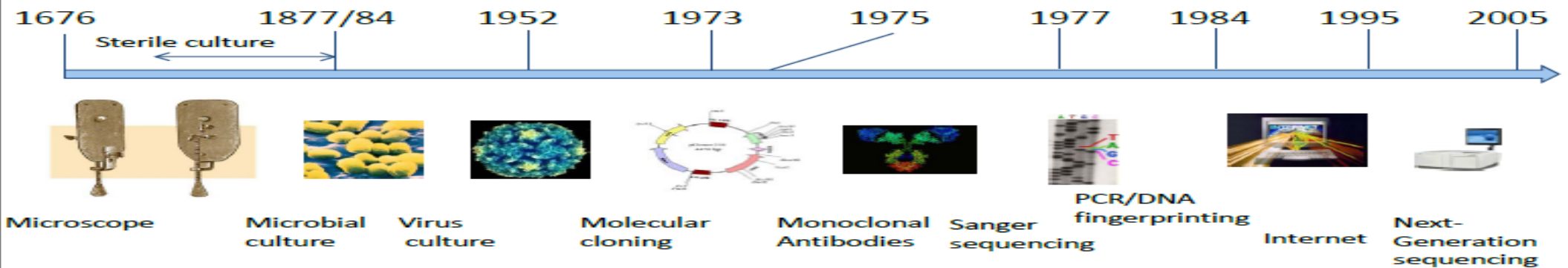


Surveillance at the molecular level: the good, the bad and the evil

Technology to support infectious disease surveillance



European Mobile Lab, MSF EVD Treatment Centre, Gueckedou, Guinea, March 2014



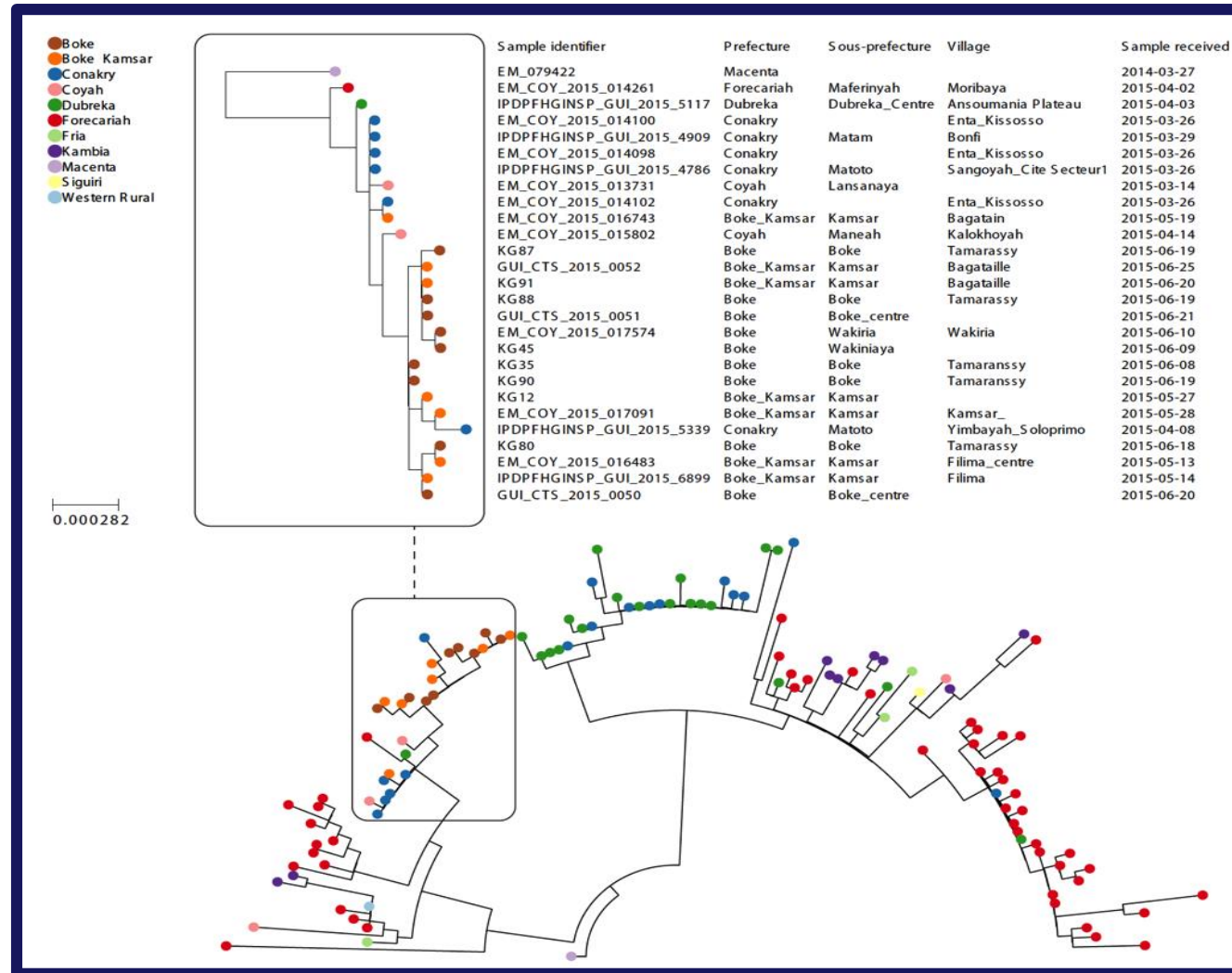
Real Time Sequence Data: contact tracing, mutation rate and understanding sexual transmission



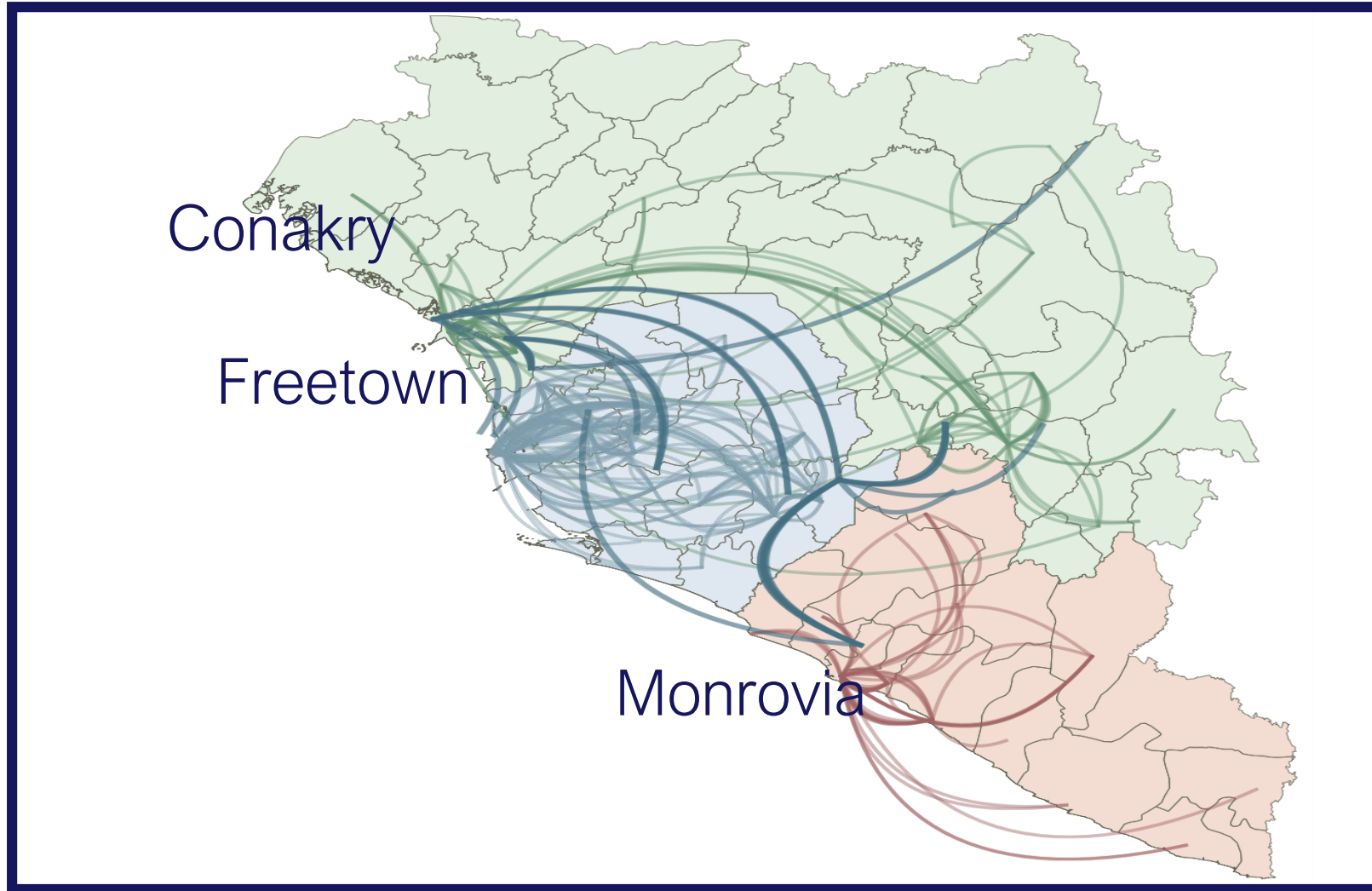
European Mobile Laboratory: real time genetic sequencing



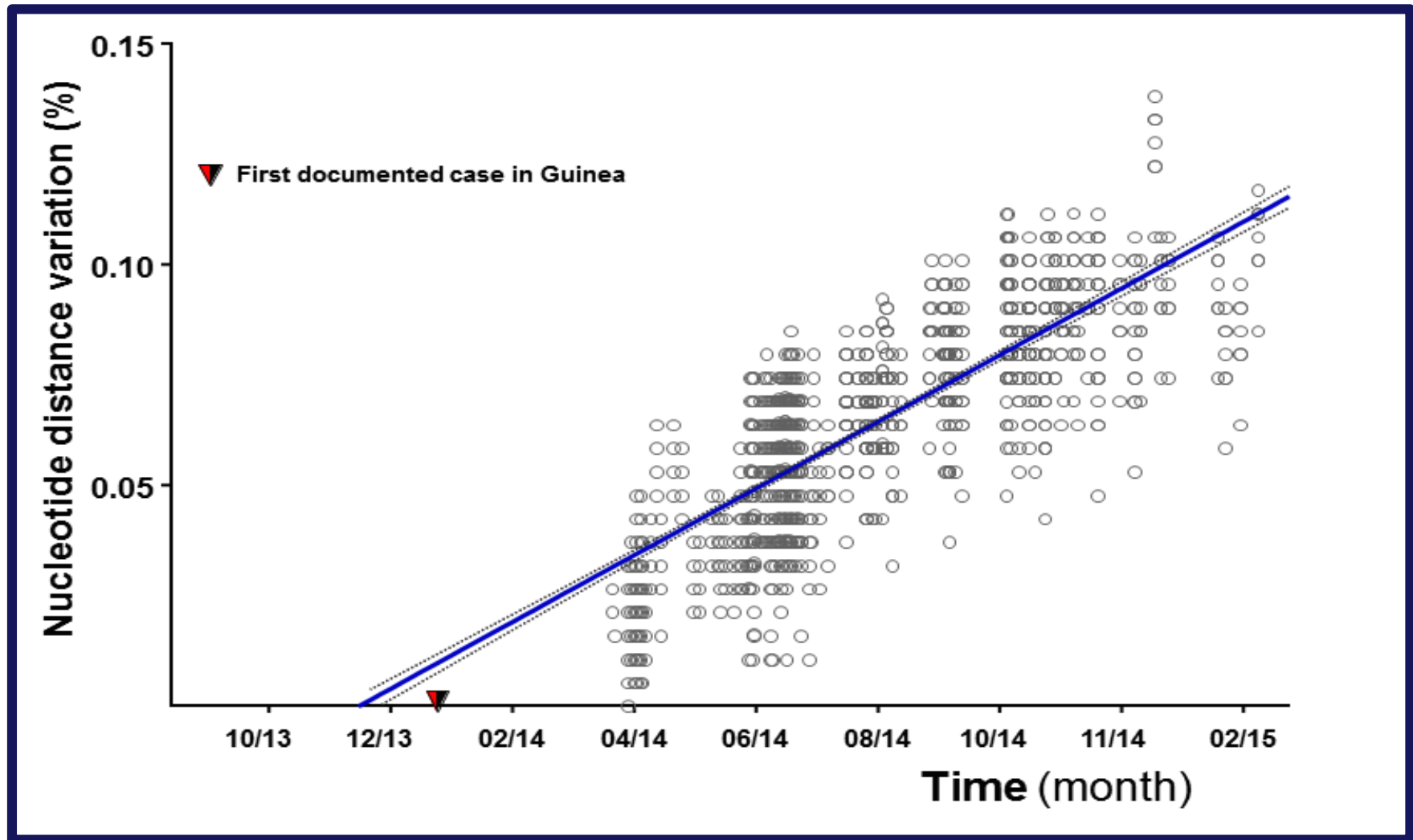
Ebola surveillance and contact tracing across prefectures, Guinea, 2014 - 2016



Ebola surveillance and contact tracing across international borders, West Africa, 2014 - 2016



