

# ***FILOVIRIDAE***

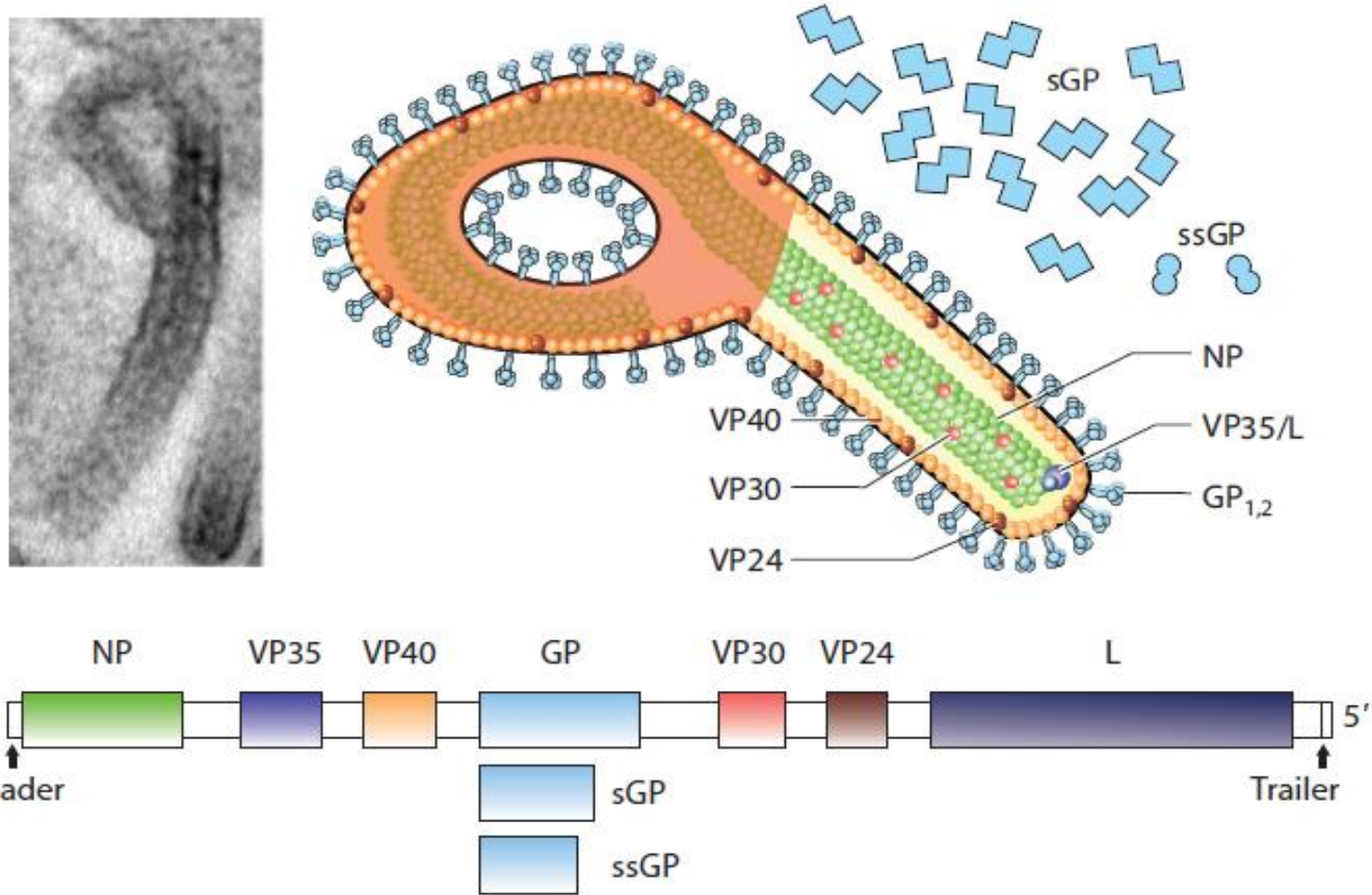
# INTRODUCTION

- **The species of filoviruses cause severe hemorrhagic fevers in humans, with high mortality rate**
- **All filovirus outbreaks have occurred in Africa, except for the initial Marburg outbreak**
- **In public mind considered “doomsday virus” or ultimate biological weapon**
- **Filoviruses are not highly contagious and prevention of direct contact with patients or their body fluids is sufficient to block the infection**
- **One reason for larger number of victims in some outbreaks has been a delay in recognizing the emergence of a filovirus against other febrile illnesses. (Over 2 weeks until specific symptoms.)**

- **The pace of filovirus research has been slower than many other viruses**
  - **Remote locations**
  - **Unexpected occurrence**
  - **Brief duration of epidemics**
  - **Lab studies must be handled in BL4 laboratory**

# VIROLOGY

- Family of *Filoviridae* has three genera, *Ebolavirus*, *Marburgvirus* and *Cuevavirus* (Only in bats)
- The name derives from the Latin *filum* (thread) reflecting filamentous morphology
- *Ebolavirus* has five subspecies:
  - Zaire
  - Tai Forest
  - Bundibugyo
  - Sudan
  - Reston (from Philippines)
- Sequence analysis indicate that Marburg and Ebola viruses diverged from a common ancestor and are evolving slowly over time



**Figure 1**

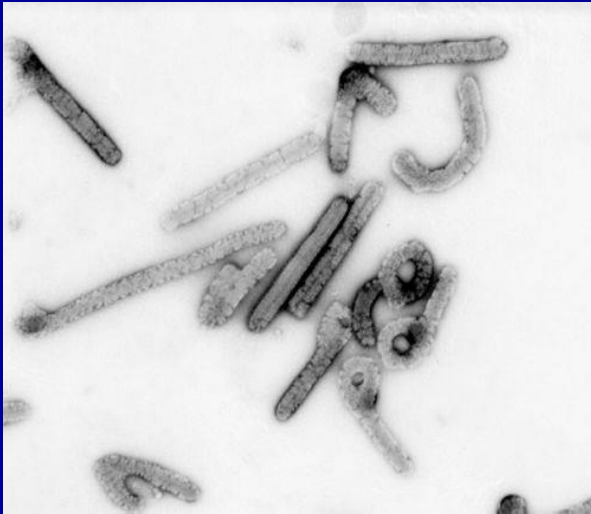
Ebolavirus structure indicating various proteins and the genes that code for them. The genome displays the following structure: 3'-leader → *nucleoprotein (NP)* gene → *viral protein (VP)* 35 gene → *VP40* gene → *glycoprotein (GP)* gene → *VP30* gene → *VP24* gene → *polymerase (L)* gene → 5'-trailer.

## Ebola virus



Courtesy of F. A. Murphy, School of Veterinary Medicine, University of California, Davis

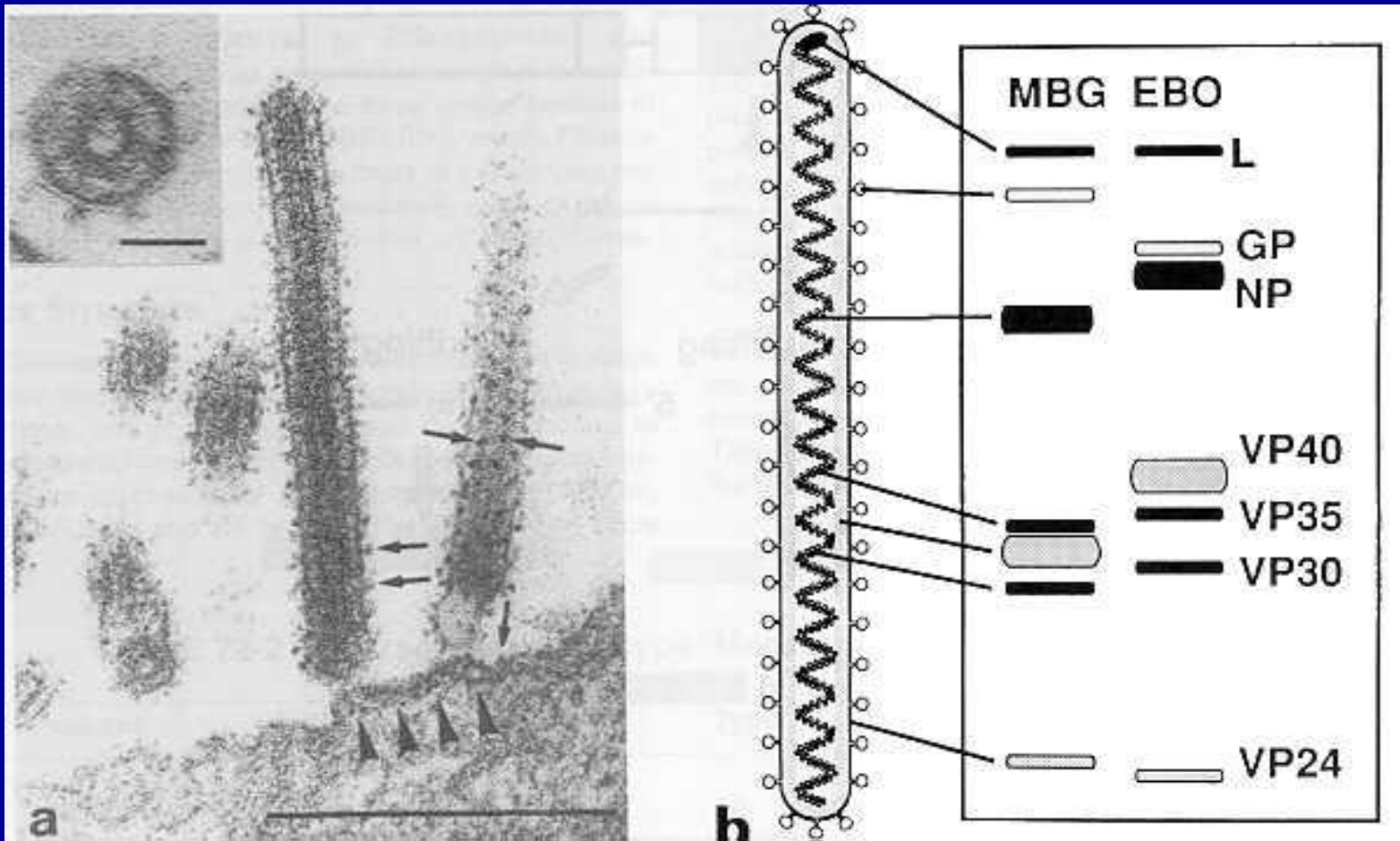
## Marburg virus



Courtesy of CDC

- **Virions have a constant diameter ~80nm, but vary in length from 800 to 14 000nm**
- **Occasionally circular, branched and U-shaped forms**
- **The receptor site is found on the cells of the cornea, conjunctiva, throat and tunica interna. That is why close contact is contagious.**
- **If you are caring for someone and you wipe the sweat off your forehead you are likely to get it.**
- **No cure.**
- **50% to 80% mortality rate.**

