

**ADDIS ABABA UNIVERSITY,
COLLEGE OF VETERINARY MEDICINE & AGRICULTURE,
DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY & VETERINARY PUBLIC
HEALTH (MIVP), BISHOFTU**

Handouts for the Course, Zoonosis(VeLT-4162), VLT Year- III, Second Semester (2019/2020)
Instructor: Professor Ashwani Kumar (50% share)

Impact of Zoonoses on Health and Economy

Human health is linked to animal health and production. This link between human and animal populations, and with the surrounding environment, is particularly close in developing regions where animals provide transportation, draught power, fuel and clothing as well as proteins (meat, eggs and milk). In both developing and industrialized countries, however, this can lead to a serious risk to public health with severe economic consequences.

The negative effects of zoonoses:

- Loss of man-hour and man-day
- Loss of labor productivity
- Monetary loss
- Adverse effect on morale of personnel
- Reduced travel and tourism to the affected areas
- Reduced livestock and food production
- Death and destruction of affected animals
- Restriction on and reduction in international trade of animals
- Loss of country's economy

Zoonoses and public health

Zoonotic diseases and human health are matter of particular concern because people, especially in developing and underdeveloped countries, are in direct close contact with domestic animals, and often not very far from wild animals. Their activities such as working with animals, in their sheds, improper disposal of waste material from animal sheds, skinning of infected animals, slaughtering of diseased animals, disposal of infected diseased animals, and poor personal hygiene practices have been one of the important risk factors.

Infectious diseases account for 29 out of the 90 major causes of human mortality and morbidity and 25% of global deaths. About 75% of the new diseases that have affected humans have been caused by pathogens originating from an animal or from products of animal origin. Many of these diseases have the potential to spread through various means over long distances and to become global problems.

Every year millions of people get sick because of foodborne zoonoses such as Salmonellosis and Campylobacteriosis which cause fever, diarrhoea, abdominal pain, malaise and nausea. Other

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bacterial zoonoses are anthrax, brucellosis, infection by verotoxigenic *Escherichia coli*, leptospirosis, plague, Q fever, shigellosis and tularaemia. Every year, 2.2 million people in less developed countries (LDCs), most of them young children, die from diarrhoeal diseases, chiefly of zoonotic origin, caused by *Campylobacter* or *Salmonella*.

Cysticercosis/Taeniasis is caused by a parasite which infects swine and can cause seizures, headache and many other symptoms in humans. Other parasitic zoonoses are trematodosis, echinococcosis/hydatidosis, toxoplasmosis and trichinellosis.

In addition a number of well-known and preventable animal diseases that can be transmitted to humans (i.e. zoonoses) such as rabies, brucellosis, leishmaniasis and echinococcosis continue to occur in many countries especially in the developing world where they mostly affect the poorest segment of the human population. They cause a serious amount of deaths and millions of people affected every year.

Almost all persons infected by rabid animals will die if not treated. Dogs are responsible for most human deaths.

Other viral zoonoses are avian influenza, Crimean-Congo hemorrhagic fever, Ebola and Rift Valley fever. Recent emerging zoonosis is infection due to Covid-19.

Economic impact of zoonoses

- Besides apart from mortality and morbidity, zoonoses are responsible for huge economic losses, particularly in dairy animals, meat, milk and other foods and products of animal origin.
- Many zoonoses cause disabling diseases in humans. A sick or disabled person cannot work as much or as well as a healthy person, hence will not earn enough money to buy proper food or healthy livestock. Unhealthy livestock produce less food. The result is less food to eat and less food to sell - less income again and not enough money.
- All major zoonotic diseases prevent the efficient production of food of animal origin, particularly of much-needed proteins, and create obstacles to international trade in animals and animal products. They are thus an impediment to overall socioeconomic development.
- The World Bank has estimated losses of USD 200 billion (direct and indirect) caused by zoonoses during 2000 and 2010. According to an OECD report (2011), a severe avian influenza pandemic could cause the death of 70 million people and decrease global GDP by 4.8%.
- The OIE estimates that morbidity and mortality due to animal diseases cause the loss of at least 20% of livestock production globally. This represents at least 60 million tons of meat and 150 million tons of milk with a value of approximately USD 300 billion per year.

Social impact

The social impacts can be expressed as indirect health consequences or behavioral changes, changes in societal values and changes in social standing and can be felt at the individual, family or community level.

Poverty is an important factor governing the risk of zoonotic diseases in rural and urban areas. Poverty can increase exposure to such infections and reduces the chances of getting rid of diseases. Poor communities are often trapped in a never-ending cycle known as "the devil's circle of poverty".

Poor people are more likely to suffer from zoonotic diseases for several reasons:

- Poor education: poor knowledge of disease existence, motivation to prevent them.
- Poor sanitary conditions: more chances of getting infection.
- They buy cheaper animals that are often less healthy animals. Cheaper meat is never the healthiest meat and often has not been inspected, therefore, can make the people ill.
- Poor veterinary and public health services: lack of veterinary services worsens the situation in rural communities for those who cannot pay for private services.
- Poor people are often less well-nourished and less healthy and are more susceptible to infectious disease in general and zoonoses in particular.
- Lack of money to set up a good health system for both animals and humans.

Close contact between domestic animals and humans is a characteristic of rural settings. Animals kept for food production in these areas. It is this close association that makes zoonotic diseases so vitally important in rural areas.

Social customs: Dogs and cats are kept as pets in both developing and developed countries. Often these animals are kept unrestricted and unsupervised, without adequate care and vaccinations. Rabies is the most important zoonosis transmitted especially through dog bites. Streets and parks in many cities in developed countries are polluted with dog faeces, posing major problems for city councils. Apart from dogs and cats, inexperienced owners often also keep exotic animals. These animals need to be kept under stringent hygienic conditions to prevent transmission of zoonotic diseases to their handlers (such as salmonella and parasitic diseases).

Socio-economic, cultural and religious factors have been observed to play an important role in the transmission of zoonotic diseases. The hydatid disease in man is more prevalent in Turkana people of North-western Kenya, where as per their religious customs the human dead bodies are exposed to hyenas and dog, thus perpetuating the transmission of the disease. Similarly, the Muslim belief of the unclean less of dogs have been responsible for the observed reduced incidence of the disease in Muslim Arabs as compared to Christian Arabs in Lebanon.

The disease is of much greater incidence in certain occupational workers like shoemakers and shoe-repairs in Lebanon, mainly related to their practices of dipping hides of animals in a decoction of dog faeces for preparing leather.

It has also been observed that certain modes of recreation in man (sea side sports, camping, tourism, mountaineering, hunting, fishing etc.) have resulted in bringing man in close contact with hydatid endemic foci and increasing the chances of his acquisition of the disease.

In the community setting in some African countries, new infections of Ebola virus were related to the ministrations of funeral rites, which involve ritual cleansing of the cadaver and removal of hair, finger nails, toe nails and clothing before burial. People visiting or taking care of infected persons in their homes or in hospitals also risk being exposed to Ebola infections.

Impact on productivity and animal health

Among the factors influencing the productivity and profitability of livestock, animal diseases including zoonoses deserve special attention because they diminish the capacity of the animal to achieve its inherent potential level of production, for any given feeding and management regimen.

Animal diseases lead to significant losses and decrease productivity

Animal diseases kill around 18% of the livestock population in low-income countries (World Organization for Animal Health [OIE]).

In LDCs (least developed countries), mortality rates in traditional village livestock systems average 20% to 22% for calves, 7% for cattle aged over 12 months, 22% to 24% for lambs and kids, 15% for adult sheep and goats, 40% to 50% for piglets and 50% for chickens aged 0 to 6 months. Cattle farmers lose as many animals as they sell.

Not only do animal diseases cause deaths, they also lead to production losses and indirect losses, which are generally far greater. Very few studies provide information on total losses in low-income countries caused by animal diseases. However, the information available for transmissible diseases confirms that losses are heavy. For instance,

An epizootic of Rift Valley fever in Somalia in the early 2000s had little impact on the country's livestock but, when Saudi Arabia banned Somalian livestock imports to protect its human and animal population, it drove down livestock prices on local markets, causing Somalian livestock producers to lose US\$47 to US\$55 million.

RICKETTSIA & ZOONOSES

General Features

The rickettsia are bacteria which are obligate intracellular parasites. They are considered a separate group of bacteria because they have the common feature of being spread by arthropod vectors (lice, fleas, mites and ticks). The cells are extremely small (0.25 μ in diameter) rod-shaped, coccoid and often pleomorphic microorganisms. *Rickettsia* cannot live in artificial nutrient environments and are grown either in tissue or embryo cultures (typically, chicken embryos are used).

The family Rickettsiaceae is taxonomically divided into three genera:

- *Rickettsia* (11 species)
- *Coxiella* (1 species)
- *Ehrlichia* (2 species)

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- *Bartonella* (3 species)

A. *Rickettsia* species are carried by many ticks, fleas, and lice, and cause diseases in humans. Based on serology, the *Rickettsiae* are grouped as below:

- Spotted fever Group
- Typhus Group
- Scrub typhus Group

All three of these contain human pathogens.

1. Spotted Fever Group

- Rocky Mountain spotted fever caused by *Rickettsia rickettsii*
- Rickettsial pox: *Rickettsia akari*
- Canadian typhus: *Rickettsia canada*
- Mediterranean spotted fever: *Rickettsia conorii*
- Siberian tick typhus: *Rickettsia siberica*
- Queensland tick typhus: *Rickettsia australis*

2. Typhus Group

- Murine/Endemic typhus: Fleaborne typhus): Caused by *Rickettsia mooseri (typhi)*
- Epidemic typhus (Brill- Zinsser disease) and Louse-borne typhus): Caused by *Rickettsia prowazekii*

3. Scrub typhus (or Chigger fever): *Rickettsia tsutsugamushi*

B. Genus *Coxiella*: *Coxiella burnetii* causes Q-fever.

Q Fever

Q fever is an illness caused by the bacterium *Coxiella burnetii*. Q fever is spread to humans from infected animals. The bacteria survive for long periods in the environment as they are resistant to heat, drying and many disinfectants. Q fever is usually an acute (immediate) infection but it can sometimes lead to a chronic (long-term) illness. Acute Q fever can cause a severe flu-like illness that is sometimes associated with hepatitis (inflammation of the liver) and pneumonia. Chronic Q fever most commonly results in inflammation of the heart (endocarditis) and people who already have heart valve disease are at increased risk.

Symptoms

Many infected people have no or few symptoms. People who do become sick often have a severe flu-like illness. Symptoms begin about 2-3 weeks after exposure and typically include:

- high fevers and chills
- severe sweats
- severe headaches, often behind the eyes
- muscle and joint pains
- extreme fatigue (tiredness)

If untreated, symptoms can last from 2-6 weeks. Most people make a full recovery and become immune to repeat infections. Occasionally, people develop chronic infections which affect the heart (endocarditis) or the liver (hepatitis). Some people develop chronic fatigue (post-Q fever)

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fatigue syndrome), which can last for many years after the initial infection. Symptoms of chronic Q fever may occur up to two years after the initial infection.

Spread

People usually get infected by breathing in infected aerosols or dust when working with infected animals, animal tissues, or animal products. The main carriers of the disease are farm animals such as cattle, sheep and goats but other animals such as kangaroos, bandicoots, domestic pets such as dogs and cats can also be infected. Pigs are not known to carry the disease.

Infected animals often have no symptoms and can shed the bacteria into their urine, faeces or milk. High concentrations of the bacteria are found in the placenta (birth by-products). Q fever can be contracted by inhaling dust from wool, hides, straw or grass that contains the Q fever bacteria. Common activities where people are exposed include birthing calves and shearing. There is also a potential risk of contracting Q fever by ingestion of unpasteurised milk from an infected animal.

Spread of Q fever from person to person has been reported but is extremely rare. Contaminated work clothing may be a source of infection.

People at at risk

- abattoir and meat workers (including contractors who visit these facilities)
- farmers and shearers
- stockyard workers and animal transporters
- veterinarians, veterinary assistants and veterinary students
- agriculture college staff and students (working with high-risk animals)
- laboratory workers (working with the bacteria or with high-risk veterinary specimens)

Horticulturists or gardeners in environments where dust, potentially contaminated by animal urine, faeces or birth products, is aerosolised (e.g. lawn mowing) may also be at risk. People commencing work in or visiting these industries are at high risk of contracting the disease. Some long-term workers in these industries become immune to the disease without becoming sick.

Prevention

A vaccine (Q-Vax®) is available to protect people against Q fever. Vaccination is recommended for all people who are working in, or intend to work in, a high-risk occupation. Workplaces at risk should have a vaccination program.

People must be screened and tested before they are vaccinated against Q fever.

People who work with animals or materials that may carry the Q fever bacteria should use appropriate protective equipment and be aware of the steps required to stop the spread of the bacteria. The risk of Q fever can be further reduced by:

- washing the hands and arms thoroughly in soapy water after any contact with animals
- washing animal urine, faeces, blood and other body fluids from the work site and equipment, and disinfecting equipment and surfaces where practicable
- properly disposing of animal tissues including birthing products
- minimising dust in slaughter and animal housing areas
- keeping yard facilities for sheep and cattle well away from domestic living areas
- removing clothing that may carry the bacteria before returning to the home environment
- wearing a mask when mowing lawn or gardening in areas where there are livestock or native animals.

People who are unimmunised should not be allowed to visit high-risk work areas such as abattoirs.

Diagnosis

The initial suspicion of a Q fever diagnosis is based on clinical symptoms and signs. Blood tests are required to confirm the diagnosis with repeated testing after two weeks.

Treatment

Q fever is treated with antibiotics, usually in the tablet form. A cardiac assessment, which may include echocardiography, is required to assess whether there are underlying abnormalities of the heart valves which increase the risk of developing chronic Q fever endocarditis. Chronic Q fever infection requires prolonged treatment with antibiotics.

Ringworm (Dermatophytosis)

The dermatophytes are a group of closely related fungi that have the capacity to invade keratinized tissue (skin, hair, and nails) of humans and other animals to produce an infection, dermatophytosis, commonly referred to as ringworm. Infection is generally cutaneous and restricted to the nonliving confide layers because of the inability of the fungi to penetrate the deeper tissues or organs of immune-competent hosts & feed on keratin, the material found in the outer layer of skin, hair, and nails. Reactions to a dermatophyte infection may range from mild to severe as a consequence of the host's reactions to the metabolic products of the fungus, the virulence of the infecting strain or species, the anatomic location of the infection, and local environmental factors.

Etiology

The etiologic agents of the dermatophytoses are classified as following in three genera,

Epidermophyton spp:-The type species is *Epidermophyton floccosum*.

Microsporum spp:-The type species is *Microsporum audouinii*.

Trichophyton spp:-The type species is *Trichophyton tonsurans*.

Transmission

These fungi live for an extended period as spores in soil. Ringworm is highly contagious and can spread multiple ways. Humans and animals can contract ring-worm after direct contact with this soil. The infection can also spread through contact with infected animals or humans. The infection is commonly spread among children and by sharing items that may not be clean.

Ringworm is an example of a zoonotic disease. Cats are among the most commonly affected animal. Dogs, cows, goats, pigs and horses can spread ringworm to humans and other animals via direct contact or contact with objects the infected animal has touched such as bedding, grooming articles, saddles, furniture, carpeting etc.

Risk factors

As described previously, it is possible to acquire ringworm from a variety of places and circumstances. The greatest risk factor is coming in contact with an affected individual or a contaminated surface. Areas of frequent sweating as well as skin fold such as those in the groin

or between the toes are commonly affected. Also from contaminated items as toilet articles, clothing, pool surfaces, showers and locker rooms, transmission is favorable.

Symptoms

The medical term for ringworm is *tinea*. Depending upon the site of infection, tinea has been named differently with different symptoms.

Tinea corporis: When fungus affects the skin of the body, it often produces the round spots of classic ringworm. Sometimes, these spots have an "active" outer border as they slowly grow and advance. Sometimes, scaling, crusting, raised areas, or even blister-like lesions can appear, particularly in the active border.

Tinea cruris: Tinea of the groin ("jock itch") tends to have a reddish-brown color and extends from the folds of the groin down onto one or both thighs.

Tinea faciei (faciale): ringworm on the face except in the area of the beard. On the face, ringworm is rarely ring shaped. Characteristically, it causes red scaly patches with indistinct edges.

Tinea manus: ringworm involving the hands, particularly the palms and the spaces between the fingers. It typically causes thickening (hyperkeratosis) of these areas, often on only one hand.

Tinea pedis: Athlete's foot may cause scaling and inflammation with itching and burning irritation in the toe webs, especially the one between the fourth and fifth toes. Another common form of tinea pedis produces a thickening or scaling of the skin on the heels and soles. This is sometimes referred to as the "moccasin distribution." Occasionally, tinea causes blisters between the toes or on the sole. Aside from athlete's foot, tinea pedis is known as tinea of the foot or, more loosely, fungal infection of the feet. Tinea pedis is an extremely common skin disorder. It is the most common and perhaps the most persistent of the fungal (tinea) infections. It is rare before adolescence. It may occur in association with other fungal skin infections such as tinea cruris (jock itch).

