OUTBREAK OBJECTIVES

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Recognize the steps taken by epidemiologists when investigating infectious disease outbreaks

OUTBREAK?

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- Your role as Chief Medical Officer of the WHO is to identify and so contain infectious disease outbreaks.
- You have received a report of what may be a new infectious agent causing a rapid onset infection characterised by high transmissibility and exceptionally high mortality.
- The source of the infection is not stated in the report but the person-person infection rate is high and fears are a pandemic might develop due to the close proximity of an international airport.
- Your mission is to travel to the outbreak location and identify the causative agent of the infection.

EPIDEMIOLOGY EXPLAINED

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- Outbreak is a term used in epidemiology to describe an occurrence of disease greater than would otherwise be expected at a particular time and place.
- An epidemic occurs when new cases of a certain disease, in a given human population, and during a given period, substantially exceed what is expected based on recent experience.
- A pandemic is an epidemic of infectious disease that has spread through human populations across a large region and affects a substantial number of people.
- There are two types of epidemic outbreak:

1) In a common source outbreak, the affected individuals had exposure to a common agent e.g. a particular water tap.
2) In a propagated outbreak, the disease spreads person-to-person.

REPORT UPDATE

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- You have arrived by UN helicopter at the main hospital in the capital city of West African country of Ziberia.
- This countries health care system is poorly developed and non-centralised
- Your assistant informs you that cases have increased in number by 20% since your first report (now 12 hours old) and there are currently 1300 suspected cases in the main hospital and around 200 spread over the 7 WHO-sponsored local field clinics
- All flights out of Ziberia have now been cancelled and UN security forces have secured the airport
- Your mission now is identify the causative agent of the infection
- Your request an updated report on the disease characteristics:

DISEASE CHARACTERISTICS 1: DESCRIBING THE OUTBREAK DISEASE

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- The International Classification of Diseases (ICD) is a health care classification system created by the WHO (www.who.org) that provides codes to classify diseases
- Severity of illness is defined as the extent of organ system failure of a patient and is classified as minor, moderate, major, or extreme.
- Disease duration can encompass several stages: a short acute disease, a longer chronic disease, a flare-up, a progressive disease, or a cure.
- The extent of a disease within a patient, whether it is localized, disseminated, or systemic also determines its severity and duration.
- In an infectious disease, the incubation period is the time between infection and the appearance of symptoms, the latency period is the time between infection and the ability to spread to another person
- Viral latency is the time the virus hides in the body in an inactive state

DISEASE CHARACTERISTICS 2: INFECTIOUS DISEASE STAGES

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- First phase is characterized by lack of symptoms or very few symptoms.
- As the pathogen starts to reproduce actively, symptoms intensify. Bacterial and viral infections can cause the same kinds of symptoms but there are some differences which are used diagnostically.
- The last phases are characterized by decline in symptom severity until their disappearance, or worsening of symptoms, organ failure and death.
- Even if a patient recovers they may continue to be a source of infection.

PATIENT SYMPTOMS

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- Fever: >38C
- Sore throat
- Severe headache, muscle pain.
- Weakness.
- Fatigue.
- Diarrhoea.
- Vomiting.
- Abdominal (stomach) and joint pain.
- Symptoms appear 3-21 days after infection
- High mortality rate due to tissue necrosis, haemorrhage and infection

TREATMENT

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- Treatment directions not clear: antibiotic/antivirals do not improve clinical outcomes
- IV rehydration, symptomatic treatment (blood transfusions) improves survival.
- Current fatality rate in main hospital site is 50% vs 77% (average) in field clinics

http://www.who.int/features/2014/liberia-mobile-ebola-lab/en/

RAPID INFECTION DIAGNOSIS IS VITAL TO SAVING LIVES

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- Early and reliable diagnosis is paramount to managing outbreaks so that patient isolation, contact tracing, and infection control procedures can be implemented in a timely fashion.
- Because infection spread occurs before symptom manifestation any diagnostic uncertainty or delay may expose individuals who are not infected.
- Delays in initial recognition of the outbreak may delay appropriate local, national, and international responses, with devastating consequences.

