EBOLA VIRUS DISEASE IN PREGNANCY:

clinical histopathologic and immunohistochemical findings

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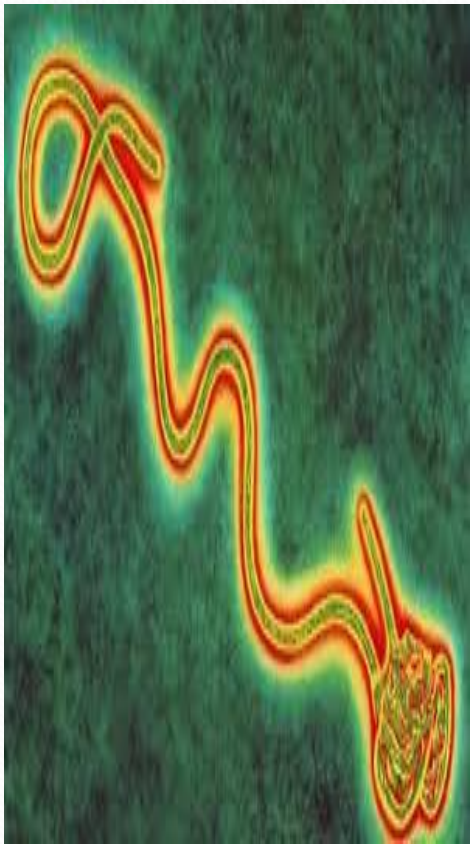
Thanks to …

- Atis Muehlenbachs, Olimpia de la Rosa Vázquez, Daniel G. Bausch, Ilana J. Schafer, Christopher D. Paddock, Jean Paul Nyakio, Papys Lame, Eric Bergeron, Andrea M. McCollum, Cynthia S. Goldsmith, Brigid C. Bollweg, Miriam Alía Prieto, Robert Shongo Lushima, Benoit Kebela Ilunga, Stuart T. Nichol, Wun-Ju Shieh, Ute Ströher, Pierre E. Rollin, Sherif R. Zaki

For such great article!

EBOLA VIRUS (EVD)

- An infectious
- Generally fetal disease marked by
  - fever
  - Severe internal bleeding
- Spread throughout contacts with
  - Body fluids by Filovirus (Ebola Virus)
- HOST
  - Unknown



BACKGROUND

Named because of Ebola River

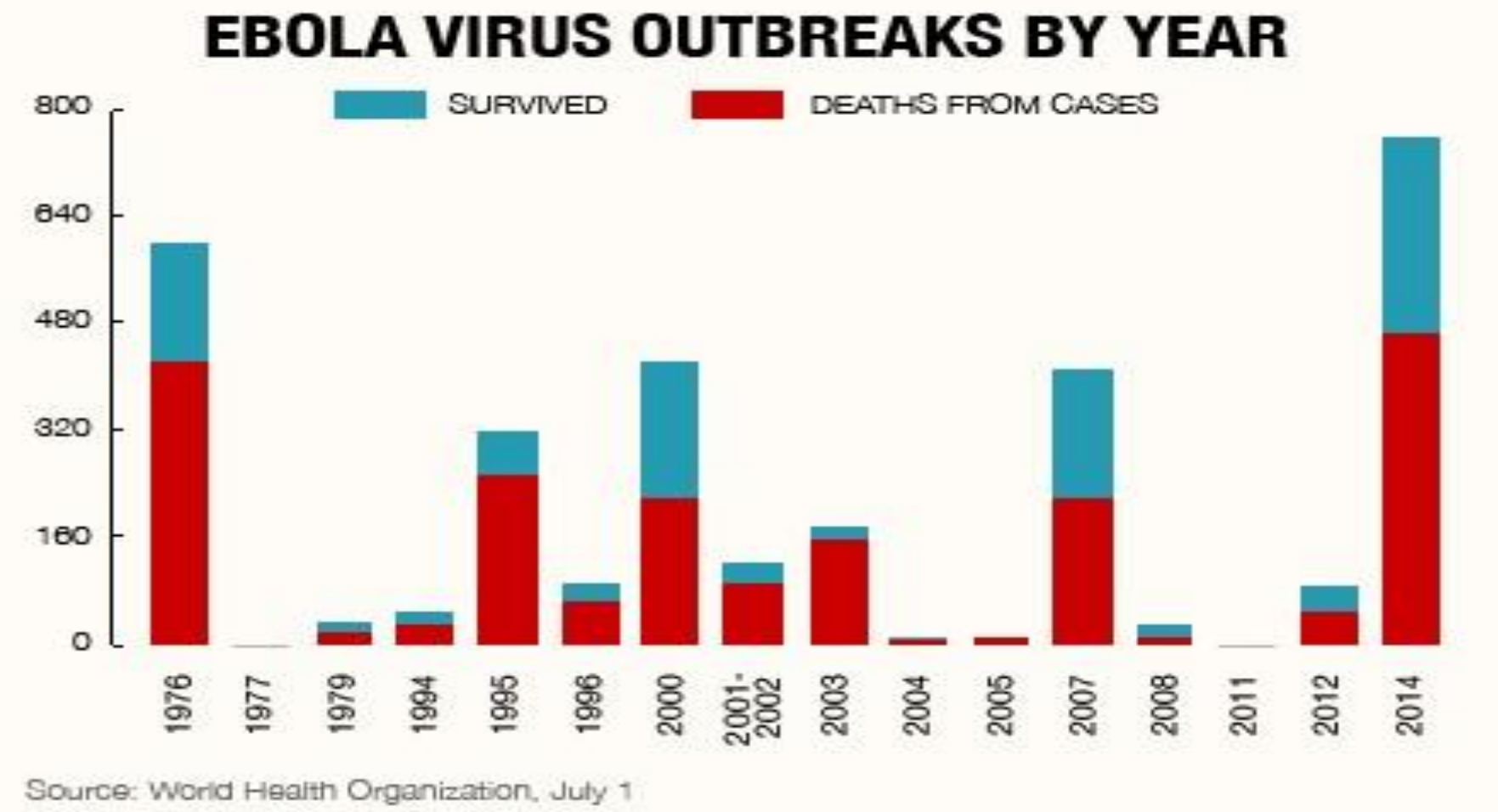
FIRST APPEARANCE OF EVD

- In Sudan and Zaire in 1976

FIRST OUTBREAK

- In Sudan
  - Infected over 284 people
  - Killing 53% of victim
  - Another strain appeared
  - Infected another 318 people
  - Mortality rate was 86%

AFFECT OF EBOLA VIRUS 1976-2014



Species of Ebolaviruses

Ebolaviruses are closely related to species in the genus Marburgvirus, which was discovered in 1967, and the two are the only members of the Filoviridae that cause epidemic human disease. Five species of ebolaviruses—known as Zaire ebolavirus, Sudan ebolavirus, Taï Forest ebolavirus, Reston ebolavirus, and Bundibugyo ebolavirus, named for their outbreak locations—have been described. The viruses are known commonly as Ebola virus (EBOV), Sudan virus (SUDV), Taï Forest virus (TAFV), Reston virus (RESTV), and Bundibugyo virus (BDBV).

Ebola virus disease (EVD) and Marburg virus disease are caused by viruses of the Ebolavirus and Marburgvirus genera (family Filoviridae). Here, we collectively refer to Ebola virus (EBOV), Sudan virus (SUDV) and Bundibugyo virus (BDBV) all within the Ebolavirus genus as ebolaviruses. Filovirus infection during pregnancy is associated with maternal hemorrhage, preterm labor, miscarriage, and maternal and neonatal death. Supplementary Table 1 presents a summary of the scientific literature to date; maternal death occurred in 102 of 125 reported cases (82%), and there was uniform loss of offspring, whether by miscarriage, stillbirth, or neonatal death. Of the 18 live births, the longest survival was 19 days.

Despite the severity of filovirus infection in pregnancy for both mother and child, very little is known regarding pathogenesis. Fetal-placental viral tropism has been hypothesized due to recent observations during the 2013–2016 West Africa EBOV outbreak: pregnant women were noted to survive EVD and clear virus from the blood without fetal loss during acute infection and to deliver stillbirths in the subsequent weeks and months with relatively high EBOV RNA levels in placental and fetal tissue swab specimens [7–10]. We report clinical, histopathologic, and immunohistochemical findings of SUDV and BDBV infections in 2 pregnant women and their offspring that help shed light on the pathogenesis of fetal infection and loss in EVD.

METHODS

- Patients

Two pregnant women with EVD were cared for in Ebola treatment centers during ebolavirus outbreaks in Gulu, Uganda, in 2000 [11, 12] and Isiro, DRC, in 2012 [13] (Schafer, unpublished data). Specimens were collected and evaluated during the course of the outbreak responses.

- Ebolavirus Diagnostic Testing

SUDV reverse transcription–polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assays (ELISAs) in Gulu and BDBV RT-PCR assays in Isiro were performed as previously described [14, 15] in field laboratories run by the Viral Special Pathogens Branch (VSPB), Centers for Disease Control and Prevention (CDC; Atlanta, Georgia). BDBV immunoglobulin M (IgM) and immunoglobulin G (IgG) ELISAs were performed by the VSPB in Atlanta.