Tinea barbae: Ringworm of the bearded area of the face and neck, with swelling and marked crusting, is often accompanied by itching, sometimes causing the hair to break off. In the days when men went to the barber daily for a shave, tinea barbae was called barber's itch.

Tinea capitis: Ringworm of the scalp commonly affects children, mostly in late childhood or adolescence. This condition may spread in schools. Tinea capitis appears as scalp scaling that is associated with bald spots (in contrast to seborrhea or dandruff, for instance, which do not cause hair loss).

Tinea unguium: Finally, fungal infection can make the fingernails and, more often, the toenails yellow, thick, and crumbly. This is referred to as fungal nails or onychomycosis.

Epidemiology

These pathogenic fungi are found worldwide, and all domestic animals are susceptible. In developed countries, the greatest economic and human health consequences come from dermatophytosis of domestic cats and cattle. A few dermatophyte species are soil inhabitants (geophilic), and cause disease in animals that are exposed while digging or rooting. Other species are host-adapted to people (anthropophilic).

The zoophilic species are transmitted primarily by contact with infected individuals and contaminated fomites such as furniture, grooming tools, or tack.

The most important animal pathogens worldwide are *M. canis*(cat, dog), *T. equinum* & *M. equinum*(horse), *T. verrucosum*(cattle, sheep, dromedary, *M. gallinae*(fowl).These species are zoonotic, especially *M canis* infections of domestic cats and *T. verrucosum* of cattle and lambs.

Diagnosis

Often, the diagnosis of ringworm is obvious from its location and appearance. Circular bare patches on the skin suggest the diagnosis but no lesion is truly specific to the fungus (similar patches may result from allergies, sarcoptic mange, and other conditions)

Skin scrapings for microscopic examination and a culture of the affected skin can establish the diagnosis of ringworm. If the diagnosis is unclear, a potassium hydroxide (KOH) preparation of a skin scraping can be reviewed under the microscope to confirm the diagnosis of a fungus as given below.

Direct microscopic examination although false negative in 5 to 15% of cases in ordinary practice, is a highly efficient screening technique, of hairs or skin scrapings may enhance clinical suspicion by demonstrating characteristic hyphae or arthrospores in the specimen. The technique is more useful in diagnosing dermatophytosis in large animals than in small animals. Hairs (preferably white ones) and scrapings from the periphery of lesions are examined for fungal elements in a wet preparation of 20% KOH that has been gently warmed or incubated in a humidity chamber overnight. And examined under X400 magnification for fungal structures.

Culture:-Fungal culture is the most accurate means of diagnosis. Dermatophyte test medium (DTM) may be used in a clinical setting. Incubation at room temperature is sufficient except when culturing for *T verrucosum* from food and fiber animals. Dermatophyte growth is usually apparent within 3–7 days but may require up to 3 weeks on any type of DTM. Dermatophytes growing on DTM cause the medium to change to red at the time of first visible colony formation. Dermatophyte fungi have white to buff-colored, fluffy to granular mycelia. Saprophytic contaminant colonies are white or pigmented and almost never produce an initial color change on DTM.

Definitive diagnosis and species identification require removal of hyphae and macroconidia from the surface of the colony with acetate tape and microscopic examination with lactophenol cotton blue stain

Many typical isolates of common dermatophytes can be identified directly from primary isolation media, particularly, Sabouraud glucose agar and potato glucose or potato flake agar. Identification characters include colony pigmentation, texture, and growth rate and distinctive morphological structures, such as microconidia, macroconidia, spirals, pectinate branches, pedicels, and nodular organs .

Treatment

Ringworm can be treated topically (with external applications) or systemically (for example, with oral medications):

Topical treatment: When fungus affects the skin of the body or the groin, many antifungal creams can clear the condition in around two weeks. Examples of such preparations include

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those that contain clotrimazole, miconazole, ketoconazole, econazole, naftifine, and terbinafine. It is usually necessary to use topical medications for at least two weeks.

Systemic treatment: Some fungal infections do not respond well to external applications. Examples include scalp fungus and fungus of the nails. To penetrate these areas and for particularly severe or extensive disease, oral medications can be used.

For a long time, the only effective antifungal tablet was griseofulvin. Now, other agents are available that are both safer and more effective. These include terbinafine, itraconazole and fluconazole. Oral medications are usually given for a three-month course.

Prevention and control

Fungi thrive in moist, warm areas, such as locker rooms, tanning beds, swimming pools and in skin folds; accordingly, those that cause dermatophytosis may be spread by sharing sporting equipment, towels, or clothing.

- Avoid sharing clothing, sports equipment, towels, or sheets.
- Washing clothes in hot water with fungicidal soap after suspected exposure to ringworm.
- Avoid walking barefoot; instead wear appropriate protective shoes in locker rooms and sandals at the beach.
- After being exposed to places where the potential of being infected is great, one should wash with an antibacterial and anti-fungal soap or one that contains tea tree oil, which contains terpinen-4-ol
- Avoid touching pets with bald spots as they are often carriers of the fungus.

Control may be accomplished by educating infected individuals not to expose others by walking barefoot near swimming pools, locker rooms, and public showers and by not sharing footwear. Frequent cleaning and disinfecting floors of public baths, swimming pools, etc.

ASPERGILLOSIS

The fungus, *Aspergillus* is responsible for mycosis and mycotoxicosis. Mycosis is the development of fungus in or on the body of host. These fungi grow in different types of foods and produce mycotoxins.

- **Myco-toxicosis** is the ingestion of food stuffs contaminated with myco-toxins resulting into in-toxicosis.
- **Mycotoxins** are toxic metabolites that are produced by microscopic filamentous fungi.

Aspergillus Mycosis

Infectious Agent : *Aspergillus fumigatus* , *Aspergillus parasiticus*

Host: The fungus is ubiquitous. People with weakened immune systems or lung diseases are at a higher risk. Animal hosts in rehabilitation are generally wild birds such as raptors or waterfowl.

Transmission: *Aspergillus*, the mold that causes aspergillosis, is very common both indoors and outdoors, so most people breathe in fungal spores every day. While under stress in a captive facility, rehab animals are more likely to develop the fungal disease. They in turn shed the spores of the fungus and the workers may inhale them.

Symptoms: .

In birds, respiratory signs. Most healthy people have no trouble resisting infection. However, this is not true for anyone who has been debilitated by illness, other diseases, or has been on long term antibiotic, antimetabolite, or corticosteroid therapy.

In humans different types of aspergillosis are prevalent with different symptoms. The allergic bronchopulmonary aspergillosis (ABPA) are similar to asthma symptoms; symptoms of allergic *Aspergillus* sinusitis include: Stiffness, Runny nose, headache, reduced ability to smell; aspergilloma (“fungus ball”) include cough, coughing up blood, shortness of breath, chronic pulmonary aspergillosis is manifested as weight loss, cough, fatigue, shortness of breath while invasive aspergillosis is manifested by fever, chest pain, cough, coughing up blood, shortness of breath,

Diagnosis

- Medical history, risk factors, symptoms, physical examinations, and lab tests to make diagnosing of aspergillosis.
- Imaging tests such as a chest x-ray or a CT scan of lungs or other parts of body depending on the location of the suspected infection.
- Tissue biopsy, in which a small sample of affected tissue is analyzed in a laboratory for evidence of *Aspergillus* under a microscope or in a fungal culture.
- A blood test can help diagnose invasive aspergillosis early in people who have severely weakened immune systems.

Prevention

- Good hygiene and good ventilation. At least 12 air exchanges per hour are recommended in any room where susceptible birds are housed.
- Waterfowl are not to be housed on wood shavings because the fungus will thrive in them when wet.
- Moldy grains and food stuffs are not fed or stored.
- Necropsies are performed with masks as are treatments on suspect patients.
- Spray necropsy birds down with a disinfectant to matt feathers and reduce aerosolized lint and debris.

Aspergillus Mycotoxicosis

Three important mycotoxins are produced by the genus *Aspergillus*, viz., Aflatoxin (*Aspergillus flavus*; *A. parasiticus*), Ochratoxin (*A. ochraceus*) and Sterigmatocystin (*Aspergillus nidulans*, *A. versicolor*) resulting in mycotoxicosis. Among these aflatoxicosis due to Aflatoxin occurs widely and is described as below.

Aspergillosis

Types of foods involved:

- Peanuts, barley, coconut, cotton seeds: invariably mycotoxins are present.
- Corn, millet, rice, oat, sorghum, beans, dry fruits: mycotoxins have been isolated.
- Soyabean: very rarely involved. Natural toxin production is rare. Presence of calcium, magnesium, boron in soyabeans inhibit toxin production by fungi.
- Peanuts are used for animals in the form of peanut cakes or pea nut butter. Oil is extracted from peanut and the remaining is concentrated as cakes to be fed to animals. Oil may have some toxin, but in cakes, the toxins are concentrated.
- Fortifying the food with peanuts cakes means fortifying the feed with toxin.

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Factors favoring toxin production by moulds.

- Substrate
- Fungal strain variation
- Genetic susceptibility of host plant or commodity or composition of commodity:
- Structural integrity of commodity
- Temperature: Wide range of temperature for growth of fungi. 20-30°C good for growth but may not be optimum for toxin production.

Major types of aflatoxins and their metabolites

At least 14 different types of aflatoxin are produced in nature. Aflatoxin B₁ is considered the most toxic, Aflatoxin G₁ and G₂ are produced exclusively by *A. parasiticus*.

Aflatoxins M₁, M₂ were originally discovered in the milk of cows that fed on moldy grain. These compounds are products of a conversion process in the animal's liver.

Standard for allowable contamination of commodities destined for human and animal consumption.

- Human foods are allowed 4-30 ppb aflatoxin, depending on the country involved.
- In contrast, grains for animal feed in the United States are allowed 300 ppb aflatoxin, because this concentration not only provides protection against acute aflatoxicosis but also is low enough to allow most of the grain produced to be traded.

Illness due to aflatoxins

Humans

High-level aflatoxin exposure produces an acute hepatic necrosis, resulting later in cirrhosis or carcinoma of the liver. Acute hepatic failure is made manifest by hemorrhage, edema, alteration in digestion, changes to the absorption and/or metabolism of nutrients, and mental changes and/or coma.

Chronic, subclinical exposure does not lead to symptoms as dramatic as acute aflatoxicosis. Children, however, are particularly affected by aflatoxin exposure, which leads to stunted growth and delayed development. Chronic exposure also leads to a high risk of developing liver cancer. A strong synergy is observed between aflatoxin and hepatitis B virus (HBV) and hepatitis C virus (HCV) agents for liver cancer.

Animals

Turkeys are extremely susceptible to aflatoxicosis. Aflatoxin has potential to lead to liver disease in dogs; however, not all dogs exposed to aflatoxin will develop liver disease. Toxic level in dog food is 100–300 ppb and requires continuous exposure/consumption for a few weeks to months to develop aflatoxicosis.

Prevention

There is no specific antidote for aflatoxicosis.

Symptomatic and supportive care tailored to the severity of the liver disease may include intravenous fluids with dextrose, active vitamin K, B vitamins, and a restricted but high-quality protein diet with adequate carbohydrate content

Possible intervention strategies

During Production

- The management can be used to minimize contamination, and the practice of inoculating the fields with non-aflatoxigenic strains of fungi may shortly be a new tool in the battle to prevent economic loss.
- Insect damage in the field can be controlled by pesticides or by cultural practices;
- Harvesting is usually done without machinery, and drying should be carried out very efficiently.

Storage

- To preserve quality in storage, it is necessary to prevent biological activity through adequate drying (<10% moisture), elimination of insect activity that can increase moisture content through condensation of moisture resulting from respiration, low temperatures, and inert atmospheres.

Processing

Three main approaches exist: dilution, decontamination, and separation.

- Dilution: the easiest means of satisfying the requirement is to mix grain low in aflatoxin with grain exceeding the regulated limits.
- Decontamination: Treatment with ammonia, alkaline substances, and ozone can denature aflatoxins, but whether this change is permanent is not clear.
- Separation: separating contaminated grain from the bulk. This approach depends on the heavy contamination of only a small fraction of the seeds, so that removing those leaves a much lower overall contamination. A major portion (80%) of the toxin is often associated with the smaller and shriveled seed, and thus screening can lower the overall concentration in the bulk. Further removal of aflatoxin-contaminated seeds may be achieved by color sorting, which, in the case of peanuts, is most effective when the seeds are blanched.

TAENIASIS:

Parasitic infection of man due to a large Tapeworm that lives in small intestine of man. It is an obligatory parasite of man and thus an example of obligatory cyclozoonoses. It is a true zoonosis which means the life cycle of this parasite is not completed without man. Under natural transmission cycle, there is no other definitive host

Causative Agent

Taenia saginata: The adult worm which lives in small intestine of man is white and semitransparent; measuring 5-12 meters.

Taenia solium: The adult worm which lives in small intestine of man and is 2-6 meters.

Larval stage of *Taenia saginata* is *Cysticercus bovis* while of *Taenia solium*: *C. cellulosae*

Types of Cysticercosis: Infection due to larval stage is called as Cysticercosis

- Bovine Cysticercosis
- Porcine Cysticercosis
- Human Cysticercosis

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- *Cysticercus bovis* mainly occurs in cattle and found in the masseter muscles, shoulder muscles, heart, tongue, diaphragm, esophagus, adipose tissue, liver, lungs and lymph nodes.
- *Cysticercus cellulosae*, mainly occurs in pigs and commonly found found in the masseter muscles, shoulder muscles, heart, tongue, diaphragm, esophagus, adipose tissue, liver, lungs and lymph nodes. In the tongue, skeletal muscles and sometimes in organs.

Shape of cysts

- *Cysticercus* is round or oval in shape and when fully developed consists of scolex invaginated into small fluid filled vesicle
- Cysts may be viable or dead
- Dead degenerated or calcified cysticerci: clearly form identifiable white spots and have fibrotic lesions,
- Viable cysticerci : Pinkish-red in color.
- True viability of the cyst can be ascertained by keeping the cysts in bile of cattle overnight. Viable cysts evaginate while the dead ones remain intact.

Porcine cysticercosis

Cysticercus cellulosae: Mainly occurs in pigs

Semitransparent, opalescent white, and elongate oval in shape and may reach a length of 0.6 to 1.8 cm

Common Sites: Tongue, Heart, diaphragm, internal masseter, neck, shoulder, intercostals and abdominal muscles.

Occasionally: Liver, lungs, kidney, eye and brain

Meat having cysticerci

Pork having cysticerci (*C. cellulosae*) is called as *measly pork*.

Beef having cysticerci (*C. bovis*) is called as *Measly Beef*

Human cysticercosis

Due to autoinfection or poor personal and environmental hygiene, man may also be infected with the eggs of *Taenia solium*

Eggs develop into larvae which may migrate even to brain and produces a condition called as neurocysticercosis (NCC) which is worldwide prevalent

Host Range

Definitive host: T. saginata & T. solium live exclusively in small intestine of man.

Intermediate host:

T. saginata mainly cattle, (deer, reindeer, llama, buffalo, giraffe and antelope)

T. solium: Mainly pigs.

(Rats, cats, dogs, sheep, cattle, deer, monkeys and man)

Clinical features of the disease in man

- Due to adult parasite (Human Taeniasis)
- Due to larval stage of *T. solium* (Human Cysticercosis)

Clinical manifestation of infection with the adult worm are frequently asymptomatic but may lead to nervousness, insomnia, anorexia, loss of weight, abdominal pain and digestive disturbances. The mobile gravid segments may make their way to unusual sites such as appendix, uterus or biliary tract and may cause serious disorders.

Clinical manifestation of infection with the larval stage: Due to larvae of *T. solium*

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Somatic disease

- Cysts in the muscles cause myositis, fever, muscle swelling and later to atrophy and fibrosis
- Ophthalmic Cysticercosis : visual difficulties, retinal edema, hemorrhage, a decreased vision or even a visual loss
- Subcutaneous cysts : formation of firm, mobile nodules, which are sometimes painful.

4. Neurocysticercosis:

Cysts in brain:

- 60% of the patients are having these cysts in brain
- headaches, nausea, vomiting, lethargy and altered mental status.
- Psychic symptoms, including epileptic seizures
- The cysts can persist in brain from 2-10 years.

Cysts in meninges

Clinical features in animals

There are no specific features of the infection in animals and the disease is diagnosed during post mortem examination.

In cattle and pigs

No specific features of the infection and the disease is diagnosed during slaughter or at post-mortem.

However, in cattle, heavy infestation by the larvae may cause myocarditis or heart failure.

Epidemiology

Man is universally susceptible. The mode of transmission for *T. saginata* is by ingestion of raw or inadequately cooked beef containing the cysticerci. For *T. solium* i) by ingestion of raw or inadequately cooked pork containing the infective larvae, or ii) by direct transfer of eggs in faeces of a person harbouring an adult worm to his own or another's mouth, or indirectly through ingestion of food or water contaminated with eggs, resulting in somatic cysticercosis. Man can also be infected with *T. solium* eggs from contaminated vegetables.

T. saginata is not directly transmitted from man to man but *T. solium* may be; eggs of both species are transmitted in the environment as long as man harbours the worm in the intestine, sometimes 30-40 years; eggs may remain viable for months.

