Not all virus filters are able to consistently assure a RF of >4 logs. Virus breakthrough is also a phenomenon common to all filter types (discussed later)

* Based on data generated at ViruSure for studies between 2005 and 2015 (>400 experiments)

Virus Filtration- Different Types of Filters

Virus clearance filters are broadly classified into two categories:

Filters that provide removal of large viruses:

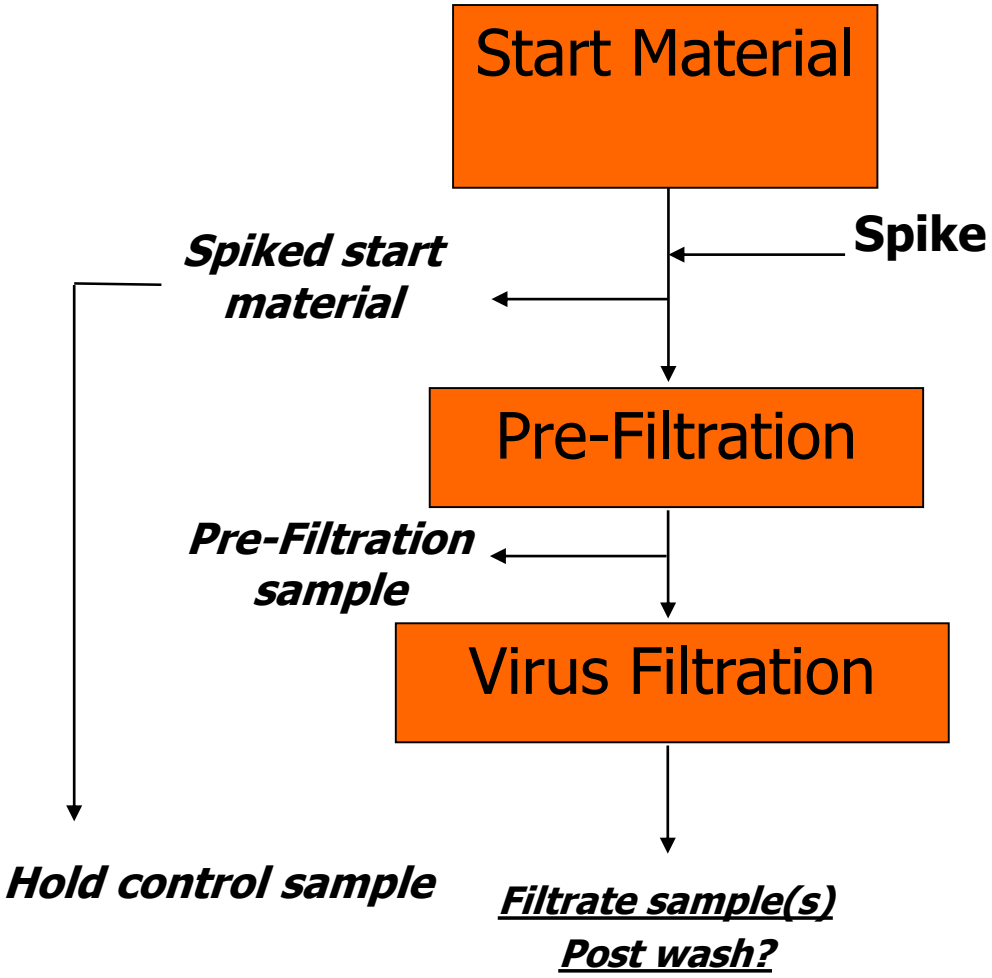
e.g. (~80–100 nm enveloped retroviruses)

Filters that provide >4 $\log_{10}$ removal of small and large viruses:

e.g. (~18–24 nm non-enveloped parvoviruses

i.e. “Parvo-grade”

Viral Filtration Studies: General Design



- Wherever possible spike prior to pre-filtration
- Inclusion of a pre-filter is important to control for the aggregation status of the virus
- Filtrate samples may be collected as a single sample or in fractions to determine where virus breakthrough occurs
- Post-wash sample may be collected separately or pooled together with the filtrate sample

Downscaling of the Process

Accurate and validated process downscaling is the foundation upon which the virus clearance data is generated:

- Manufacturer must demonstrate comparability with the manufacturing process
- Critical process parameters must be controlled (e.g. pH, Temperature, Pressure, Protein concentration)
- Pressure breaks should be considered for virus filtration to mimic possible scenarios that might happen in manufacturing

Virus Filtration- Robustness Discussions

Virus Filtration: Worst-Case Conditions?

Several factors have been identified that could impact on the robustness of a virus filtration step:

- Volume filtered- virus breakthrough occurs at higher frequency at larger volumes
- Matrix- different matrices can yield different reduction factors when evaluating the same filter type
- Pressure- lower pressure can result in higher levels of virus breakthrough. Pressure release can also occasionally result in virus breakthrough depending on the length of the pause
- Virus load- virus breakthrough occurs at higher frequency above a given virus load inside the filter

Fractionation of the Filtrate

By collecting the filtrate sample in fractions, it may be possible to get more information to support the robustness of the filtration step by determining when virus breakthrough becomes more significant:

- Generally virus breakthrough tends to occur at high levels late in the filtration
- Such data can help in defining manufacturing specifications and in handling manufacturing deviations

Volume Filtered?- An example of Virus Breakthrough with PPV

	Cumulative Log_{10} RF for Process			
	1-10ml	11-20ml	21-30ml	31-40ml
Experiments 1 (spiking at ~9 logs total PPV)				
Run 1	-	-	-	1.65
Run 2	-	-	-	1.69
Experiments 2 (virus load limited to 6 logs total PPV)				
Run 1	-	-	-	2.35
Run 2	-	-	-	1.99
Experiments 3 (spiking at ~9 logs total PPV)				
Run 1	4.58	3.31	2.35	1.60
Run 2	≥4.75	2.92	2.55	1.70

Data generated with Competitor D filters; i.e. volume to filter area can be very critical for some filters, less critical for other filter types where only low level virus breakthrough is occasionally observed

Pressure Break and Virus Breakthrough

Recent data has demonstrated that if virus filtration is interrupted (i.e. resulting in a drop in pressure within the filter) then following re-start of the filtration virus breakthrough has been occasionally observed:

- Such a scenario might represent a worst case condition for virus filtration
- In virus clearance studies however, pressure release is a standard procedure when performing the post-wash (different to manufacturing where often the post-wash is applied without any break in pressure)- this would represent worst case
- Virus breakthrough is more likely where a high virus load has been applied to the filter
- Data from pressure release studies can support manufacturing deviations, e.g. where a pump or pressure vessel fails during manufacturing

Must Robustness be Performed with all Viruses?

Although not generally required for Phase 1 studies, for licensed products regulatory authorities like to see more data for the filtration step to demonstrate robustness:

- Must an evaluation of robustness be performed for all viruses (large and small)?
  - Generally, authorities will accept robustness studies performed with only the smaller model virus selected for the study (i.e. normally Parvovirus)
  - Some authorities have even stated that they would accept the log reduction factor obtained with Parvovirus as applicable to larger viruses (e.g. Retrovirus)

Summary

Emerging viruses continue to challenge the safety of biopharmaceutical products:

- All product types are affected as contaminations in blood-derived and recombinant derived products has demonstrated
- Controlling the risks requires a good understanding of which viruses might be of risk:
  - Apply careful sourcing of materials
  - Apply the most appropriate testing stategy to further control the risks
  - Ensure that effective and ***robust*** steps are built into the manufacturing process

Questions?