# MARBURGH VIRUS



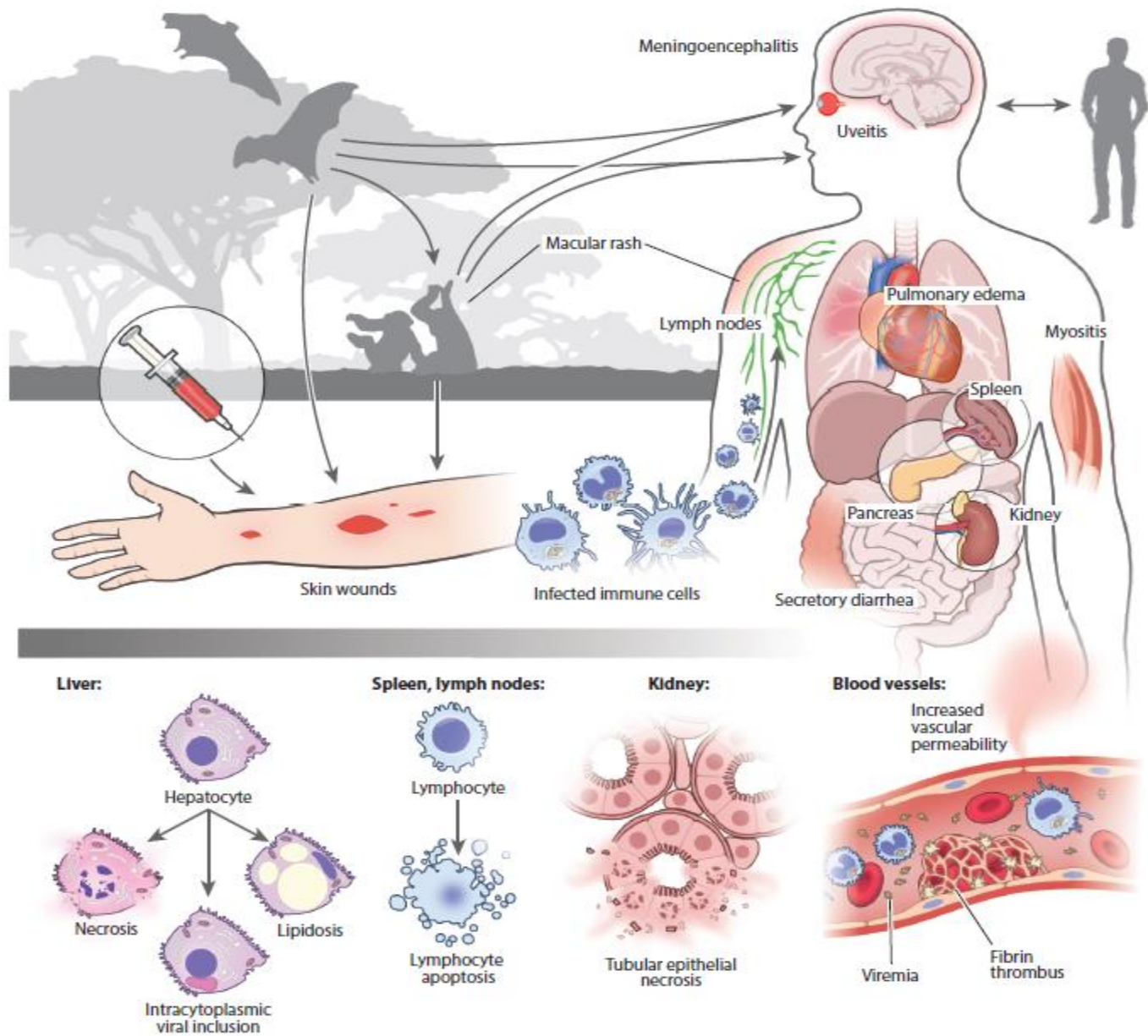
# VIRUS LIFE CYCLE



# Mechanism of transmission

- **Most person-to-person transmissions occur through direct physical contact with virus-containing body fluids – blood, urine, vomit, feces, and probably sweat.**
- **Once it is on the hands it is easily spread to the eye.**
- **Bush meat is infectious as it is slaughtered.**
- **There is no sign that airborne spread occurs**
- **There is no evidence that filoviruses are transmitted between humans by arthropod vectors**

- Scientists believe that the first patient becomes infected through contact with an infected animal, such as a fruit bat or primate (apes and monkeys), which is called a spillover event. Person-to-person transmission follows and can lead to large numbers of affected people. Primates are also affected by Ebola, and multiple spillover events occur when people butchered or ate infected primates. See TED Talk on bush meat if you really want to get depressed.
- When an infection does occur in humans, the virus can be spread in several ways to others. Ebola is spread through direct contact (through broken skin or mucous membranes in, for example, the eyes, nose, or mouth) with blood or any other body fluids of a person who is sick with Ebola
- Air dropped bat feces is **very dangerous**.
- objects (like needles and syringes) that have been contaminated with the virus
- Ebola is not spread through the air or by water, or in general, by food. (except bushmeat)



**Figure 3**

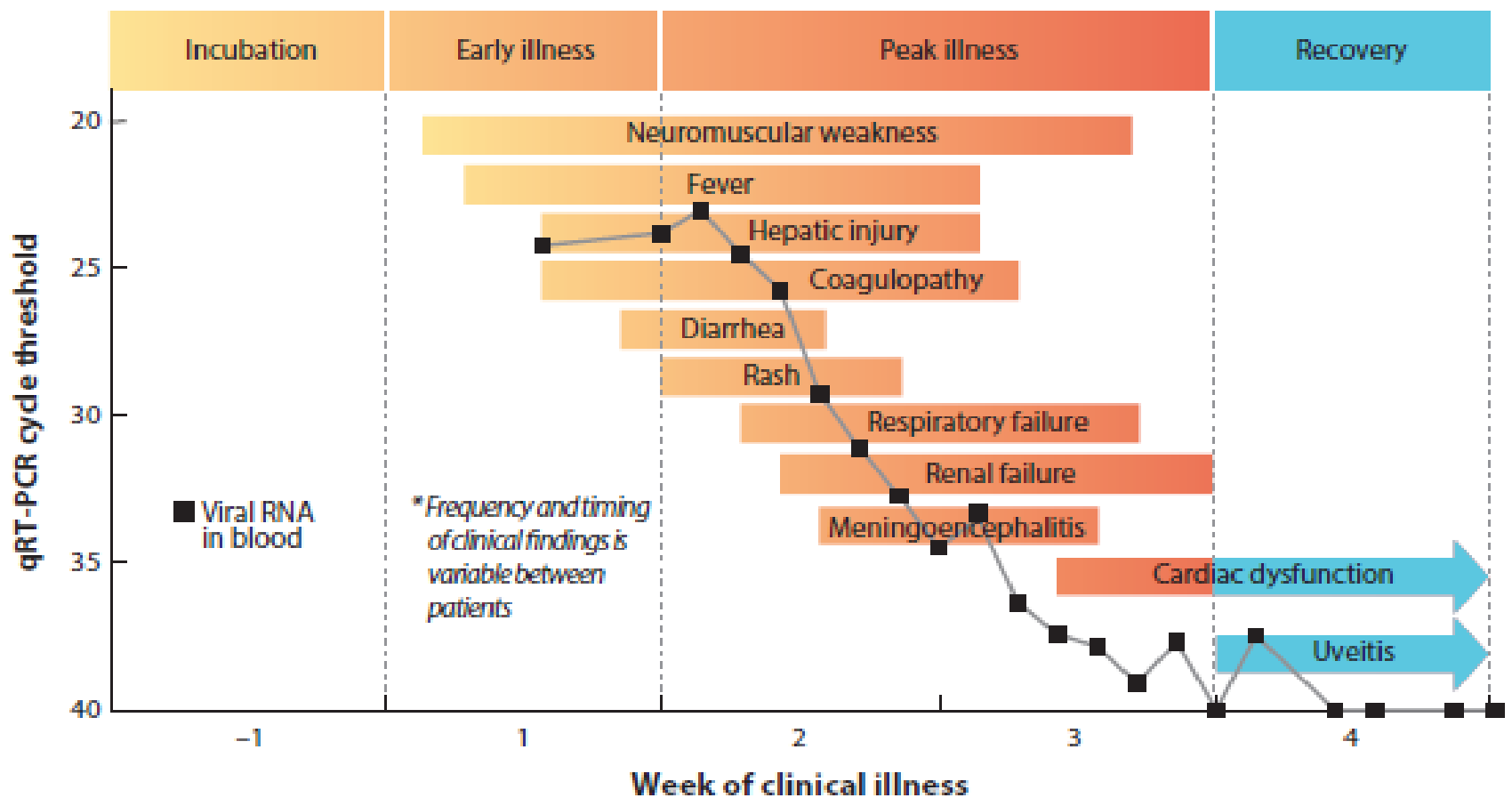
The transmission and pathogenesis of ebolavirus infection. Zoonotic, nosocomial, or person-to-person transmission of ebolavirus leads to viral infection of mononuclear phagocytes, which transport the virus to regional lymph nodes. Virus replication is followed by viremia with widespread viral dissemination, leading to tissue and vascular damage.

- Ebola virus has been found in semen for up to 3 months after recovery. Abstinence from sex (including oral sex) is recommended for at least 3 months. If abstinence is not possible, condoms may help prevent the spread of disease.
- It is in other body fluids as well.