ELISA

- Rapid blood tests detect specific RNA sequences by reverse-transcription polymerase chain reaction (RT-PCR) or viral antigens by *enzyme* –*linked immunosorbent assay* (ELISA).

- Most acute infections are determined through the use of polymerase chain reaction testing (PCR).

- Virus is generally detectable by RT-PCR between 3 to 10 days after the onset of symptoms.

Histopathologic Analysis, Immunohistochemical Analysis, and Transmission Electron Microscopy

❖Placenta (Gulu and Isiro), fetal tissues (Gulu), and a postmortem skin biopsy (Isiro) were collected and placed in 10% neutral buffered formalin and transported to the CDC, where the samples were processed using standard histological methods. The identification and scoring of malarial parasite pigment was performed as previously described [16]. Immunohistochemical analysis for ebolavirus antigens was performed using a polymer-based indirect immunoalkaline phosphatase detection system for colorimetric detection (Biocare Medical, Concord, California). Rabbit polyclonal antisera against EBOV, SUDV, and Reston virus and EBOV hyperimmune mouse ascitic fluid (courtesy of Thomas Ksiazek, VSPB, CDC), previously shown to detect SUDV and BDBV antigens, were each used at a 1:1000 dilution with appropriate positive and negative controls [17]. On-slide embedding and transmission electron microscopy was performed as previously described [18].

RESULTS. Patient 1

- Patient 1, Gulu, Uganda, 30-year-old housewife

Symptoms: asthenia, anorexia, abdominal pain, nausea, vomiting, diarrhea, and dry cough - presented with a 1-day of illness. Has been pregnant for 28 weeks. Next day, she had temperature of 36.7°C, here heart rage was 120 beats/minute, and her respiratory rate was 24 breaths/minute, with an oxygen saturation level of 92% by pulse oximetry. Her blood tested positive for SUDV by both ELISA antigen assay and nested RT-PCR.

On day four of illness, the patient spontaneously delivered a dead but apparently morphologically normal fetus and placenta. The degree of vaginal bleeding did not seem abnormal for a stillbirth. Over the next 3 days, the patient complained of joint pain and swelling, throat and chest pain, persistent dry cough, dyspnea, and, briefly, hiccups. Her wrists and knees were visibly swollen and tender to the touch, and pulmonary rales persisted. She was consistently febrile. Disease severity peaked at day 7 of illness, when vital signs were an axillary temperature of 37.8°C, a heart rate of 128 beats/minute, a respiratory rate of 30 breaths per minute, and an oxygen saturation level of 90%. She gradually improved, and she was discharged on day 13 with normal vital signs and all symptoms resolved.

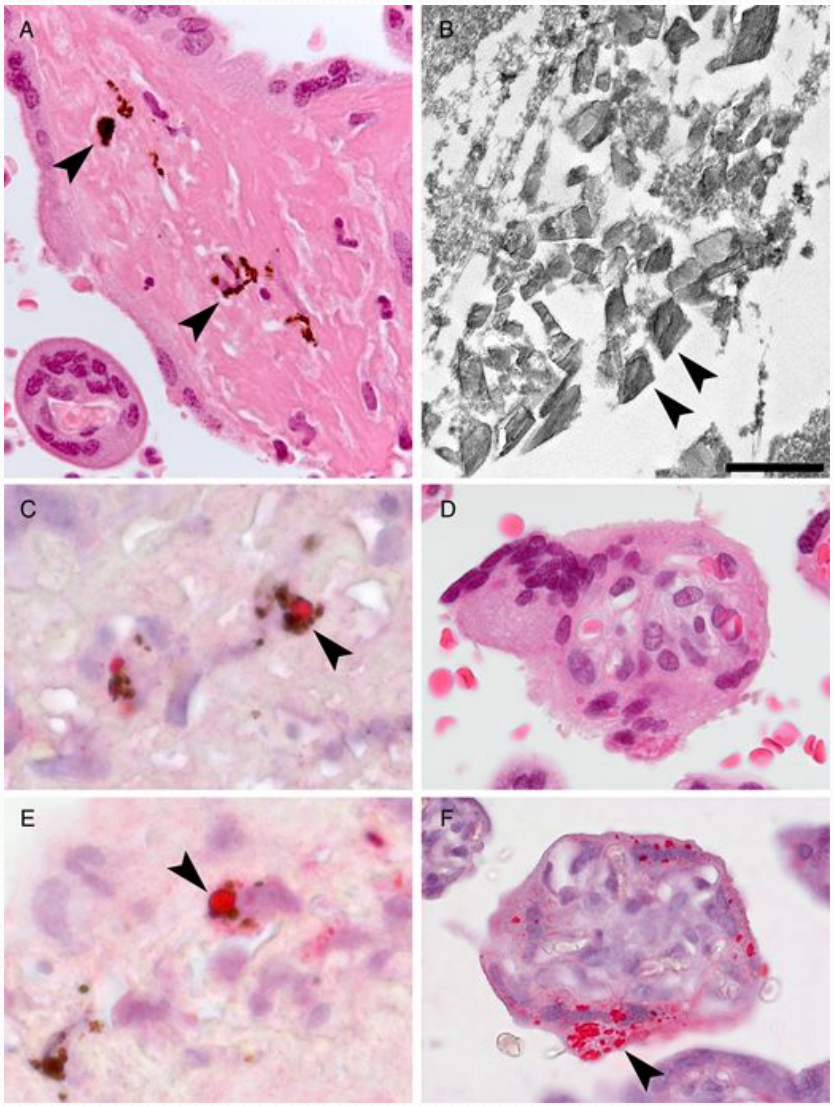
Pathologic Findings

The placenta had mild subchorionitis and a moderate amount of malarial parasite pigment (hemozoin) in fibrin and within macrophages embedded in fibrin (Figure 1A). No parasitized erythrocytes or malarial intervillous inflammatory infiltrates were present. By electron microscopy, hemozoin crystallites were identified (Figure 1B), but no ebolavirus virions were seen. The umbilical cord was normal.

Immunohistochemical analysis revealed ebolavirus antigen in the placenta, primarily within areas of fibrin deposition, localized to embedded maternal mononuclear cells, including malarial parasite pigment–laden macrophages (Figure 1C). Focal immunostaining was seen within the syncytiotrophoblast (Figure 1D). The decidua, fetal placental villous stroma, amnion, and umbilical cord were negative by immunohistochemical analysis, and no tissue necrosis or viral inclusions were noted.

Fetal tissues (lung, heart, liver, spleen, kidney, skin, and bone marrow) were well preserved with minimal autolysis, were normal for gestational age, and had no necrosis or viral inclusions. All fetal tissues were negative by immunohistochemical analysis.

Placental findings from patient 1.
A, Hemozoin (malarial parasite pigment) in fibrin (arrowheads).
B, Transmission electron microscopy showing malarial parasite hemozoin crystallites (arrowheads); no ebolavirus virions were identified. Scale bar = 500 nm.
C, Colocalization of ebolavirus antigen (arrowheads) with malarial parasite pigment.
D, Serial sections by hematoxylin-eosin staining (upper) and immunohistochemical staining (lower) showing ebolavirus antigen (arrowhead) localized to the syncytiotrophoblast.



RESULTS. Patient 2

- Patient 2, 29-year-old housewife, who was transferred from a health center because of suspicion of EVD by a local clinician who was aware that her relative died recently. She was admitted to the Ebola treatment center on day 4 of illness with fever, fatigue, headache, abdominal pain (with uterine contractions), anorexia, dysphagia, vomiting, diarrhea, and muscle and joint pain. Her last menstrual period date was unknown, but she was initially estimated to be 7 months pregnant. Conjunctival injection was noted. Her heart rate was 80 beats/minute, and her respiratory rate was 20 breaths/minute. Her cervix was 50% effaced with a 4-cm dilation, and fetal movement was normal.

On day 5 of illness, her cervix was 100% effaced with an 8-cm dilation, and she was treated with oxytocin. A malaria rapid diagnostic test was positive, and AL was continued. That night (day 6 of illness), spontaneous vaginal delivery of a live-born male infant occurred without assistance. The degree of vaginal bleeding did not seem abnormal for a normal delivery, although she had had an episode of black stool some hours later. She was treated with oxytocin, ergometrine, intravenous fluids, and cefixime, and Plumpy′nut (Nutriset) was provided. On day 7, the mother's condition rapidly deteriorated, with wheezing, drowsiness, weakness, and a temperature of 38.5°C. Antibiotics were switched to ceftriaxone. On day 8, she became comatose and died. A postmortem skin sample was collected from the mother as part of the routine outbreak response protocol [17].