Besides contamination by man, birds especially sea birds and coprophagous beetles may disseminate the eggs.

A person can have mixed infection with *T. saginata* and *T. solium*.

Diagnostic procedures in animals

1. Routine meat inspection:

- Two deep cuts in the external and one deep cut in the internal muscles of mastication.
- The cut surfaces of the muscle and the tongue are inspected visually.
- The pericardial surface of the heart is inspected, then the heart muscle incised lengthwise to open the ventricles and to cut through the intraventricular septum.
- When one or more cysts are found there is a requirement for further cuts with specific reference to predilection sites e.g. diaphragm and inspection of offal.

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Judgment

Heavy” infestation

- Lesions in two of the usual inspection sites i.e. masseter muscles, tongue, oesophagus, heart, diaphragm or exposed musculature .
- Two sites during incisions into the shoulder and into the rounds.

Carcass and viscera of heavily infested animals are condemned

In moderate or light infestation small number of cysticerci including dead or degenerated cysts are present.

Localized infestation: there is a requirement to store the carcass at a temperature:

- *Not exceeding -7°C for not less than 21 days or*
- *At a temperature not exceeding -10°C for not less than 14 days before release for human consumption.*

the carcass is conditionally passed

2. Other methods of diagnosis in animals

Immunodiagnostic methods like Indirect-haemagglutination test; indirect immune-fluorescence; Skin reaction; Radioimmunoassay; Complement fixation test; ELISA; PCR

Diagnostic procedures in man

- Demonstration of *T.saginata* and *T.solium* eggs (oval, 30x20u brown in color) and gravid segments in faeces.
- Radiologically, the cestodes may sometimes be demonstrated in the intestine.

Treatment

There is no specific chemotherapy for human cysticercosis; surgical excision is the only satisfactory treatment. For adult parasites, niclosamide, quinacrine hydrochloride are effective. Praziquantel and benzimidazole derivative (albendazole) have been found fairly promising particularly in cattle.

Prevention and control

1.Massive chemotherapy of infected individuals,

- Niclosamide,
- Quinacrine hydrochloride ,
- Praziquantel
- Traditional herbal remedy called ‘Kosso’.

2.Improving sanitation

3.Educating people

4.Avoid use of sewage effluent for pasture irrigation without adequate treatment.

5.Processing of meat

- Freezing
- <-5°C for >360 hr, i.e. 15 days/
- <-10°C for >216 hr, i.e. 9 days /
- <-15°C for >144 hr, i.e. 6 days.
- Heating
- >56°C core temperature >1 sec,
- Irradiation (100Krad death, 40Krad inhibition for development),

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- Pickling meat in 25% salt solution for 5 days

Preventive measures in animals

- Restrict the access of the cattle to surface drinking water and by supplying them with fresh water
- Avoid the access of pigs to latrine or to human faeces.
- Competent meat inspection must be made compulsory
- Anthelmintic treatment of infected animals
- Premises disinfection

For human cysticercosis

- Surgical treatment or chemotherapeutic drugs or both should be applied.
- Albendazole is preferable over praziquantel due to its lower cost.
Corticosteroids and anticonvulsants do not reduce CSF and brain drug levels.
- Surgical treatment includes direct excision of ventricular cysts, shunting procedures, and removal of cysts via endoscopy

ECHINOCOCCOSIS

Caused by a parasitic tapeworm: Echinococcus

Species causing human infection:

E. granulosus

E. multilocularis

E. oligarthrus

E. vogelli

The disease in man is due to its larval stage, hydatid cyst, which means cyst with water like fluid and thus the name, hydatidosis. It has following types:

Classic hydatid disease

- Caused by *E. granulosus*.
- This species is adapted to dogs and a variety of domestic and sylvatic animal intermediate hosts.
- A major public health and economic problem.

Alveolar hydatid disease by *E. multilocularis*.

- Final and intermediate hosts are foxes and rodents respectively.
- Potential exposure to man is not great.
- However, if it occurs, it is one of the most lethal parasitic infections to occur in humans.

Polycystic form of hydatid disease by *E. vogeli*.

Life cycles of *E. oligarthrus* and *E. vogeli* are limited to sylvatic animals. Limited to central and South America

Hydatid cyst

- Cyst is filled with fluid.
- Cyst consists of an inner germinative layer of cells supported externally by laminated membrane of variable thickness. Surrounding the cyst, there is another layer due to host reaction.

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- Small secondary cyst, called brood capsules, bud internally from germinal layer which produces multiple protoscolices.
- A protoscolex is a scolex with the rostellum and suckers.

Types of hydatid cysts

- Sterile hydatid cysts: Brood capsules and protoscolices in the cysts are not formed.
- Fertile hydatid cysts: Cysts with brood capsules and protoscolices.
- Unilocular cysts: Cystic stage *E. granulosus* and most common in food animals
- Multilocular cysts : Cystic stage of *E. multilocularis*. Primary cyst gives to others by continuous exogenous budding, to produce a larval mass made of hundreds of contiguous vesicle that may occupy more than the one half of the invaded hepatic lobe.

Size of the hydatid cyst

- In animals: varies from a marble to small football, usually of the size of goose egg. Early forms appear as white nodules, as yet containing no fluid: may be seen in the liver 4 weeks after ripe eggs are ingested. The cysts are 2.5 mm in size and contain fluid : after 8 weeks of taking ripe eggs.
- Hydatid cysts are 15-20 mm in diameter: after 6 months and only then do they produce scolices and brood capsules and become infective.
- The hydatid cysts develop slowly, taking months to increase in size to 5-10 cm although some, especially in man, may reach 50 cm in diameter

An average-sized hydatid cyst may contain as 2 million protoscolices. Bovine livers of 91-113 Kg and pig liver of 50 Kg have been recorded. Such liver are markedly cirrhotic and cause ascites.

In humans, the slowly growing hydatid cysts may attain a volume of many liters and contain many thousands of protoscolices. The fluid is highly allergic.

Hydatid cysts in liver is always associated with marked fibrous tissue reaction which may be 13 mm thick. Affected liver are enlarged depending upon number and size of the cysts.

Sites of cysts

Liver and lungs are the commonest sites. In sheep lungs are affected as often as liver, the commonest form being unilocular fertile cysts; in the ox lung is affected more often than liver, usually with small unilocular cysts; in pigs and horse liver is the most frequent site of infection.

Source of infection to man

- Ingestion of the ova of dog tapeworm
- Contamination of the hairs of the dog's coat with ova from feces: most common source
- Dogs may pass ova from anus by licking
- Hand to mouth transfer of tapeworm eggs by handling sheep fleeces of sheep contaminated by sheepdog feces

Clinical features in man:

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- The clinical signs are caused by mechanical pressure exerted by the cyst on the surrounding tissue and hence vary with the size and location of cyst.
- Cysts of moderate size are generally asymptomatic and may cause mild abdominal heaviness.
- Large cysts, however, cause mechanical obstruction.
- Physical examination reveals hepatomegaly
- Jaundice may ensue from pressure on major biliary ducts.
- Fever, malaise, headache and eosinophilia are generally associated with the clinical disease.
- In pulmonary form, there is often coughing with or without haemoptysis(spitting of blood) Coughing with membrane from a ruptured cyst.
- Liver lesions are sometimes painful
- Cranial cysts may produce nervous symptoms
- If the cyst is ruptured inside the body, there can be secondary infection and/or cause fatal anaphylaxis.

Clinical features in animals

Generally few, if any, clinical signs despite severe levels of infection. Large cyst in liver cause ascites. Liver becomes cirrhotic. Adult worms in the dogs rarely cause problems except enteritis in heavy manifestations. The disease is usually detected during slaughter.

Epidemiology:

It is a cyclo-zoonotic infection. In India, the infection is prevalent widely in all parts of the country but the prevalence rates vary from place to place and species to species. In some areas the disease is found to affect a high (89%) proportion of cattle population. The prevalence of echinococcosis shows a marked variation in different species of domestic animals: 17.82-31.9%(cattle), 11.3-48.1% buffaloes, 2.75-30.5% sheep, 2.6-21% goats and 3,52% swine. In dogs the parasite can be found in as many as 16.30% of the animals.

Reports concerning the occurrence of human hydatidosis in India have been appearing in the literature from time to time. The infection is high between 16-30 years of age. The infection ratio between human female and male is 30:40.

Socio-economic, cultural and religious factors have been observed to play an important role in the transmission of this disease in man. The disease is more prevalent in Turkana people of North-western Kenya, where as per their religious customs the human dead bodies are exposed to hyenas and dog, thus perpetuating the transmission of the disease. Similarly, the Muslim belief of the unclean less of dogs have been responsible for the observed reduced incidence of the disease in Muslim Arabs as compared to Christian Arabs in Lebanon.

The disease is of much greater incidence in certain occupational workers like shoemakers and shoe-repairs in Lebanon, mainly related to their practices of dipping hides of animals in a decoction of dog faeces for preparing leather.

It has also been observed that certain modes of recreation in man (sea side sports, camping, tourism, mountaineering, hunting, fishing etc.) have resulted in bringing man in close contact with hydatid endemic foci and increasing the chances of his acquisition of the disease.

Diagnosis:

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1. Clinically, abdominal radiography, choangiography and liver scanning together with individual history diagnose the disease.
2. Casoni's skin test, and intradermic allergic test using filtered hydatid fluid as antigen is a screening test of some value for estimating the prevalence of this disease.
3. Serological tests, such as, CFT, the latex agglutination test, the fluorescent antibody test, immuno-electroporesis etc. are helpful for studying the Epidemiology of the disease.

Diagnosis in animals

In the food animals the diagnosis is normally made postmortem

Confirmation is made at PM or by demonstration of adult *E. granulosus* in the feces of dogs purged with praziquantel or other taenicide.

Prevention and control

- Health educational efforts for improving personal hygiene in the handling of dogs as pets and companion
 - The prevention of dogs from gaining access to raw offal and the proper disposal of the offal is an essential control measure.
 - Reducing the dog population (Control of stray dogs).
 - Minimizing dogs' role in the transmission by mass treatment (praziquantel: 5 mg/kg body weight).
 - Control of environmental contamination by dog feces.
 - Awareness of the public especially the farmers in relation to responsible dog ownership
 - Disposal of carcass of dead animals, especially sheep, properly and immediately by deep burial or incineration
8. Efficient meat inspection procedure with effective control of rejected meat and offals is an essential control measure.
9. Surgical removal of the cysts is a conventional practice of treatment especially in case of human patients.

TOXOPLASMOSIS

-
- Disease caused by arc (toxoon) shaped organisms
- *Toxoplasma gondii*, an intestinal coccidian of felids is the causative agent
- Cats are the key host
- Socioeconomically important because of human sufferings and long term care of children with mental retardation and blindness are enormous

Stages of parasite:

- Adult parasite
- unsporulated oocyst : passed in the feces
- sporulated oocyst: infective stage in the environment. Each is having two sporocysts and each of it has 4 banana shaped sporozoites
- Bradyzoites: Resting stage
- Tachyzoites: multiplying stage

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Life cycle

Hosts:

Definitive: Cats, both wild and domestic, are the only definitive hosts

Intermediate : Animals like sheep, goat, pigs, poultry and humans

Life cycle in two stages

Asexual stage: in intermediate and definite host

Sexual stage: occurs in definite host

Clinical features in man:

- It may occur as a *congenital* or an *acquired infection*.
- Pregnant women -- not themselves unusually prone to *T. gondii* infection or clinical disease
- Congenital infection may occur following maternal infection acquired during the first trimester of pregnancy are more severe than those acquired in the second and third trimester.

Congenital infection occurs by the transplacental transfer of *Toxoplasma*. There may be severe fulminating infection resulting in abortion, miscarriage, stillbirth or the birth of a baby with manifestations of acute toxoplasmosis. In the benign forms the disease may become manifest in infancy, childhood or even adult life. Infection in first trimester is associated with more severe lesions. Congenital infection may be manifested by anaemia, jaundice, hepatosplenomegaly, lymphadenitis, and retinochoroditis. In neurological form of congenital infection there may be retinochoroiditis, convulsions and intracranial calcification.

Disease in congenitally infected children

(i) Mild disease may consist of slightly diminished vision only

(ii) Severely diseased children: full tetrad of signs including retinochoroiditis, hydrocephalus, convulsions, and intracerebral calcification. Of these, hydrocephalus is the least common but most dramatic lesion of toxoplasmosis.

Testing of all pregnant women for *T. gondii* infection is compulsory in some countries

Postnatally Acquired Toxoplasmosis

By ingesting food and water contaminated with oocysts from infected cat feces, or by ingesting tissue cysts in under cooked or uncooked meat.

Lymphadenitis is the most frequently observed clinical form. Postnatally Acquired Toxoplasmosis

-
- Lymphadenopathy may be associated with fever, malaise, rash, fatigue, muscle pain, sore throat and headache. It is also a common cause of chorioretinitis
- Although the condition may be benign, its diagnosis is vital in pregnant women because of the risk to the fetus.
- Encephalitis is the most clinically important manifestation of toxoplasmosis in immunosuppressed patients.

Toxoplasmosis is a major cause of death among patients with AIDS.

Pregnant women -- while not themselves unusually prone to *T. gondii* infection or clinical disease, parasite invasion across the placenta can result in severe neurological abnormalities in the fetus; congenital toxoplasmosis is a leading cause of birth defects.

A minority may experience temporary parasitaemia and may have low-grade fever, malaise, and lymphadenopathy.

Acquired infection in man is usually less severe and can present with a varied clinical picture. Lymphadenopathy is the commonest manifestation and may be accompanied with fever, malaise, and rash. It is also a common cause of chorioretinitis.

Disease in animals:

Severe disease is caused by *T. gondii* in many species of animals.

This includes:

- Cats of any age can die of toxoplasmosis, kittens and those with depressed immunity are the most likely.
- embryonic death and resorption, fetal death and mummification, abortion, stillbirth and neonatal death in goats and sheep.
- Outbreaks of toxoplasmosis in pigs cause higher mortality in young pigs than in adult pigs.
- . Sporadic and widespread outbreaks of toxoplasmosis occur in rabbits, mink, birds and other domesticated and wild animals.
- Among companion animals, fatal toxoplasmosis may occur in dogs that are immunosuppressed following infection with concurrent distemper virus.
- No clinical disease has been observed in cattle

Epidemiology:

T. gondii has the distinction of being one of the most cosmopolitan parasites known with more than 180 countries reporting its occurrence.

Toxoplasmosis is transmitted in two ways: acquired or congenital. Animal houses, gardens, fields and pastures are frequently contaminated by *Toxoplasma* oocysts excreted by the infected cats. After primary infection, a cat can shed oocyst for about two weeks and millions of these can be present in a single sample of stool. Oocysts are resistant to most ordinary environmental conditions and can survive under moist conditions for months and even years. Coprophagous invertebrates like cockroaches and flies can spread oocysts mechanically. The sporulated oocysts can also be carried by wind, water, insects, birds and fomites from one place to another. Oocysts are resistant to environmental stresses and possess a remarkable transmission due to their small size.

The edible parts of food animals especially those of sheep, goat and swine contain the tissue cysts of *T.gondii*. The viable cysts can persist for life in animals. Raw or undercooked meat from infected animals is therefore, a serious threat to human health. Milk of goats has been found to contain the parasite and can also act as a source of infection especially for the human infants. Cow milk, however, has not been found to pose a similar risk. Carnivores and man are usually infected by the ingestion of tissue cysts.

Congenital transmission involves passage of tachyzoites through placenta to the developing foetus. Infections due to laboratory and autopsy accidents and handling of infected meat in homes and slaughterhouses have been reported.

Diagnosis:

Demonstration and isolation of parasite

- Biopsy material (lymph nodes and muscles) or impression smears can be used for detection of the parasite.
- Toxoplasma can be isolated from patients by inoculation of laboratory animals. Mice are highly susceptible.
- The inocula used are secretions, heparinized blood, CSF, biopsy material and products of conception in cases of abortion.
- Samples suspected of contamination are treated with antibiotics (1000units/ml penicillin; 10ug/ml streptomycin) and allowed to stand at room temperature for an hour.

Detection of T. gondii oocysts in the environment

- Contamination of the environment by oocysts is widespread, as oocysts are shed by domestic cats and other members of the Felidae. Cats may excrete millions of oocysts after ingesting as few as 1 bradyzoite or 1 tissue cyst, and many tissue cysts may be present in one infected mouse.
- Oocysts are detected in the feces of infected cats by concentration methods (e.g. flotation in high density sucrose solution) because too few may be present to be detected by direct smear.
- For definitive identification, T. gondii oocysts should be sporulated and then bioassayed in mice to distinguish them from other related coccidians.
- T. gondii oocysts have been isolated from soil.
- Bioassay of soil samples by feeding to pigs and chickens may be more sensitive than direct determination of oocysts in soil since pigs can be infected by feeding as few as one oocyst .
- Detection of T. gondii oocysts in water which however, is more difficult .

Serology:

Paired serological tests (methylene blue dye test, IHA, CF, agglutination, IF and ELISA) can determine the infection status of animal/man.