## Clinical Presentation and Clinical Course (CDC)

- Patients with EVD generally have abrupt onset of fever and symptoms typically 8 to 12 days after exposure. Initial signs and symptoms are nonspecific and may include **elevated body temperature or subjective fever, chills, myalgias, and malaise**. Due to these nonspecific symptoms, Ebola can often be confused with other more common infectious diseases such as malaria, typhoid fever, and, pneumonia. By this time the person could infect a whole village.
- Patients can progress from the initial non-specific symptoms after about 5 days to develop gastrointestinal symptoms such as severe watery diarrhea, nausea, vomiting and abdominal pain. Patients often have conjunctival injection. Seizures may occur, and cerebral edema has been reported. Frank hemorrhage is less common; only 18% of patients. Patients may develop a rash by day 5 to 7 (usually involving the neck, trunk, and arms) that can desquamate. Pregnant women may experience spontaneous miscarriages.

- **Patients with fatal disease usually develop more severe clinical signs early during infection and die typically between days 6 and 16 of complications including multi-organ failure and septic shock**
- In non-fatal cases, patients may have fever for several days and improve, typically around day 6. Patients that survive can have a prolonged convalescence.
- Fatality rate in West Africa is 71%.



**Figure 5**

Clinical course of a typical case of severe Ebola virus disease. Detection of viral RNA in blood by quantitative reverse transcription polymerase chain reaction (qRT-PCR) (*line graph*), believed to represent viremia with infectious virions, occurs shortly after the onset of clinical illness. As disease progresses, sequential organ failure ensues despite declining viral RNA in blood. New or persistent clinical findings occur during recovery, following clearance of viral RNA in the blood. Figure adapted with permission from Reference 135.

# PATHOGENESIS

- Ebola virus enters the patient through mucous membranes, breaks in the skin, or Gi tracy and infects many cell types, including monocytes, macrophages, dendritic cells, **endothelial cells**, fibroblasts, hepatocytes, adrenal cortical cells and epithelial cells.
- Ebola migrates from the initial infection site to regional lymph nodes and subsequently to the liver, spleen and adrenal gland. Although not infected by Ebola virus, lymphocytes undergo apoptosis resulting in decreased lymphocyte counts.
- Hepatocellular necrosis occurs and is associated with dysregulation of clotting factors and subsequent coagulopathy. Adrenocortical necrosis also can be found and is associated with hypotension and impaired steroid synthesis.
- **Ebola virus appears to trigger a release of pro-inflammatory cytokines with subsequent vascular leak and impairment of clotting ultimately resulting in multi-organ failure and shock. (CDC)**

- **Simultaneous occurrence of massive cytolysis, fluid shifts, hemorrhage, and tissue ischemia resulting from obstruction of capillary blood flow by microthrombi, all contribute to the fatal outcome**

# IMMUNOLOGY

- **Much evidence indicate that early IFN- $\alpha/\beta$  response is critical in determining the outcome**
  - Typically interferons slow down initial replication to give time for adaptive immune responses
- **Filoviruses can block the effect of interferons permitting rapid dissemination of the virus**
- **It may be that those who survive have the longest incubation period prior to the onset of illness. There may be a genetic component to this.**
- **Virus specific IgM was present in all ill individuals in Ebola Zaire 1995 outbreak who survived and in only half of those who died**

# PREVENTION AND TREATMENT

- There are no vaccines – however, monkey studies are very promising showing protection against infection even after single injection (2003) – human phase I trials undergoing - suggest that human vaccines are possible
- Prevention by using protective clothing etc. to block human-to-human transmissions (pictures to follow)
- **Filoviruses are quite stable and can stay infectious in aqueous solutions at room temperature for weeks, tolerate repeated freezing and thawing. Cadavers are contagious.**
- Can be inactivated by heating at 60°C for 30 min, or treatment with e.g. bleach, formaldehyde or phenolic disinfectants
- There are no effective treatments currently available.

- Symptoms of Ebola are treated as they appear. The following basic interventions, when used early, can significantly improve the chances of survival:
  - Providing intravenous fluids (IV) and balancing electrolytes (body salts)
  - Maintaining oxygen status and blood pressure
  - Treating other infections if they occur
- Recovery from Ebola depends on good supportive care and the patient's immune response. People who recover from Ebola infection develop antibodies that last for at least 10 years, possibly longer. It isn't known if people who recover are immune for life or if they can become infected with a different species of Ebola. Some people who have recovered from Ebola have developed long-term complications, such as joint and vision problems.

**Experimental therapies and vaccines are under development, but we need new outbreaks to test them. Several are in trials.**

- **Passive infusion from recovered patients was successful in saving some people.**
- **During an outbreak there are a lot of survivors around who can donate their antibodies.**

# EPIDEMIOLOGY

- **Outbreaks occur sporadically and unpredictably in Central Africa**
- **Outbreaks typically gain international media attention when person-to-person spread has taken place within a hospital**
- **Most outbreaks result from an initial transfer of virus to a human from the unknown natural source**
  - **Bats**
  - **Infected gorillas, chimpanzees, forest antelopes (both dead or alive)**
- **Then a chain of person-to-person infections occur when people are exposed to fluids of sick victims**

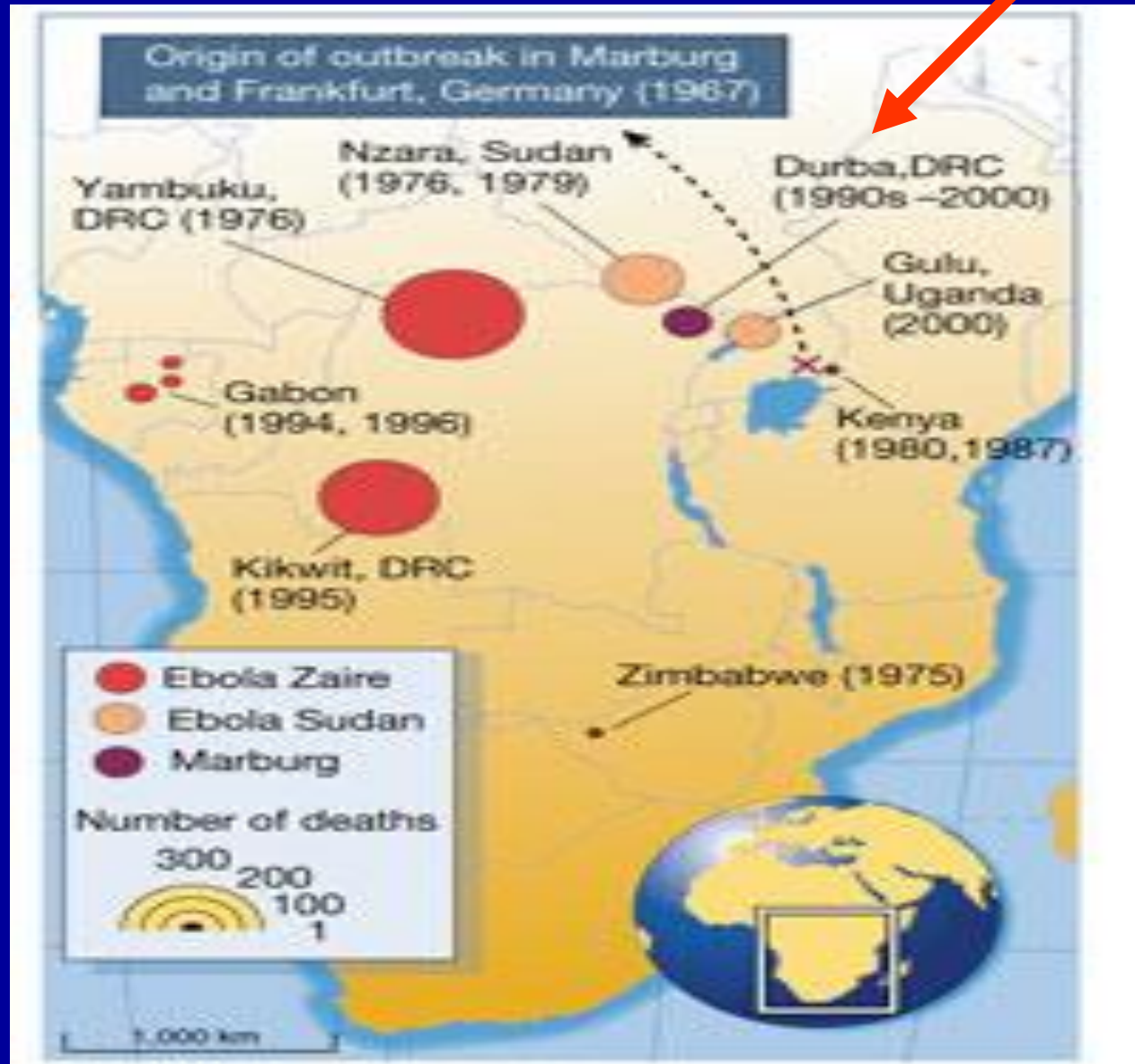
- **Calculated death rates may have increased the lethality of these viruses since fatal cases are more likely to be registered than mild or asymptomatic**
- **Filovirus infections have occurred in Africa for millennia where spread was limited by the absence of hospitals and the use of traditional quarantine methods**
- **Sporadic cases may still continue to escape detection**

# Marburg virus

- **The first filovirus epidemic occurred in the center of Europe in the summer of 1967**
- **Vaccine plant workers in Marburg and Frankfurt in Germany, and in Belgrade, Yugoslavia became severely ill with a previously unknown febrile disease – in some cases associated with hemorrhagic manifestations**
- **All victims had a contact with African Green monkeys imported from Uganda – some of the monkeys were also suffering from severe illness**
- **25 workers and 6 medical personnel became ill – 7 died**

- **No further cases of Marburg infection have been detected in nonhuman primates**
- **Until 1998, only six additional human cases were observed, all of them in Africa**
- **The first large-scale outbreak was detected in Democratic Republic of Congo in November 1998**
  - **Durba - gold mining area**
  - **A total of 141 cases were identified with 82% mortality rate**
  - **Few patients gave a history of recent close contact with ill person – suggesting that repeated exposure of virus from unknown resource was taking place**
  - **Marburg virus seems to be endemic to Durba mining area – the source is a mystery**

# Durba mining area



- **2004–2005 : Angola**

- **Outbreak in Uige Province in October 2004**

- **As of 2 April 2005, the Ministry of Health reported a cumulative total of 163 cases, of which 150 were fatal**

- **Most cases detected in other provinces have been linked directly to the outbreak in Uige**

- **At the request of the government, international assistance, coordinated by WHO, was organized to help contain the outbreak**

# N Angola



# N Africa



- **2005 Marburg haemorrhagic fever outbreak .**
- **The disease spread particularly among people exposed to the Marburg virus during home care or at funerals, via contact with body fluids of those who died from the disease**
- **The dangerous use of home-based injections was also identified as a major cause of the outbreak's spread**
- **As of 23 August 2005, the Ministry of Health in Angola reported a total of 374 cases, including 329 deaths (CFR 88%) reported countrywide**

## Bringing the Marburg outbreak under control

2005



In the Northern Angolan province of Uige, an outbreak of Marburg haemorrhagic fever begins to be brought under control. More than 300 people have died, but the number of new cases has declined considerably since the peak in early April. Health workers and the community are still on high alert for the disease.