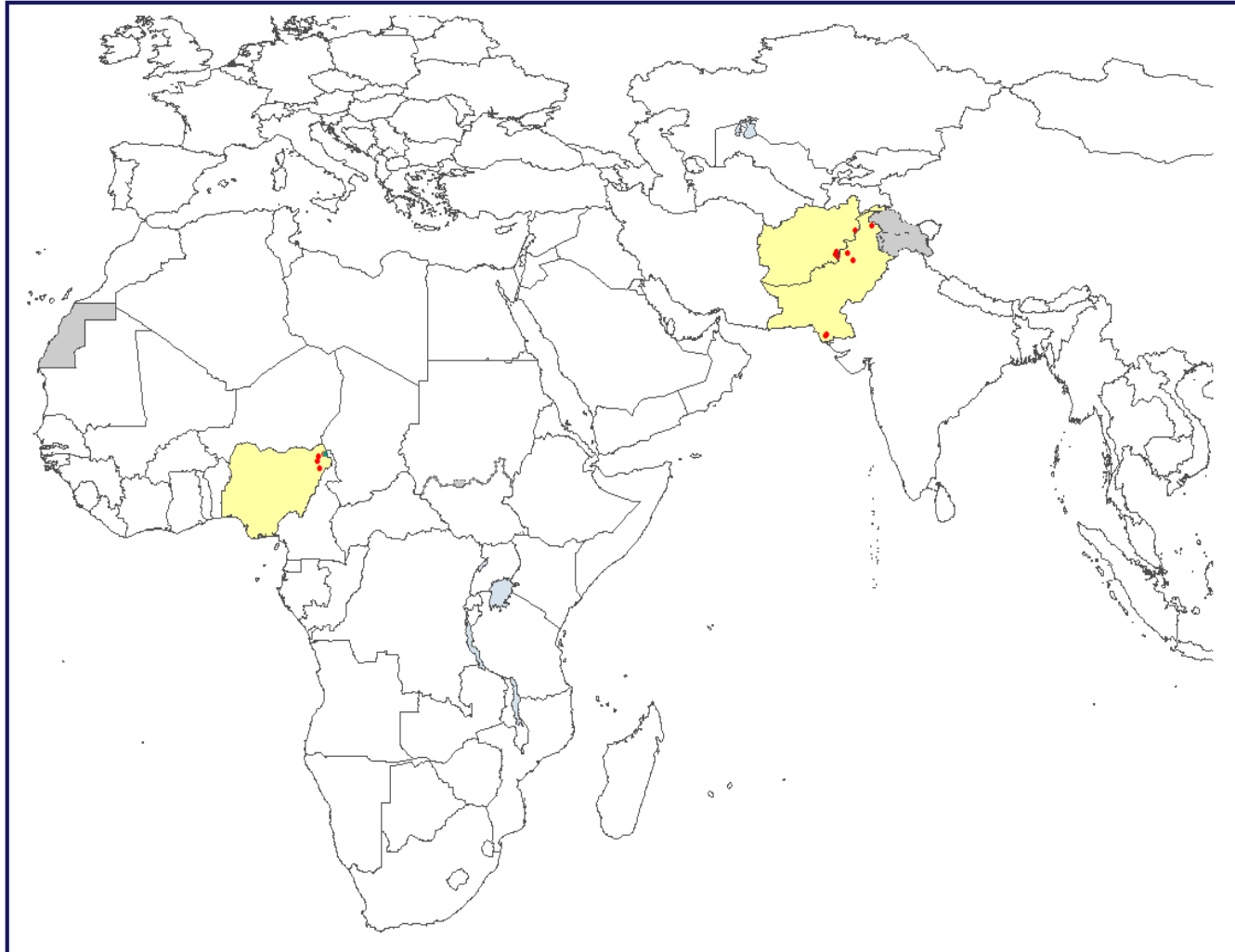
Ebola surveillance: prediction of outbreak origins and rate of evolution



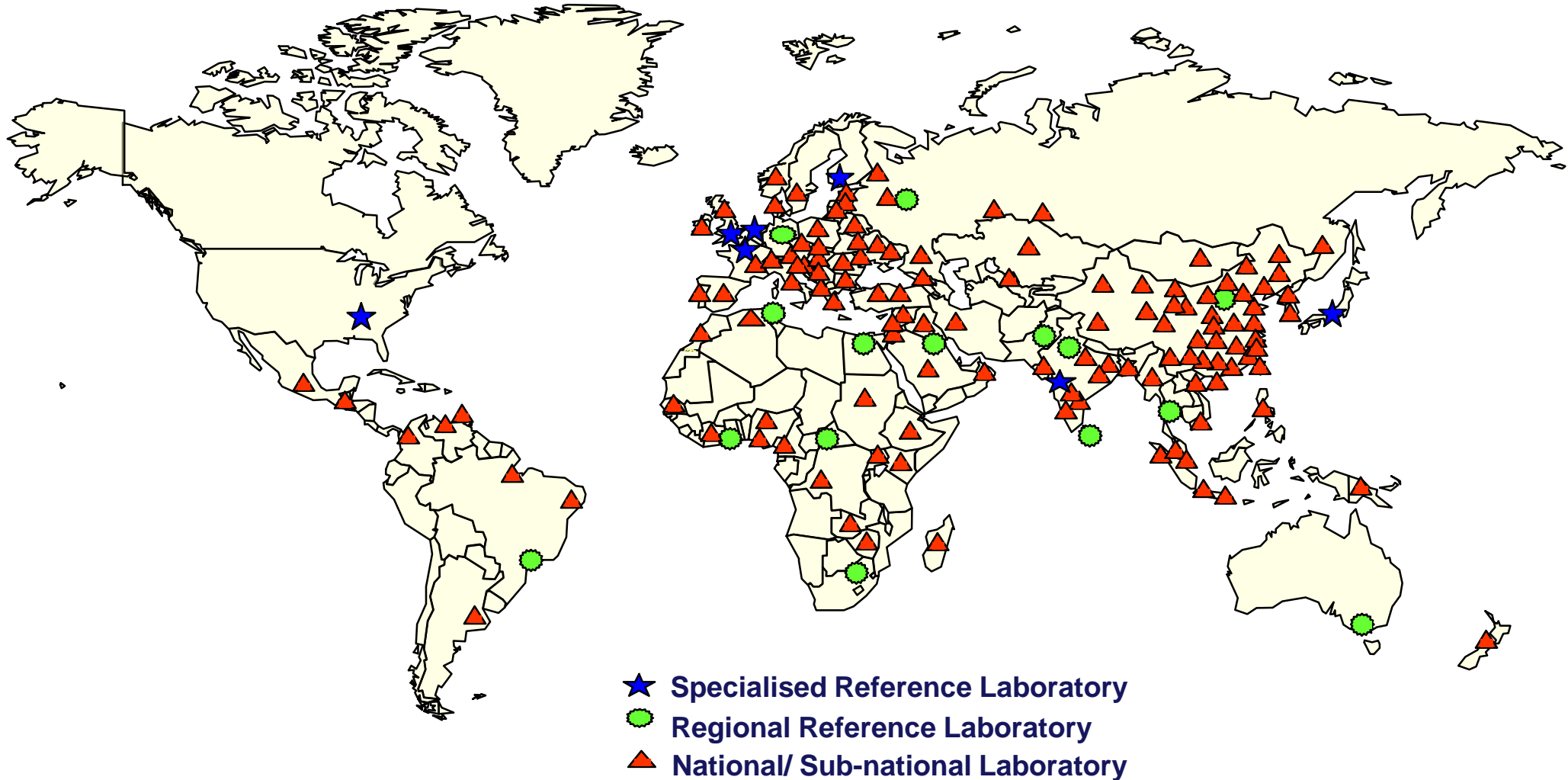
Surveillance at the molecular level: the good

- **Powerful supplement to surveillance when laboratory findings linked to epidemiology and outbreak understanding and control**

Polio Eradication: cases reported through surveillance, 23 May – 22 November 2016



Polio eradication strategy 3: surveillance of acute flaccid paralysis



Polio epidemiologists supporting surveillance

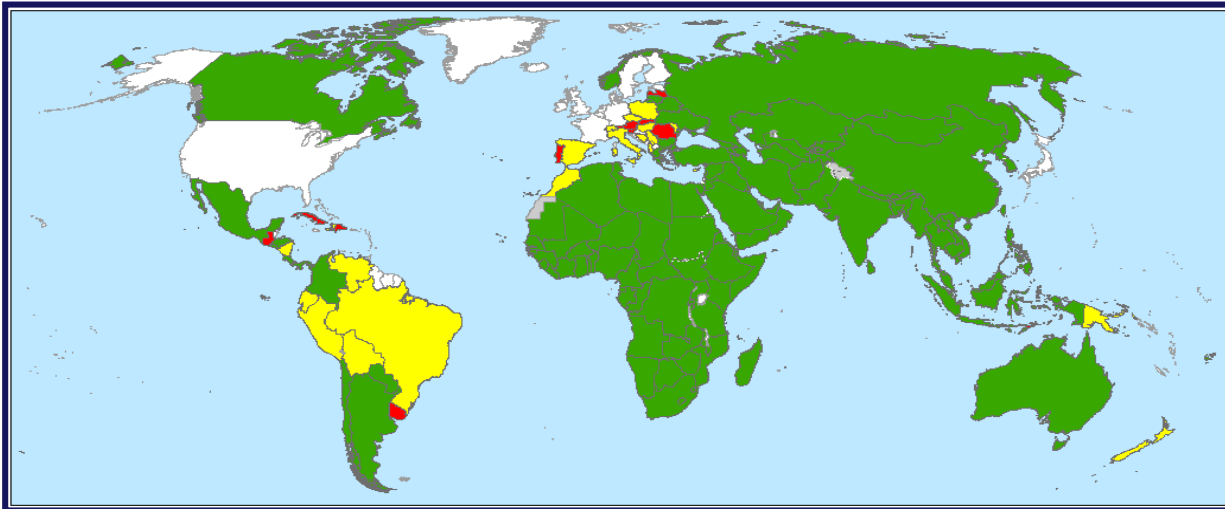
- Specially trained polio surveillance officers: in central government and provinces/districts**
 - each with driver with vehicle**
 - per diem for staff and driver for 10 field days per month**
 - fuel, local maintenance of vehicle**
 - real-time communication (cell or satellite phone, laptop)**

Acute flaccid paralysis (AFP) surveillance system: targets

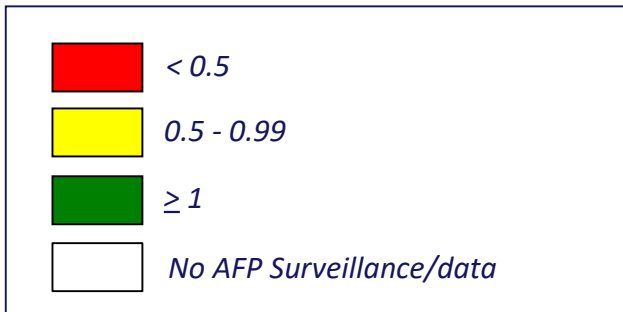
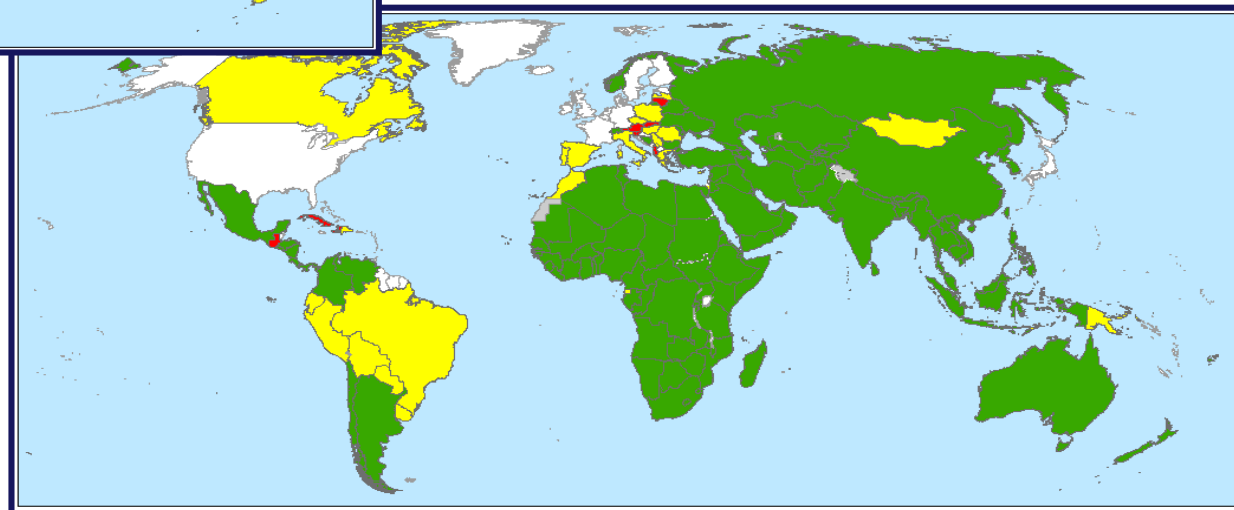
- **AFP reporting (*AFP reporting rate*)**
 - > 1 report < 15 years of age per 100 000
- **Specimen collection (*stool collection rate*)**
 - > 80% samples collected within 14 days of onset