Prevention and Control:

1. Proper cooking of meat is essential. Heating meat to 66°C kills the organisms.
2. The hazards inherent in handling raw meat in slaughter houses and in the homes are well recognized and measures such as hand washing or wearing of rubber gloves should be adopted.
3. Freshly passed oocysts in cat's faeces are non-sporulated and therefore non-infective. Proper disposal at this stage by incineration or flushing down the toilet obviates the risk of infection
4. Control of diet of pet cats (prevent ingestion of raw meat, wild birds and rodents) and elimination of strays.
5. Sand and soil represent a durable source of infection; therefore children's play areas should be made inaccessible to cats.
6. Pet cats shedding oocysts can temporarily be isolated and treated with sulphadiazine and pyrimethamine. Immunoprophylaxis has been attempted in animals using phenol killed toxoplasma and irradiated toxoplasma with some success.

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7. As the foetus and neonates represent the most vulnerable section, avoidance of infection by pregnant women is very important. Routine serological check up in antenatal clinics and treatment of infected mothers would be a valuable step in protecting the foetus.

LEISHMANIASIS

Leishmania : A protozoa

Two morphological forms:

Promastigote:

- Spindle shaped body having a flagellum near the anterior end.
- Occurs in the gut of sandfly and artificial culture.
- Infective stage which is introduced into the skin by the bite of fly.

Amastigote (Leishman Donovan bodies, LD bodies):

- Ovoid shape with one blunt end.
- Found in the mononuclear cells of the skin or reticuloendothelial system in vertebrates

Types of Leishmaniasis

1. Visceral leishmaniasis(Kala- azar)

- Caused by *L. donovani*
- Lesions from skin metastasizes throughout the reticuloendothelial system

2. Cutaneous leishmaniasis

- Caused by *Leishmania tropica*
- Primary lesion in skin and infection limits to it.

3. Mucocutaneous leishmaniasis(Espundia)

- Caused by *L. brazileinsis*
- Metastasis in lymph glands, skin and mucocutaneous junction

Visceral leishmaniasis

Clinical features:

- I-period: few months to two years, optimum is 4 months
- Discomfort below the left costal margin from the enlarged spleen
- Weak and emaciated appearance but with good diet and clean tongue
- Temperature of 102F but quite unaware that he has fever
- Fever is like enteric fever but no toxemia and appetite is good
- Onset of malaria like fever with chills and rigors but unaffected by antimalarial drugs
- Irregular fever may continue for a prolonged period.

Post Kala-azar Dermal Leishmanoid

- This condition appears after about one year of treatment of visceral leishmaniasis with sodium antimony compounds.
- There are skin lesions: erythematous patches, depigmented patches or nodules over the face, forearms, inner aspects of thighs.
- LD bodies are present in the lesions
- The phenomenon is the result of immune response of the host.
- Eruptions disappear during relapse and reappear with recovery from infection.
- Delayed hypersensitivity is positive in such cases.

Epidemiology

- Man to man transmission by the bite of sand fly, *Phlebotomous aargentipee*(vector).
- Young adults and adolescents mainly affected. Children can also suffer.
- Disease affect low-socio economic group of people
- It is house based
- Overcrowding, ill ventilation, collection of organic matter inside the house facilitate transmission
- Alluvial soil, dense vegetation, high altitutde and high humidity(above 70%) facilitate epidemics
- Reservoir: Canines(Dogs in urban region, jackals in sylvatic transmission)
- Rodents have been found in Kenya

Laboratory Diagnosis

- Direct Demonstration of parasite
- Microscopic examination of the bone marrow, spleen or liver biopsy for LD bodies.
- Culture of bone marrow aspirate, liver and spleen biopsy material to NNN medium, incubate at 22C for a week and examine for promastigote

2. Indirect evidence

- 1. Leucopenia with relative neutropenia.
- 2. Aldehyde test
- 3. Demonstration of antibodies

Aldehyde test

- Principal: Marked production of globulin by plasma cells in Kala-azar. The test detects these globulins.
- Method:
- 1 ml serum + one drop of 40% formaldehyde
- Mix and leave for 2 hrs.
- Positive test: Opacity of egg white color along with jelly formation within 20 min to 2 hrs
- The test is good for surveillance.
- Not good as diagnostic test because test is positive only after 2-3 months of infection and becomes negative after 6 months of cure.

Demonstration of antibodies

- Complement Fixation test
- Indirect Fluorescent Antibody test(IFT)
- ELISA
- Leishmania Skin Test(Delayed hypersensitivity reaction)

Cutaneous Leishmaniasis

- Incubation period: Few weeks to six months. In some cases it may be one to two years.
- The lesion begins as a small nodule, which ulcerates in majority of the cases. The parasite is found along the red margin.
- The sore heals spontaneously.
- The sores are distributed on exposed parts of the body, particularly on the face and extremities.

- Two species of sandflies: *Phlebotomus paptasi* and *Phlebotomus sergenti* transmit this type leishmaniasis.

Dogs act booster animal during epidemic

Mucocutaneous Leishmaniasis

- *Leishmania braziliensis* produces single or multiple, seldom self-healing ulcers in the primary cutaneous stage. The condition is accompanied by involvement of lymphatic. Untreated primary ulcers lead to mucocutaneous leishmaniasis.
- The metastatic lesions spread along the lymphatic, producing nodules is essential form of the disease

Tapir Nose

- A condition in which nasal and pharyngeal cartilages are destroyed, lips and nose swell.
- The condition may be painful or painless with secondary infection.
- Mucocutaneous leishmaniasis is a serious type of condition.
- The primary lesions are similar to cutaneous leishmaniasis in the mucosa of upper respiratory tract.
- In two third of the patients, lesions restricted to nose.
- In rest of cases, pharynx, larynx, palate and lips also involved. The lesion progresses as: Small crust-----ulceration-----erosion-----destruction of tissues. Nasal septum may be destroyed.

Prevention, Control and Eradication of Zoonotic Diseases

Control: The reduction of disease incidence, prevalence, morbidity or mortality to a locally acceptable level as a result of deliberate efforts; continued intervention measures are required to maintain the reduction. Example: diarrhoeal diseases.

□ **Prevention** primarily covers measures to prevent the occurrence of disease, such as by reductions of risk factors.

It also means to arrest disease progress and reduce its consequences once established. These are known as secondary and tertiary prevention that arrest or retard existing disease and its effects through early detection and appropriate treatment; or to reduce the occurrence of relapses and the establishment of chronic conditions through, for example, effective rehabilitation.

Eradication: Permanent reduction to zero of the worldwide incidence of infection caused by a specific agent as a result of deliberate efforts. Example: smallpox.

Methods of prevention and control

- Interruption of transmission

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Instructor: Professor Ashwani Kumar (50% share)

- Controlling reservoir of infection
- Protecting susceptible population

1. Interruption of transmission

1.1. Changing some components of environment to prevent the infectious agent from patient or carrier from entering the body of susceptible host, e.g., chlorination of water will interrupt cholera agent from water to host.

1.2. Food borne zoonotic diseases: Hand washing, adequate cooking, prompt refrigeration of food and withdrawal of contaminated food. Establishment of food hygiene like hygiene in animal production; hygiene of slaughter; hygiene in handling and processing food stuffs.

1.3. When vector borne: control measures primarily at the vector and its breeding places.

1.4. Droplets or droplet nuclei transmission: Early diagnosis and treatment, personal hygiene and proper handling of secretions and excretions.

1.5. Ensure safety of animal products (wool, hides, bones, fat, others) by hygiene during collection, storage, processing, transport.

1.6. Ensure safety or use of animal carcasses and washes by proper animal carcass disposal and excreta/garbage disposal.

2. Controlling reservoir of infection: The **reservoir** for infectious agents is the principal habitat where a specific infectious agent lives and multiplies. The reservoir is necessary for the infectious agent either to survive, or to multiply in sufficient amount to be transmitted to a susceptible host, e.g. **soil** is the reservoir for *Clostridium tetani* or **water**, the reservoir for *Legionella pneumophila*.

Elimination of the reservoir may be easy with animal reservoir (e.g. bovine tuberculosis, brucellosis) but not possible in humans.

General measures of reservoir control comprise:

2.1. Early diagnosis: This is the first important step.

2.2. Notification: Once disease has been detected or even suspected, it should be notified to the local health authority that will control measures. Some diseases are notifiable which are considered to be of serious menaces (hazards) to public health.

2.3. Isolation: It means separation for the period of communicability, of infected persons or animals from others in such places as to prevent or limit the direct or indirect transmission. Isolation is also possible by “ring immunization” i.e. encircling the infecting population with a barrier of immune one through whom the infection is unable to spread.

2.4. Treatment: The objective is to kill the infectious agent when it is still in the reservoir, i.e. before it is disseminated. Treatment reduces the communicability of disease, cut short the duration of illness and prevent the development of secondary cases. Treatment can be individual

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treatment or mass treatment. Inadequate treatment may induce drug resistance and affects the control by chemotherapy.

2.5.Quarantine: It has been defined as the limitation of freedom of movement of such animals or persons exposed to disease for a period of time no longer than the longest usual incubation period of the disease. This will prevent the contact of infected from uninfected population.

3.Protecting susceptible population

3.1.Active immunization: Several vaccines are available for animals and human. Rabies is a good example.

3.2.Passive immunization: Useful only when exposure to exposure has just occurred or is possible within the next few days. Immunity is short and variable 1-6 weeks.

3.3.Chemoprophylaxis

3.4.Eliminating the pathogen from its animal reservoir(s). In some countries, livestock diseases such as bovine and porcine brucellosis and bovine tuberculosis have been eradicated, and the prevalence of *Salmonella* in poultry has been significantly reduced.

3.5.Vaccination.

3.6.Flea and tick control.

3.7.Periodic testing for enteric parasites or other pathogens, and other disease control measures in domestic animals can also protect people.

3.8.Non specific measures: Improvement in the quality of life like better housing, water supply, sanitation, nutrition.

4.Surveillance is defined as the ongoing systematic collection, analysis, interpretation, and dissemination of outcome-specific data essential to the planning, implementation, and evaluation of public health practice.

Veterinary surveillance: As frontline healthcare providers, veterinarians assist with the recognition, diagnosis, reporting, and **control of zoonotic disease in animals**. When an unusual zoonotic disease trend or outbreak is recognized, veterinarians can assist the investigation through enhanced surveillance for animal disease. Health alerts typically include information on veterinary occupational risks as well as symptoms, diagnosis, and reporting protocols for the disease in animals. There should be veterinary alert systems, as in developed countries, for rapid notification of zoonotic or animal disease outbreaks.

Zoonosis (VeLT4162)

CVMA-AAU, 2020



Gezahegne Mamo (DVM, MSc, PhD, Associate Professor -VPH)

College of Veterinary Medicine and Agriculture

Addis Ababa University

Email: gezahegne.mamo@aau.edu.et

Zoonosis (plu. Zoonoses)

- **Zoonoses are diseases and infections that are naturally transmitted between vertebrate animals (sometimes by a vector) and humans (WHO)**
- **Out of 1,415 pathogens known to infect humans, 61% were zoonotic. About 75% of the new (emerging) diseases that have affected humans over the past 10 years have been caused by pathogens originating from animal or from products of animal origin**
- **Zoonotic diseases are an emerging public health problems and have high overall impact on human health, animal health and hence on livelihood and ecosystem**
- **Among those zoonoses recognized today as particularly important are anthrax, plague, brucellosis, Bovine tuberculosis, leptospirosis, salmonellosis, spotted fever caused by Rickettsiae, rabies, several common arthropod borne viral infections (arboviral infection), certain parasitic diseases, especially cysticercosis, hydatid disease, trypanosomiasis and toxoplasmosis , emerging zoonotic diseases-Ebola, SARS, Avian flu, MERS, Zika others**

History of Zoonosis

- **Rudolf Virchow (1821-1902)** a German physician and pathologist coined the term "zoonosis" to indicate an infectious disease that is passed between humans and animals. He became interested in the linkages between human and veterinary medicine while studying a roundworm, *Trichinella spiralis*, in swine.
- He said, "between animal and human medicine there are no dividing lines--nor should there be."
- WHO in 1959 defined that Zoonoses are "those diseases and infections which are naturally transmitted between vertebrate animals and man".



Classification of Zoonosis

● **Classification mainly based on**

□ **According to the etiological agents:**

- **Bacterial zoonoses e.g. anthrax, brucellosis, plague, leptospirosis, salmonellosis, lyme disease**
- **Viral zoonoses e.g. rabies, arbovirus infections, yellow fever, influenza, Ebola, zika**
- **Rickettsial zoonoses e.g. murine typhus, Q-fever**
- **Protozoal zoonoses e.g. toxoplasmosis, trypanosomiasis, leishmaniasis**
- **Helminthic zoonoses e.g. echinococcosis (hydatid disease), taeniasis, schistosomiasis**
- **Fungal zoonoses e.g. aspergillosis, histoplasmosis, cryptococcosis,**
- **Ectoparasites e.g. scabies, myiasis**

Classification of Zoonosis

According to the mode of transmission

Direct zoonoses

These are transmitted from an infected vertebrate host to a susceptible host (man) by direct contact, by contact with a fomite or by a mechanical vector. The agent itself undergoes little or no propagative or developmental changes during transmission, e.g. rabies, anthrax, brucellosis, leptospirosis, toxoplasmosis.

Cyclozoonoses

These require more than one vertebrate host species, but no invertebrate host for the completion of the life cycle of the agent, e.g. echinococcosis, taeniasis.

Metazoonoses

These are transmitted biologically by invertebrate vectors, in which the agent multiplies and/or develops and there is always an extrinsic incubation (prepatent) period before transmission to another vertebrate host e.g., plague, arbovirus infections, schistosomiasis, leishmaniasis.

Saprozoonoses

These require a vertebrate host and a non-animal developmental site like soil, plant material, pigeon dropping etc. for the development of the infectious agent e.g. aspergillosis, cryptococcosis, histoplasmosis,

Classification of Zoonosis

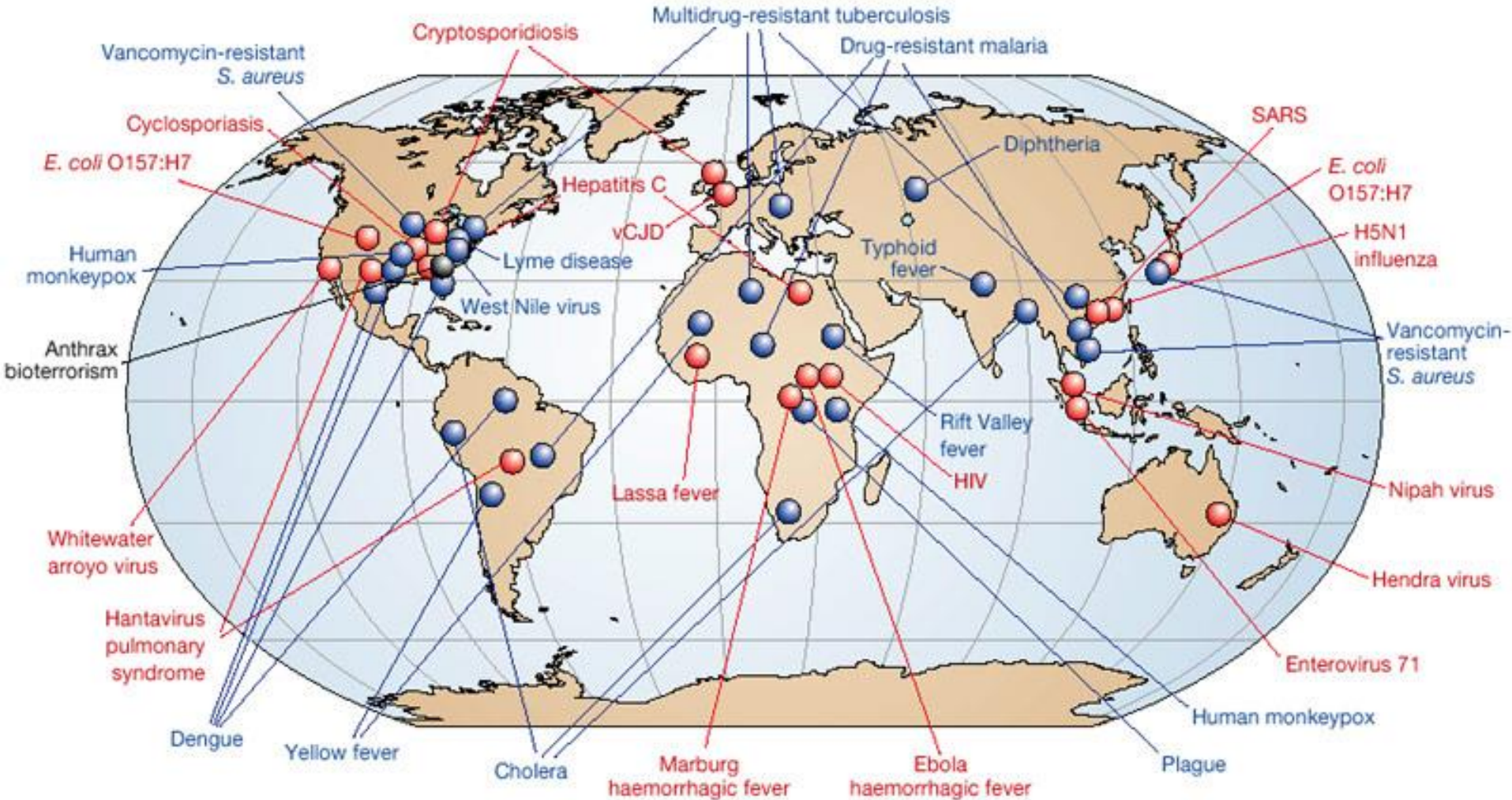
□ According to the reservoir host:

- **Anthropozoonoses:** Infections transmitted to man from lower vertebrate animals e.g. rabies, leptospirosis, plague, arboviral infections, brucellosis and Q-fever.
- **Zooanthroponoses/reverse zoonosis:** Infections transmitted from man to lower vertebrate animals e.g. streptococci, staphylococci, diphtheria, enterobacteriaceae, human tuberculosis in cattle and parrots.
- **Amphixenoses:** Infections maintained in both man and lower vertebrate animals and transmitted in either direction e.g. salmonellosis, staphylococcosis

Emerging and Re-emerging Zoonosis

- **Emerging infectious disease is a one that is caused by a newly discovered infectious agent or by a newly identified variant of a known pathogen, which has emerged and whose incidence in humans has increased during the last two decades and is threatening to increase in the near future**
- **A re-emerging infectious disease is a one which was previously controlled but once again has risen to be a significant health problem.**
It also indicates a disease which was formerly confined to one geographic area, has now spread to other areas

Emergence of infectious diseases



Morens et al., 2004, Nature 430, 242-49

The Coronavirus Epidemic

COVID-19 CORONAVIRUS OUTBREAK
Last updated: February 19, 2020, 13:05 GMT

Coronavirus Cases:

75,317

Deaths:

2,012

Recovered:

15,072



SITUATION IN NUMBERS total and new cases in last 24 hours

Globally

93 090 confirmed (2223 new)

China

80 422 confirmed (120 new)
2984 deaths (38 new)

Outside of China

12 668 confirmed (2103 new)
76 countries (4 new)
214 deaths (48 new)

ACTIVE CASES

58,233

Currently Infected Patients

46,177 (79%)
in Mild Condition

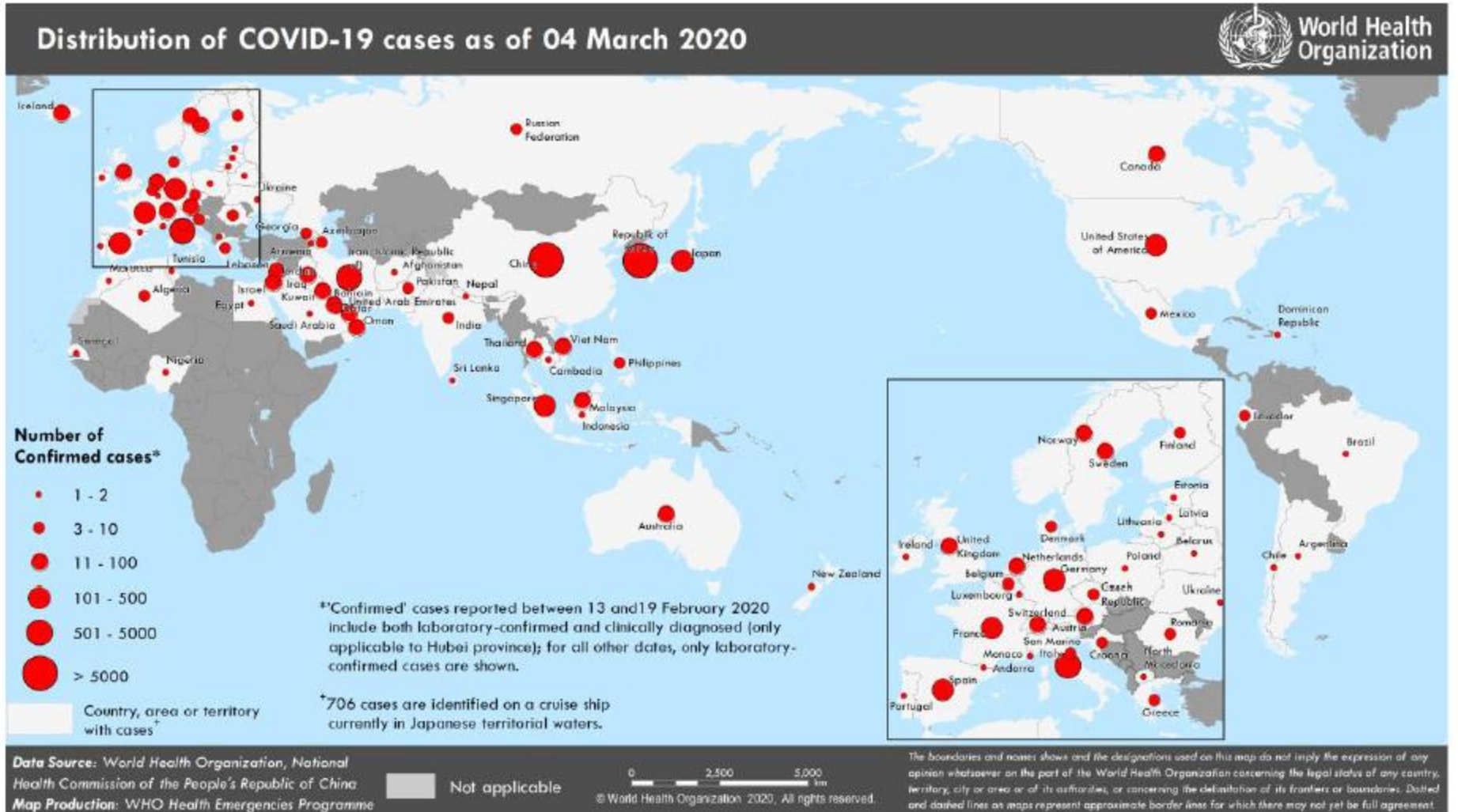
12,056 (21%)
Serious or Critical

WHO RISK ASSESSMENT

China	Very High
Regional Level	Very High
Global Level	Very High

2,012 (12%)
Deaths

Figure 1. Countries, territories or areas with reported confirmed cases of COVID-19, 04 March 2020



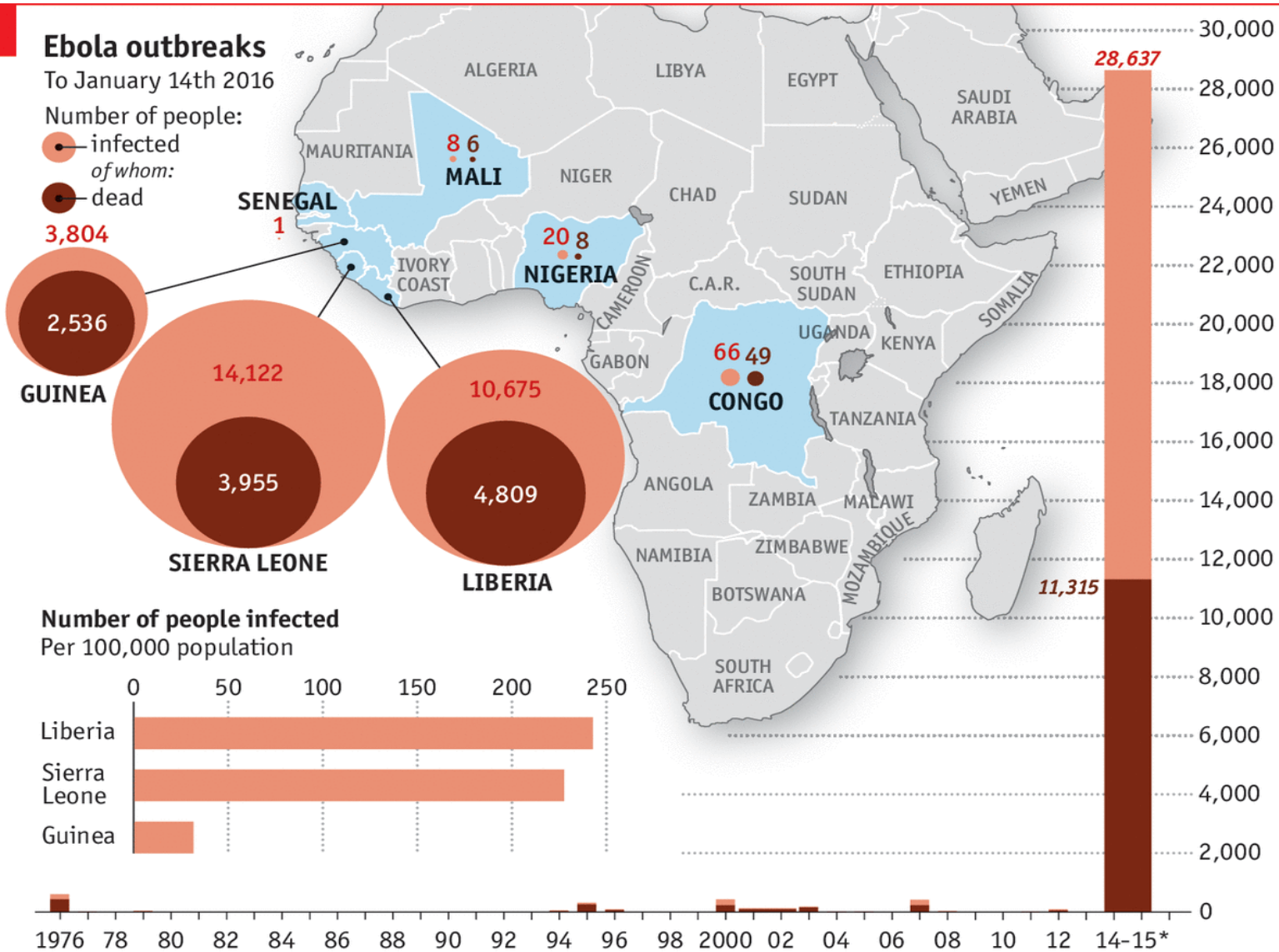
Ebola in Africa: the end of a tragedy?

Ebola outbreaks

To January 14th 2016

Number of people:

- infected
- of whom:
- dead



Sources: WHO; UN; *The Economist*

*Includes cases in Italy, Spain, Britain and the United States. Excludes Congo

Economist.com

<http://www.economist.com/blogs/graphicdetail/2016/01/daily-chart-12>

Gezahegne M-CVMA Zoonosis VLT III 2020

Reasons for zoonotic disease emergence

❑ Ecological changes in man's environment

- With the expansion of human population, man is forced to exploit the virgin territories and natural resources which increases exposure to potential pathogens in the new territories

❑ Genetic drift and shift (influenza)

❑ Modification of the immunological status of populations

- ❑ Change in susceptibility of populations; vaccination

❑ Handling animal by-products and wastes (occupational hazards)

- Q-fever in abattoir and rendering plant workers, tick borne diseases in wood cutters, salmonellosis in food processors, bovine tuberculosis in farmer

Reasons for zoonotic disease emergence

❑ Increased trade in animal products

- Countries which import hides, wool, bone meal, meat, etc. from an area where some of the zoonoses are endemic, are likely to introduce the disease into their territories, e.g. salmonellosis, foot and mouth disease, anthrax, Newcastle disease etc.

❑ Increased density of animal population

- Animals may carry potential risk of increased frequency of zoonotic agents in man e.g. dermatophytosis, tuberculosis, brucellosis etc.

❑ Transportation of virus infected mosquitoes

- Aircraft, ship, train, motor and other vehicles bring the viruses in to a new area, e.g. yellow fever, Chikungunya fever, dengue fever, Zika virus etc.

❑ Cultural anthropological norms

- Consumption of raw animal products (raw meat, milk or blood) as a cultural habit expose the population to zoonotic diseases eg. Brucellosis in pastoral communities

People at higher risk of zoonotic diseases

- **Poverty affected communities**
- **Pastoralists and people with close contact with livestock**
- **People with low population immunity (HIV, age)**
- **Areas of altered climate and increased vector range**
- **Areas of political unrest and population migration**
- **Natural disaster affected areas**
- **Areas of deforestation and altered biodiversity**
- **People affected by global trade and travel**

Bacterial Zoonotic diseases

 **Zoonoses can be classified by infectious agent type: Bacteria, Viruses, Parasites, Fungi, Prion**

- **Bacterial Zoonosis:**

- **Zoonotic tuberculosis, Anthrax,, Brucellosis , Salmonellosis, *Escherichia coli* O157:H7, Campylobacteriosis, Leptospirosis,**

Zoonotic Tuberculosis

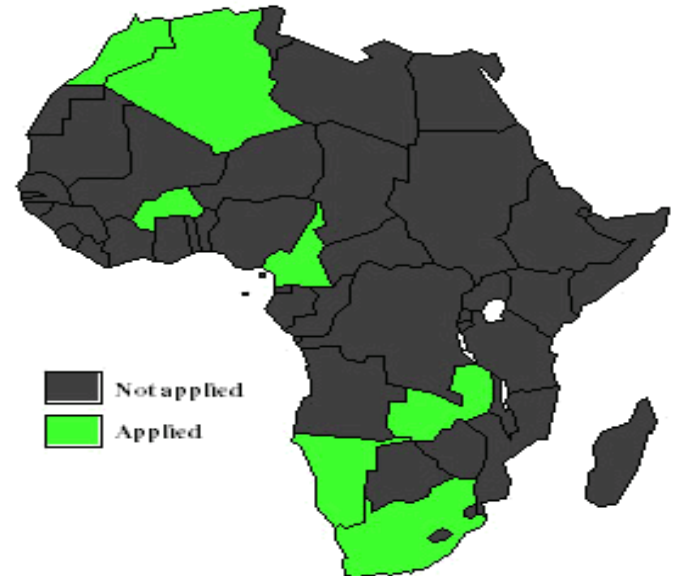
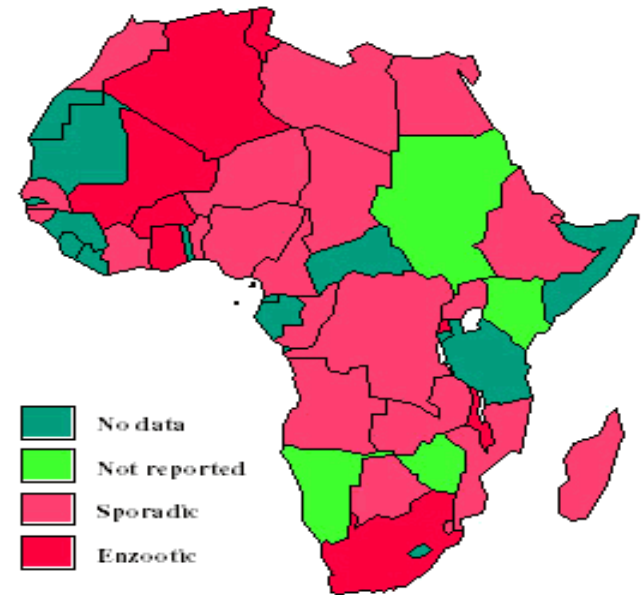
- Zoonotic tuberculosis is human tuberculosis caused by *Mycobacterium bovis* which originated from animals
- *M. bovis* – a wide host range affect domestic and wild animals, and humans
 - Cattle
 - Humans and non-human primates
 - Goats, sheep, cats, dogs, pigs, buffalo, badgers, possums, deer, elk, bison, horses, foxes, hares, ferrets, antelope, camels, llamas, alpacas

Causes of Human TB

- **Developed countries estimates**
 - *M. tuberculosis*: 99% of cases
 - *M. bovis*: 1% of cases (at present)
 - Prior to pasteurization (1908) – estimated 10-30% of human TB cases due to *M. bovis* in US
- **Developing countries**
 - Unknown, but *M. bovis* likely to account for higher percentage of disease where organism is enzootic in cattle and pasteurization is not widely used (e.g., parts of Africa)
 - Presently, 10-15% HTB caused by *M. bovis* (Ashford *et al.*, 2001)
 - Poor countries, which are high burden for TB, rely on AFB smear – do not perform cultures so difficult to differentiate

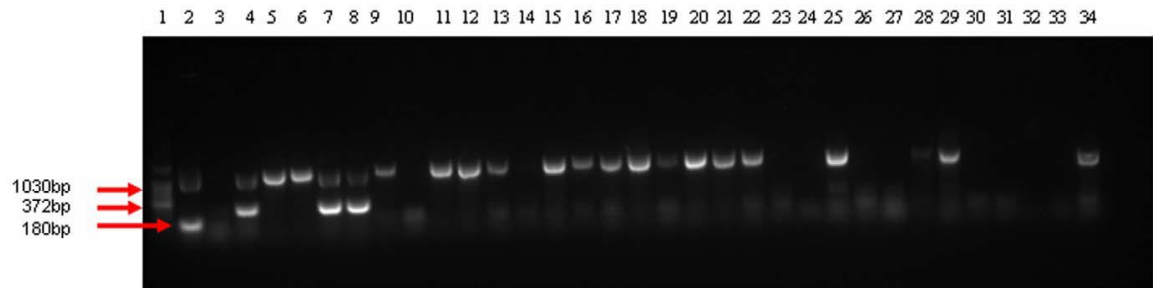
Zoonotic tuberculosis

- In Africa, nearly 85% of livestock and 82% of the human population live in areas where the disease is endemic or partially controlled
- **Human disease caused by *M. bovis* has been confirmed in African countries.**
- **Egypt (6.4%), Nigeria (3.9%), Tanzania (36% from Lymph node biopsy-LNB)- Cosivi *et al.*, 1998**
- **Ethiopia (17%- Kidane *et al.*, 2002 from LNB in Butajira)- controversial –**
-Recently, Gumi *et al.*, 2012- 1.73%- Borna from PTB, Mamo *et al.*, 2013- 1.3%-Afar from PTB cas