LAB DIAGNOSIS

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- Every morning and evening, blood samples from patients in the main hospital Clinic are brought to the mobile WHO laboratory.
- Field clinic patient samples are provided each morning
- Patient-patient contact suggests that the infectious agent is present in most body fluids of those infected, so all analyses were completed in a portable biological safety hood so that lab workers avoid any direct contact with blood.
- No growth on bacterial culture diagnostic plates of blood samples
- Patient plasma filter sterilised through a 0.2 mm filter is cytotoxic towards cultured human cell lines showing the infectious agent is too small to be bacterial or protozoal
- You order a range of tests:

VIRUS DIAGNOSTIC TESTS

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- Confirmation that symptoms are caused by a virus infection are made using the following diagnostic methods:
- Reverse transcriptase polymerase chain reaction (RT-PCR) assay
- Antibody-capture enzyme-linked immunosorbent assay (ELISA)
- Serum neutralization test
- Electron microscopy
- Virus isolation by cell culture

LAB INVESTIGATIONS

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Extracting genetic material from a blood sample – infectious agent transmission possible from blood so protective aseptic techniques are key to prevent technical staff infections.

CLINICAL LAB REPORT 1

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INVESTIGATION AIM:

IDENTIFY INFECTIOUS AGENT IN CURRENT ZAIRE OUTBREAK

REPORT DATE	PROJECT NAME		PREPARED BY	
Date	ZIBERIA/WHO OUTBREAK INVESTIGATION		LABORATORY MANAGER: *insert name here*	
Diagnostic task	**Replicate test?**	**Send results to:**	**Sample origin**	**Results return**
TESTS 1-3 BACTERIAL AND VIRUS ISOLATION TESTS	Yes but Subject to sample & Lab time availability	Email/text results at once to local WHO contacts & Outbreak Clinic lab liaison officers	Outbreak Field Clinics	As soon as possible

BACTERIAL AND VIRUS DIAGNOSTIC TEST RESULTS

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SAMPLES OF BLOOD SAMPLES WERE TESTED FOR THE PRESENCE OF BACTERIA AND VIRUSES USING PLATE CULTURE AND TRANSMISSION ELECTRON MICROSCOPY.

FIG 1

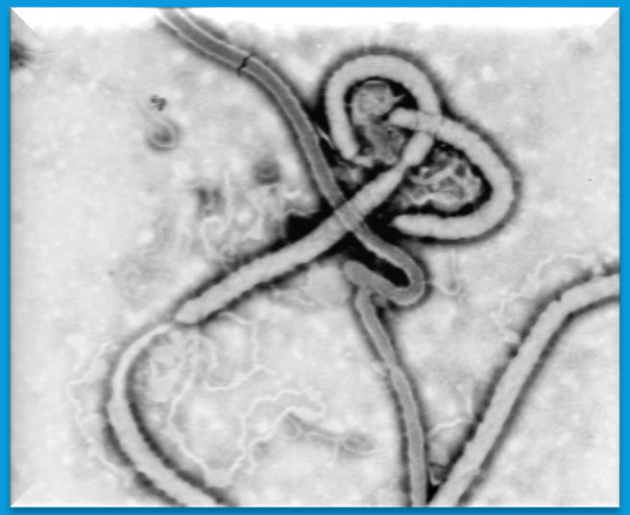

FIG 2

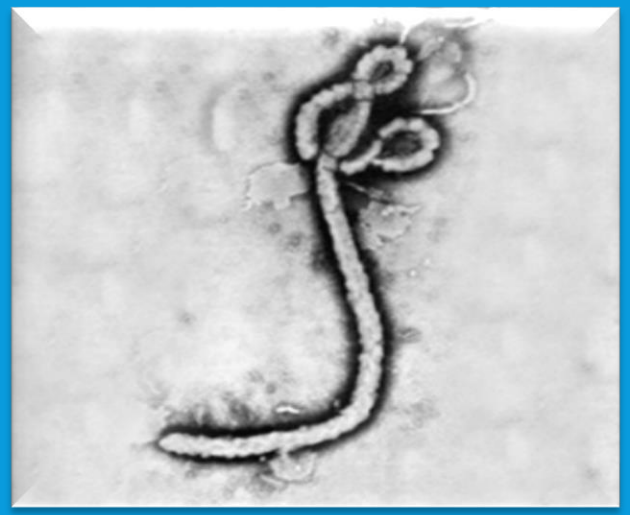

FIG 3

CONCLUSIONS

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1. BACTERIAL CULTURE OF BLOOD SAMPLES ON A VARIETY OF CULTURE MEDIA SUITABLE FOR GROWTH OF BLOOD-BOURNE BACTERIAL PATHOGENS (NUTRIENT AGAR IS SHOWN IN FIG 1) DID NOT PRODUCE ANY COLONIES ON ANY OF THE CULTURE PLATES.
2. TRANSMISSION ELECTRON MICROSCOPY (TEM) OF BLOOD FROM PATIENTS PRODUCED FILAMENTOUS VIRUS-LIKED STRUCTURES AT MAGNIFICATION X50000 (FIGS 2 & 3). TEM STRUCTURES NOT FOUND IN ANY SAMPLES OF BLOOD FROM NON-INFECTED PATIENT CONTROLS.
3. SEARCHING OF DATABASE OF VIRAL TEM IMAGES (FIGS 2 & 3) SHOWS STRUCTURAL HOMOLOGY WITH HAEMORRHAGIC VIRUSES