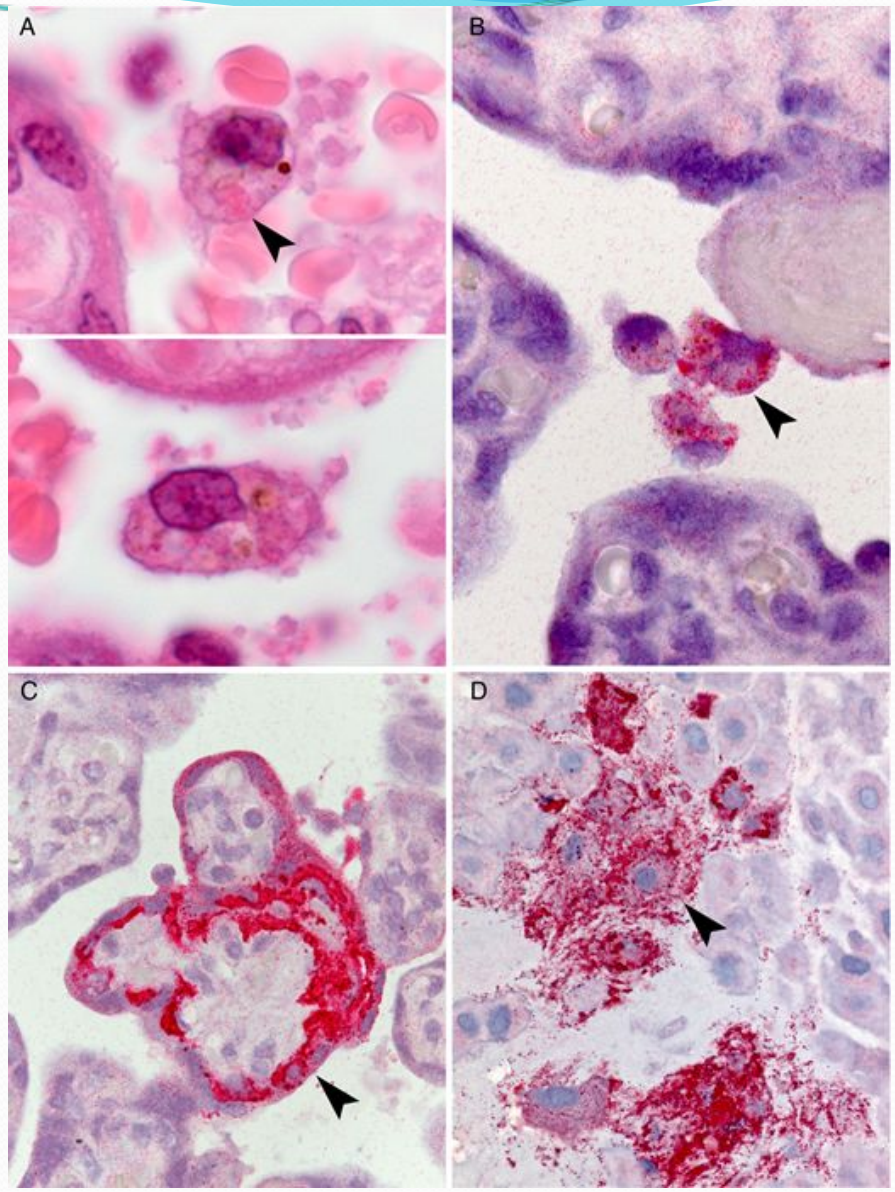
The infant appeared healthy at birth, with Apgar scores of 8/10/10, and was clinically assessed to be at term on the basis of examination of the nails and soles of the feet. Infant formula was provided, although the baby may have briefly breastfed immediately after delivery. A placental sample was collected to evaluate for BDBV. Blood collected at 1 day of age (the second day of life) was positive for BDBV by RT-PCR, with a cycle threshold (Ct) of 29.2. Over the next few days, the baby was noted to be quiet and inactive. He became febrile (temperature, 38.5°C) on day 4 of age, and repeat testing of the blood revealed an RT-PCR Ct of 17.9 with negative IgM and IgG ELISA results. Over the next few days, the baby had hematemesis and bloody stools. He developed respiratory distress and coma and died on the seventh day of age (eighth day of life). No postmortem specimens were collected from the infant.

Pathologic Findings

In the placenta, scattered atypical maternal macrophages were seen within the intervillous space. These cells had degenerate-appearing nuclei, cytoplasmic blebs, and small eosinophilic cytoplasmic granules, suggestive of viral inclusions (Figure 2A). The placenta was otherwise normal, and the placental membranes and umbilical cord were not sampled. No malarial parasite pigment or parasitized erythrocytes were seen. No virions were seen by transmission electron microscopy.

Ebolavirus antigen was seen by immunohistochemical analysis within the circulating large atypical maternal mononuclear cells (Figure 2B). Antigen was also present in multiple foci within the villous syncytiotrophoblast (Figure 2C), frequently most intense at the basal aspect. Fetal stromal and endothelial cells were negative by immunohistochemical analysis. In the basal plate, immunostaining was prominent within the extravillous trophoblast (Figure 2D) ,with scattered additional cell types likely representing decidual and maternal mononuclear cells. Focally, the lining cells of the maternal vessels of the basal plate (likely endovascular trophoblasts) were positive. Within the placenta, fetal stromal tissue, including villous blood vessels, was negative by immunohistochemical analysis. The postmortem maternal skin specimen was morphologically normal and immunohistochemically negative.

Placental findings from patient 2. A, Circulating atypical maternal macrophages with vacuolated cytoplasm and eosinophilic cytoplasmic granules suggestive of viral inclusions (arrowhead). By immunohistochemical staining, ebolavirus antigen was found to localize to circulating maternal macrophages (B), syncytiotrophoblast (C), and intermediate trophoblast within the basal plate (D; arrowheads).

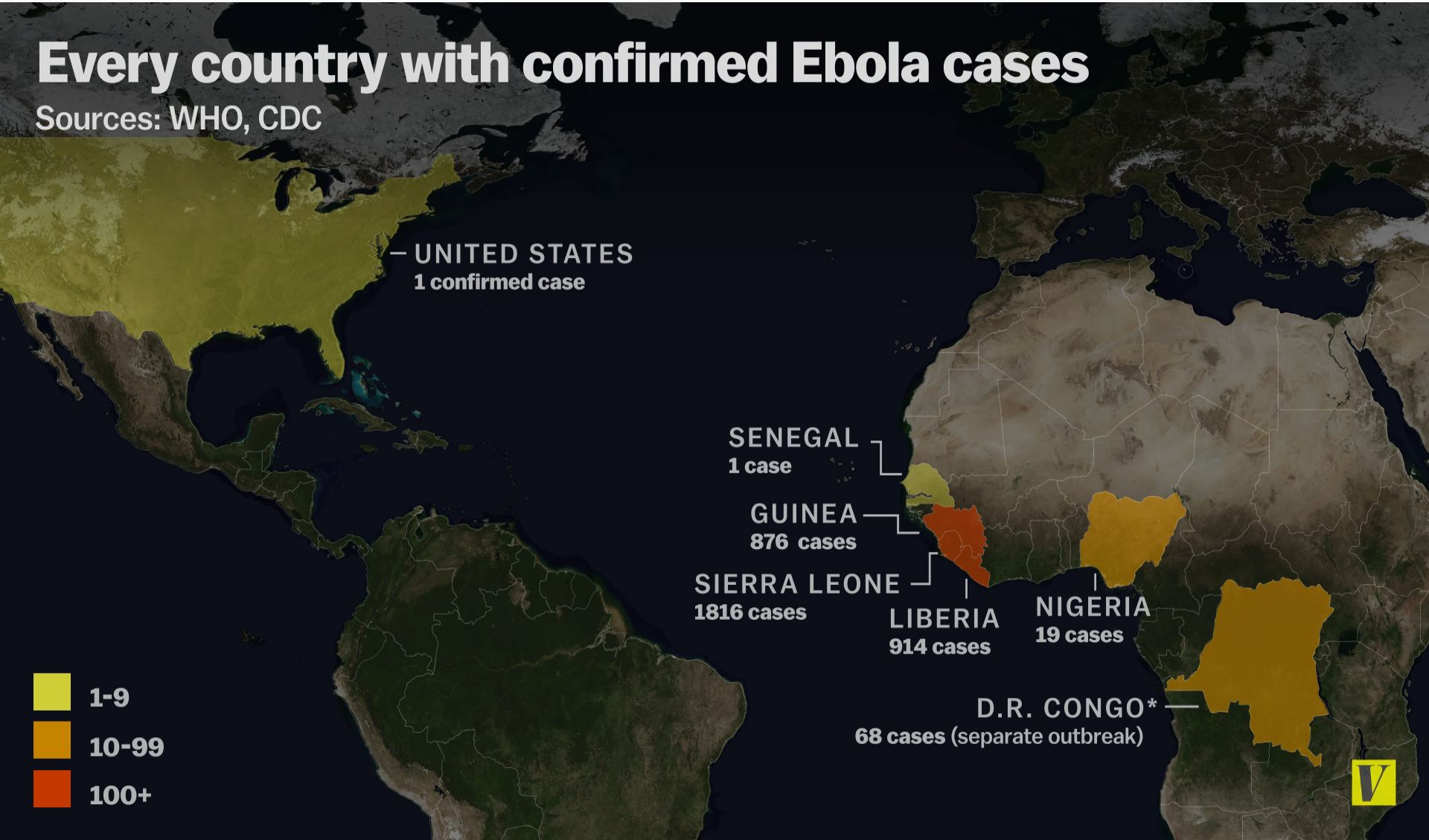


DISCUSSION

Vertical transmission of pathogens can be by transplacental, transvaginal, or by breastfeeding routes. Placenta sampling provides the opportunity to study disease processes in living patients and gain insights regarding the mode and mechanism of vertical transmission. In this study, SUDV or BDBV antigen was noted in fetal trophoblast cells, suggesting that these viruses can infect and potentially cross the placental epithelial barrier, resulting in transplacental infection of the fetus. Transplacental infection of the fetus by EBOV has been previously documented in stillbirths by PCR analysis of amniotic fluid, fetal blood, and fetal swab specimens [7, 8]. The immunoprotective role of the placenta may promote the persistence of virus observed in these cases even after virus has been cleared from maternal blood [8, 9].