Dr Mark Katz from the Centers for Disease Control and Prevention, USA, takes a sample from a woman for diagnostic testing. The woman has had contact with several members of her immediate family who later died of Marburg haemorrhagic fever.



The woman and her son are taken to the isolation ward at the Uige Provincial Hospital to await their test results. Good news: they both test negative.



In the isolation ward at Uige Provincial Hospital, a doctor from Médecins sans Frontières cares for a patient who has the Marburg virus. The fatality rate for Marburg is extremely high — and almost everyone who gets the disease dies



In the town of Songo — 50 km from Uige — it is market day. Local volunteers spread messages about Marburg haemorrhagic fever and the importance of safe injections. A "Safe Injection Campaign" was initiated after health workers discovered unsafe injection use in homes, private clinics and among traditional healers. Re-using dirty needles and syringes can easily spread the Marburg virus

In outbreaks of viral haemorrhagic fevers, including those caused by Marburg virus, unprotected exposure to dead bodies is a significant cause of further spread. Here, mobile teams in Uige, Angola, prepare for the safe transport and burial of fatal cases of Marburg haemorrhagic fever.



# Images from the outbreak of Marburg haemorrhagic fever in Uige Province, Angola 2005



Mobile PCR lab

# 2007: Marburg Hemorrhagic Fever Outbreak in Uganda

- On July 27, 2007, CDC was notified of a suspect case of Marburg. A blood specimen taken from **the only fatal patient, a miner at a local lead and gold mine**, was received by CDC on Friday, July 27, 2007. The specimen tested positive for Marburg virus.
- A 6-person CDC team consisting of three medical officers, a mammologist, and two microbiologists arrived in Uganda on August 10, traveling to the town of Ibanda in Kamwenge province, near the site of the mine where the exposures are believed to have occurred. **The team initiated an investigation by capturing bats and other animals at the site of the mine in an effort to further identify the animal host of the Marburg virus**, and by tracing human contacts in communities near the mine.

## 2008: Marburg hemorrhagic fever, imported case – Netherlands ex Uganda, July

- On July 10, 2008 CDC was notified by the European CDC about a case of Marburg hemorrhagic fever in a woman from The Netherlands. The woman had recently returned from traveling in Uganda. On one occasion the woman had contact with a bat in a cave in the Maramagambo forest in Western Uganda (at the southern edge of Queen Elizabeth National Park), and became ill after returning to The Netherlands.
- Laboratory testing revealed evidence of Marburg virus. The patient died on Thursday July 11, 2008 in the morning.

## 2008: Marburg hemorrhagic fever, imported case – United States

- On January 22, 2009, CDC retrospectively diagnosed a case of **Marburg hemorrhagic fever in a U.S. traveler**, who returned from Uganda in January, 2008. The patient developed illness four days after returning to the U.S., was hospitalized, discharged, and fully recovered.

- The recovered patient had visited the “python cave” in Maramagambo Forest, Queen Elizabeth Park, western Uganda, same place the Dutch tourist visited. This is a popular destination among tourists to see a cave inhabited by thousands of bats.
- Both patients likely acquired their infections as a result of contact with cave-dwelling fruit bats, which are capable of harboring Marburg virus. Marburg virus is a zoonotic virus that like can also be transmitted through direct contact with a symptomatic patient or materials contaminated with infectious body fluids.
- Without a zoonotic reservoir it can't persist in North America. If the infected bats get into Central America look out.





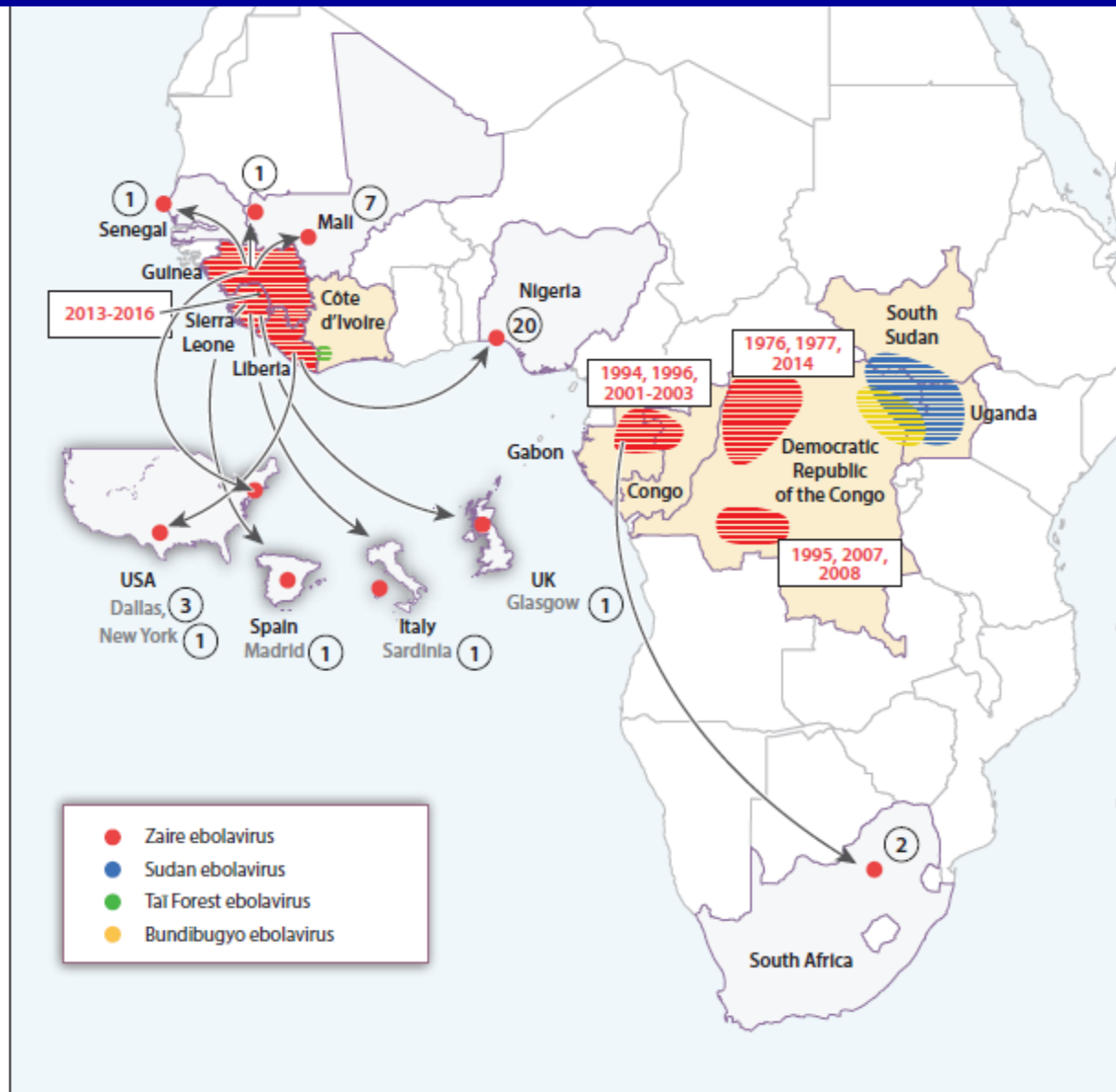






# **EBOLAVIRUS Epidemiology**

- **Ebolavirus has five subspecies:**
  - **Zaire**
  - **Sudan**
  - **Tai Forest**
  - **Reston (from Philippines)**
  - **Bundibugyo**

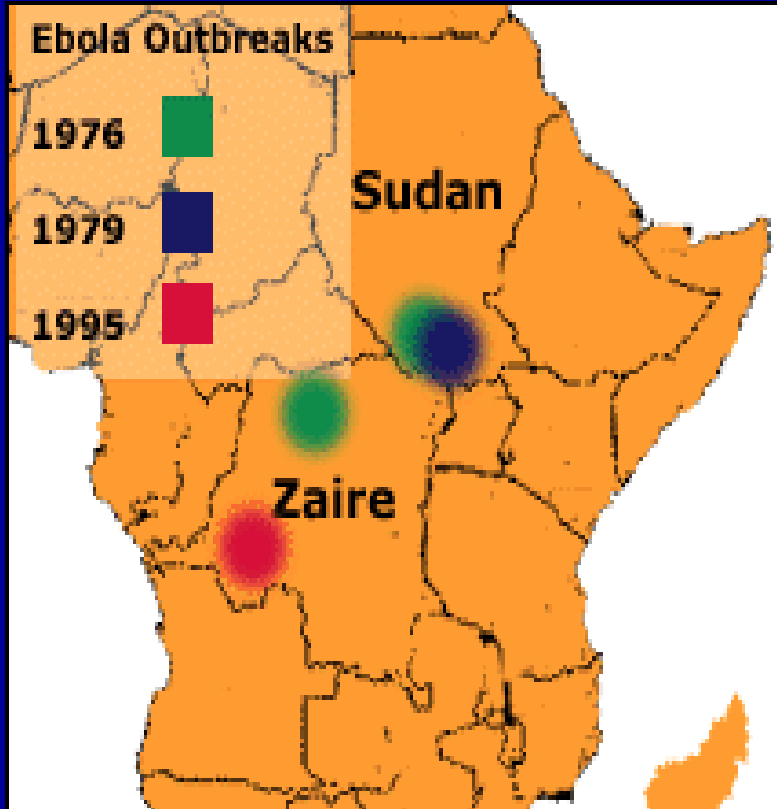


**Figure 2**

Geographic locales of known ebolavirus outbreaks in West and Central Africa (from 1976 to August 2016). The map shows the occurrence of all known human outbreaks associated with four different ebolavirus species across the equatorial belt within the African continent. The circled numbers indicate EVD cases resulting from introductions of EBOV-infected persons from Guinea, Sierra Leone, Liberia, or, in one instance, from an individual infected in Gabon during the 2013–2015 West African epidemic.