CLINICAL LAB REPORT 2

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INVESTIGATION AIM:

IDENTIFY AGENT IN CURRENT ZAIRE INFECTION OUTBREAK

REPORT DATE	PROJECT NAME	PREPARED BY
Date	ZIBERIA/WHO OUTBREAK INVESTIGATION	LABORATORY MANAGER: *insert name here*

REPORT FINDINGS:

TASK	REPLICATE	SEND RESULTS TO:	SAMPLE ORIGIN	RESULTS RETURN
Virus diagnostic tests	Yes but Subject to sample & Lab time availability	Email/text results at once to local WHO contacts & Outbreak Clinic lab liaison officers	Outbreak Field Clinics	As soon as possible
TEST 1: TRANSMISSION ELECTRON MICROSCOPY (TEM) OF BLOOD SAMPLES				

DIAGNOSTIC TEST RESULTS & CONCLUSIONS

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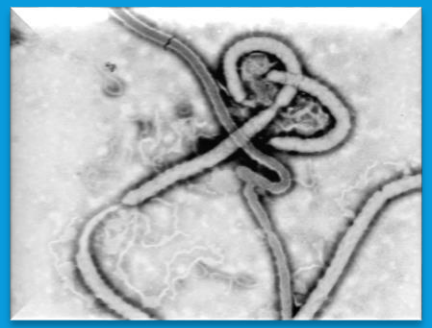

FIG 1

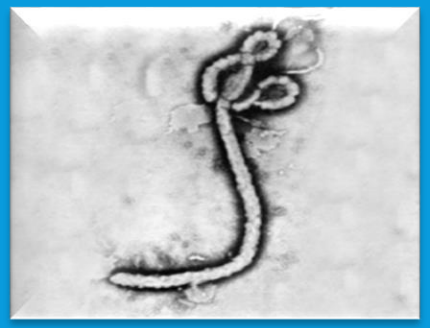

FIG 2

CONCLUSIONS

1. TEM OF BLOOD SMEAR PRODUCED FILAMENTOUS VIRUS-LIKED STRUCTURES AT MAGNIFICATION 50,000 (FIGS 1 & 2). TEM STRUCTURES NOT FOUND IN ANY SAMPLES OF BLOOD FROM NON-INFECTED PATIENT CONTROLS.
2. DATABASE SEARCHING OF TEM IMAGES SHOWS STRUCTURAL HOMOLOGY WITH HAEMORRHAGIC VIRUSES

CLINICAL LAB REPORT 3

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INVESTIGATION AIM:

IDENTIFY AGENT IN CURRENT ZAIRE INFECTION OUTBREAK

REPORT DATE	PROJECT NAME	PREPARED BY
Date	ZIBERIA/WHO OUTBREAK INVESTIGATION	LABORATORY MANAGER: *insert name here*

REPORT FINDINGS

TASK	REPLICATE	SEND RESULTS TO:	SAMPLE ORIGIN	RESULTS RETURN
TEST4: PCR ANALYSIS OF BLOOD SAMPLES. USE OF PCR PRIMERS FROM KNOWN HAEMOLYSIS CAUSING BLOOD BORNE PATHOGENS	Yes but Subject to sample & Lab time availability	Email/text results at once to local WHO contacts & Outbreak Clinic lab liaison officers	Outbreak Field Clinics	As soon as possible

PCR DIAGNOSTIC TEST RESULTS & CONCLUSIONS

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NUCLEIC ACID WAS EXTRACTED FROM BLOOD SAMPLES AND RNA WAS ISOLATED. THIS WAS USED IN PCR REACTIONS USING THE BACTERIA AND VIRUS PRIMERS SHOWN. REACTION MIXES WERE RUN ON AN AGAROSE GEL CONTAINING ETHIDIUM BROMIDE AND VIEWED USING UV LIGHT (FIG 4)