Several human pathogens can efficiently penetrate the placental barrier and infect the fetus, including some herpesviruses, human immunodeficiency virus, Zika virus, Treponema, and Toxoplasma. The trophoblast is the major cellular barrier to fetal infection, and it comprises 2 major types: the villous trophoblast, which is directly exposed to maternal blood, and the extravillous trophoblast, which invades the maternal decidua and directly contacts maternal cells, including lymphocytes and decidual stromal cells. In this study, both the syncytiotrophoblast (in both patients) and the extravillous trophoblast (in the patient from Isiro) demonstrated ebolavirus antigen by immunohistochemical analysis.

COUNTRIES AFFECTED WITH EVD



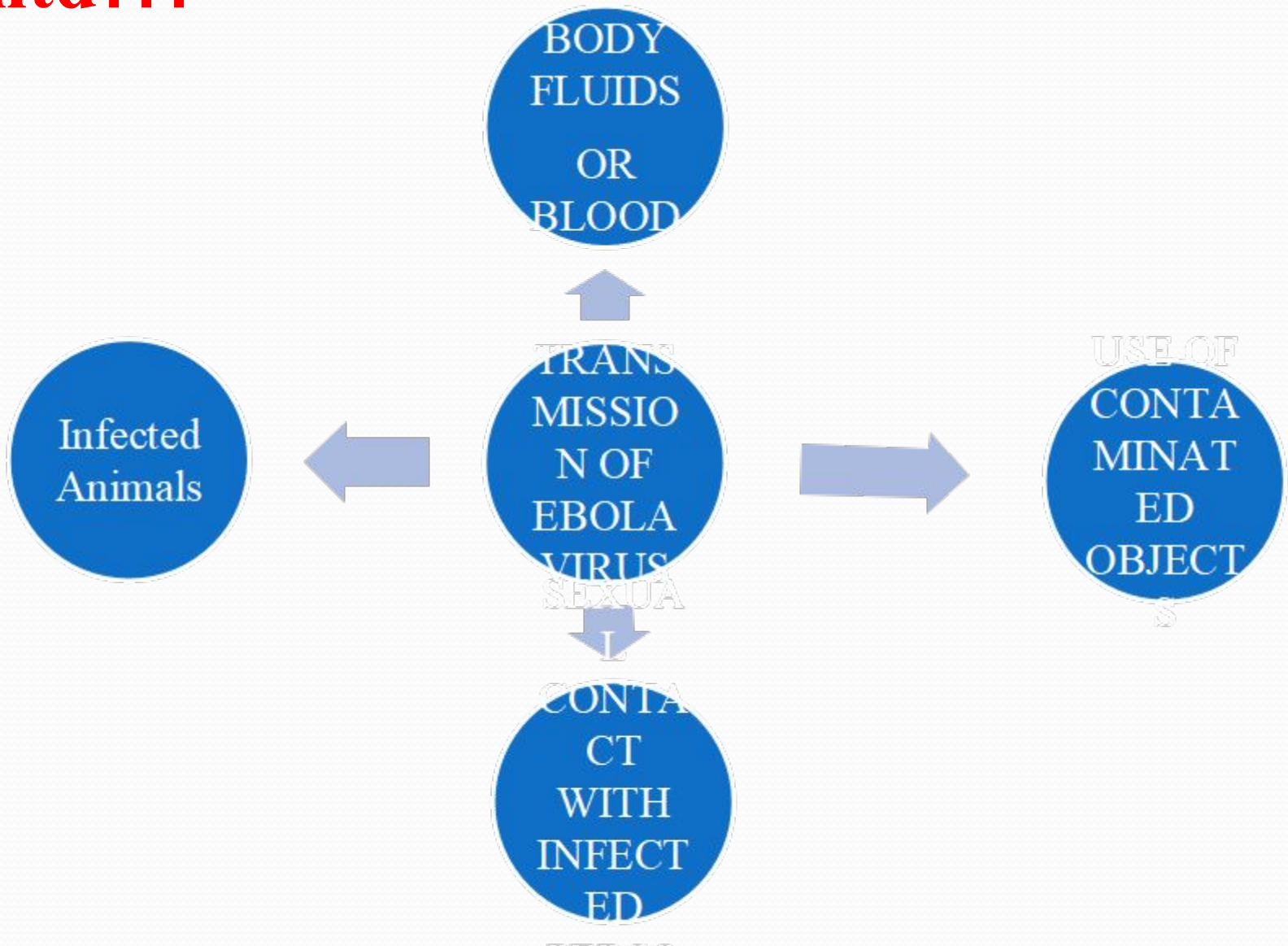
Transmission of Ebola virus

Natural reservoir host of Ebola virus has not yet been identified

The manner in which the virus first appears in a human at the first of its outbreak is unknown

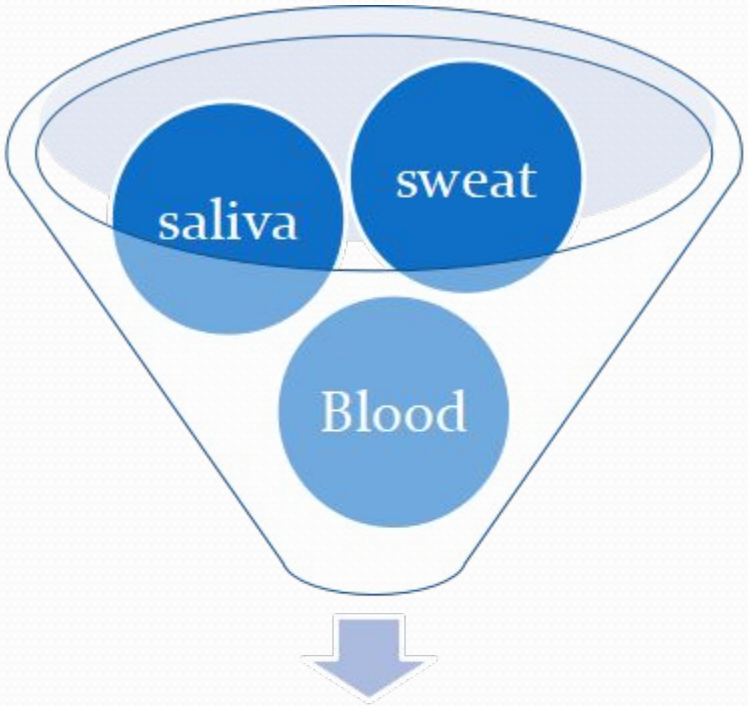
Researchers believed that the first patient becomes infected through contact with an infected animals

Contd…


BODY FLUIDS OR BLOOD
Infected Animals
TRANS MISSIO N OF EBOLA VIRUS
USE OF CONTA MINAT ED OBJECT S
SEXUA L
CONTA CT WITH INFECT ED