Polio Surveillance: Non-polio AFP Rate

Oct 2014 – Sep 2015



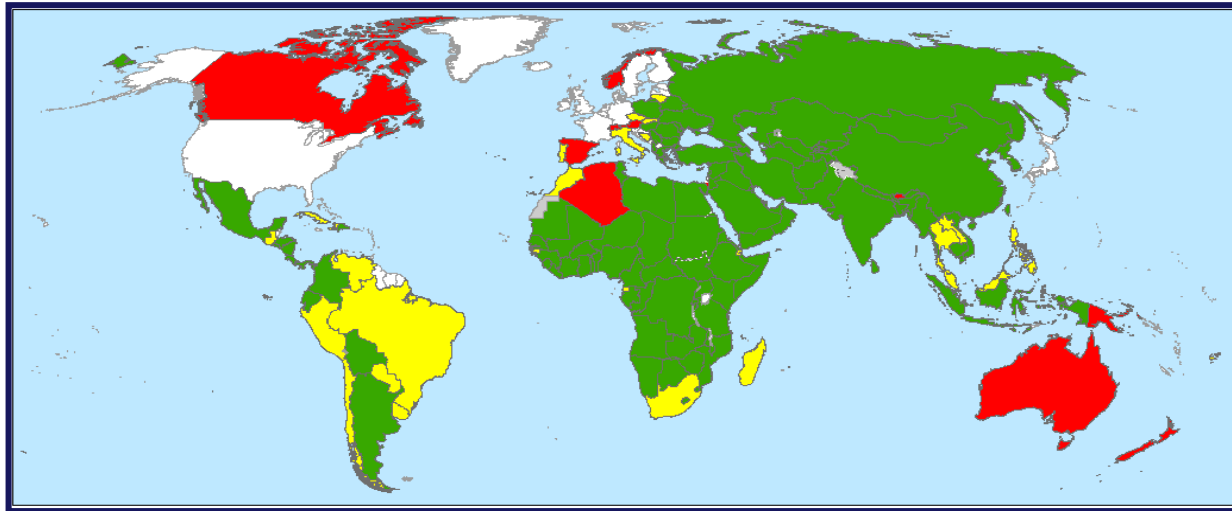
Oct 2015 – Sep 2016



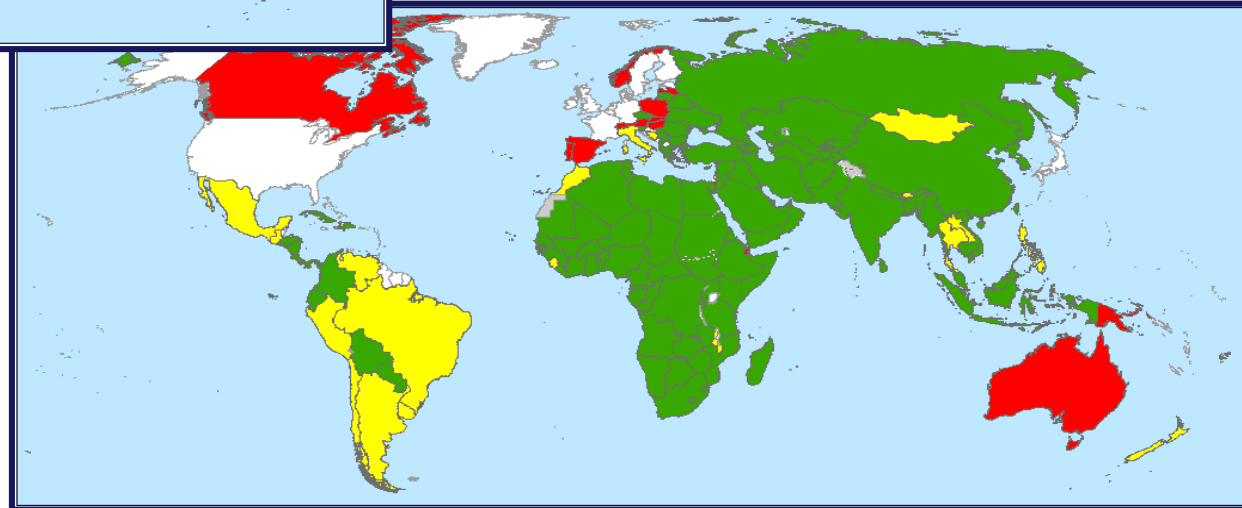
November 2016

Polio Surveillance: Stool Collection Percentage

Oct 2014 – Sep 2015



Oct 2015 – Sep 2016



 < 60%

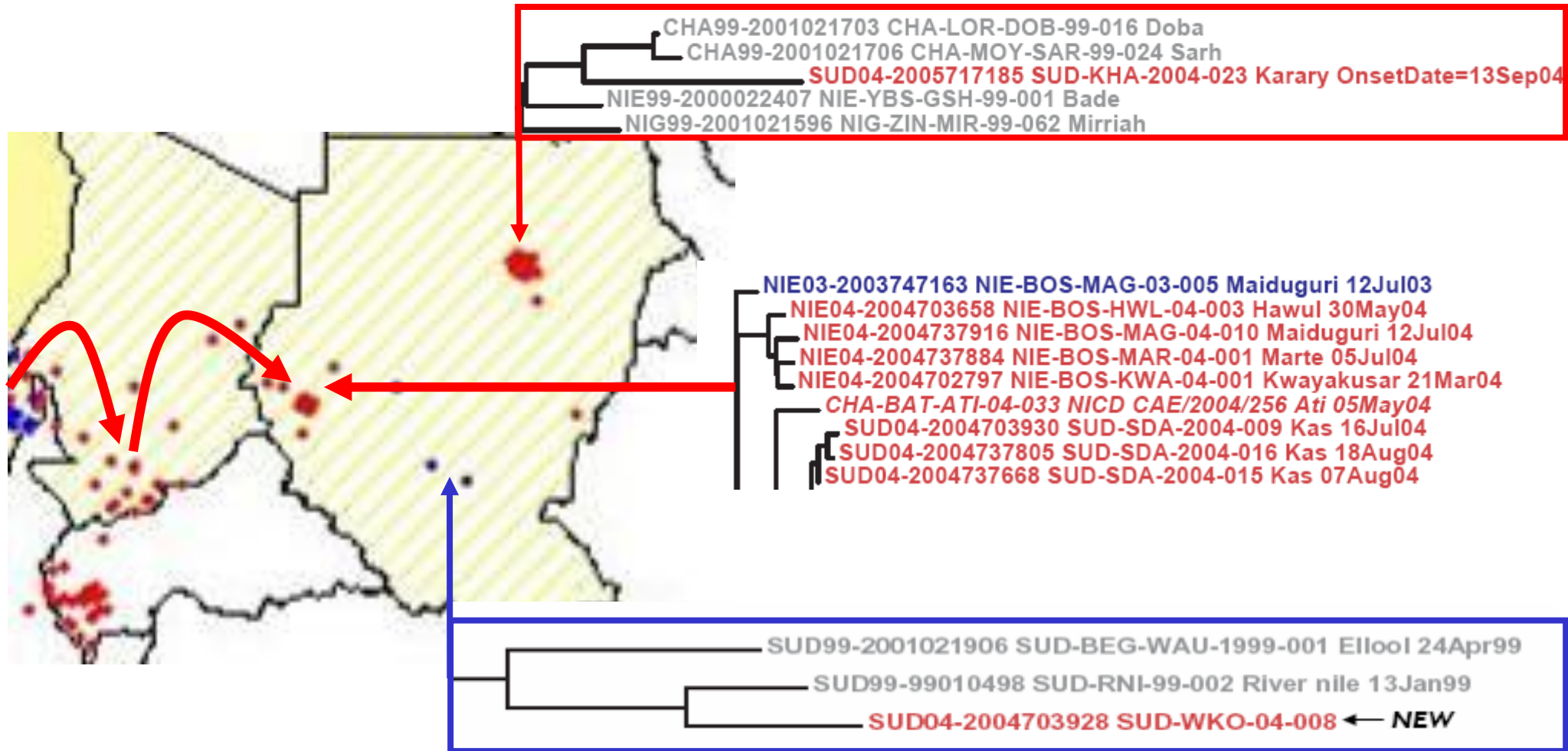
 60% - 79.99%

 ≥ 80%

 No AFP Surveillance/data

November 2016

Application: molecular surveillance/epidemiology



viruses linked to common ancestors, West and Central Africa, 2004

Progress in polio eradication, West Africa, 2008-2010

2008

2009

2010

Genetic Clusters of Poliovirus 1

2008

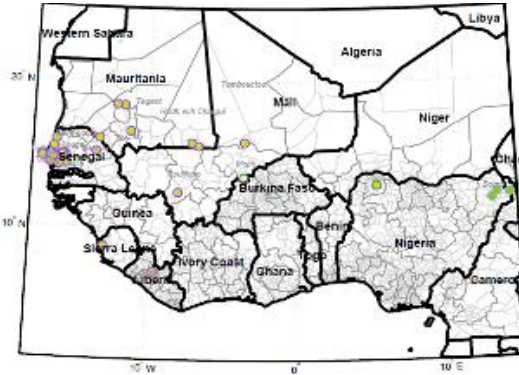
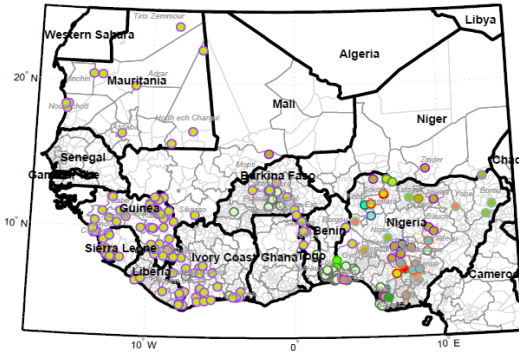
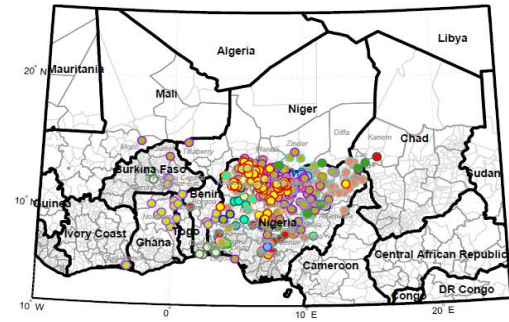
2009

2010

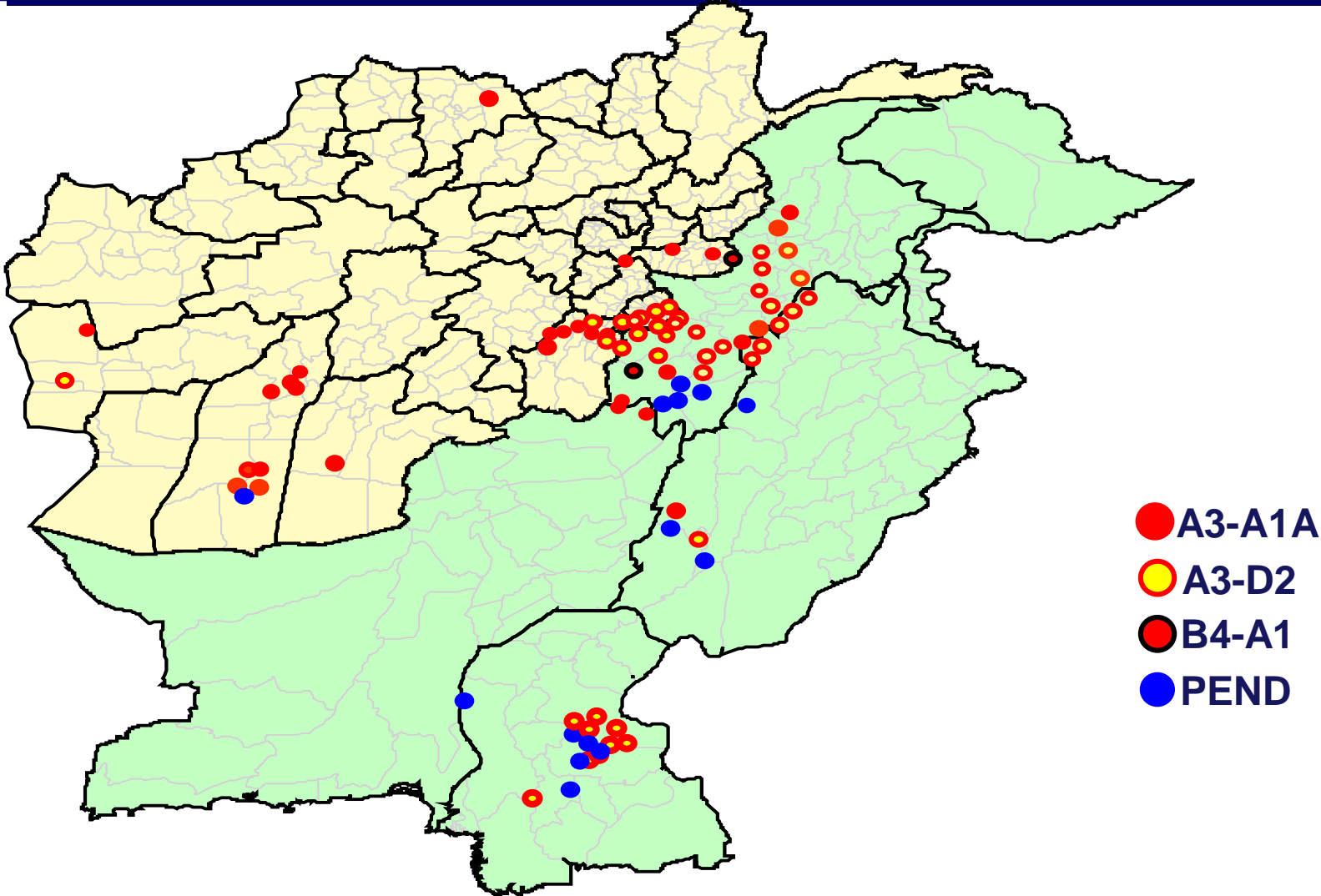
- B4B1C1A
- B4B1C1B
- B4B1C2A
- B4B1C2B
- I1C1B1A
- I1C1B1B
- I1C1B1C
- I6C2B1B
- I6C2B2B1
- I6C2B2B2
- I6C2B3B
- I6C2B4A1
- I6C2B4A2A
- I6C2B4A2B
- I6C2B4A3A
- I6C2B4A3B
- I6C2B4B1
- I6C2B4B2
- I6C2B4C1A
- I6C2B4C1B
- I6C2B4C2
- I6C2B4C3
- I6C2B4C4
- I6C2B4C5
- I6C2B4C6A
- I6C2B4C6B
- I6C2B4C7
- I6E2
- J1A
- J1B1
- J1B2

- B4B1C1B
- B4B1C2A
- I1C1B1A
- I6C2B1B
- I6C2B2B1
- I6C2B4A1
- I6C2B4A2A
- I6C2B4A2B
- I6C2B4A3A
- I6C2B4C1A
- I6C2B4C1B
- I6C2B4C2
- I6C2B4C3
- I6C2B4C4
- I6C2B4C5
- I6C2B4C6A
- I6C2B4C6B
- I6C2B4C7