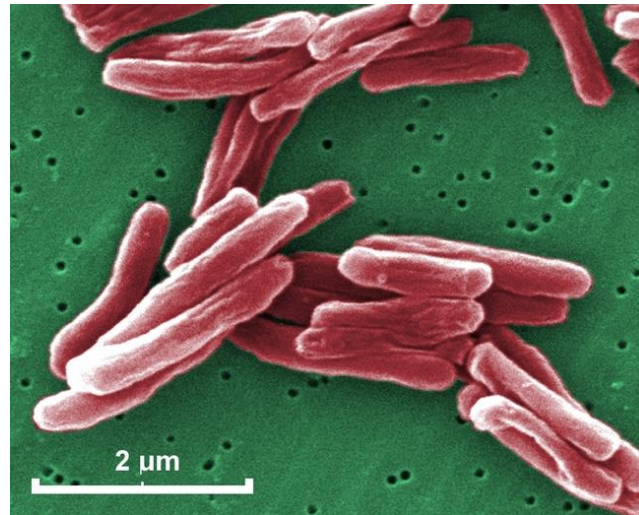
Manifestations of Tuberculosis Caused by *M. bovis* in human

- ***M. tuberculosis* disease and *M. bovis* disease in humans are clinically and radiographically indistinguishable**
- **Differentiation depends upon laboratory isolation and identification**
 - Culture based biochemical tests
 - Molecular techniques- Confirmatory



Genus: *Mycobacterium*

- **Phylum: *Actinobacteria*, Family: *Mycobacteriaceae*, Genus: *Mycobacteria***
- **~198 species of *Mycobacteria*** (<http://www.bacterio.net/mycobacterium.html>)
- **Thick cell wall composed of lipid**
- **All species in the genus *Mycobacterium* show ~ 94.3% sequence similarity of genes in the DNA**



SEM 15549x Photo: Janice Haney Carr

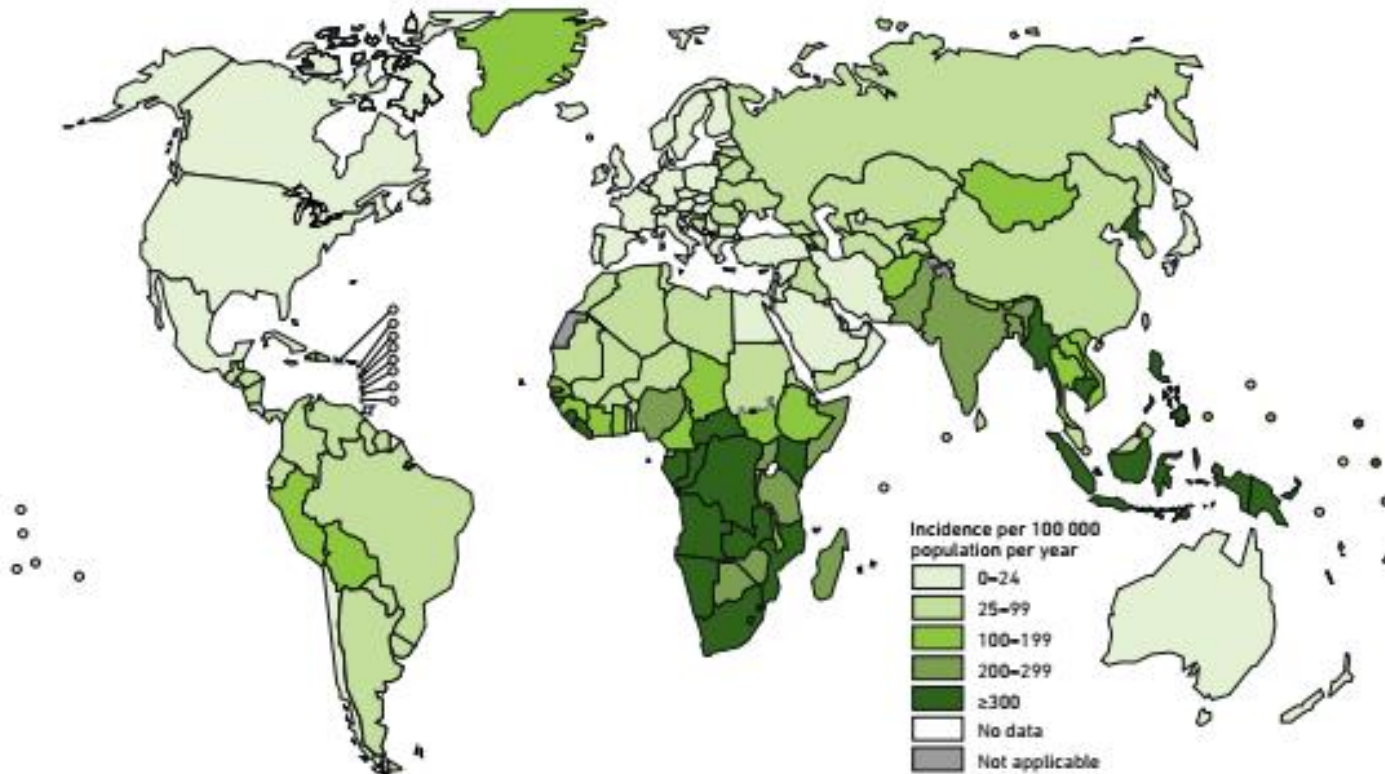
***Mycobacterium tuberculosis* complex**

- *Mycobacterium tuberculosis*, *M. africanum*, *M. bovis*, *M. caprae*, *M. microti*, *M. pinnipedii*, *M. canettii*
- High genetic similarity (99.95%) at a nucleotide level
- Differ with respect to host range, pathogenicity, limited phenotypic and /or genotypic traits
- Major public health problem
- *M. bovis* has the largest host range (domestic and wild animal, human)

Mycobacterium tuberculosis complex

- Approximately 1.7 billion (23%) individuals of the world population estimated with latent TB (Houben and Dodd, 2016- PLoS Med , Vol 13)
- About 10 million new cases and 1.3 million TB death in 2017 (WHO, 2018)

Estimated TB incidence rates, 2017



(WHO, 2018)

Epidemiology of BTB in Ethiopia (2000-2016)

B. Sibhat et al.

Preventive Veterinary Medicine 147 (2017) 149–157

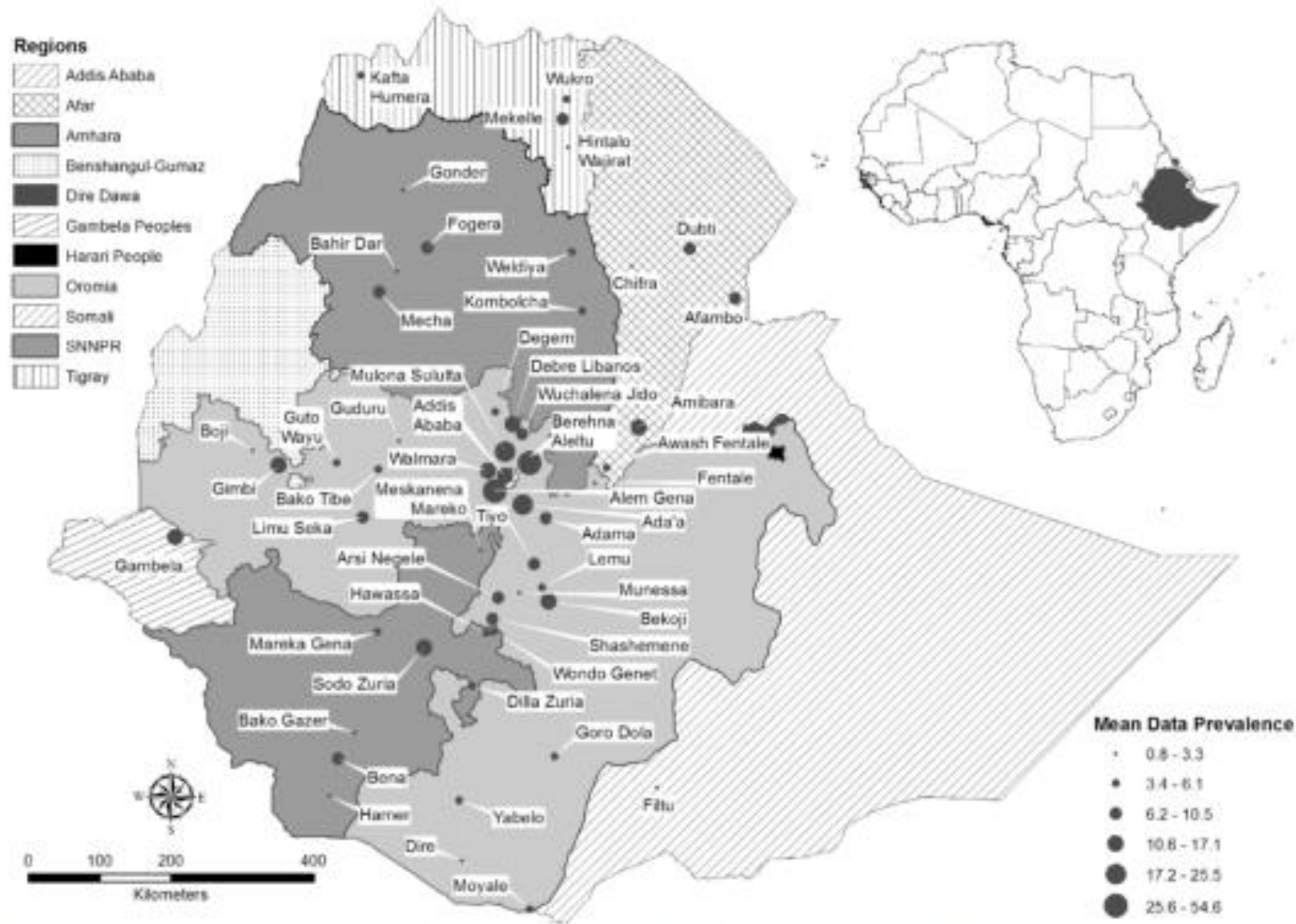


Fig. 4. Distribution and mean prevalence of bovine tuberculosis in districts of Ethiopia. Most of the studies were conducted in Addis Ababa, Amhara, Oromia and Southern Nations and Nationalities Peoples regions while no valid published study was obtained from Benishangul-Gumuz, Harari and Dire Dawa regions. On the other hand, few studies were undertaken in Afar, Gambella, Somali and Tigray regions. Variable animal level prevalence of BTB were recorded in the districts of the regions ranging from 0.8% to 54.6%; the highest prevalence being reported in intensive farms in and around cities while the lowest prevalence being recorded in grazing animals in rural areas.

Source of pathogenic mycobacteria

● Usually infected animals

● *Mycobacterium bovis* – respiratory discharge, faeces, milk, urine and semen

● *M. avium* and *M. paratuberculosis* – shed in faeces

● *M. tuberculosis* – mainly respiratory discharges

● Wild animal reservoirs of *M. bovis*

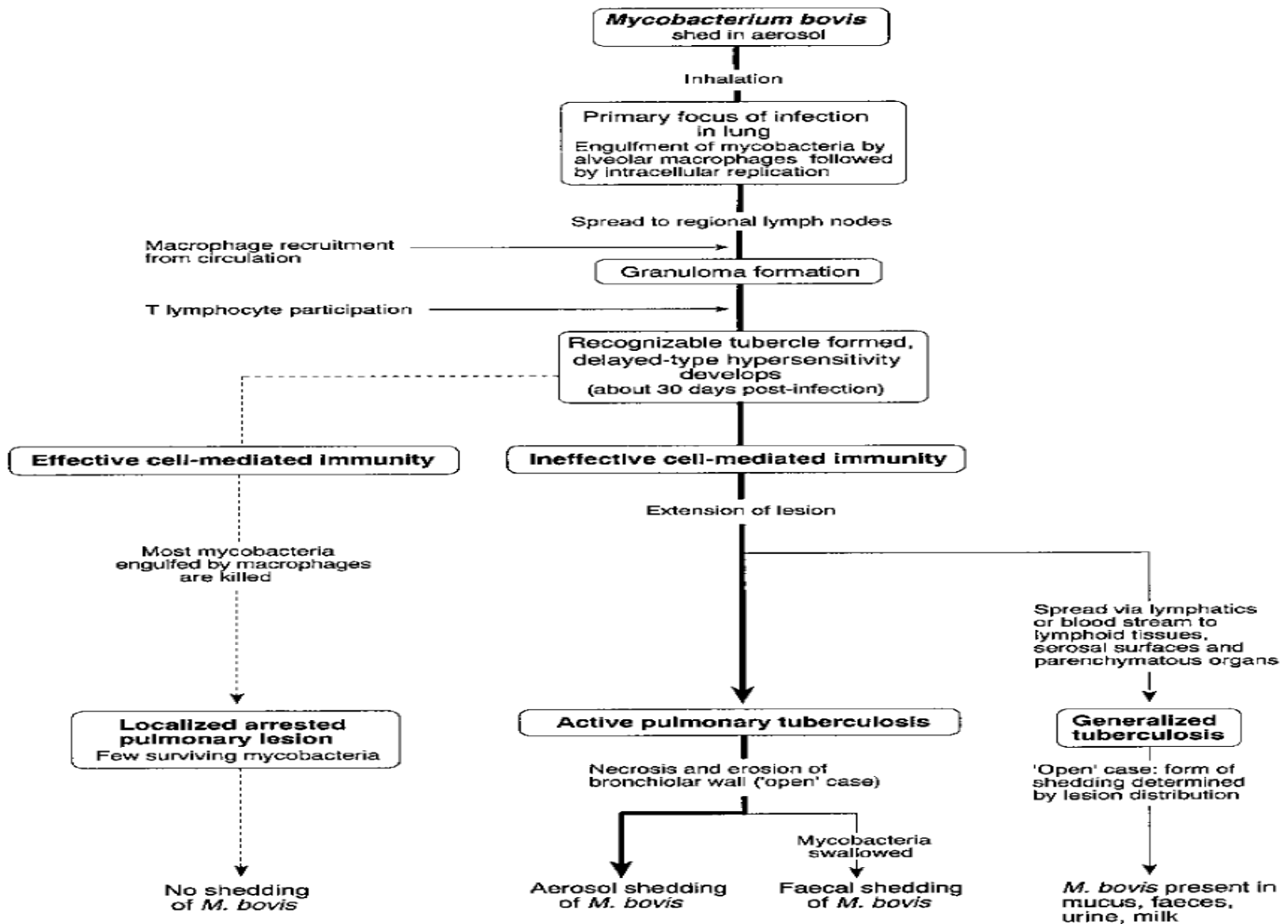
● Badgers in Europe , Brush-tailed opossums in New Zealand, Cape buffalo in East Africa, deer in Europe and America