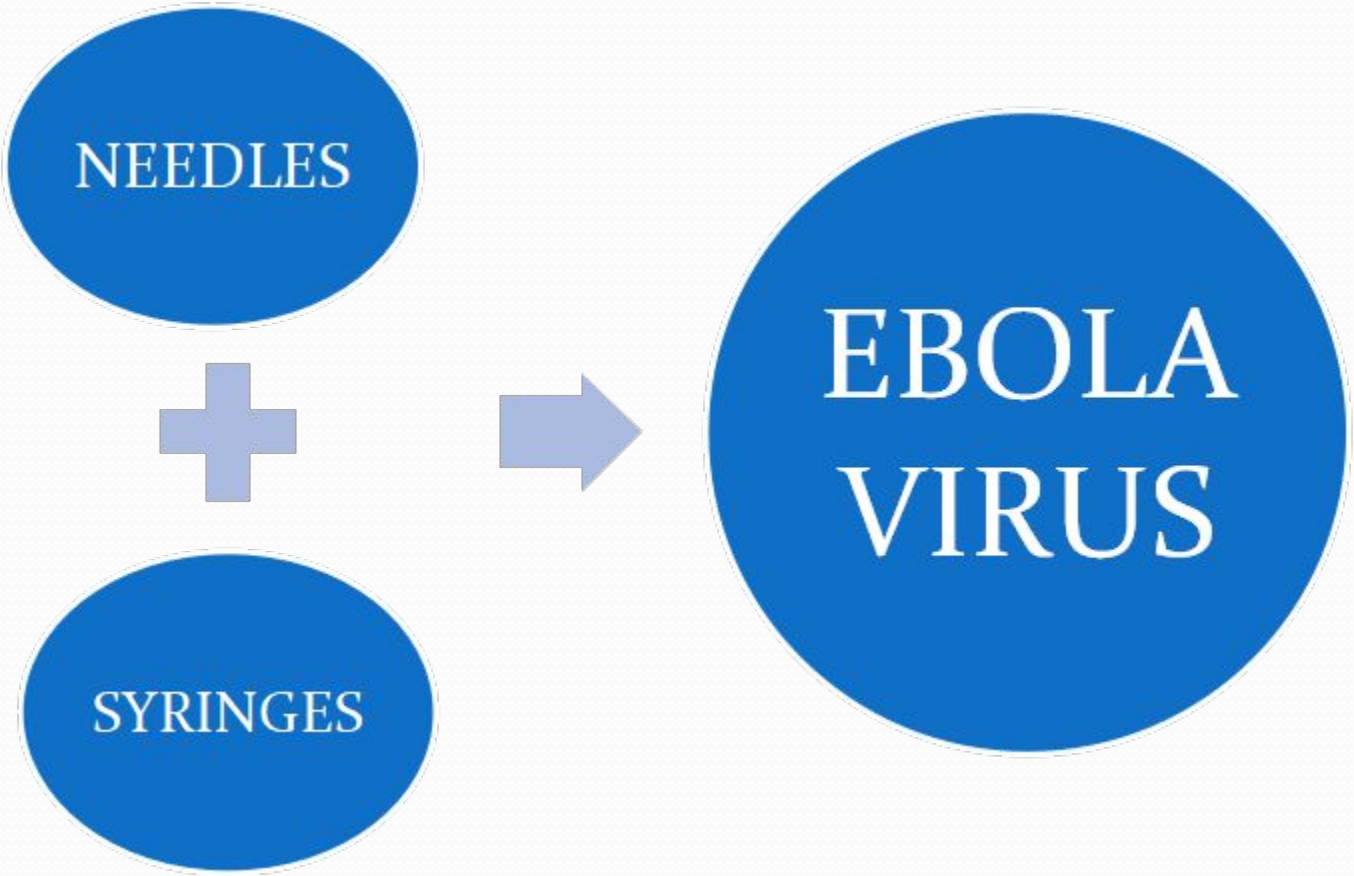
Types of Body Fluids That involves in transmission of Ebola virus

- BODY FLUIDS



Thanks for Attention!

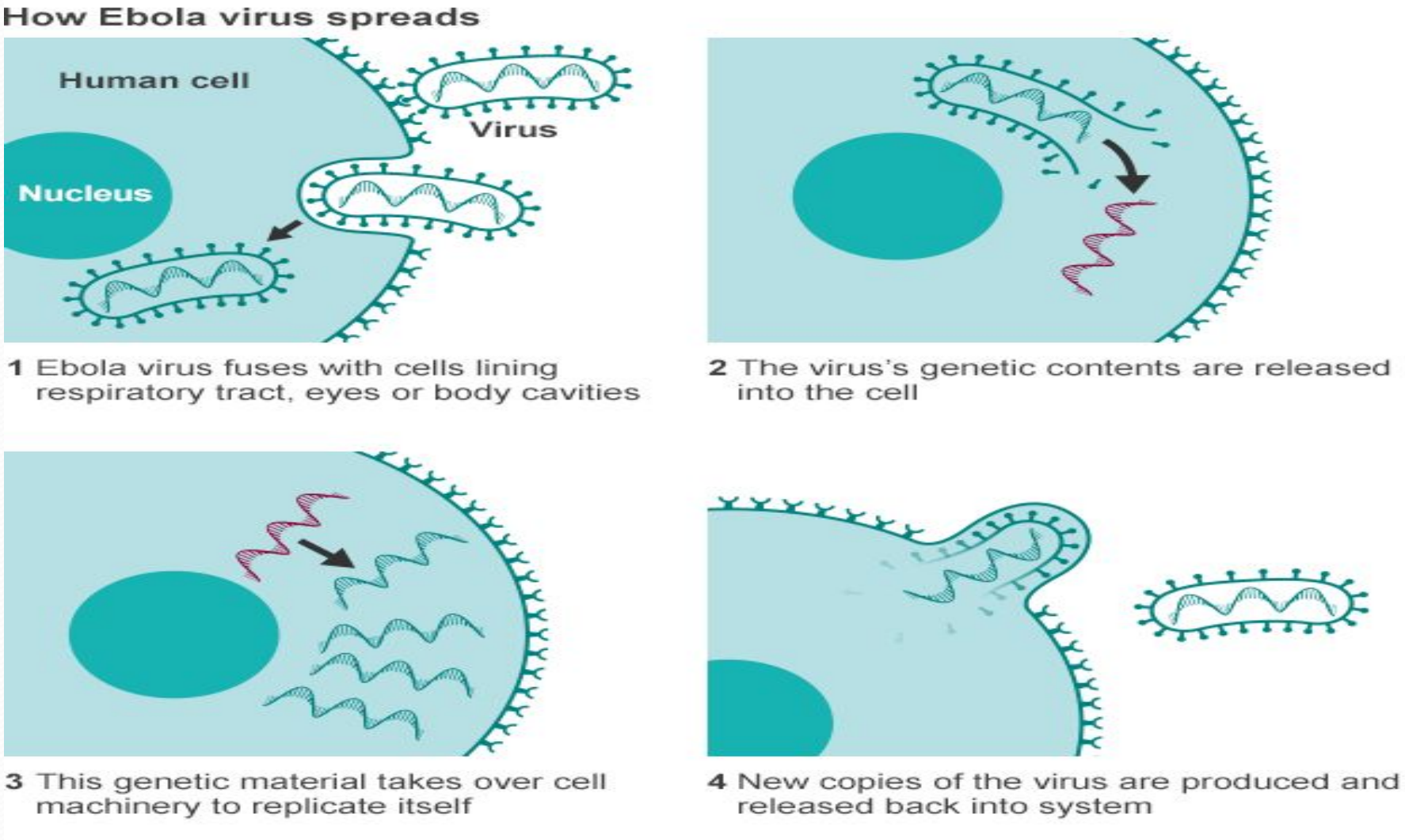
CONTAMINATED OBJECTS THROUGH WHICH EBOLA VIRUS TRANSMITS



IN AFRICA,EBOLA VIRUS MAY BE SPREAD THROUGH BUSHMEAT

TRADITIONAL AFRICAN RITUALS PLAYED ROLE IN TRANSMISSION OF VIRUS

EBOLA VIRUS ENTRES INTO THE HUMAN'S CELL



OTHER WAYES IN WHICH EBOLA VIRUS CAN TRANSMIT

- TOUCHING THE SOILED CLOTHES OF INFECTED PERSON
- HAVING SEXUAL CONTACT WITH INFECTED PERSON
- HANDLING UNSTERILISED MEDICAL EQUIPMENT

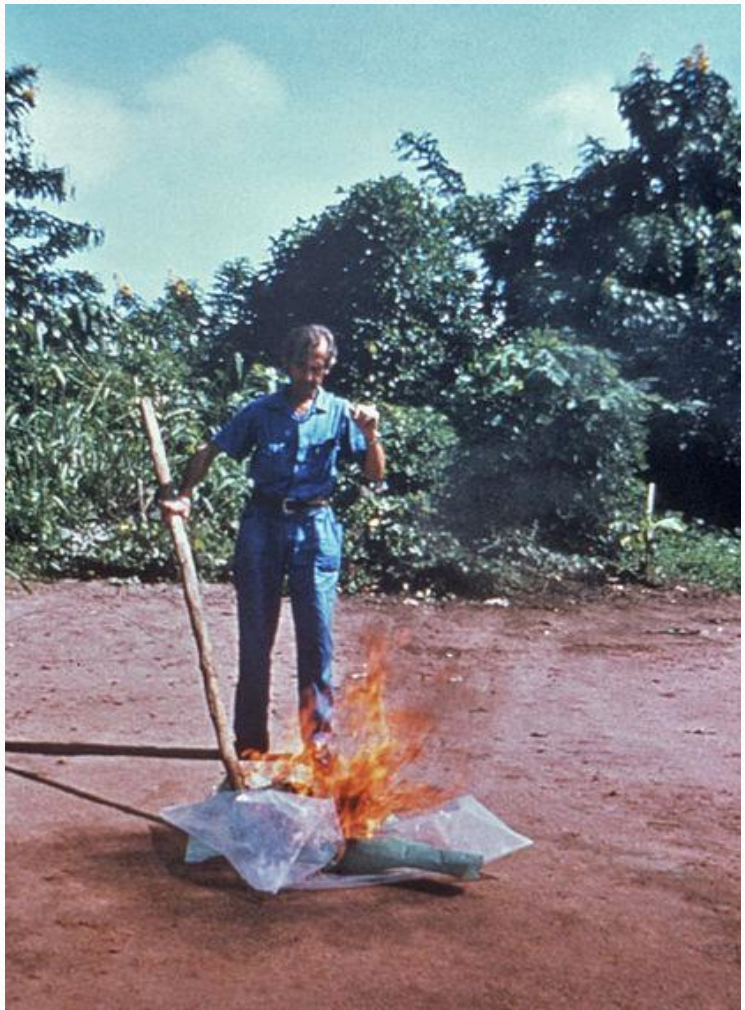
MASS CREMATION HAVE BEEN SANCTIONED BY THE GOVERNMENT IN LIBERIA IN BID TO HELP TO HALT THE DEADLY VIRUS