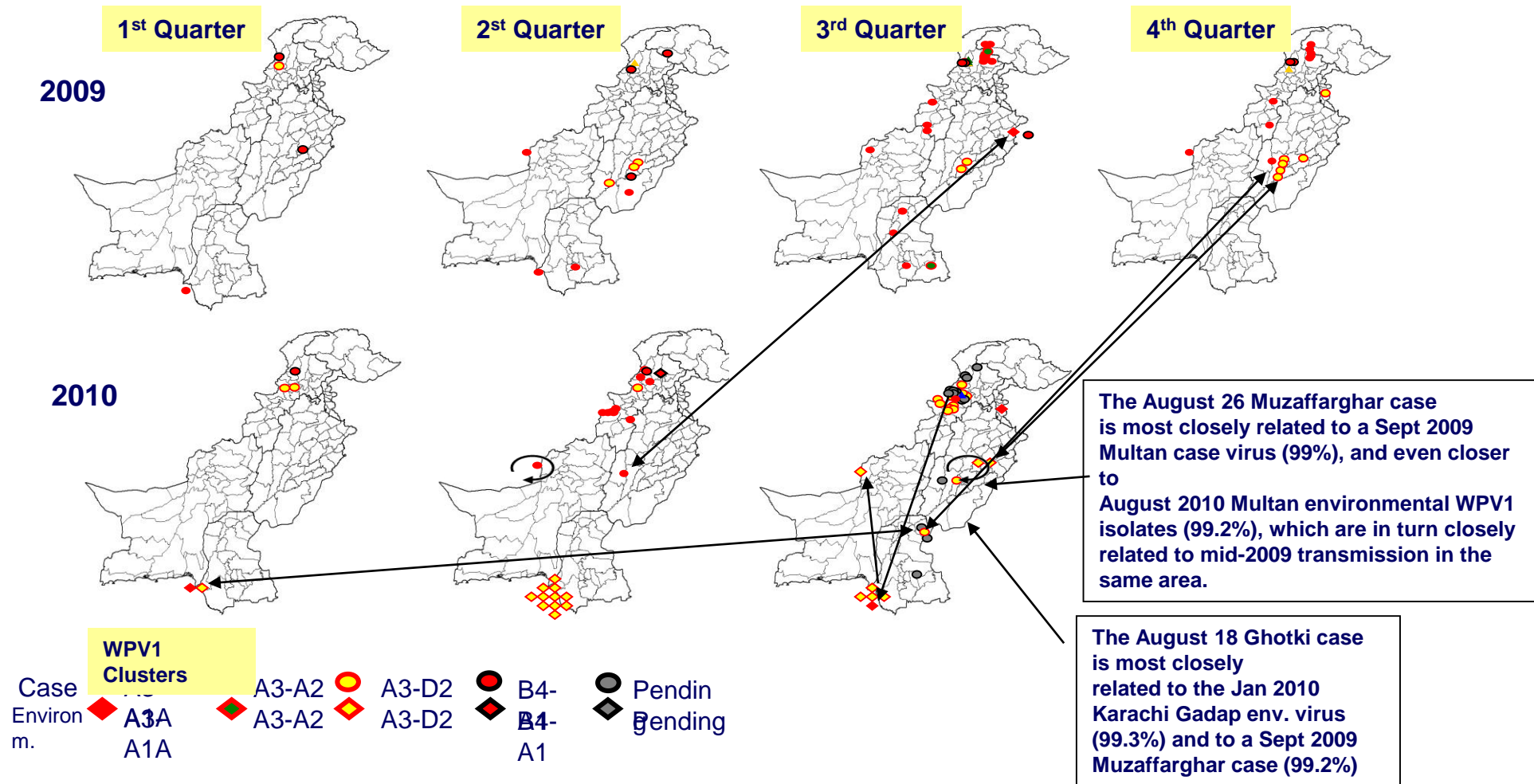
- I6C2B4A2A
- I6C2B4A2B
- I6C2B4A3A
- I6C2B4C1A



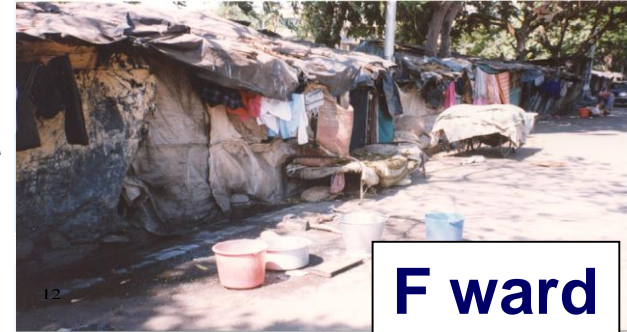
Afghanistan & Pakistan P1 isolates by genetic cluster 2010



WPV1 by genetic cluster and quarter, Pakistan 2009 and 2010



Environmental polio surveillance, open sewage, Mumbai, India



Photos courtesy Jagadish Deshpande, Entero Virus Research Centre, Mumbai

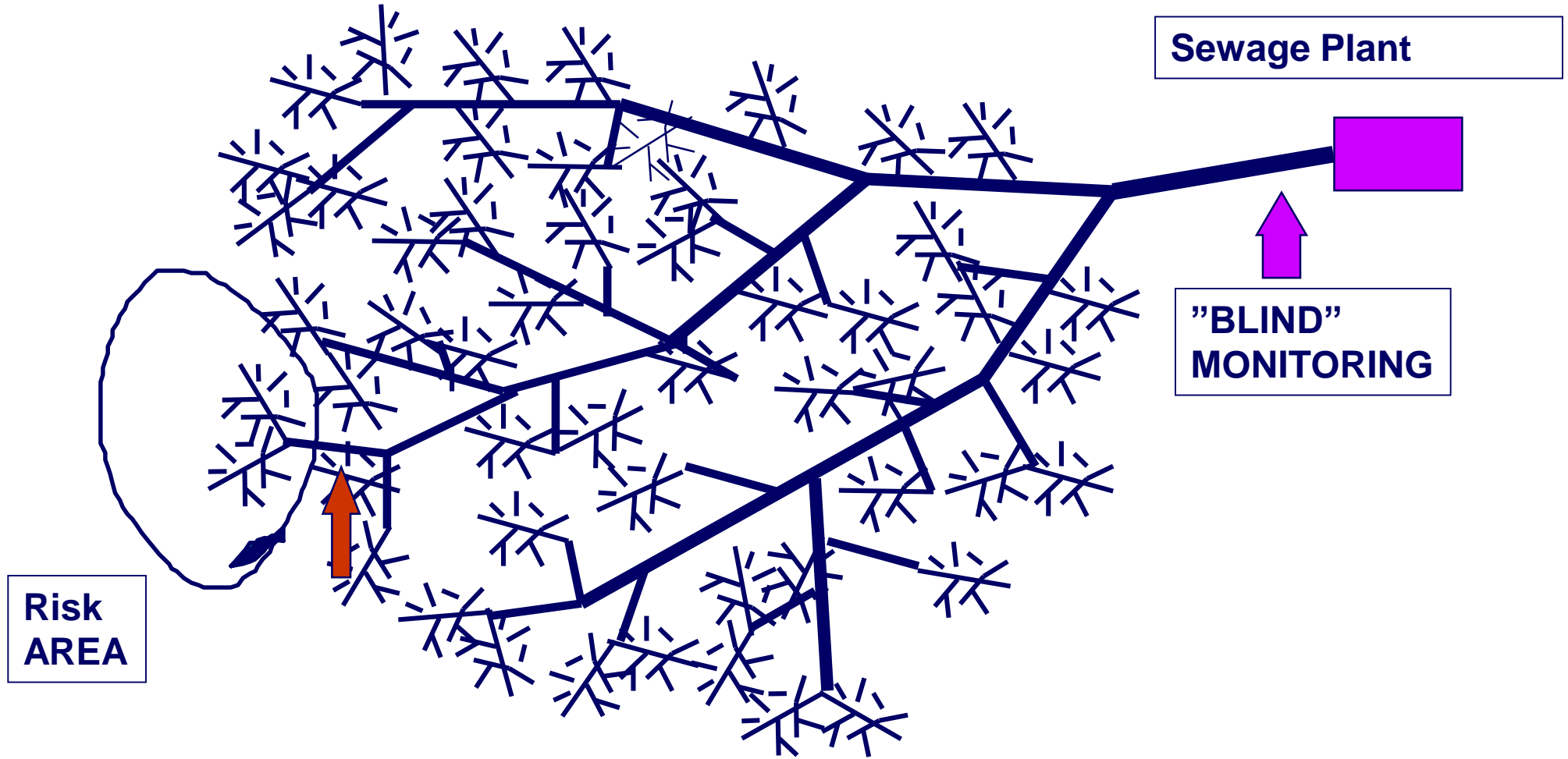
Polio virus Detected In Sewage in Mumbai, India 2005-2009

Ward	Total Samples*	Total Polio	Jan		Feb		Mar		Apr		May		Jun		Jul		Aug		Sep		Oct		Nov		Dec		Date of Last Poliovirus					
			Samples	Polio	Samples	Polio	Samples	Polio	Samples	Polio	Samples	Polio	Samples	Polio	Samples	Polio	Samples	Polio	Samples	Polio	Samples	Polio	Samples	Polio	Samples	Polio						
Year 2005																																
F	50	2	4	0	4	0	5	0	4	1	4	0	5	0	3	0	4	0	4	0	4	0	5	0	4	1	14-Dec-05					
G	50	7	4	1	4	0	5	0	4	0	4	0	5	0	3	0	4	0	4	0	4	3	5	1	4	2	27-Dec-05					
M	50	7	4	0	4	0	5	0	4	2	4	0	5	0	3	0	4	3	4	1	4	1	5	0	4	0	19-Oct-05					
Total	150	16	12	1	12	0	15	0	12	3	12	0	15	0	9	0	12	3	12	1	12	4	15	1	12	3						
Year 2006																																
F	15	0	4	0	4	0	5	0	2	[Redacted]															-							
G	15	2	4	2	4	0	5	0	2																24-Jan-06							
M	15	0	4	0	4	0	5	0	2																-							
Total	45	2	12	2	12	0	15	0	6																							
Year 2007																																
F	35	4	[Redacted]							5	1	4	3	4	0	5	0	4	0	5	0	4	0	4	0	4	0	20-Jun-07				
G	35	3								5	0	4	3	4	0	5	0	4	0	5	0	4	0	5	0	4	0	4	0	4	0	20-Jun-07
M	35	5								5	0	4	0	4	1	5	0	4	1	5	0	4	1	5	0	4	2	4	1	4	0	21-Nov-07
Total	105	12								15	1	12	6	12	1	15	0	12	1	15	0	12	1	15	0	12	2	12	1	12	1	
Year 2008																																
F	53	2	5	1	4	0	4	0	5	0	4	0	4	0	5	0	4	0	4	0	5	0	4	1	5	0	19-Nov-08					
G	53	7	5	0	4	3	4	3	5	0	4	0	4	0	5	0	4	0	4	1	5	0	4	0	5	0	10-Sep-08					
M	53	23	5	3	4	4	4	2	5	4	4	4	4	0	5	2	4	1	4	1	5	1	4	1	5	0	26-Nov-08					
Total	159	32	15	4	12	7	12	5	15	4	12	4	12	0	15	2	12	1	12	2	15	1	12	2	15	0						
Year 2009																																
F	48	3	4	1	4	0	4	0	5	1	4	1	4	0	5	0	4	0	5	0	4	0	4	0	4	0	1	0	20-May-09			
G	48	0	4	0	4	0	4	0	5	0	4	0	4	0	5	0	4	0	5	0	4	0	4	0	4	0	1	0	-			
M	48	0	4	0	4	0	4	0	5	0	4	0	4	0	5	0	4	0	5	0	4	0	4	0	4	0	1	0	-			
Total	144	3	12	1	12	0	12	0	15	1	12	1	12	0	15	0	12	0	15	0	12	0	12	0	3	0						

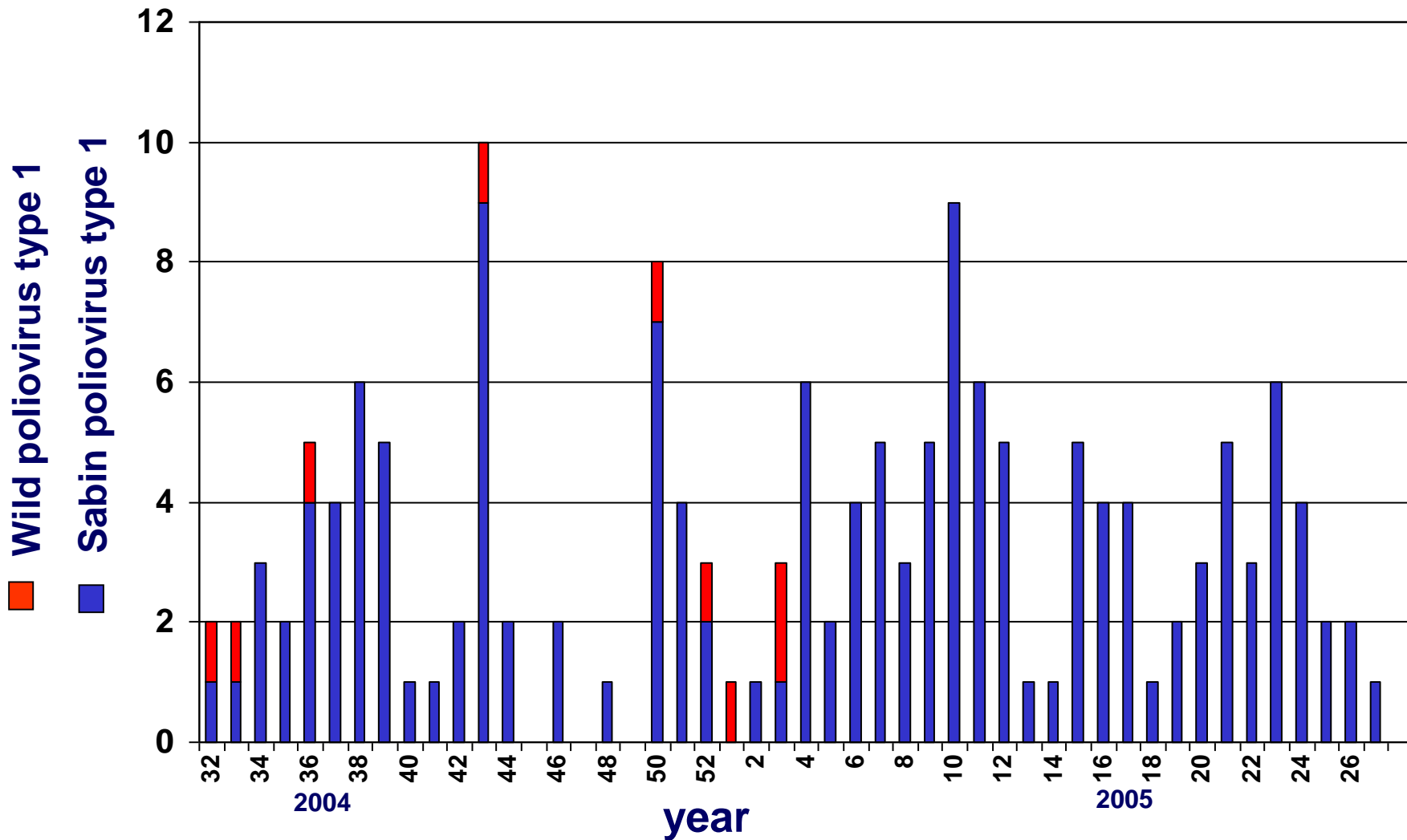
•WPV introductions from UP and Bihar with limited circulation in Mumbai
 •VDPVs detected for the first time in 2009