● No information on Ethiopian wildlife reservoir

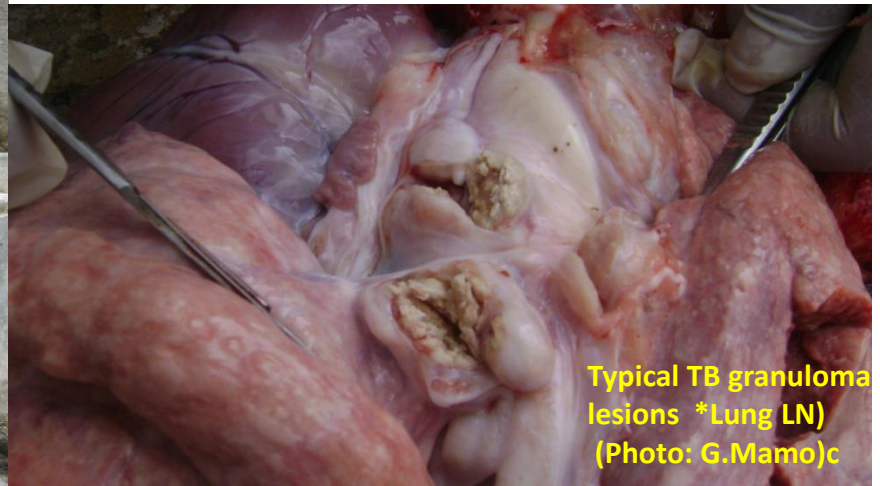
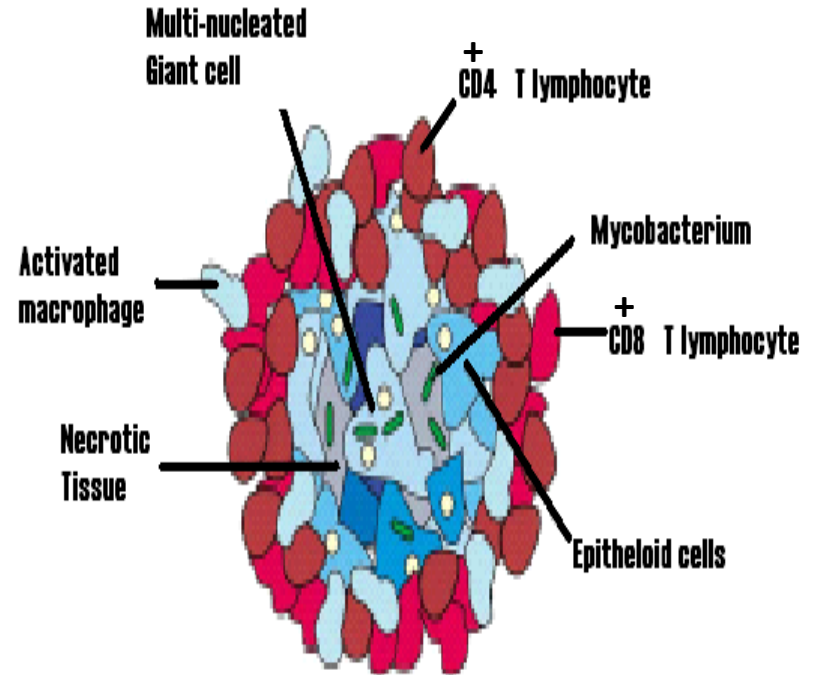
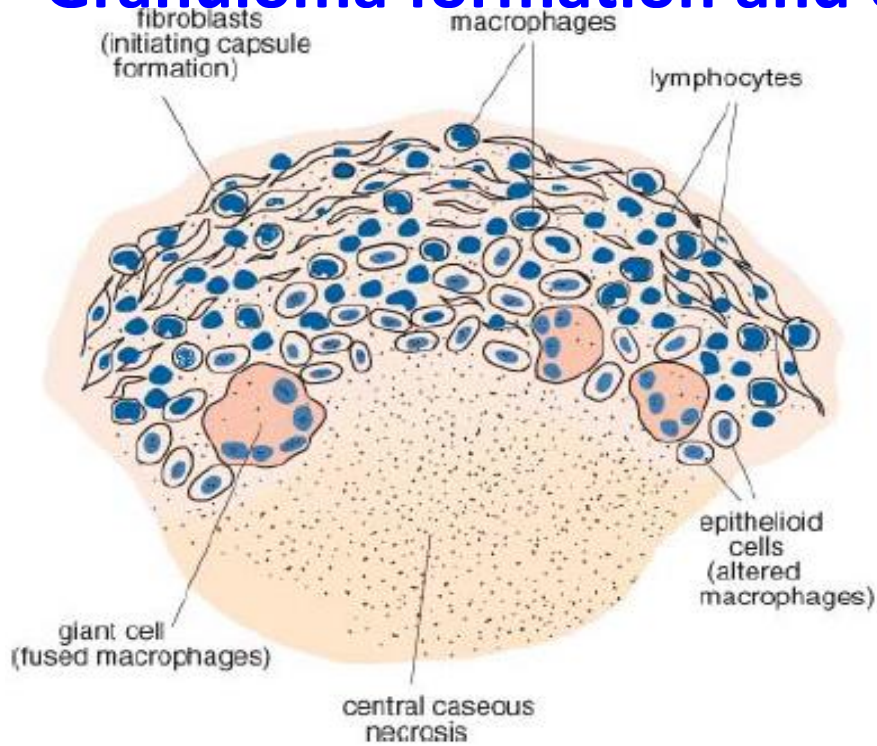
● Mycobacteria are resistant to physical influences , will retain their viability in soil and dried faeces for many months

● Atypical (opportunistic) mycobacteria widespread in soil, pasture, marshland and water

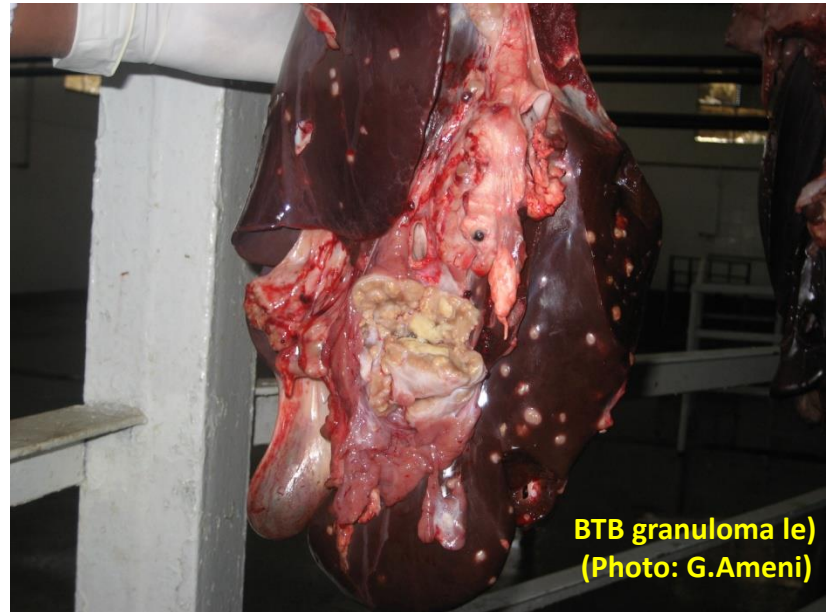
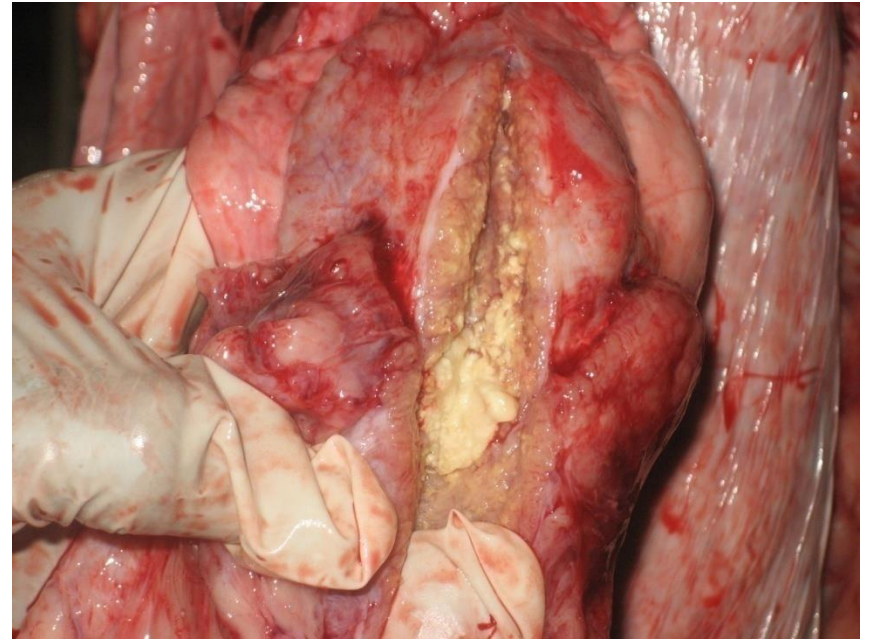
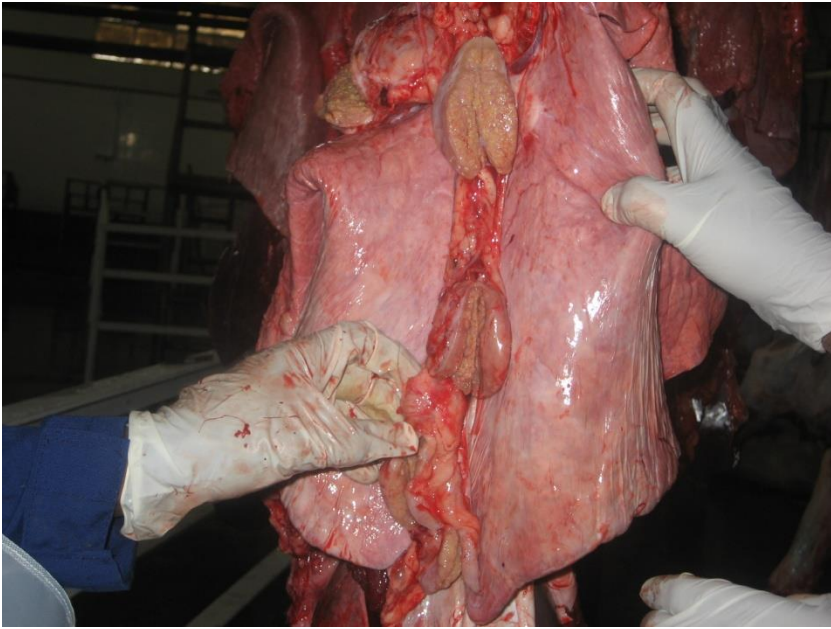
Pathogenesis *M. bovis*



Granuloma formation and caseous necrosis



Tuberculous lesions in Cattle



**BTB granuloma le)
(Photo: G.Ameni)**

Laboratory diagnosis

Mycobacteria causing TB and atypical mycobacteria

Specimens

- From live animals – aspirates from cavities, lymph nodes, biopsies, tracheobronchial lavages, and milk
- From dead animals – tuberculous lesions from lung, lymph nodes and other tissues

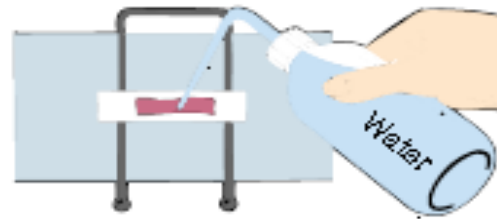
Direct microscopy

- The Ziehl-Neelsen (acid-fast) stain is used to stain smear from lesions and other specimens

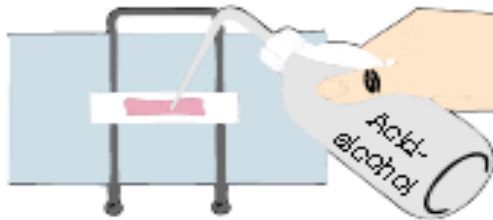
ZN (acid-fast) staining



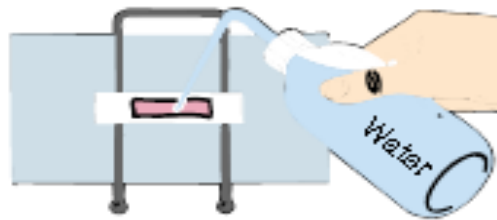
(a) Apply carbol-fuchsin to saturate paper and heat for 5 minutes in an exhaust hood



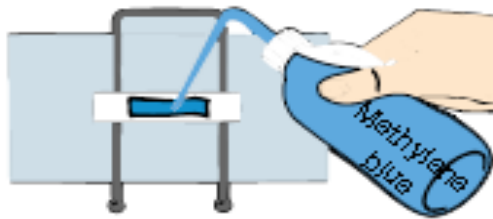
(b) Cool and rinse with water for 30 seconds



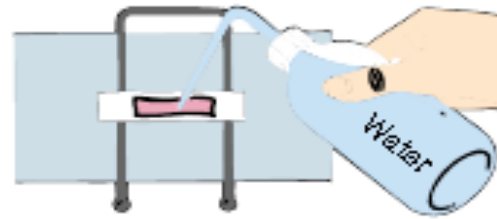
(c) Decolorize with acid-alcohol until pink (10-30 seconds)



(d) Rinse with water for 5 seconds



(e) Counterstain with methylene blue for about 2 minutes

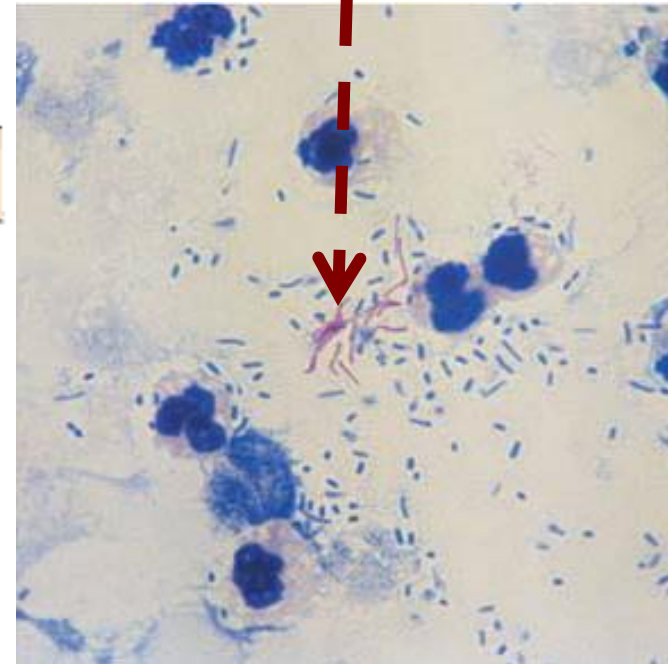


(f) Rinse with water for 30 seconds



(g) Blot dry with bibulous paper

AFB +ve



Laboratory diagnosis

Mycobacteria Isolation

🌐 Preliminary procedures which are necessary to recover slow-growing mycobacteria

- Selective decontamination- to reduce fast-growing contaminating bacteria such as 5% oxalic acid, 2-4% NaOH, 1% quaternary ammonium compound, hexadecylpyridinium chloride (HPC)
- Digestion of mucus – to release mucin-trapped mycobacteria from sputum for growth in culture medium such as zephiran-trisodium phosphate
- Centrifugation of specimen- allow to concentrate small numbers of mycobacteria for smear or culture

🌐 Media for mycobacteria

- Egg-based Lowenstein-Jensen (LJ) , Stonebrinks media , Middle brook (MB) 7H10 agar
- Media are prepared as solid slants in screw-capped bottles
- Malachite green dye (0.025g/100m) – used as selective agent
- M. tuberculosis*, *M. avium* and many atypical mycobacteria require glycerol for growth
- M. bovis* require sodium pyruvate supplement for growth and glycerol inhibits growth
- M. paratuberculosis* requires mycobactin (iron supplement)
- Incubation of media at 37°C for up to 8wks -12wks
- M. leprae*, *M. lepraemurium* and mycobacteria causing bovine skin tuberculosis has not been cultured *in vitro*.

Laboratory diagnosis

Mycobacteria Identification

Colony morphology

Eugonic- characteristics 'rough, tough and buff' colonies – *M. tuberculosis* while *M. avium* colonies that break up easily

Dysgonic- sparse, thin and moist-sheen colony growth of *M. bovis* on media containing glycerol, however *M. bovis* grows well on pyruvate containing media as smooth colony

Pigment production and response to light

Chromogenic – pigment producing mycobacteria

Photochromogenic and Scotochromogenic

Non-chromogenic

Microscopic appearance – mycobacteria from culture appear red-staining, thin, long rod in ZN stain

Biochemical tests

Niacin production

Deamination of pyrazinamide

Nitrate reduction

Susceptibility to thiophen-2-carbonic acid hydrazide (TCH)



M. tuberculosis on Lowenstein-Jensen medium



Laboratory diagnosis

Table 17.2 Clinical significance, growth characteristics and biochemical differentiation of pathogenic mycobacteria.