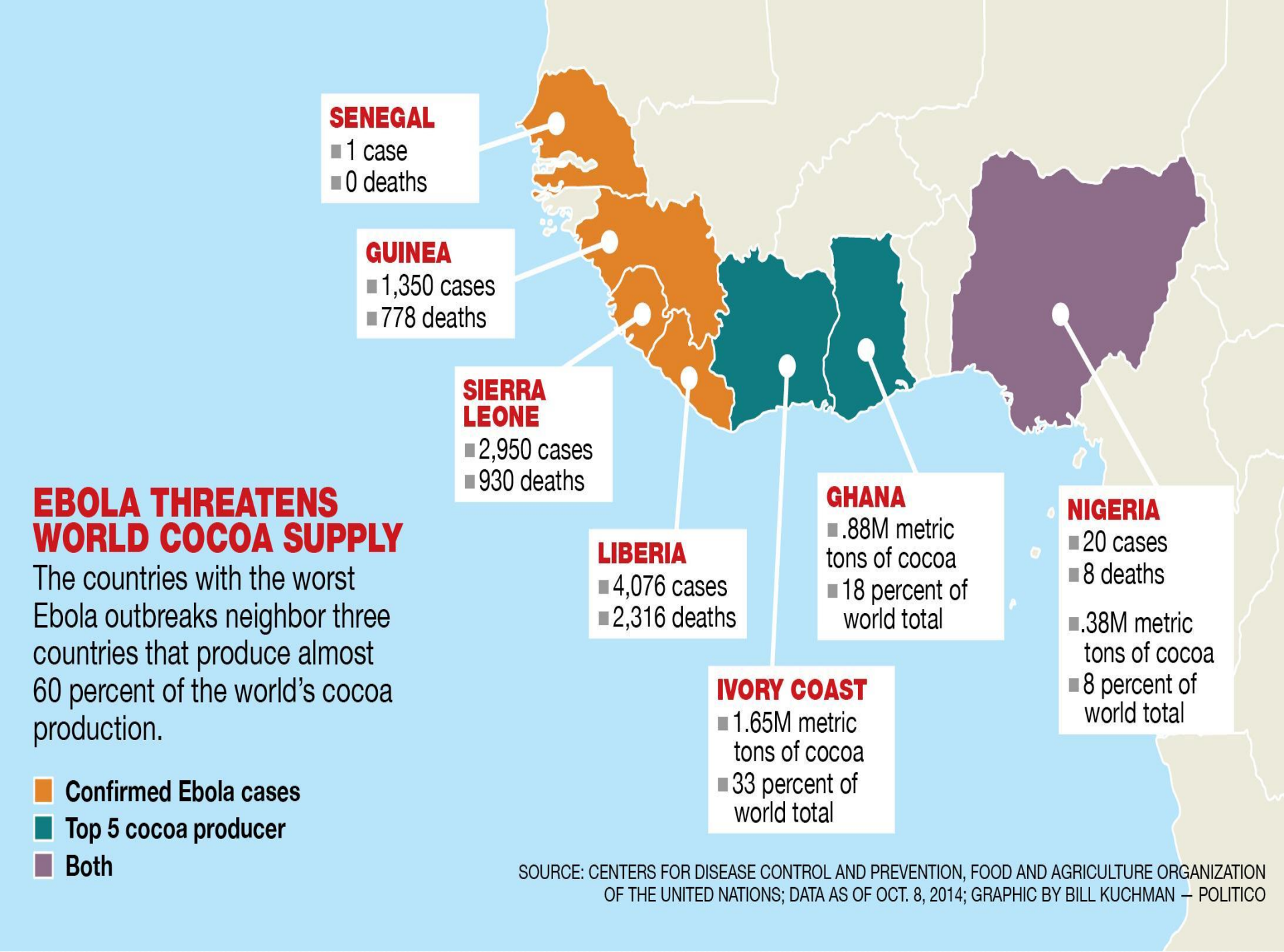
THESE SHOCKING PICTURES SHOW THE BODIES OF EBOLA VICTIMS BEING BURNED ON HUGE FUNERAL PYRE

FRUIT BATS ARE MAJOR CAUSE FOR THE TRANSMISSION OF THE EBOLA VIRUS DISEASE

UNHYGIENIC ENVIRONMENT MAY ALSO BE A CAUSE OF TRANSMISSION OF EBOLA VIRUS IN WEST AFRICA

CDC WORKER INCINERATES MEDICAL WASTE FROM EBOLA PATIENTS IN ZAIRE




SENEGAL
1 case
0 deaths
GUINEA
1,350 cases
778 deaths
SIERRA LEONE
2,950 cases
930 deaths
LIBERIA
4,076 cases
2,316 deaths
IVORY COAST
1.65M metric tons of cocoa
33 percent of world total
GHANA
.88M metric tons of cocoa
18 percent of world total
NIGERIA
20 cases
8 deaths
.38M metric tons of cocoa
8 percent of world total
EBOLA THREATENS WORLD COCOA SUPPLY
The countries with the worst Ebola outbreaks neighbor three countries that produce almost 60 percent of the world's cocoa production.
Confirmed Ebola cases
Top 5 cocoa producer
Both
SOURCE: CENTERS FOR DISEASE CONTROL AND PREVENTION, FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS; DATA AS OF OCT. 8, 2014; GRAPHIC BY BILL KUCHMAN — POLITICO

How do we know if someone is infected?

Early signs and symptoms of infections (7-9 Days)

- FEVER

If there is no fever there is no Ebola.

❖HEADACHE

Severe headaches start developing

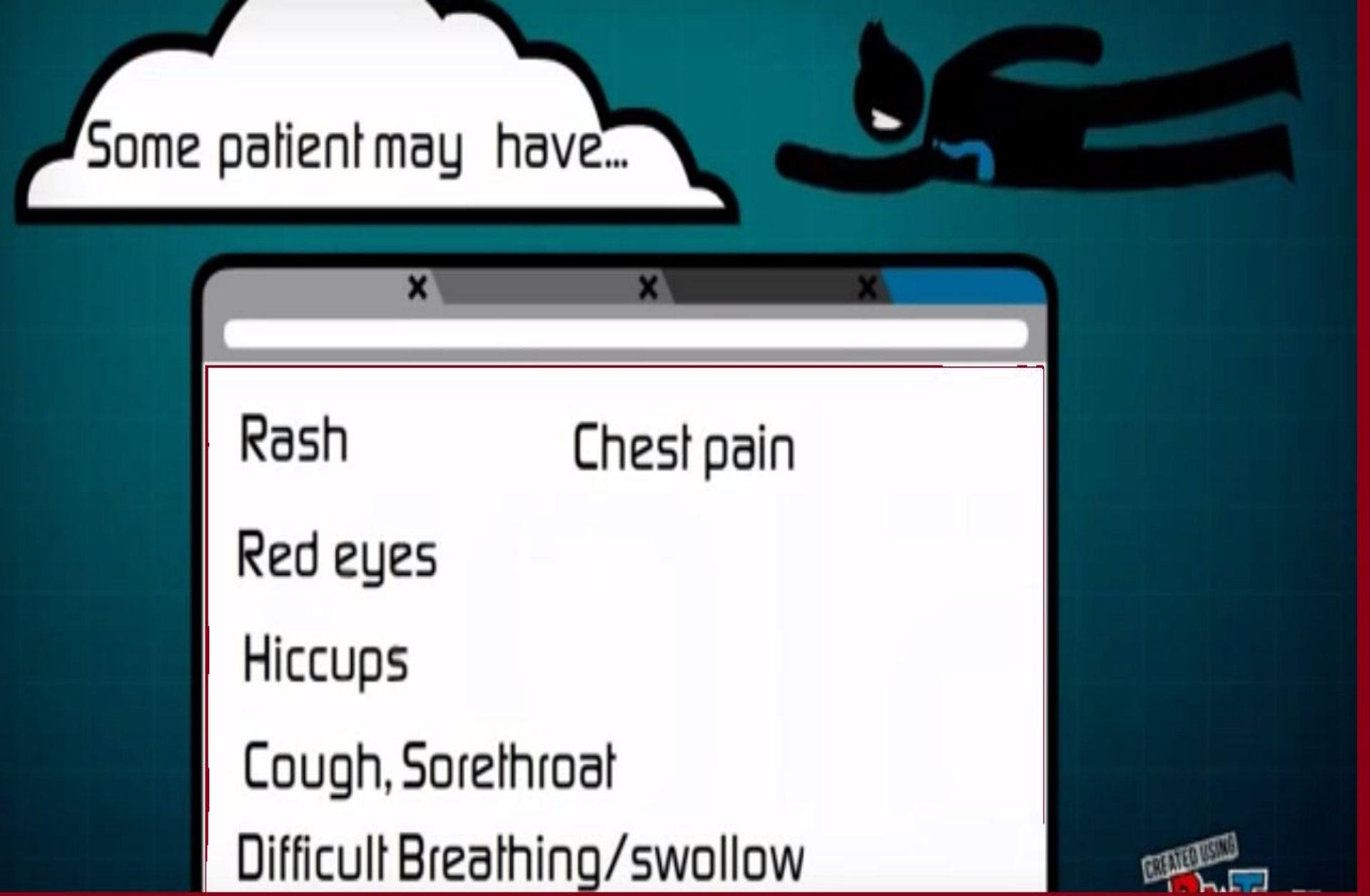
NAUSEA

Sickness in the stomach and involuntarily impulse to vomit is felt by patient.