P1 P3 Both P1 & P3
 P1 VDPV P3 VDPV

Environmental polio surveillance, sewage network, Cairo, Egypt

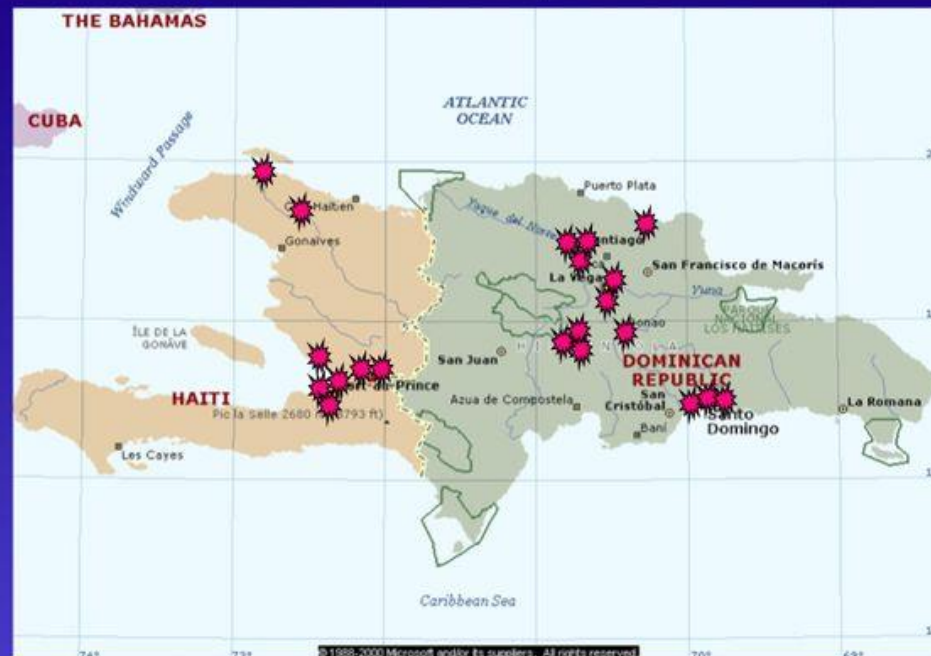


Poliovirus isolated from sewage by week, Egypt, August 2004 – August 2005



Vaccine derived polio: first recorded outbreak, Hispaniola, 2001

Areas with Confirmed Polio Cases on Hispaniola Island, 2000 - 2001*



Source: PESS/HVP
Data as of 1 July 2001



= areas with confirmed polio

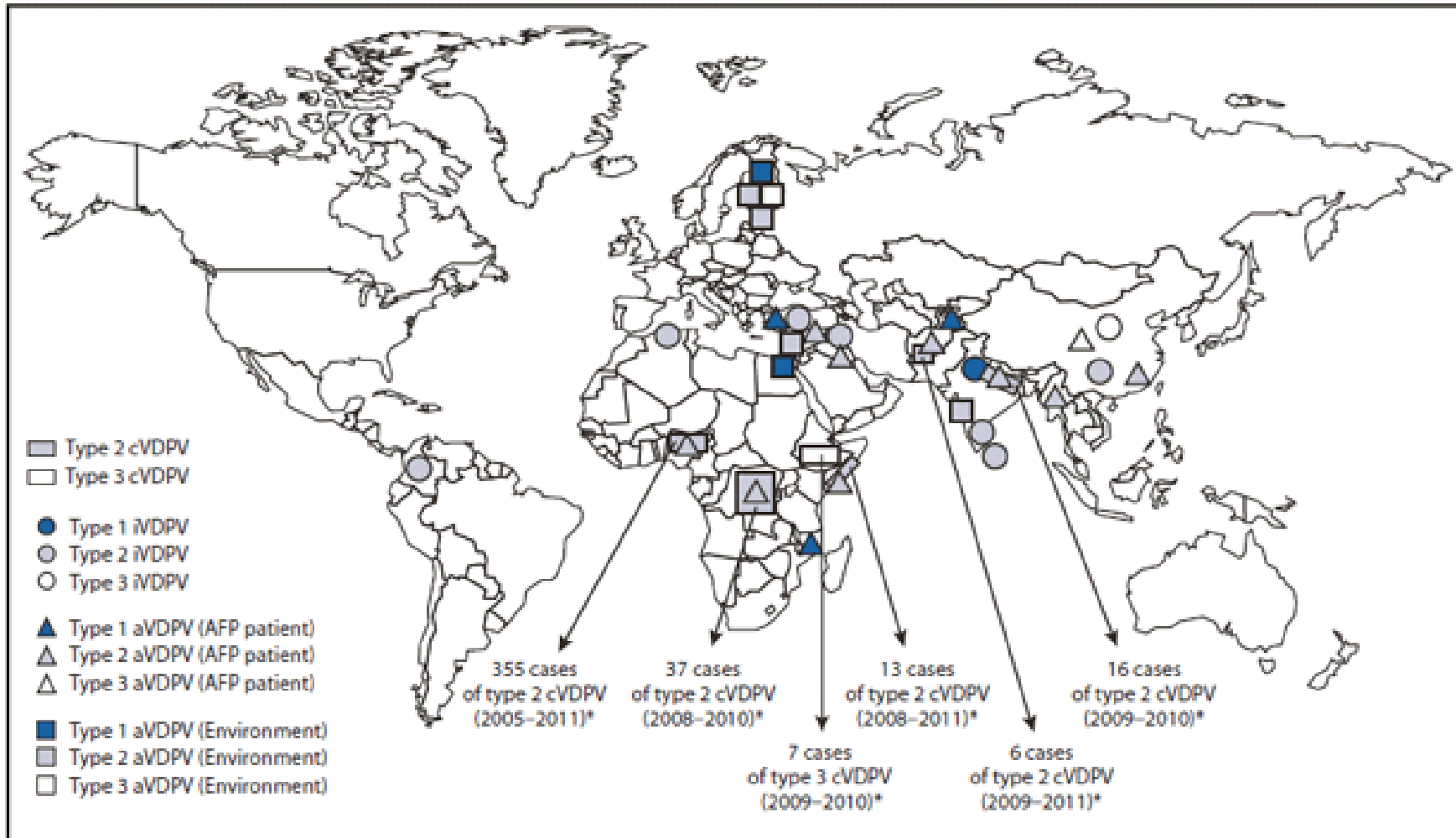
cases

Circulating vaccine derived polio virus (cVDPV): definition

cVDPV is a virus with >1% difference from parent OPV strains by full VP1 sequence homology found by AFP surveillance

- consistent with an extensive period of virus excretion or transmission**
- generally in a areas with low OPV coverage rates**

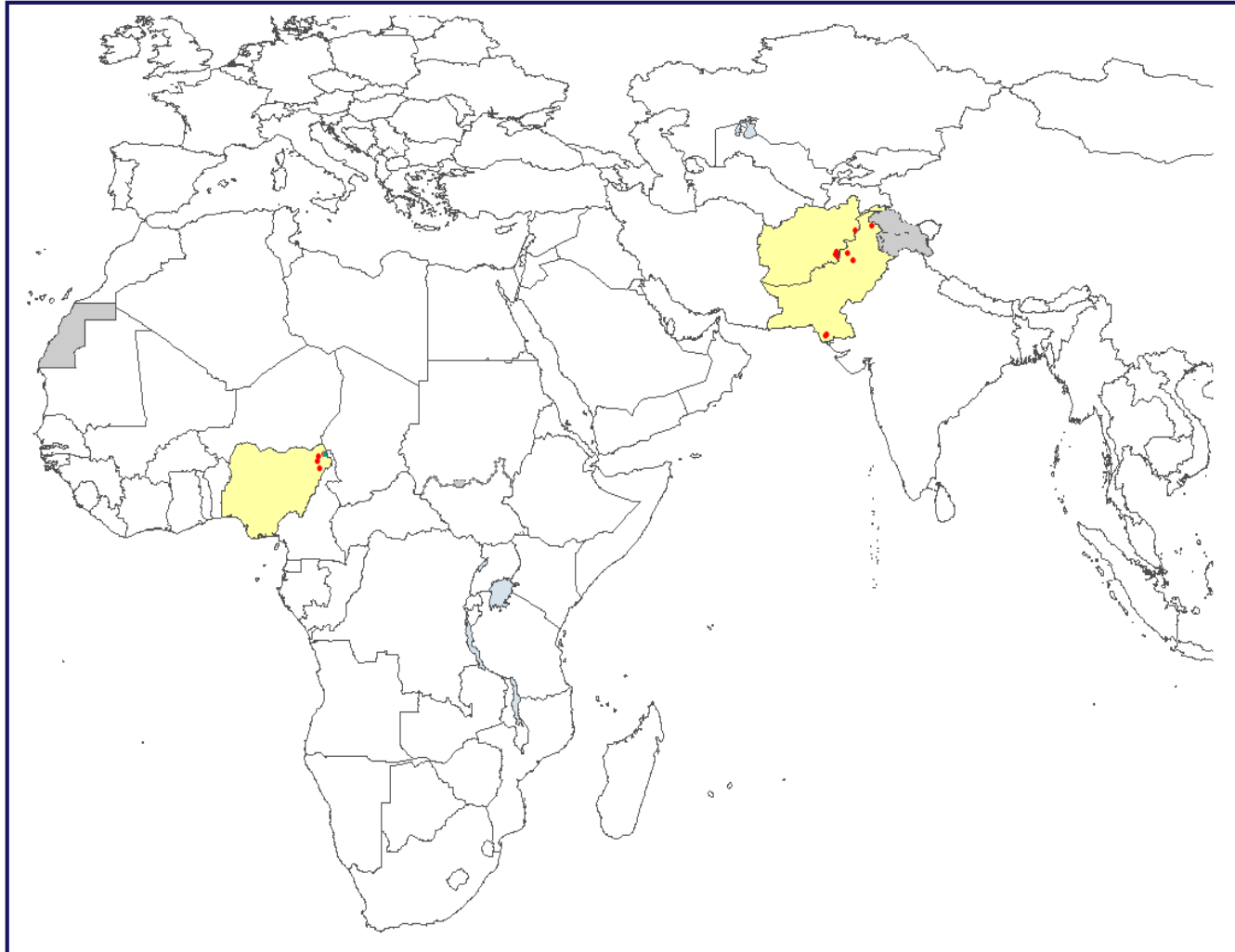
Circulating vaccine derived polio virus (cVDPV) outbreaks, 2008 - 2011



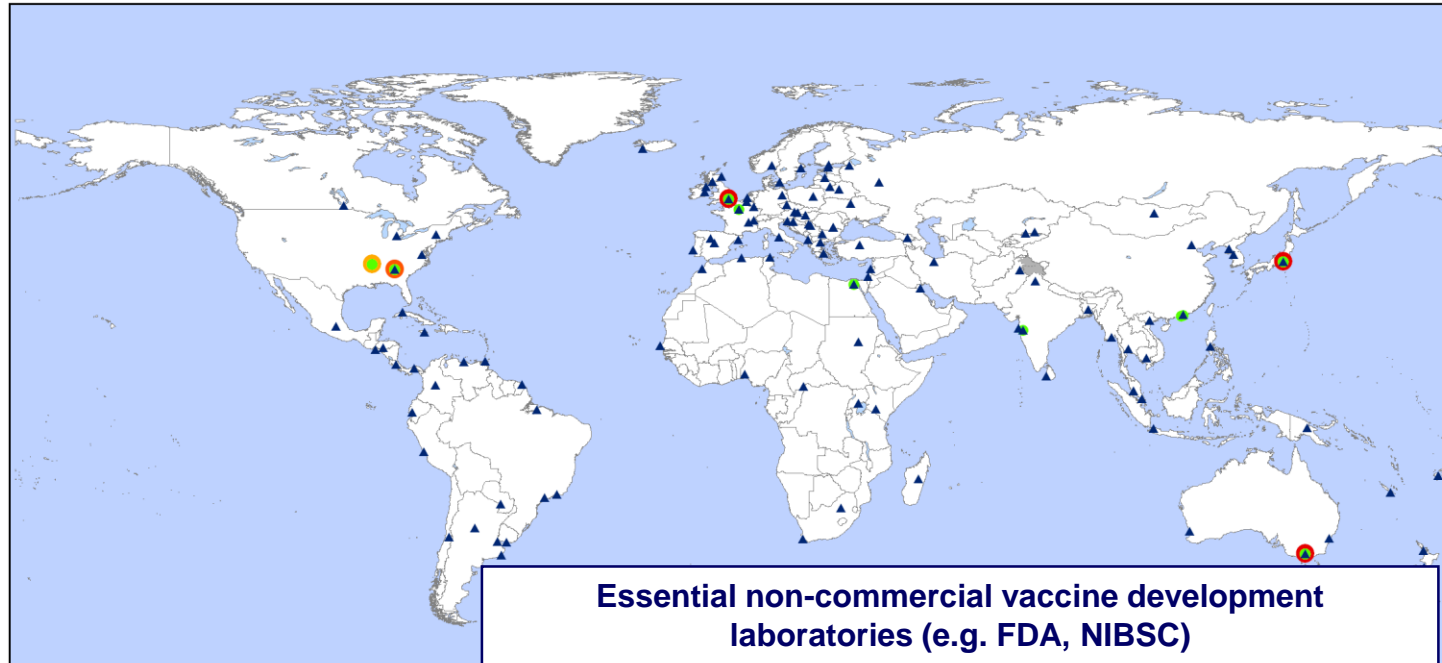
Global Circulating Vaccine-derived Poliovirus. Reported Cases, 2000 - 2016