# Ebola virus – Ebola Zaire

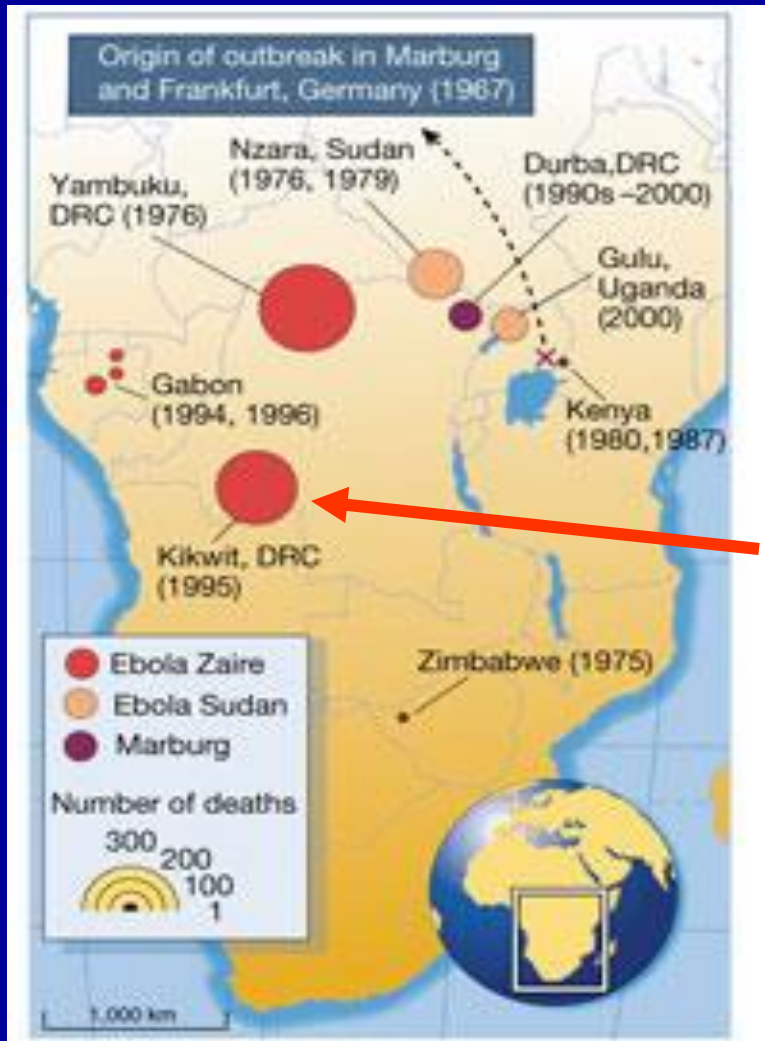
- June & September, 1976
- Two outbreaks with related viral agents – named Ebola Zaire and Ebola Sudan
- Ebola Zaire name derived from Ebola river, near outbreak epicenter
- The source of the infection was never determined



# **Ebola virus – Ebola Zaire**

- Hospitalization of sick individuals led to rapid spread of Ebola Zaire to doctors, nurses and other patients by means of nursing procedures and reused contaminated syringes**
- The outbreak ended when patients and staff fled the facility and the remaining ill patients were quarantined in their home villages**
- A total of 318 cases were recognized with an estimated 88% fatality rate**
- Few mild cases were recognized**

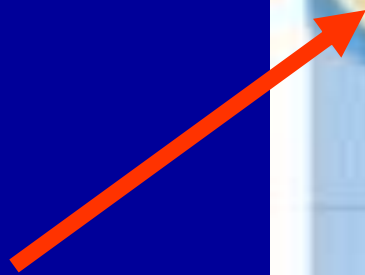
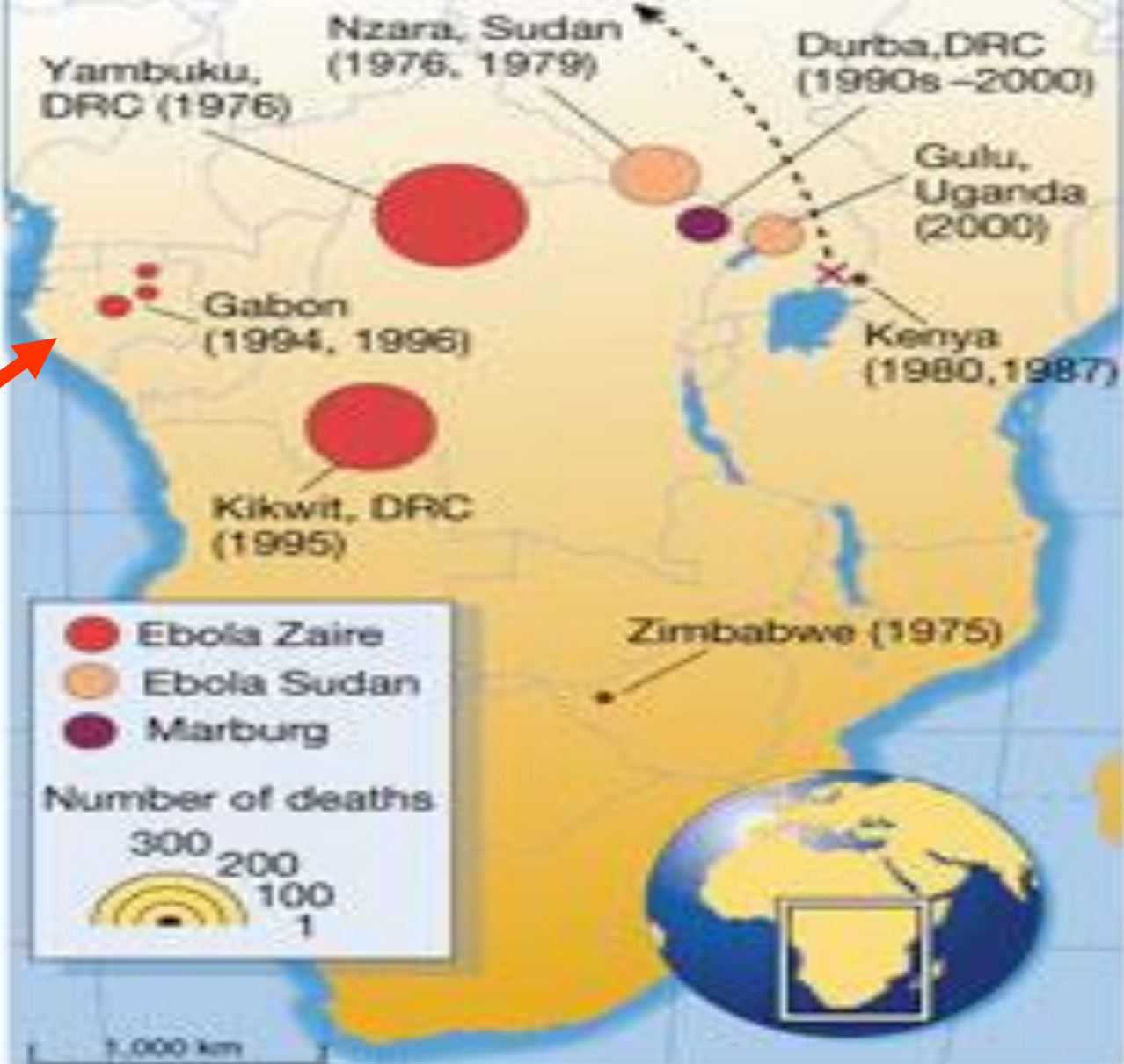
- Ebola Zaire reappeared in 1995 in Kikwit, Zaire
- Another hospital-centered epidemic
- Person-to-person transmission started on January and the disease was recognized on April when individual was admitted into hospital with fever and bloody diarrhea
- Patient underwent surgery and infected the entire operating team



- There were total of 315 cases over the course of epidemic with 244 deaths, including 80 medical workers. **80% mortality**
- A 16% secondary attack rate was found among family members: cared for patients or prepared cadavers
- Viruses isolated in the 1995 outbreak differed only 1.5% in their GP sequence from those recovered in 1976
  - This suggests that filoviruses undergo relatively few replication cycles in their unknown natural reservoir
- 2001-2002: Republic of Congo, 59 cases, **75% mortality**

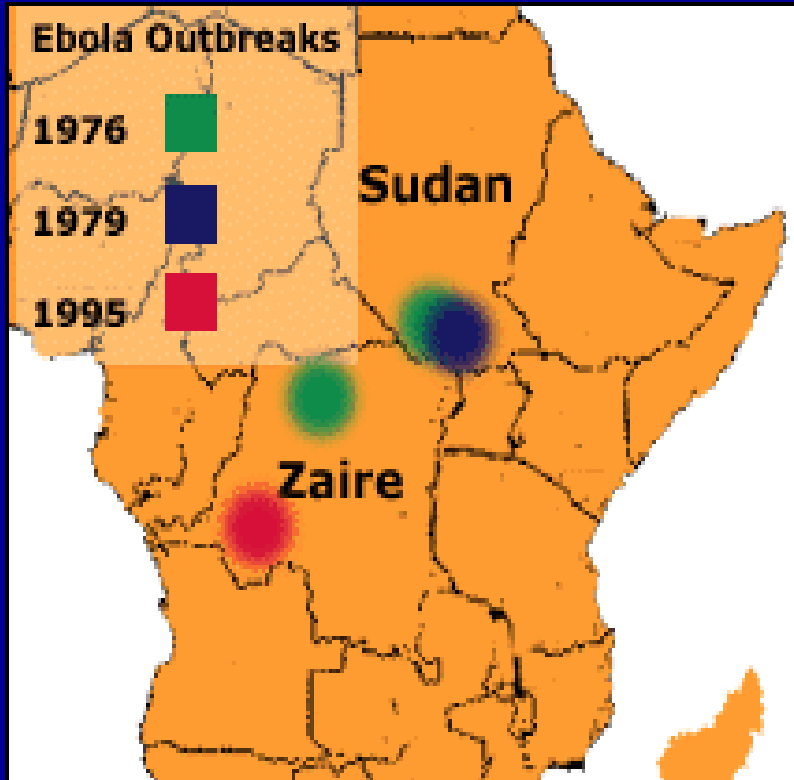
- **2002-2003: Republic of Congo, 143 cases, 86% mortality**
- **2003: Republic of Congo, 35 cases, 83% mortality**
- **Other outbreaks occurred in Gabon**
  - 1994 49 cases; 65% mortality and
  - 1996 37 cases from eating dead chimpanzee: 57% mortality,
  - 1996 60 cases, mortality 75%,
  - 2002 65 cases, 82% mortality
- **2007: Republic of Congo, 249 cases, 183 deaths – 78% mortality**