MUSCULAR PAIN

Joint and muscle pain leads to intense weakness throughout the body of the person.

❖TIREDNESS


Some patient may have...
Rash
Chest pain
Red eyes
Hiccups
Cough, Sorethroat
Difficult Breathing/swollow

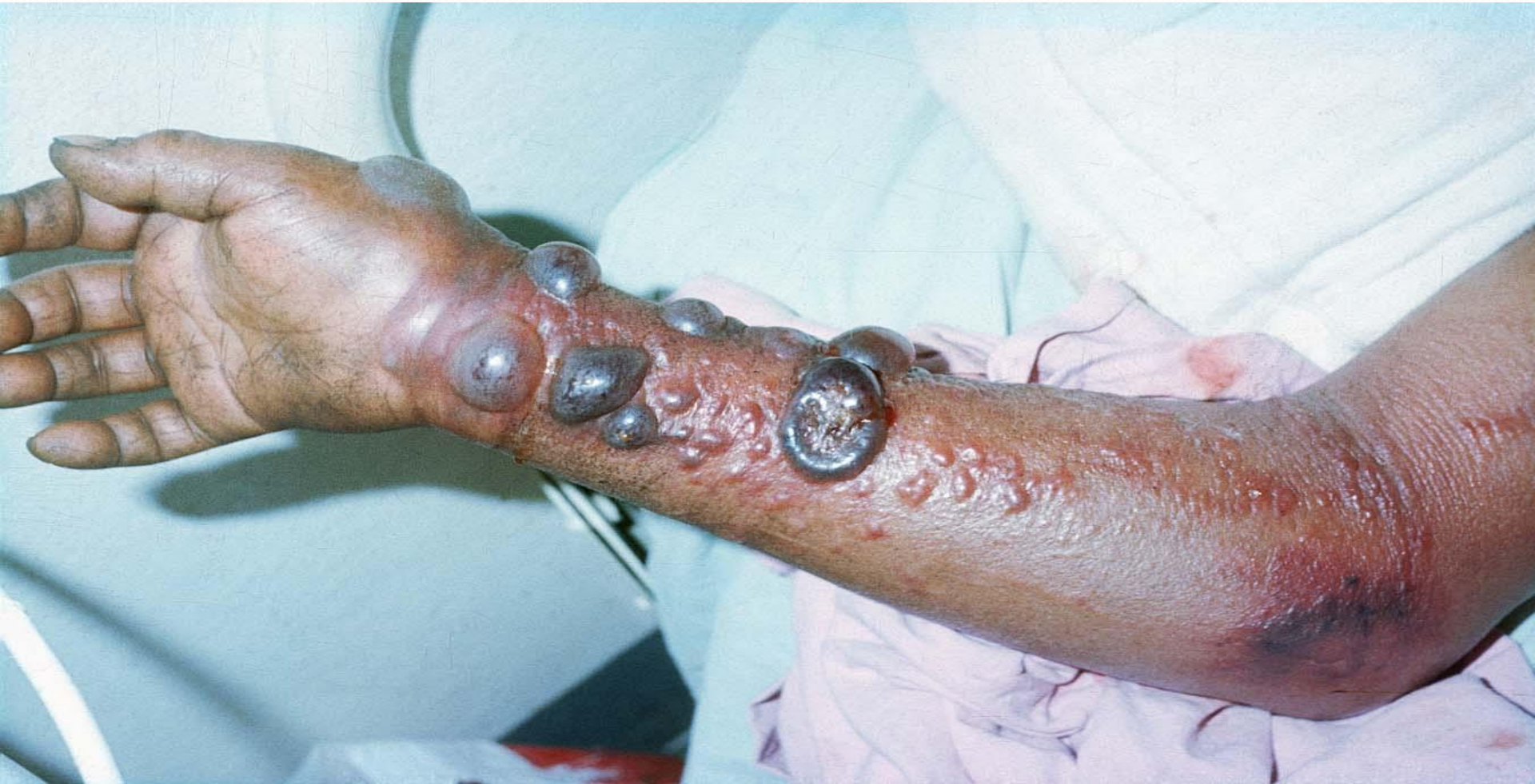
Day 10th followed by:

- Vomiting

An another major symptom to approve the person is infected by Ebola virus.

Diarrhea

Rashes



Condition worsens on day 11th

- **BRAIN DAMAGE**

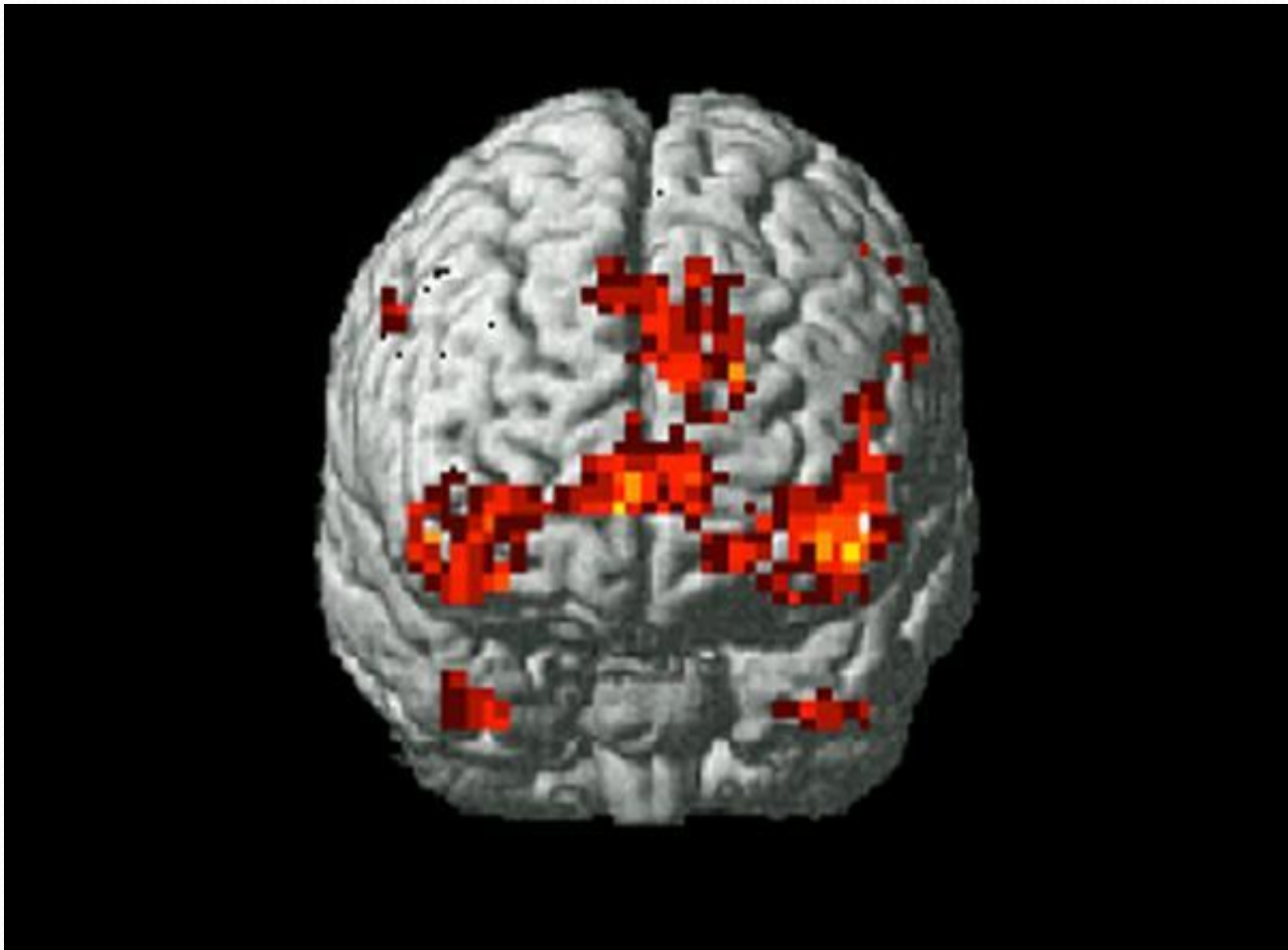
Loss of consciousness ,

Seizures,

Massive internal bleeding

leads to brain

damage.



Internal & External Bleeding

Bleeding from body
Openings (nose, gums ,gastrointestinal tract, etc) may be seen
In some patients.

Diagnosis Of Ebola Virus

How it is diagnosed?

Diagnosis before testing is completed for Ebola, test for following disease must be completed

- Malaria
- Typhoid fever
- Shigellosis
- Cholera
- Leptospirosis
- Rickettsiosis
- Relapsing fever
- Meningitis
- Hepatitis
- Other viral hemorrhagic fevers

It is difficult to distinguish EVD from other infectious diseases but it can be investigated by some methods

- Antibody-capture enzyme-linked immunosorbent assay (ELISA)
- Antigen-capture detection tests
- Serum neutralization test
- Reverse transcriptase polymerase chain reaction (RT-PCR) assay
- Electron microscopy
- Virus isolation by cell culture

Diagnostic Considerations

Although there is *no* approved specific therapy for Ebola virus.