Country	cVDPV type 1 ³																	Onset of most recent case
	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	
Laos																8	3	11-Jan-16
Madagascar															1	10		22-Aug-15
Ukraine																2		07-Jul-15
Mozambique												2						02-Jun-11
Myanmar							1	4										06-Dec-07
Indonesia						46												26-Oct-05
China					2													11-Nov-04
Philippines		3																26-Jul-01
DOR/Haiti	12	9																12-Jul-01
Total type 1	12	12	0	0	2	46	1	4	0	0	0	2	0	0	1	20	3	
Country	cVDPV type 2 ³																	Onset of most recent case
	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	
Nigeria						3	22	71	68	155	27	34	8	4	30	1	(1 ²)	28-Aug-16 ²
Guinea															1	7		14-Dec-15
Myanmar																2		05-Oct-15
Pakistan													16	48	22	2		09-Feb-15
South Sudan															2			12-Sep-14
Cameroon														4				12-Aug-13
Niger							2			2	1	1		1				11-Jul-13
Chad											1		12	4				12-May-13
Afghanistan											5	1	9	3				13-Mar-13
Somalia								1	6	1	9	1	1					09-Jan-13
Kenya													3					29-Aug-12
DRCongo									13	5	18	11	17					04-Apr-12
China													2					06-Feb-12
Yemen												9						05-Oct-11
India										15	2							18-Jan-10
Ethiopia								3	1									16-Feb-09
Madagascar		1	4			3												13-Jul-05
Total type 2	0	1	4	0	0	3	2	0	85	184	55	65	68	65	55	12	0	
Country	cVDPV type 3 ³																	Onset of most recent case
	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	
Yemen													3	1				12-Jul-13
Ethiopia										1	5							17-May-10
Cambodia						1	1											15-Jan-06
Total type 3	0	0	0	0	0	1	1	0	0	1	5	0	3	1	0	0	0	

Polio Eradication: cases reported through surveillance, 23 May – 22 November 2016



WHO global influenza surveillance network



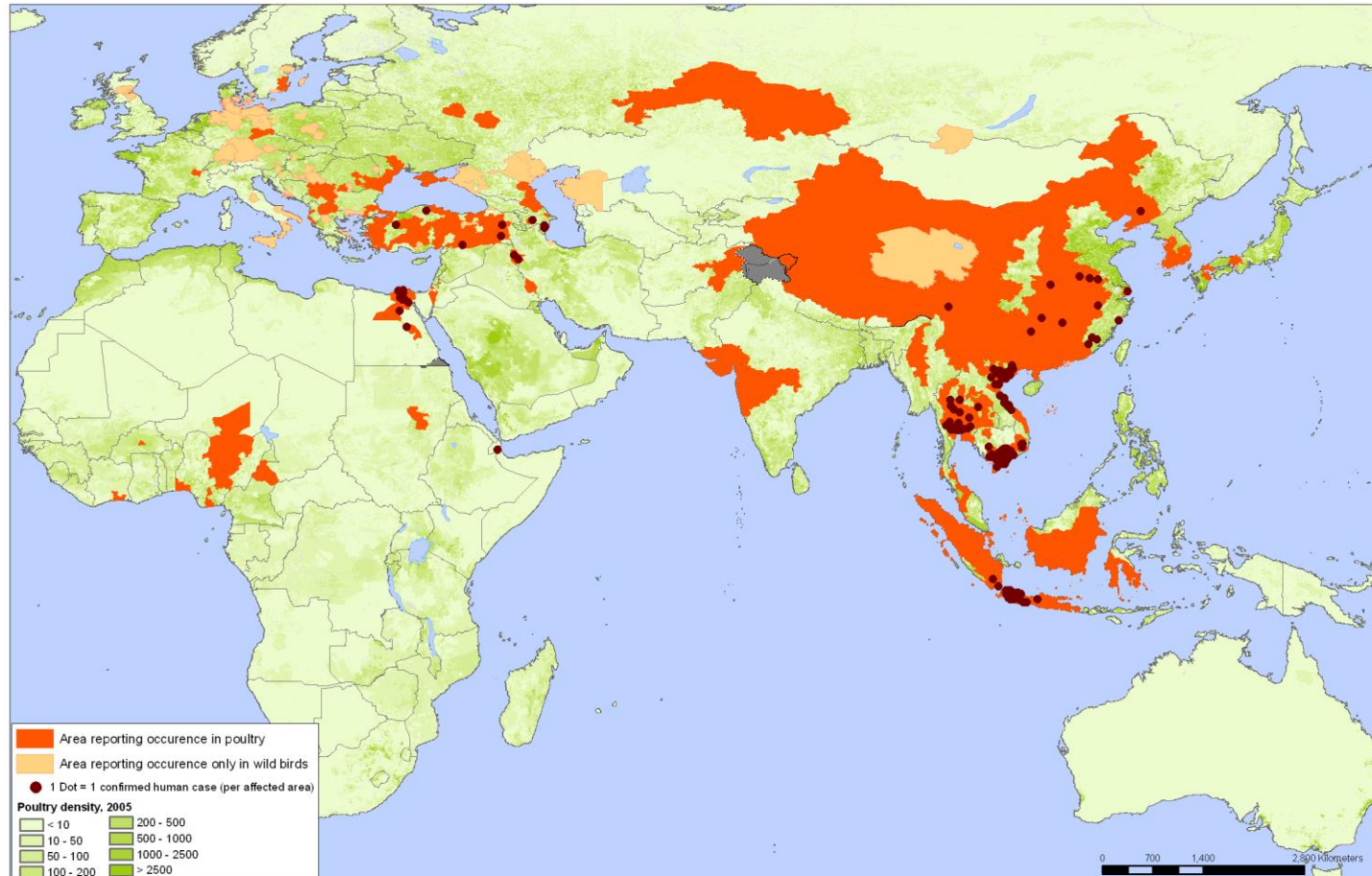
25 July 2008

- ▲ National Influenza Centres
- H5 Reference Laboratories
- WHO Collaborating Centre for Studies on the Ecology of Influenza in Animals
- WHO Collaborating Centre for the Surveillance, Epidemiology and Control of Influenza
- WHO Collaborating Centres for Reference and Research on Influenza

1997, H5N1, Hong Kong: culling of live chickens and ducks



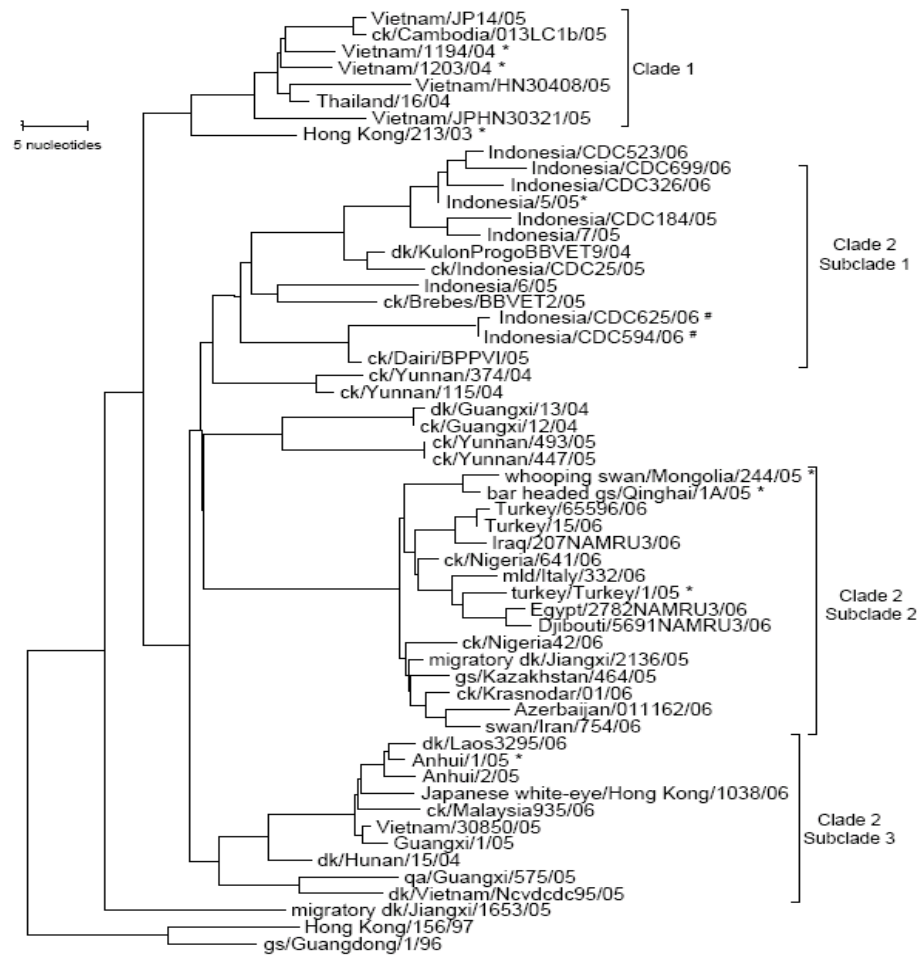
Confirmed Human and Poultry H5N1 Infections since 2003



The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement. Communicable Diseases (CDS) World Health Organization © WHO 2006. All rights reserved

Data source: World Organisation for Animal Health (OIE) and national governments/WHO/EPR/FAO
Map Production: Public Health Mapping and GIS

Risk assessment: H5N1 virus groups (clades) infecting humans since 2003



* Candidate vaccine reference viruses

Karo family cluster



Surveillance at the molecular level: the good

- Powerful supplement to surveillance when laboratory findings linked to epidemiology and outbreak understanding and control**
- Adds scientific and epidemiological understanding to risk assessment and emerging infectious diseases**

Cholera, Haiti, 2010



Cholera Haiti, 2010



Source: WHO

Conditions leading to cholera transmission, Haiti, 2010

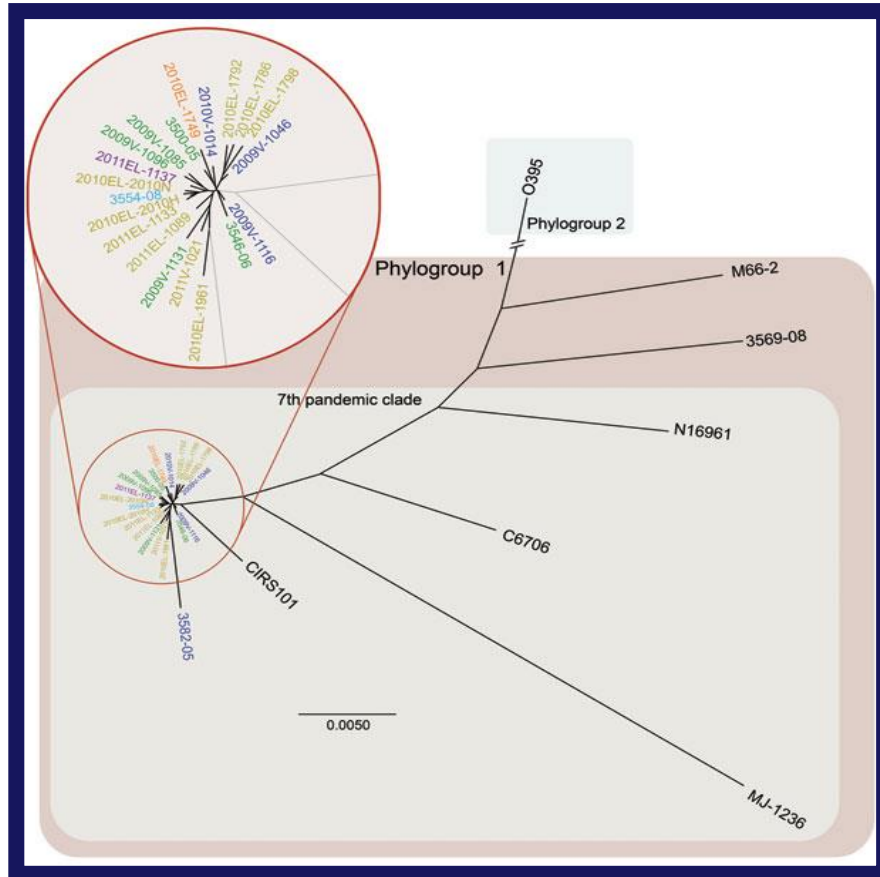


Spread of *Vibrio cholerae* 01 – Central and South America, January 1991 – November 1994

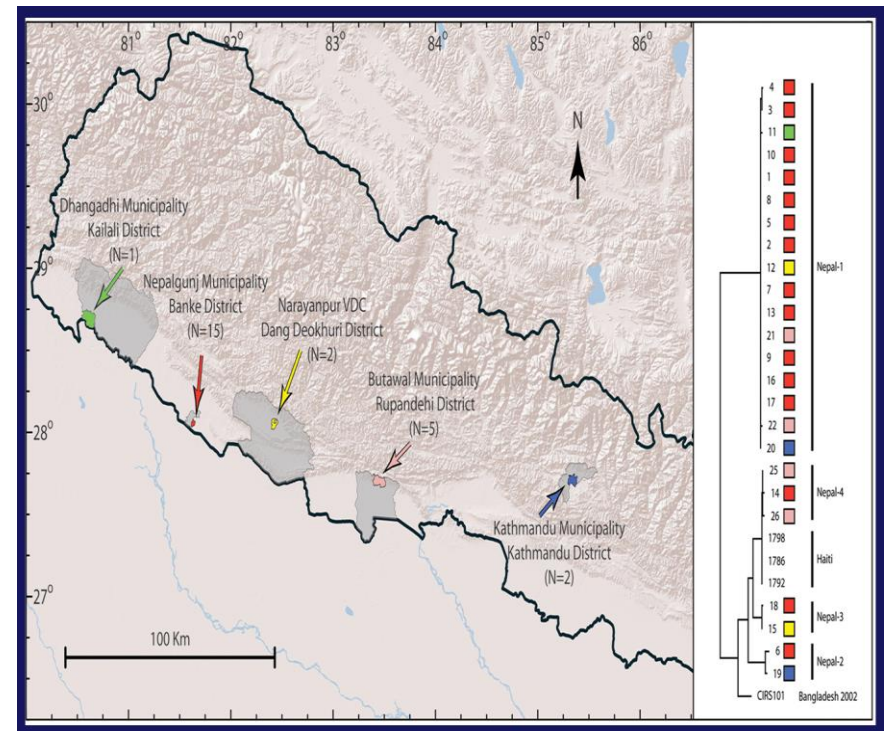


Source: WHO

Whole-genome neighbour-joining tree, *Vibrio cholerae*, Haiti, 2010 and Nepal



Haiti, 2010



Nepal, 2009

Source: CDC/WER and Hendeikson/MBio

UN Peace Keeping Forces, Annapur Camp, Haiti



Conditions leading to cholera transmission, Haiti, 2010



Cholera in Haiti, announcement of results of molecular testing, 14 November 2010

Cholera in Haiti introduced by UN Nepalese troops confirms Swedish Ambassador

By ktwop

It would seem that the cholera tap in Haiti was "turned" on by UN troops.