# Origin of outbreak in Marburg and Frankfurt, Germany (1967)



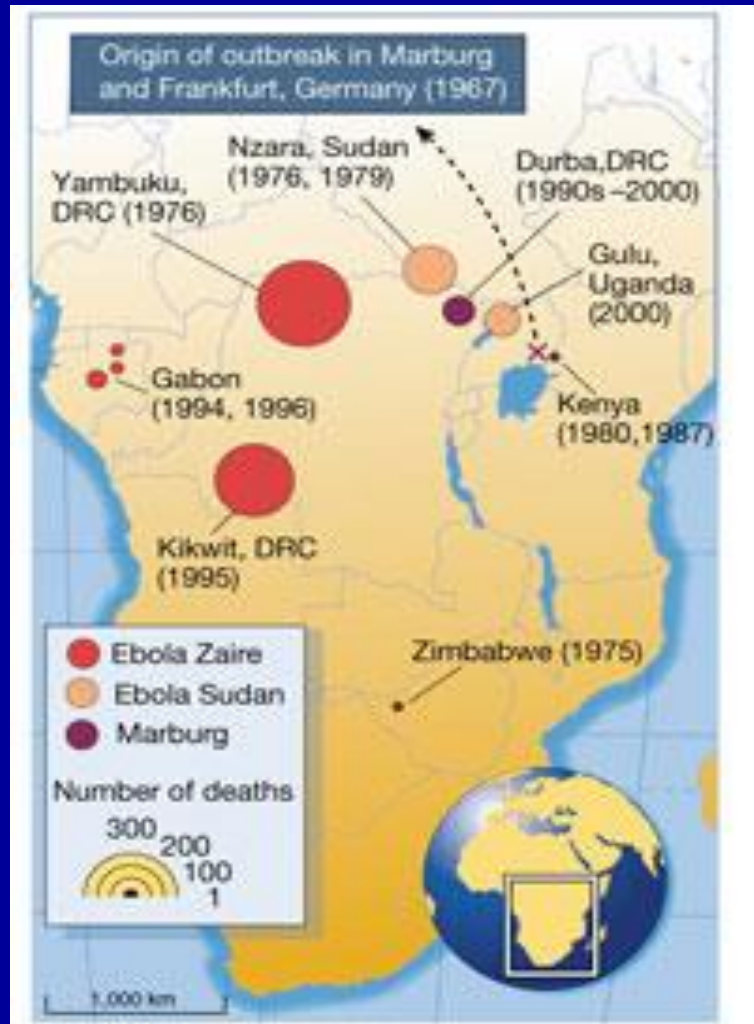
# Ebola virus – Ebola Sudan

- 1976 Sudan epidemic started among workers in cotton cloth factory
- Presence of rat infestation and bat colony in a roof suggested that either one might be the source
- Majority of cases occurred when sick patients were taken into hospital
- Total of 284 cases with 53% mortality rate



Courtesy of Don DeWitt, Bergen county academies, Hackensack, New Jersey

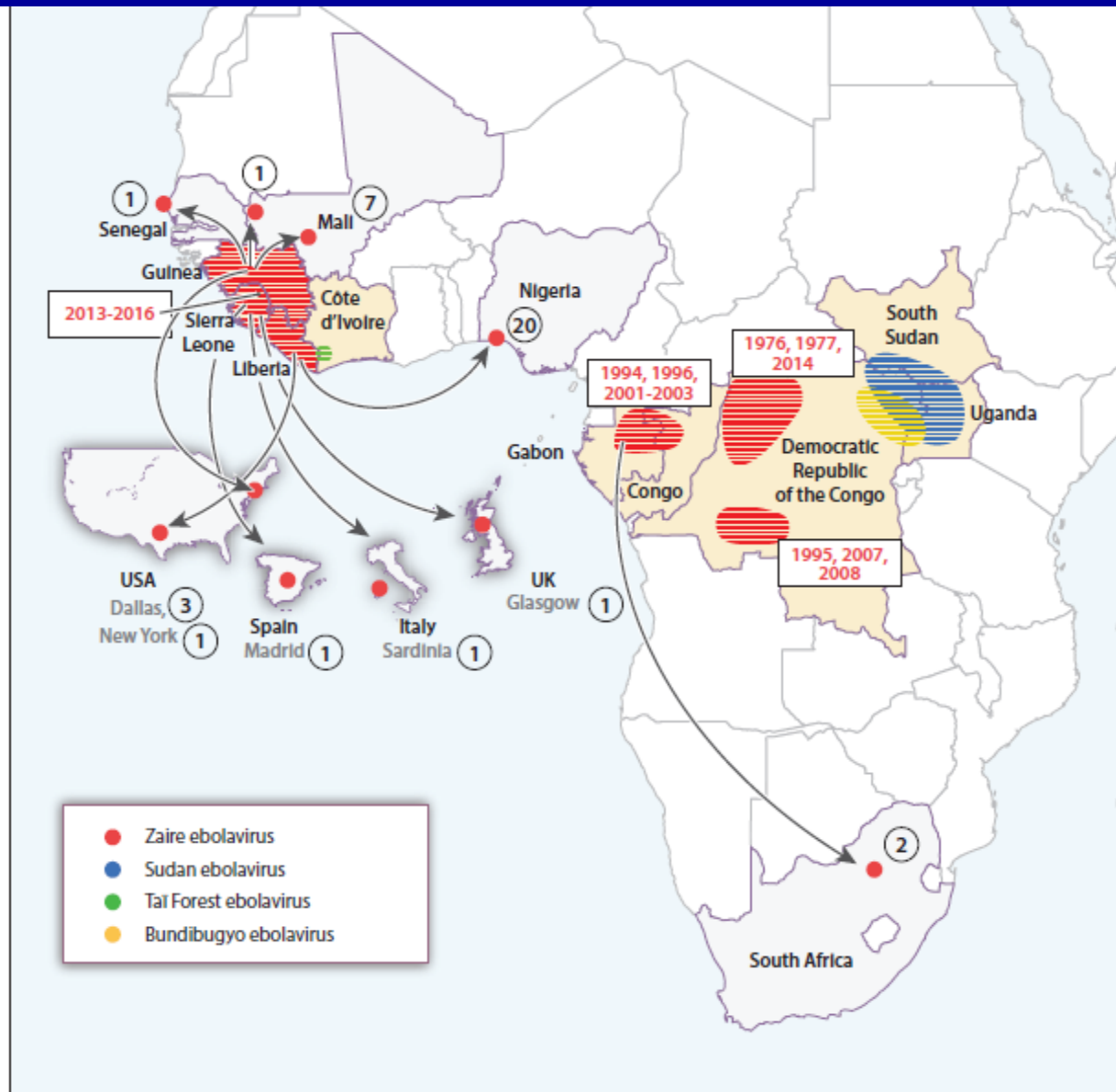
- Virus returned to the same site in 1979 causing 34 infections and 22 fatalities
- After 20 years, Ebola Sudan reappeared in October 2000 in Gulu, Uganda
- Outbreak was recognized when a number a student nurses become fatally ill
- There were total of 425 cases with 224 deaths



Courtesy of Don DeWitt, Bergen county academies, Hackensack, New Jersey

# **Ebola virus – Tai Forest**

- **Ebola Cote d'Ivoire 1994 – NOW *Ebola Tai Forest*, death of chimpanzees, investigators performed autopsy with rubber gloves – one became ill 6 days later**
  - **New subspecies of Ebola was discovered**



**Figure 2**

Geographic locales of known ebolavirus outbreaks in West and Central Africa (from 1976 to August 2016). The map shows the occurrence of all known human outbreaks associated with four different ebolavirus species across the equatorial belt within the African continent. The circled numbers indicate EVD cases resulting from introductions of EBOV-infected persons from Guinea, Sierra Leone, Liberia, or, in one instance, from an individual infected in Gabon during the 2013–2015 West African epidemic.

# **Ebola virus – Reston**

- **1989 Reston, Virginia, numerous deaths among macaques imported from Philippines**
- **Infection rapidly spread among the animals**
- **Animal caretakers exposed to the infected animals did not become ill**
- **However, later on, several of them developed antibodies to Ebola antigens – asymptomatic infection**
- **May indicate that it is not pathogenic for humans**

- **However, Reston is closely related to Ebola and is pathogenic for nonhuman primates – may cause human disease in other circumstances**
- **Similar outbreaks in animal facilities occurred in Pennsylvania (1990), Texas (1992) and Italy (1996)**