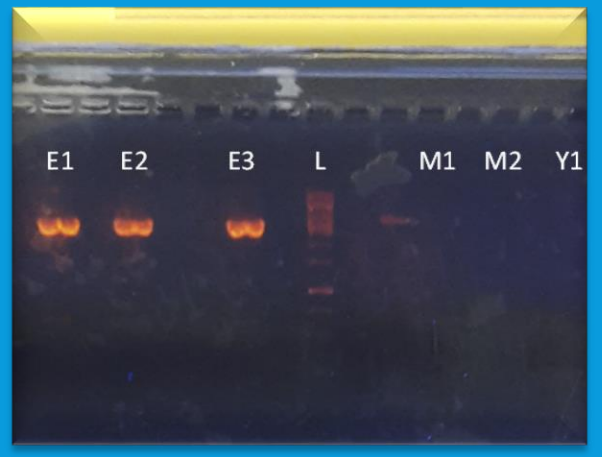

FIG 4 AGAROSE GEL SHOWING RESULTS OF PCR REACTIONS

E1: EBOLA VIRUS1

E2: EBOLA VIRUS2

E3: EBOLA VIRUS3

M1: MARBURG VIRUS1

M2: MARBURG VIRUS2

Y1: *YERSINIA PESTIS* PRIMER 1

L: DNA LADDER (SIZE MARKER)

CONCLUSIONS

PRESENCE OF A BAND IN E1, E2 AND E3 INDICATES A GENETIC SIMILARITY TO THE SOURCE OF THE PCR PRIMER. PCR PRODUCT WITH EBOLA PRIMERS BUT NOT MARBURG OF *YERSINA PESTIS* PRIMERS.

KOCH'S POSTULATES

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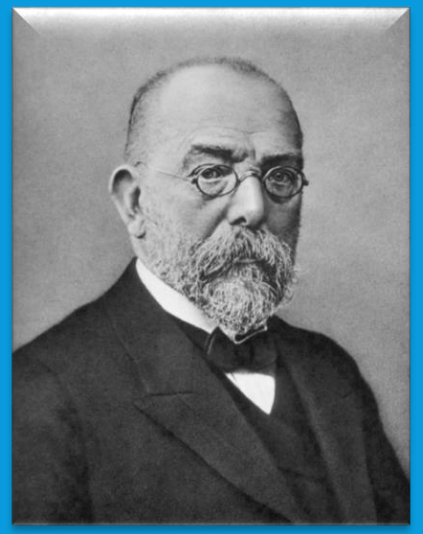
- Koch's postulates are 4 criteria designed to establish a causal relationship between a causative microorganism and an infectious disease. The postulates were formulated by Robert Koch and Friedrich Loeffler in 1884 to establish the etiology of anthrax and tuberculosis.
- The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms.
- The microorganism must be isolated from a diseased organism and grown in pure culture.
- The cultured microorganism should cause disease when introduced into a healthy organism.
- The microorganism must be re-isolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.
- With the advent of metagenomics, PCR (polymerase chain reaction) detection of nucleic acids is more often used than direct isolation of pathogenic microorganisms



Robert Koch circa 1900

METAGENOMIC SEQUENCING TO DIAGNOSE OUTBREAK SPECIES OUTBREAKS:

KOCH'S POSTULATES FOR THE 21ST CENTURY

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- A nucleic acid sequence belonging to a putative pathogen should be present in most cases of an infectious disease.
- Fewer, or no, copies of the pathogen-associated nucleic acid sequences should occur in hosts or tissues without disease.
- With improvement of the patient, the copy number of pathogen-associated nucleic acid sequences should decrease or become undetectable. With clinical relapse, the copy number should increase.
- The nature of the microorganism inferred from the available sequence should be consistent with the known biological characteristics of that group of organisms.
- The gene sequence-based forms of evidence for microbial causation of disease should be reproducible.
- Once virulence factors have been identified, it is possible to develop a vaccine against the factors: the avian flu vaccine was developed using reverse genetic techniques

TEST REPORT SUMMARY

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- You now have the test results in:
- It is clear that the outbreak is caused by a blood and other bodily fluid born infectious agent.
- It appears to be non-responsive to antibiotics and conventional anti-virals
- Bacterial plates do not culture any colonies
- Immunological tests and electron microscopy provide more information:
  -- There is an immune response to the infectious agent
  -- Electron microscopy shows a filamentous type structure in all patients
- PCR for haemorrhagic virus primers were positive

YOUR REPORT

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- You have been asked to explain to the WHO and the press the nature of the identity of the infectious agent causing the Ziberia outbreak
- Explaining how you came to your conclusion, what is the most likely identity and source of the infectious agent?
- Your report word limit is 300 words.

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