Translated freely from *Svenska Dagbladet*:

Violent riots have erupted in Haiti after rumors of the cholera infection having originated from the Nepalese UN staff - something which the UN denied. But now, the Swedish Ambassador Claes Hammar has confirmed that the rumor is true. "I've had it confirmed by a diplomatic source that the cholera comes from Nepa" he told *Svenska Dagbladet*.

Since the earthquake on 12 January, cholera has taken hold in the extreme poor living conditions in Haiti. The epidemic broke out in October and since then more than 900 people have died and 15 000 are believed to be infected.

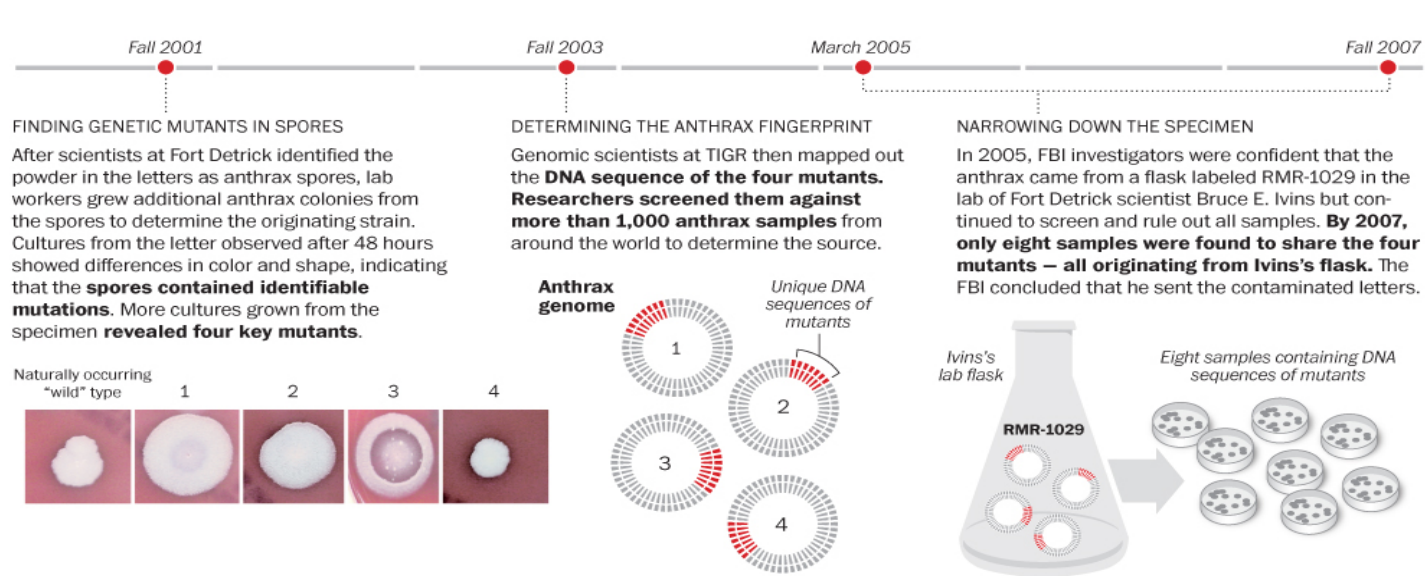
Violent riots broke out yesterday in the cities of Cap Haitien, Hinche and Milot, when hundreds of people attacked the UN mission MINUSTAH. The riots began



Demonstrations against UN Peacekeeping Forces, Haiti, 15 November 2010



Anthrax molecular analysis, 2001-2007



SOURCES: Dr. Jacques Ravel of the Institute for Genome Sciences, University of Maryland School of Medicine; U.S. Army Medical Research Institute of Infectious Diseases; Morphotype imagery from Rasko et

Public accusation of possible perpetrator and suicide

Guilt of Bruce Ivins, Accused in Anthrax Case

by Mike Wiser, PBS Frontline, Greg Gordon, McClatchy Newspapers, and Stephen Engelberg, ProPublica July 18, 2011, 8:03 p.m. 39 Comments | Republish | E-mail | Print

Update (7/19): On Tuesday, Justice Department lawyers [retracted statements that question the FBI's finding](#) that a former Army microbiologist mailed the anthrax-filled letters that killed five people in 2001.

This story was co-published with [PBS FRONTLINE](#) and [McClatchy](#).

WASHINGTON -- The Justice Department has called into question a key pillar of the FBI's case against Bruce Ivins, the Army scientist accused of mailing the anthrax-laced letters that killed five people and terrorized Congress a decade ago.

Shortly after Ivins committed suicide in 2008, federal investigators announced that they had identified him as the mass murderer who sent the letters to members of Congress and the media. The case was circumstantial, with federal officials arguing that the scientist had the means, motive and opportunity to make the deadly powder at a U.S. Army research facility at Fort Detrick, in Frederick, Md.

Tweet 102



New court documents cast doubt on the guilt of Dr. Bruce Ivins, an Army scientist who committed suicide as federal authorities prepared to charge him with killing five people by sending anthrax spores in the mail in 2001. (Frederick News Post, Sam Yu/AP Photo)

Re-examination of the evidence

NEWS

F R O M T H E N A T I O N A L A C A D E M I E S

NATIONAL ACADEMY OF SCIENCES
NATIONAL ACADEMY OF ENGINEERING
INSTITUTE OF MEDICINE
NATIONAL RESEARCH COUNCIL

Date: Feb. 15, 2011

FOR IMMEDIATE RELEASE

Science Alone Does Not Establish Source of Anthrax Used in 2001 Mailings

WASHINGTON – A National Research Council committee asked to examine the scientific approaches used and conclusions reached by the Federal Bureau of Investigation during its investigation of the 2001 *Bacillus anthracis* mailings has determined that it is not possible to reach a definitive conclusion about the origins of the anthrax in letters mailed to New York City and Washington, D.C., based solely on the available scientific evidence.

Findings of the committee's study include:

- The FBI correctly identified the dominant organism found in the letters as the Ames strain of *B. anthracis*.
- Silicon was present in significant amounts in the anthrax used in the letters. But the committee and FBI agree that there is no evidence that the silicon had been added as a dispersant to "weaponize" the anthrax.
- Spores in the mailed letters and in RMR-1029, a flask found at the U.S. Army Medical Research Institute for Infectious Diseases (USAMRIID), share a number of genetic similarities consistent with the FBI finding that the spores in the letters were derived from RMR-1029. However, the committee found that other possible explanations for the similarities -- such as independent, parallel evolution -- were not definitively explored during the investigation.
- Flask RMR-1029, identified by the U.S. Department of Justice as the "parent material" for the anthrax in the attack letters, was not the immediate source of spores used in the letters. As noted by the FBI, one or more derivative growth steps would have been required to produce the anthrax in the attack letters. Furthermore, the contents of the New York and Washington letters had different physical properties.
- Although the FBI's scientific data provided leads as to the origin of anthrax spores in the letters, the committee found that the data did not rule out other possible sources. The committee recommended that realistic expectations and limitations regarding the use of forensic science need to be clearly communicated to the public.

Surveillance at the molecular level: lack of concern of consequence

CIA organised fake vaccination drive to get Osama bin Laden's family DNA

Senior Pakistani doctor who organised vaccine programme in Abbottabad arrested by ISI for working with US agents



CIA organised fake vaccination programme in Abbottabad to try and find Osama bin Laden. Photograph: Md Nadeem/EPA

BREAKING: At Least 13 Survivors in Colombia Plane Crash - Emergency Team PMU

SPUTNIK

Pakistani police officers and rescue workers gather at the site of suicide bombing targeting a polio vaccination center in Quetta, Pakistan, on Wednesday, Jan. 13, 2016.

Attack on Pakistan Polio Clinic Recalls CIA Vaccination Ruse

© AP Photo/ Arshad Butt

MIDDLE EAST 01:06 14.01.2016 (updated 01:10 14.01.2016) Get short URL 4 827 3 3

NEWS

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16h At Least 13 Survivors in Colombia Plane Crash - Emergency Team PMU

At least 15 people were killed and 25 wounded on Wednesday in a bomb blast that targeted a polio eradication center similar to one previously used by the CIA to track terrorists in the region.

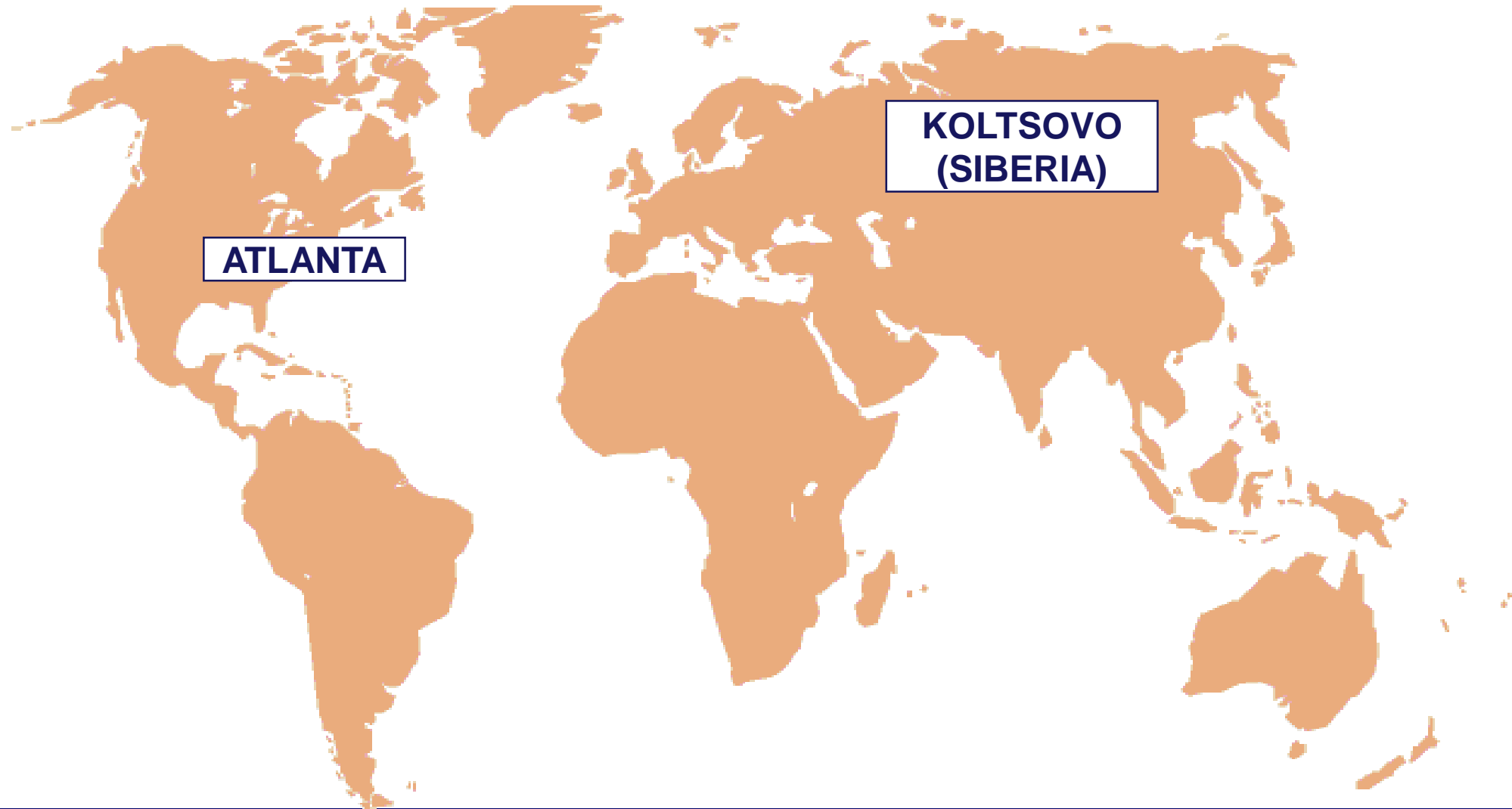
Surveillance at the molecular level: the bad

- Powerful supplement to surveillance when laboratory findings linked to epidemiology and outbreak understanding and control
- Adds scientific and epidemiological understanding to risk assessment and emerging infectious diseases
- **When used inappropriately, molecular linkages can cause harm**