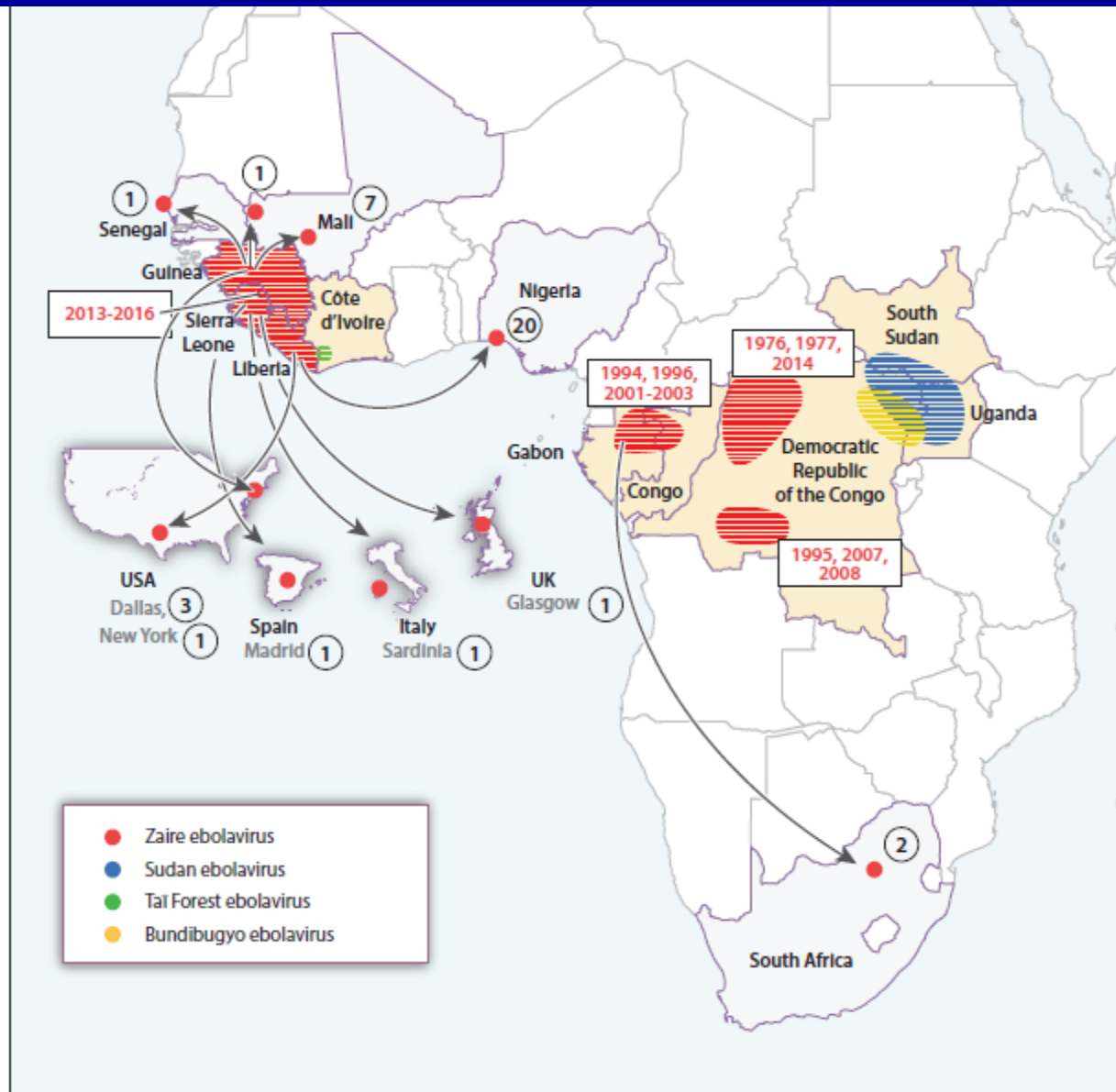
# 2008: Ebola-Reston virus detected in pigs in Philippines (CDC)

- On October 25, 2008, CDC received samples of pig tissues, from FADDL, the Foreign Animal Disease Diagnostic Laboratory on Plum Island, NY. The samples, originally collected from pig farms outside Manila, identified **Ebola-Reston virus** that infected macaques from the Philippines imported into the US for research in 1989, 1990 and 1996, and into Italy in 1992.

# Ebola Reston in pigs and humans in the Philippines (WHO)

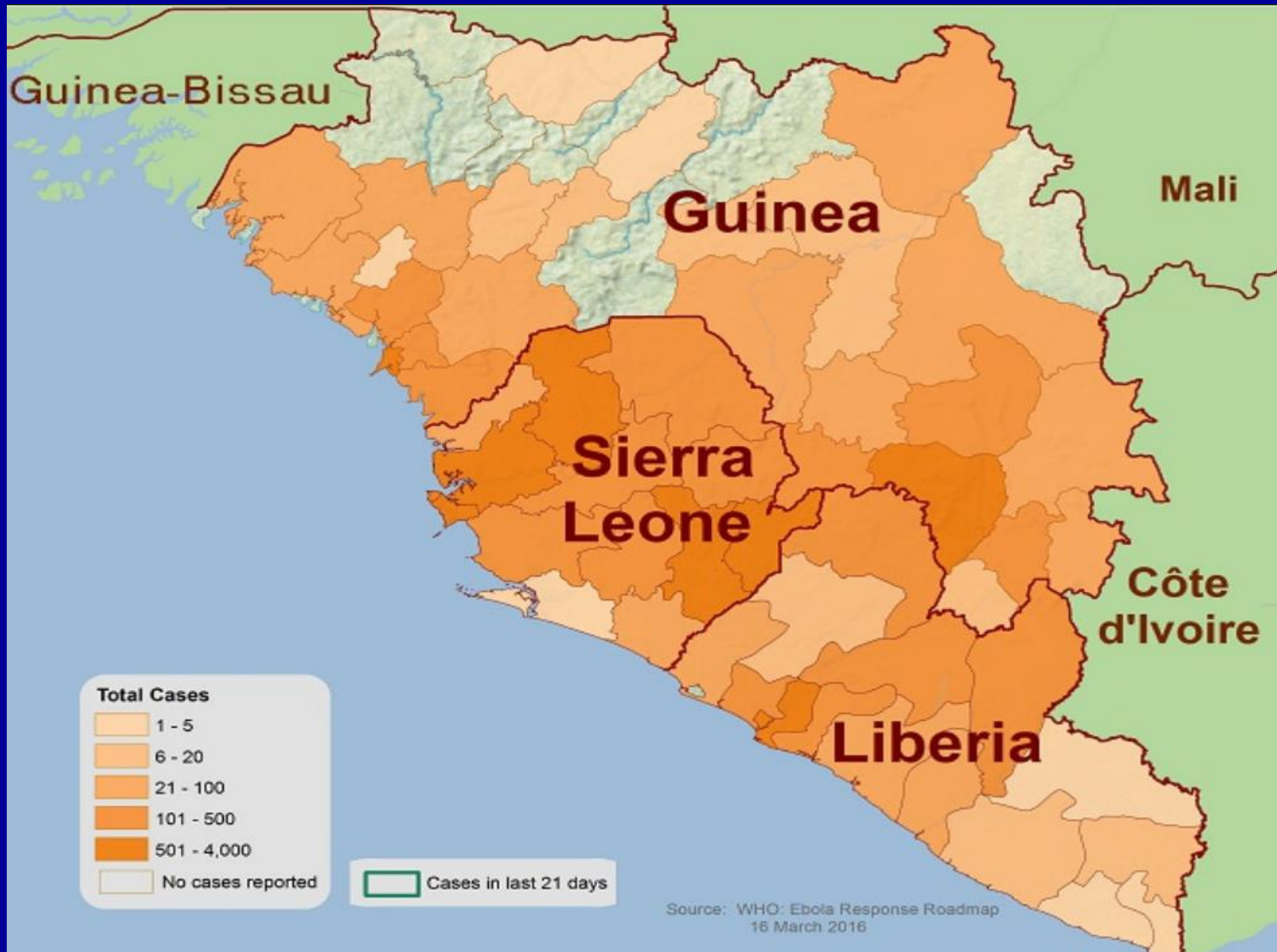
- 31 March 2009 -- On 16 February 2009, a slaughterhouse worker who has daily contact with pigs tested positive for antibodies against the Ebola Reston virus.
- This brings to six, out of a total of 141 people, who have tested positive for Ebola Reston antibodies in the Philippines. All six people who were antibody positive reported occupational exposure to pigs.
- They appear to be in good health. Pig-to-human transmission is believed to be the most likely source of infection.

# **2013-2016 EBOLA OUTBREAK**



**Figure 2**

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Source: WHO: Ebola Response Roadmap  
16 March 2016

## Case Counts\*

As of April 13, 2016

(Updated April 13, 2016)

**Total Cases (Suspected, Probable, and Confirmed): 28,652**

**Laboratory-Confirmed Cases: 15,261**

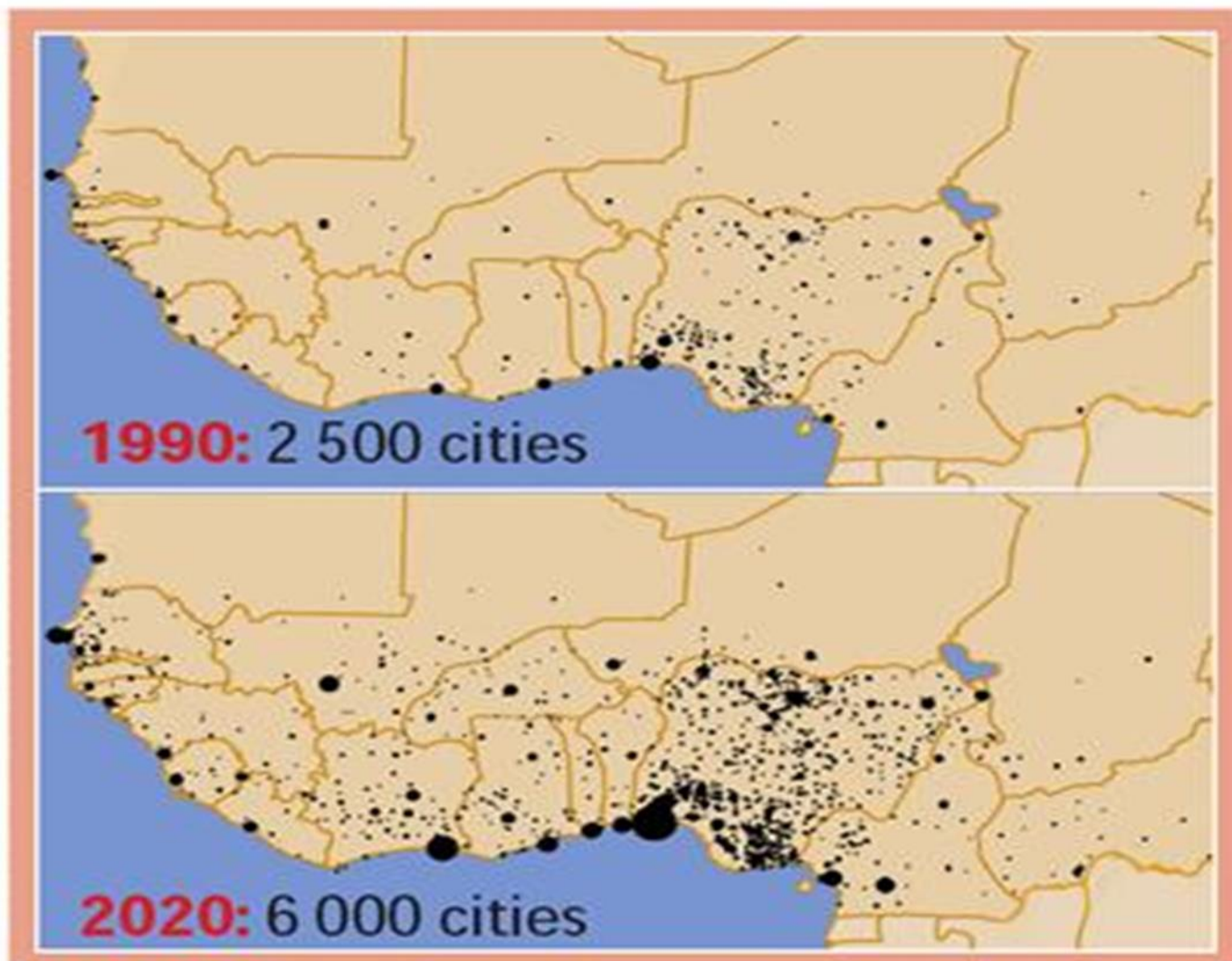
**Total Deaths: 11,325**

\*Case counts updated in conjunction with the World Health Organization updates and are based on information reported by the Ministries of Health.