- Clinical Findings - include fever of greater than 36.8 C (101.5 F) and additional signs or symptoms like severe headache, muscle pain, vomiting, diarrhea, abdominal pain

- Risk factors
  - Those who have had contact with blood, body fluids or human remains of a patient known to have or suspected to have Ebola virus disease.
  - Residence in or travel to an area where Ebola virus transmission is active.
  - Direct handling of bats, rodents or primates from endemic areas.

A high risk exposure includes any of the following

- Percutaneous or mucous membrane exposure to blood or body fluids of a person with Ebola virus disease.
- Direct skin contact with patient having Ebola virus disease without appropriate personal protective equipment.
- Direct contact with dead body without personal safety equipments in a country where an Ebola virus disease outbreak is occurring.

Diagnostic Tests

- Rapid blood tests for Marburg and Ebola virus infection are the most commonly used tests for diagnosis.

- Testing for Ebola and Marburg virus should only be performed in specialized laboratories.

Other Tests

- Antigen detection may be used as a confirmatory test for immediate diagnosis.

- For individuals, who are recovering from Ebola virus disease, PCR testing is also used to determine when a patient can be discharged from hospital setting.

- In some cases, testing for IgM or IgG antibodies to Ebola virus may also be useful to monitor the immune response over time and/or evaluate for past infection.

Stages of symptoms of Ebola virus

- Stage 1

  Headache, sore throat, fever, muscle soreness
- Stage 2

  High fever, Vomiting, Passive Behaviour
- Stage 3

  Bruising, Bleeding from nose, mouth, eyes;

  Blood in stool, Impaired liver function
- Stage 4

  Loss of consciousness, Seizures, Internal bleeding leading to death

Hospital Protocol for Ebola hit

- Handling Personal Protective Equipment (PPE)
- Removal
- Isolation
- Fluid Control
- Disinfecting
- No Needles

A new drug target for Ebola virus

- Researchers have recently developed a new drug target in the Ebola virus that could be used against it to fight the disease

- University of Utah chemists have produced a molecule known as *peptide mimic* that displays a functionally critical region of the virus that is universally conserved in all known species of Ebola

TREATMENT AND VACCINE FOR EBOLA VIRUS

- Coffee, Fermented Soy, homeopathic Spider Venom, And Vitamin C, May All Hold Promise As Anti Ebola Virus Therapies.

An Ebola Treatment Centre

- Entry point
- Ebola infection enters there to be examined by medical staff in protective gear
- Patients are into two groups based on the probability
- Low probability ward

- Patients could face a long wait until their test results from the lab come back, revealing whether or not they are infected . Patients who might not have the deadly virus are isolated from those suffering from Ebola , reducing their exposure to the infection while In the treatment centre.

High Probability Ward

- Patients suspected of having Ebola based on the initial medical examination remain here until official confirmation arrives that they have the virus . Only once the Ebola diagnosis is confirmed they transferred to another ward

❖Decontamination

- The Utah scientists designed peptide mimic of a highly conserved region in the Ebola protein that controls entry of the virus into the human host cells.

❖Dressing Room

- Dressing for a high risk area is a complex process. Medicals walk in a pairs , with the partner checking for any tears in the suit .

- The protective equipment includes a surgical cap and hood ,gogles , medical mask ,impermeable overalls ,an apron ,two sets of gloves and rubber boots

Mortuary

The mortuary is located outside the clinic but within the double fence as bodies are highly infectious .

Patient Exit

The exit on the side are for patients whose blood tests show that they do not have ebola ,or those that recover.

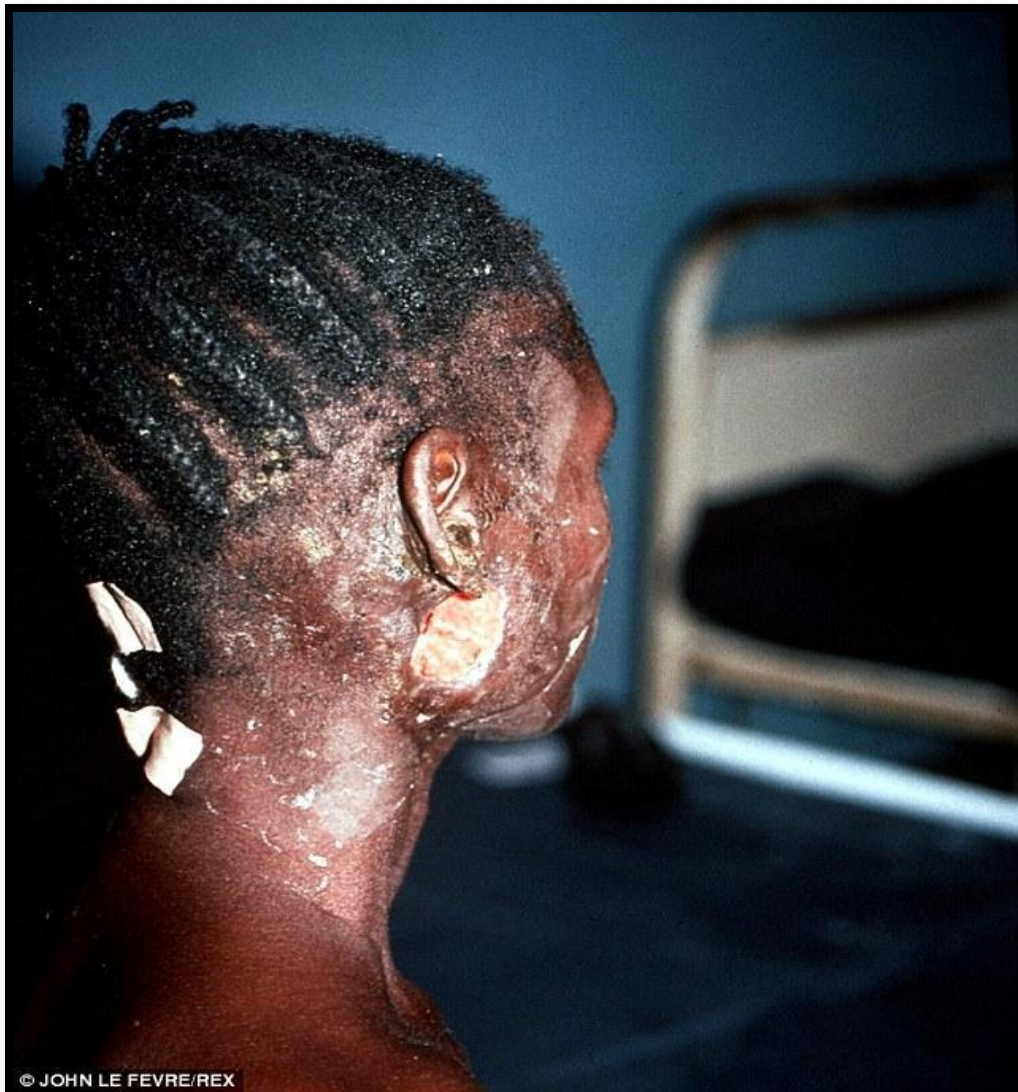
Could Statins Treat Ebola

- Statins should be considered as a possible treatment for Ebola

- Statins also have been suggested as a treatment for patient with sepsis, a condition that involves an out of control immune response similar to that seen in Ebola patients.

Canadian – Made Ebola Vaccine Starting Clinical Trials In Humans

- Experimental Canadian made Ebola vaccine is beginning clinical trials in healthy humans .
- The results are expected in December
- Clinical trails are now starting for an experimental made in Canada Ebola vaccine amid growing global concern over the disease that's left more than 4,000 people dead.




VIRUS ALERT
EBOLA
VIRUS ALERT

HOW TO PREVENT EVD

- If have sudden fever, diarrhoea, or vomiting, go to the nearest health facility
- Make no contact with Ebola affected people
- Use a special kind of clothes while treating Ebola affected people

PROTECT YOURSELF
PROTECT YOUR FAMILY
PROTECT YOUR COMMUNITY
from the Ebola virus

✓ DO

Always wash your hands with soap and

Always cook your food properly

Go to health facility anytime you have head ache, fever, pain, diarrhea, red eyes rash and vomiting

Tell everyone you meet about Ebola so they can be informed

Call for help or questions

0886520581 or 0886374733

✗ DO NOT

Do not touch people with signs of Ebola or have died of Ebola

Do not touch clothes & bed cloths of people who have died of Ebola

Do not touch vomit, saliva, urine, blood and poo poo of people who have signs and symptoms of Ebola

Do not play with monkeys and baboons

Do not eat bush meat

Do not eat plums eaten by bats

QUICK ACCESS TO APPROPRIATE LABORATORY SERVICES

PROPER MANAGEMENT SERVICES FOR WHO ARE INFECTED

PROPER DISPOSAL OF DEAD THROUGH CREMATION OR BURIAL

WORLDWIDE HELPLINE NUMBER

People who go early to the health centre have a better chance of survival.

CALL 117 WITH YOUR QUESTIONS