The Birmingham outbreak of smallpox, August 1978: the last human cases



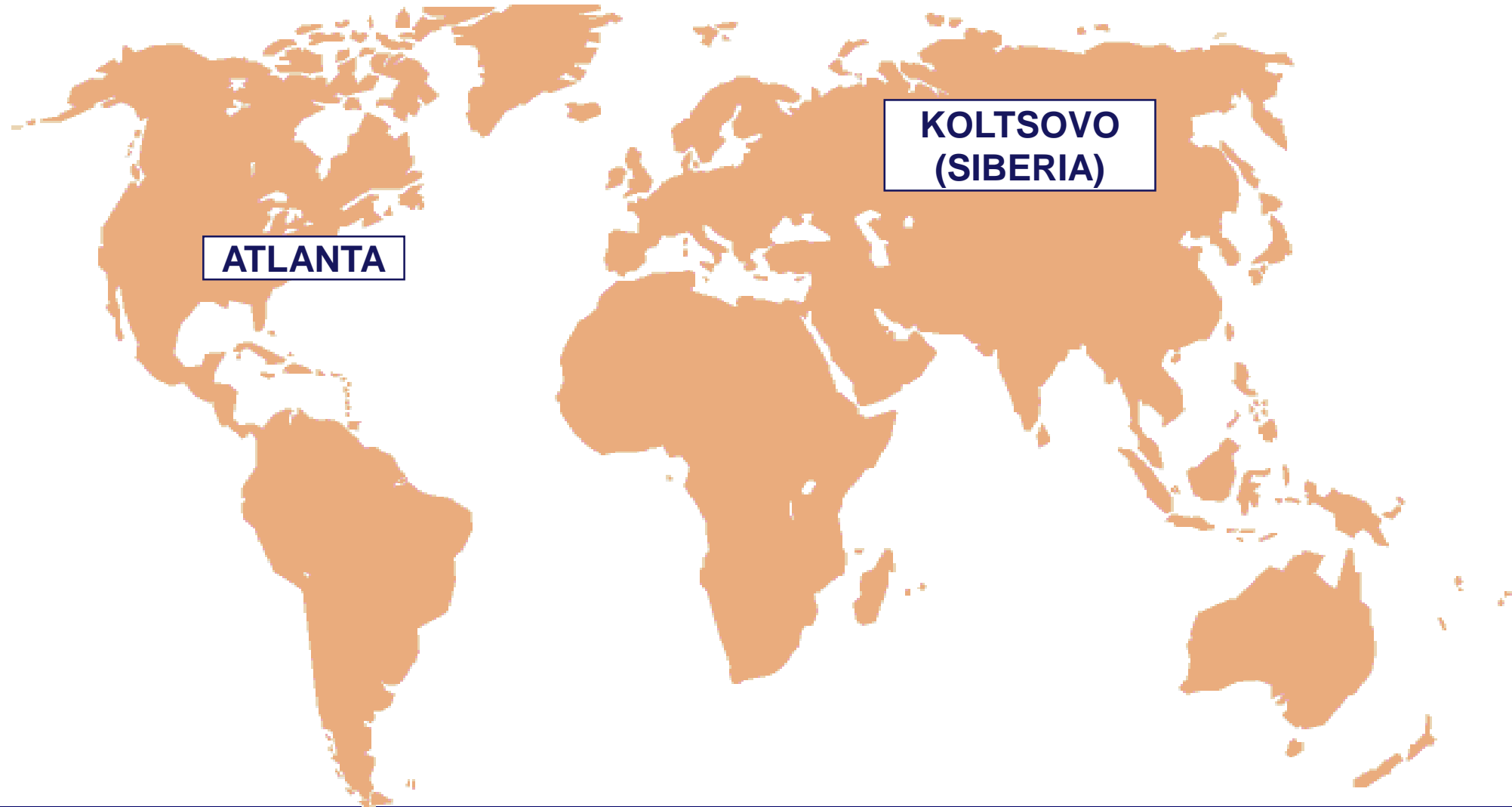
Smallpox virus: officially remains in 2 WHO Collaborating Centres



Certification of smallpox eradication, 1980

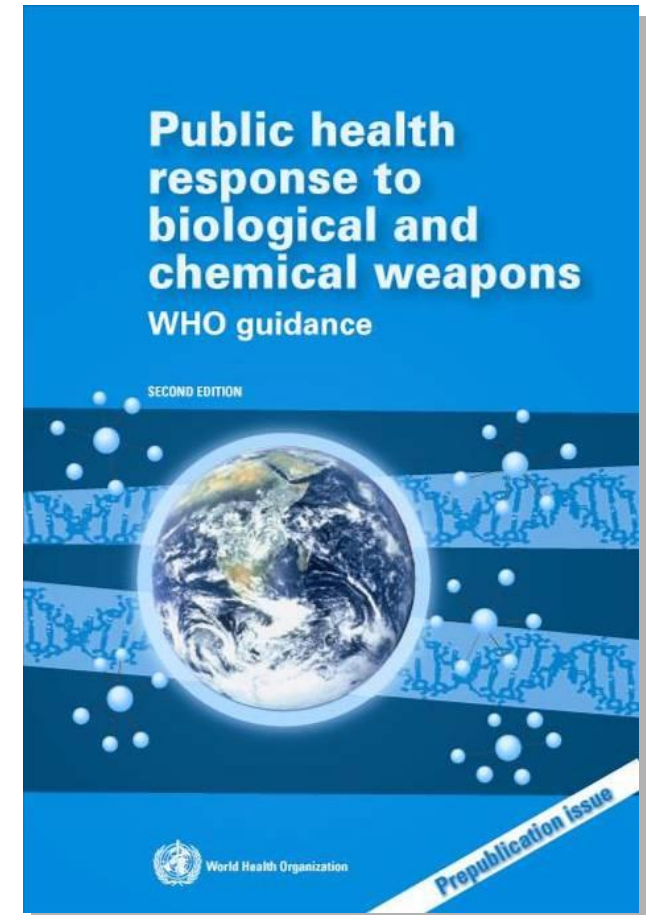


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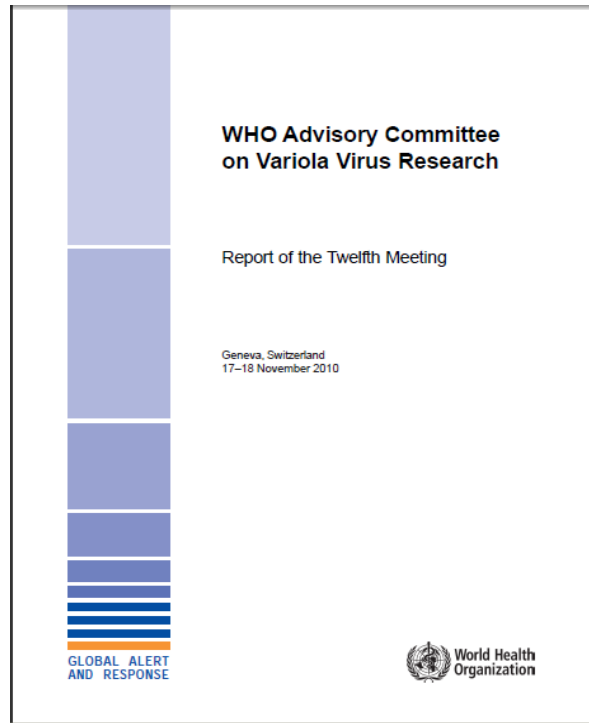


Reports of virus outside WHO repositories 2000: real or perceived threat?

- Updated WHO guidance
- Industry scaled up smallpox vaccine production
- Industrialized countries stockpiled smallpox vaccine/vaccinia immune globulin
- Intensified research on new, safer vaccines, anti-virals and diagnostics in USA and Russia

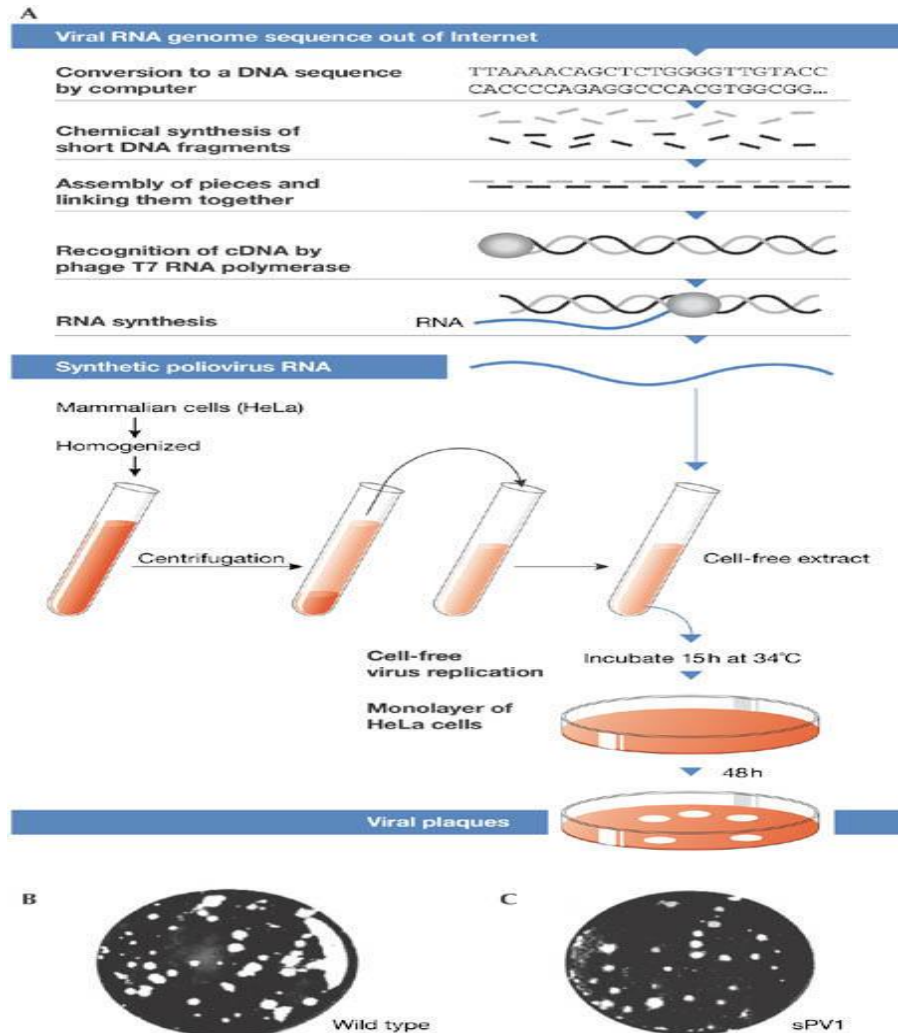


Is there a continued risk to smallpox eradication?



4.5 Dr G. McFadden presented Chapter 3, on variola genomics, which is summarized in Annex 1. The chapter concluded that "publicly available genomic information has been used by many international scientists to design highly sensitive virus diagnostics. The newly-gained understanding of the relationship between variola virus and other orthopoxviruses also provides important clues to understanding the value and limitations of animal models for human smallpox. Remarkable expansion in the technologies of DNA synthesis, sequencing, and cloning has created today's situation, where it is **now technically possible to synthesize the entire variola virus genome from scratch, using only publicly available sequence information, and to reconstitute infectious virus** using currently available techniques of molecular biology. As a result of this ability, future biodefence strategies need to incorporate new thinking regarding how best to control the application of these synthetic biology technologies."

De novo synthesis of poliovirus, Stony Brook University



Synthesis of poliovirus in the absence of natural template.

(A) Short complementary segments of synthetic DNA (oligonucleotides) are annealed, and enzymatically extended and ligated (connected). A full-length complementary DNA (cDNA) is assembled stepwise to represent the entire genetic information of the poliovirus RNA genome in the form of DNA. The cDNA is then transcribed into infectious viral RNA by a T7 RNA transcriptase. This RNA is used to seed a HeLa cell-free extract that will replicate, just like in intact cells, to form progeny virions

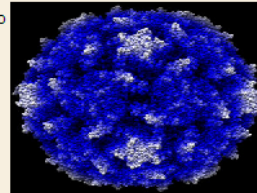
(B,C) Evidence for *de novo* synthesized virus is provided by plaque assays. Poliovirus plaques derived from synthetic virus (sPV1) and wild-type virus, respectively, are formed on monolayers of HeLa cells

De novo synthesis of poliovirus: a risk to eradication/a risk to the future?

Scientists' creation of the weak virus signals the beginning of a new era in vaccination.

A team of molecular biologists and computer scientists at Stony Brook University have designed and synthesized a new class of weakened polioviruses. They have used a synthesizing method with computer software to systematically re-code the poliovirus genome.

In order to artificially synthesize a virus the genome has to be decoded first. But decoding a polio virus that had almost (10442) possibilities isn't a simple task. Therefore, using a powerful computer algorithm, the team found particular re-codings of the genome predicted to weaken the virus.



"The researchers made hundreds of small mutations in the genome that perfectly preserved the viral proteins but changed the way those proteins were encoded by RNA (ribonucleic acid), so that pairs of amino acids were added by transfer RNAs (tRNAs) that rarely work together in normal proteins. They call the process "Synthetic Attenuated Virus Engineering," or "SAVE." The resulting virus contains completely authentic, wild-type poliovirus proteins."

The most highly decoded virus will be weakened, so it will no longer infect cells anymore. Thus a virus modified using "SAVE" technology might act as a vaccine by



Surveillance at the molecular level: the bad

- Powerful supplement to surveillance when laboratory findings linked to epidemiology and outbreak understanding and control
- Adds scientific and epidemiological understanding to risk assessment and emerging infectious diseases
- When used inappropriately, molecular linkages can cause harm
- **Molecular understanding in the wrong hands has the potential to cause evil**

Surveillance at the molecular level the bad

- Powerful supplement to surveillance with findings linked to epidemiology and understanding and control
- Adds scientific and epidemiological understanding to risk assessment and infectious diseases
- When used carelessly, molecular linkages can cause harm
- Understanding in the wrong hands has the potential to cause evil

Whatever its goal, surveillance at the molecular level is a powerful tool; it must be used ethically and with care