## WHY SUCH MASSIVE OUTBREAK?

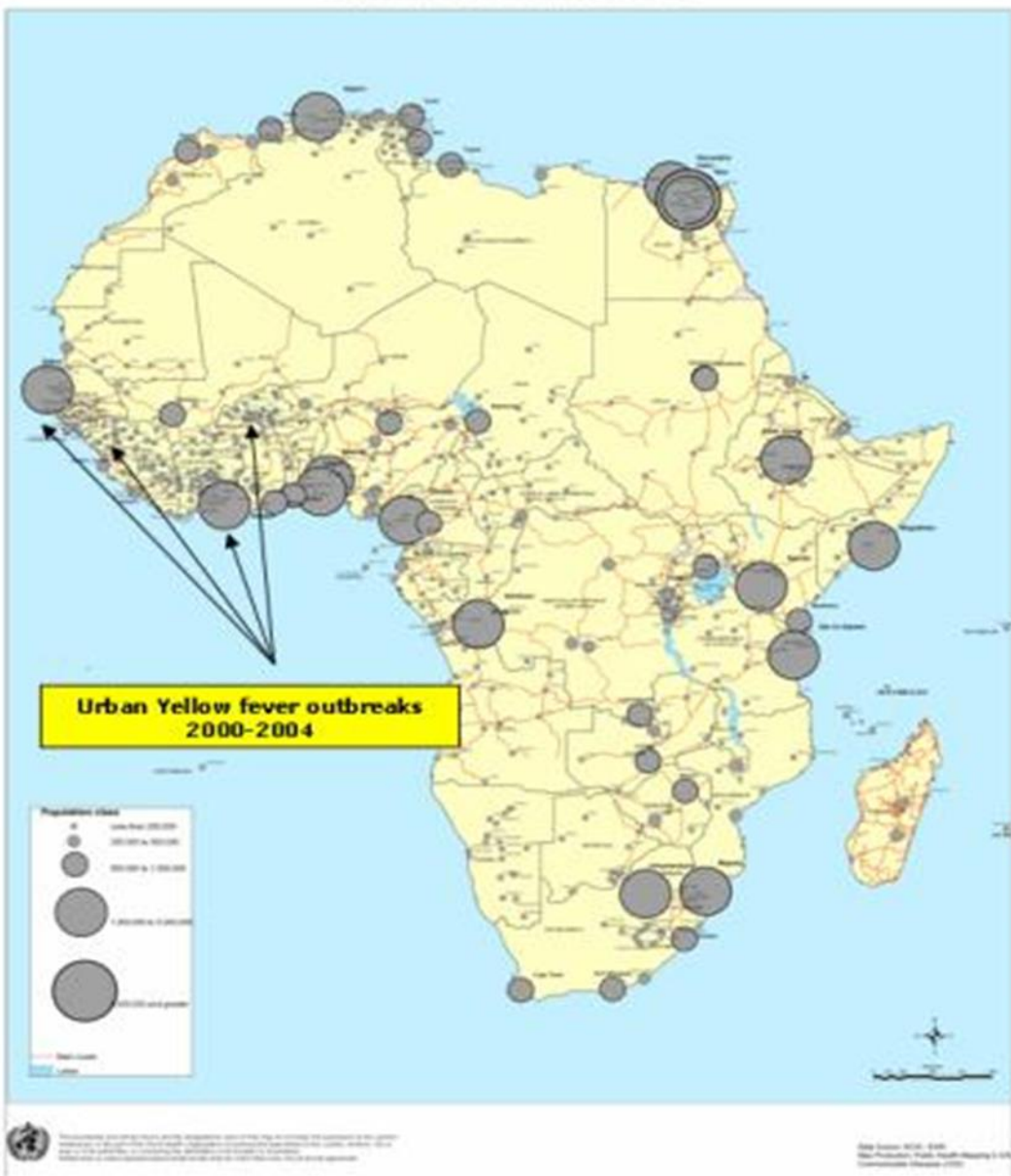
- Location, location, location.....

## Urban growth in Western Africa



(Source: OECD, 1997)

### Population of main cities in Africa



World Health Organization  
Geneva, Switzerland  
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Map Source: CIA, 2004  
Map Projection: UTM  
Scale: 1:10,000,000  
Data Source: Various

- EBOLA subspecies causing current outbreak is **ZAIRE** (typically seen in central Africa)
- We do not know how it entered into west Africa. (Bat migration?)

A major problem with containing ebola has been slow diagnosis. With a 9-11 day incubation period followed by 5 days of S & S that don't tell you much, people can be infected and infecting others for a long period. A new rapid field test has been developed.



**EBOLA**

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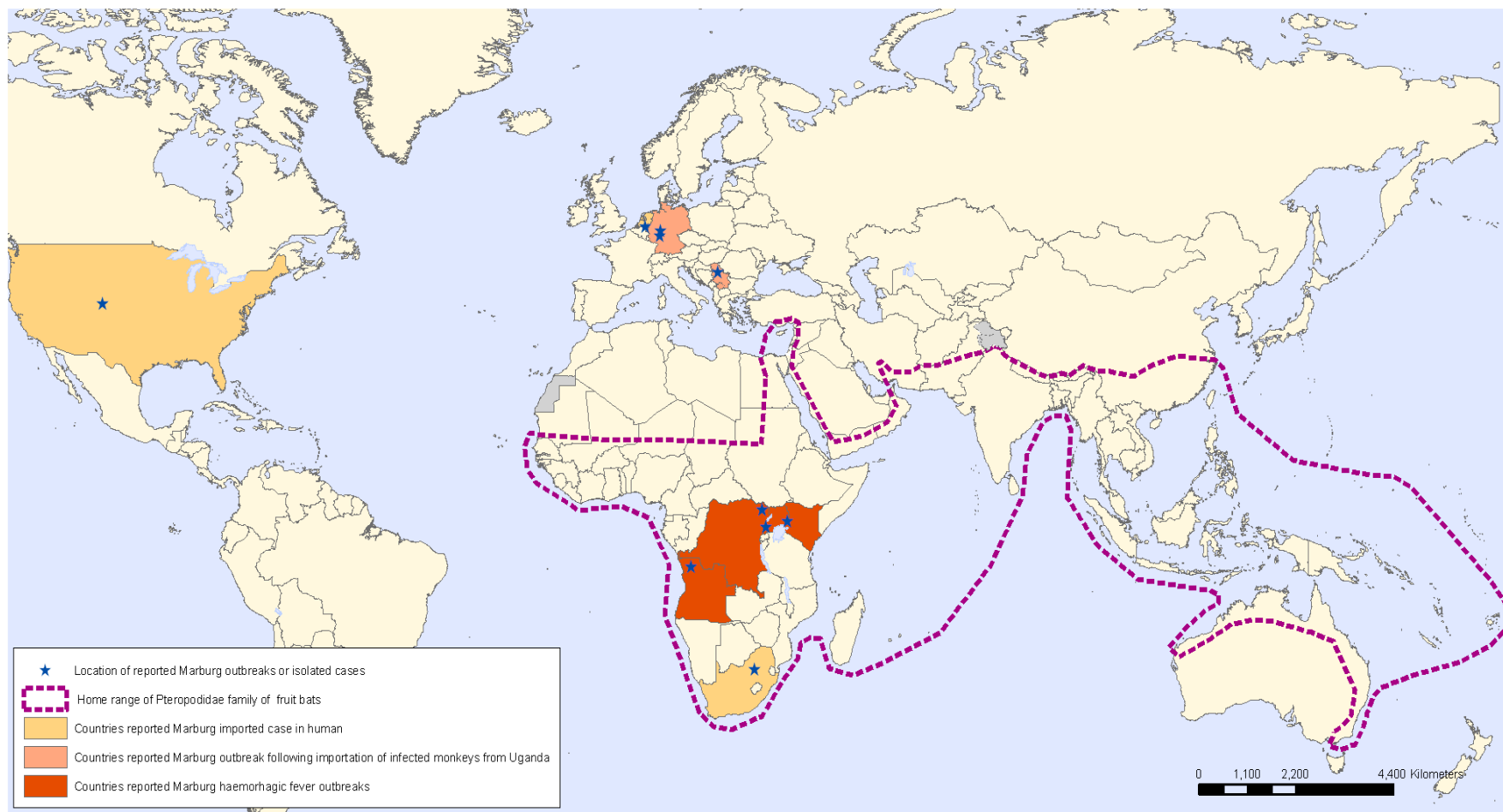
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# **TRANSMISSION - reservoir**

- **After testing samples taken from some 30 000 mammals, birds, reptiles, amphibians, and arthropods in outbreak regions antibodies and genetic fragments of Ebola or Marburg viruses have been found in bats.**
- **In Uganda in 2008, Marburg viruses were isolated from frugivorous bats.**

## Geographic distribution of Marburg haemorrhagic fever outbreaks and fruit bats of Pteropodidae Family



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.

Data Source: Global Alert and Response Department  
World Health Organization  
Map Production: Public Health Information  
and Geographic Information Systems (GIS)  
World Health Organization



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