	<i>M. tuberculosis</i>	<i>M. bovis</i>	<i>M. avium</i> complex	<i>M. avium</i> subsp. <i>paratuberculosis</i>
Significance of infection	Important in man and occasionally in dogs	Important in cattle and occasionally in other domestic animals and man	Important in free-range domestic poultry, opportunistic infections in man and domestic animals	Important in cattle and other ruminants
Cultural characteristics and requirements				
Growth rate	Slow (3-8 weeks)	Slow (3-8 weeks)	Slow (2-6 weeks)	Very slow (up to 16 weeks)
Optimal incubation temperature	37°C	37°C	37-43°C	37°C
Atmospheric requirements	Aerobic	Aerobic	Aerobic	Aerobic
Colonial features	Rough, buff, difficult to break apart	Cream-coloured, raised with central roughness, break apart easily	Sticky, off-white, break apart easily	Small, hemispherical; some pigmented
Essential growth supplement	None	None	None	Mycobactin
Effect of added glycerol	Enhanced growth (eugonic)	Growth inhibited (dysgonic)	Enhanced growth (eugonic)	
Effect of added sodium pyruvate	No effect	Enhanced growth	No effect	
Biochemical differentiation				
Niacin accumulation	+	-	-	
Pyrazinamidase production	+	-	+	
Nitrate reduction	+	-	-	
Susceptibility to TCH (10 µg/ml) ^a	Resistant	Susceptible	Resistant	

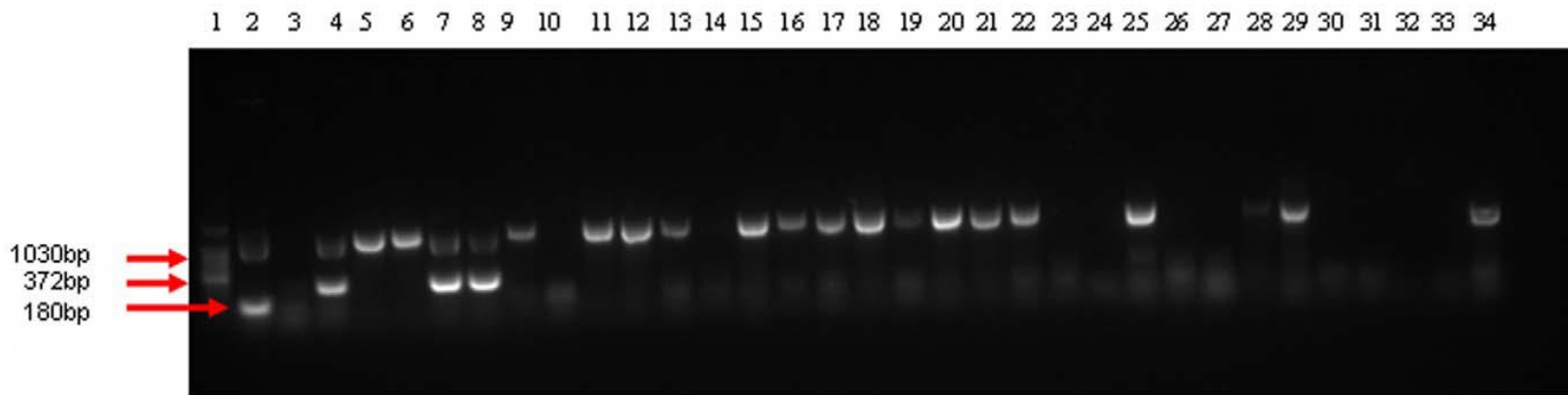
a TCH, Thiophen-2-carboxylic acid hydrazine

Laboratory diagnosis

Molecular methods of Identification of mycobacteria

🌐 Nucleic acid probe methods – for identification of *M. tuberculosis* complex, *M. avium* complex and atypical mycobacteria

📄 Multiplex PCR method , Region of Deletion (RD) PCR method, Spoligotyping method , RFLP method , MIRU-VNTR method , 16S rRNA sequencing



Multiplex PCR from camel samples (Mamo *et al.* , 2011, PLoS One Volume 6 | Issue 1 | e15862)

Spoligotyping

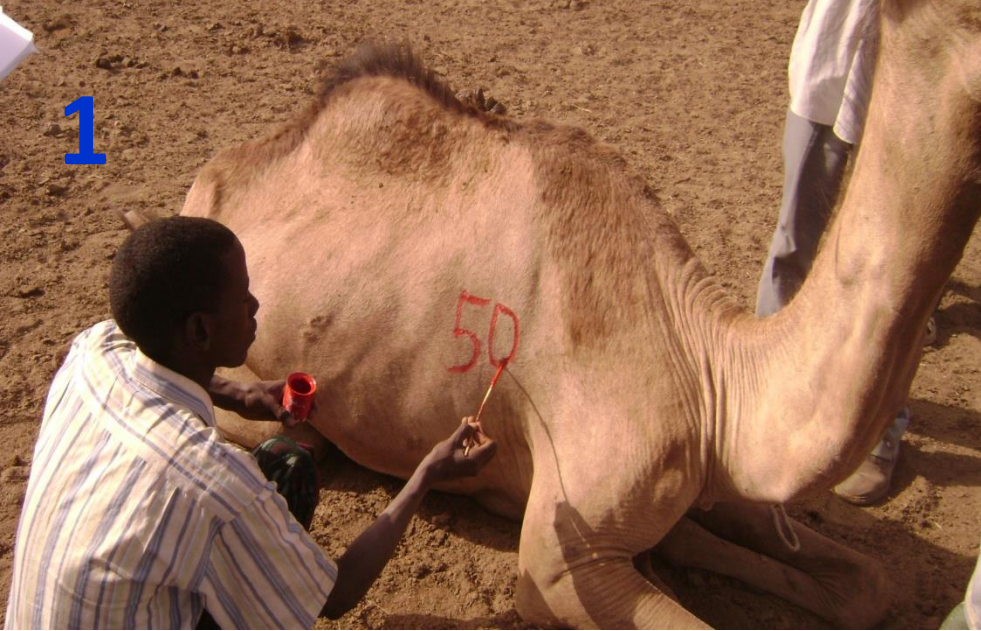


Spoligotyping results from Human PTB patients Afar-(Mamo,2014)

Field and Laboratory immunologic tests for tuberculosis

Single Comparative Intradermal Tuberculin test (SCIDT)

- Tuberculin (PPD, purified protein derivative)- prepared from *M. bovis* or *M. avium* , is a complex mixture of mainly proteins, lipids, and carbohydrates
- SCIDT involves injection of avian and bovine tuberculins simultaneously at different sites (12cms apart) into the skin of the neck
- Tuberculous animal show a delayed-type hypersensitivity reaction at the injection site in around 72hrs post-inoculation
- The reaction is characterized by thickening of the skin due to mononuclear cell infiltration – indurations of skin 4mm or more than the avian PPD injection site and the animal is termed as bovine reactor
- False positive can occur in case of infection with other mycobacteria other than *M. bovid s* (eg. *M. avium*, atypical mycobacteria)
- False negative (unresponsiveness) can also occur and is called Anergy – might be caused by different reasons- immunological, physiological status (postpartum period),



**Comparative intradermal
tuberculin test (CIDT)**

Gezahegne M-CVMA Zoonosis VLT III 2020

5 Reading after 72 hrs

Laboratory diagnosis

Serological tests

● Other tests include ELISA, gamma interferon assay using whole blood (Bovigam) have been developed

Histopathology

- In Ziehl-Neelsen (acid-fast) stained smear of scrapings from granuloma or biopsies/fine needle aspirate of the lymph nodules shows numerous long, slender, acid-fast rods are seen
- Histopathological sections stained with ZN staining shows also acid-fast mycobacteria
- Mycobacteriological culture isolation should be attempted for confirmation and characterization

Zoonotic TB in Ethiopia

- ❑ Isolation of *M. bovis* from human lymphadenitis cases in Ethiopia indicate the role of *M. bovis* in the increasing human TB cases(Kidane *et al.*, 2002)
 - ❑ *M. bovis* was found to be cause for 17.1% (6/35) TB lymphadenitis in Butajira, South Ethiopia based on PCR- but recent work showed no *M. bovis* from FNA collected from the same area – **Controversial**
- ❑ With increasing HIV infection in Ethiopia and the practice of consumption of raw animal products by most of Ethiopian population there is a potential risks of zoonosis of *M. bovis* in human
- ❑ In rural areas of Ethiopia most people drink raw milk and have very close contact with cattle (sharing shelter at night) can intensify the risk of transmission and spread of *M. bovis*
- ❑ Regassa (2005) demonstrated that cattle owned by TB patients had higher prevalence (24.3%) than cattle owned by non tuberculous owners (8.6%). And the author also noted that 16.7% of 42 human isolates were identified as *M. bovis*

Risk factors for transmission of BTB from Animal to human in Ethiopia

Close Physical contact :

- Close physical contacts between human and potentially infected animals is present, particularly in rural areas of the country
- Domestic animals enclosed in the same house with the owner and this create a potential risk for aerogenous transmission of *Mycobacterium* spp. from animals to human or vice versa.

Consumption of raw milk and meat

- The most prevalent form of the disease in man caused by *M. bovis* is extra pulmonary tuberculosis
- This is mainly due to consumption of raw milk and consumption of raw meat, fresh animal blood consumption as a cultural habit by most Ethiopian are the most important potential risk for *M. bovis* infection in Ethiopia.

Risk factors of transmission



Watering point (Afar-Halidage)
Photo: Gezahegne M.



Ape drinking from tapwater
(Photo: Gezahegne M-Afar)

Human

Domestic animals

Wild animals

Co-infection with HIV/AIDS

- **Human immune deficiency virus is the leading risk factor for tuberculosis**
- **In study conducted at Butajira, South Ethiopia, HIV infection is associated with mycobacterial species (Kidane *et al.*, 2002). The result showed that there is higher tuberculosis infection in HIV positive patients 10/11 (90.9%) than HIV negative 25/29 (86.2%).**

Lack of control Mechanisms

- **For control and elimination of BTB the implementation of test and slaughter policy has been shown to be efficient in developed countries**
- **This control strategy is not feasible to apply in most developing countries**
- **In Ethiopia, this measure can not be adopted in practice due to:**
 - **Lack of knowledge on the actual prevalence**
 - **The existing technical and financial limitations**
 - **cultural and traditional beliefs**
- **In addition, lack of practice pasteurization of milk and eating of raw meat is some of the potential risk for zoonosis of BTB in Ethiopia**
- **Few attempts have been undertaken in government owned dairy farms Eg. The Holetta Bull Breeding Center successfully controlled BTB in its farm through test and slaughter method**

Reverse zoonosis of TB



Fig. 1. A farmer in central Ethiopia discharging tobacco juice directly into the oral cavity of his cattle, a common practice in this region and a possible route of transmission of *Mycobacterium tuberculosis* from humans to cattle.

In conclusion, this study highlights the possible risk of human-to-cattle transmission of *M. tuberculosis* through the practice of mouth-to-mouth feeding of tobacco juice and/or where animals live in close contact with tuberculous humans. Epidemiological studies are ongoing to determine the impact of tobacco juice feeding on cattle health and on the potential for transmitting *M. tuberculosis* to cattle.

Reverse zoonosis of TB

Hindawi Publishing Corporation
Veterinary Medicine: An International Journal
Volume 2012, Article ID 863146, 8 pages
doi:10.1155/2012/863146

Research Article

Tuberculosis in Goats and Sheep in Afar Pastoral Region of Ethiopia and Isolation of *Mycobacterium tuberculosis* from Goat

Gezahegne Mamo Kassa,^{1,2,3} Fekadu Abebe,³ Yalalel Worku,^{2,4} Mengistu Legesse,^{1,3}
Girmay Medhin,¹ Gunnar Bjune,³ and Gobena Amen¹

¹Akkaba, Seveera, Institute of Pathobiology, Addis Ababa University P.O. Box 1176, Addis Ababa, Ethiopia

²Faculty of Veterinary Medicine, Addis Ababa University P.O. Box 34, Didero Zet, Ethiopia

³Faculty of Medicine, Institute of Health and Society, Department of Community Medicine,
International Community Health, University of Oslo, P.O. Box 1154, Blindern 04-0318 Oslo, Norway

⁴Afar Pastoral Agricultural Bureau, Afar Pastoral Region P.O. Box 79, Seveera, Ethiopia

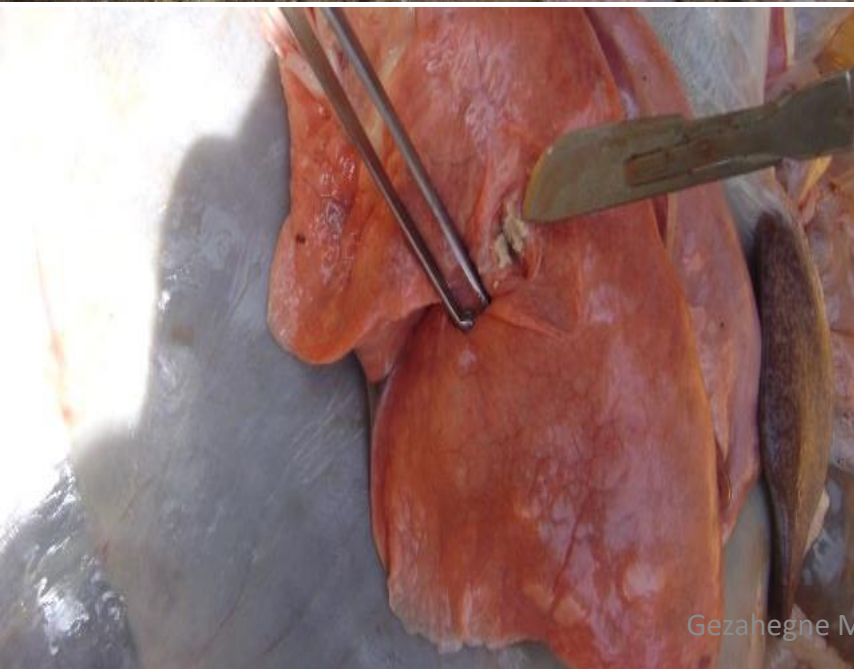
Correspondence should be addressed to Gezahegne Mamo Kassa, g.m.kassa@studmed.uio.no

Received 3 February 2012; Accepted 21 May 2012

Academic Editor: Michael D. Walsh

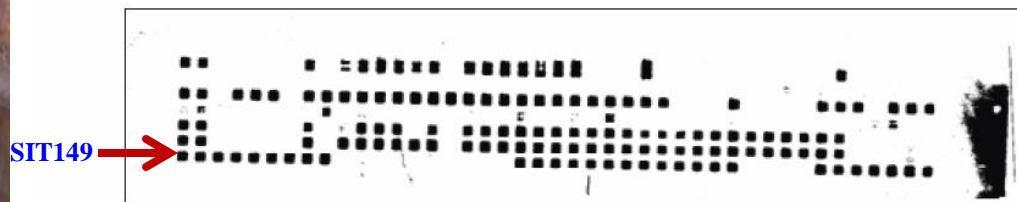
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A cross-sectional study was conducted on 2231 small ruminants in four districts of the Afar Pastoral Region of Ethiopia to investigate the epidemiology of tuberculosis in goats and sheep using comparative intradermal tuberculin skin test, postmortem examination, mycobacteriological culture and molecular typing methods. The overall animal prevalence of TB in small ruminants was 05% (95% CI 02%–07%) at 25 mm and 33% (95% CI 3%–47%) at cutoff 22 mm. The herd prevalence was 20% (95% CI 12–28%) and 49% (95% CI 37–56%) at 25 mm and 22 mm cut-off points, respectively. The overall animal prevalence of *Mycobacterium* *avium* complex infection was 23% (95% CI 2.1–3.5%) and 63% (95% CI 5.8–7.9%) at 25 mm and 22 mm cut-off points, respectively. Mycobacteriological culture and molecular characterization of isolates from tissue lesions of tuberculin reactor goats resulted in isolation of *Mycobacterium tuberculosis* (H37Rv) and non-tuberculosis mycobacteria as causative agents of tuberculosis and tuberculosis-like diseases in goats, respectively. The isolation of *Mycobacterium tuberculosis* in goat suggests a potential transmission of the causative agent from human and warrants further investigation in the role of small ruminants in epidemiology of human tuberculosis in the region.



Tuberculosis in Goat

● *M. tuberculosis* SIT149

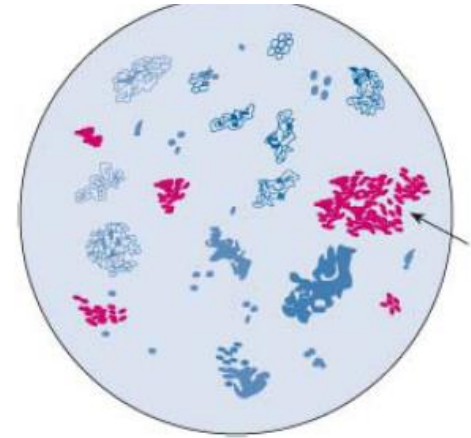


Conclusion

- In Ethiopia, there is lack nation wide information on extent of , risk factors zoonotic TB and role of *M. bovis* in human tuberculosis, hence further researches should be promoted with emphasis on pastoral area
- Feasible control measures should be designed at a national level to control BTB in livestock and reduce the risk of transmission of *M. bovis* to human
 - ❑ Implementation of pasteurization of milk before consumption
 - ❑ Detailed meat inspection in abattoirs
- Promotion of regional and international collaborations for development of efficient vaccine

Brucellosis

- *Brucella* species are small (0.6 x 0.6 to 1.5 µm), nonmotile, coccobacillary, Gram-negative bacteria
- *Brucella* are modified Ziehl-Neelsen (MZN)-positive- Since they are not decolourized by 0.5% acetic acid in the MZN staining technique and on this smear characteristically appear as clusters of red coccobacilli
- *Brucella* species include the classical important six species :
 - ❑ *B. abortus* (cattle, biovars 1-6, and 9)
 - ❑ *B. melitensis* (goats, sheep, biovars 1-3)
 - ❑ *B. suis* (pigs, reindeer and hares, biovars 1-5)
 - ❑ *B. ovis* (sheep),
 - ❑ *B. canis* (dogs)
 - ❑ *B. neotomae* (desert wood rats).
 - ❑ *B. ceti* (dolphins/porpoises), *B. pinnipedialis* (seals), *B. microti* (voles) and *B. inopinata* (reservoir undetermined)- New members included recently
- *Brucella* species are aerobic, capnophilic (requires CO₂) and catalase-positive, urease-positive except *B. ovis*
- *Brucella* species are causes of brucellosis, an infectious diseases of livestock and also human with major economic and public health importance



Brucella species and their host/clinical significance

<i>Brucella</i> species	Usual host/clinical significance	Species occasionally infected/clinical significance
<i>B. abortus</i>	Cattle/abortion, orchitis	Sheep, goats, pigs/sporadic abortion Horses/bursitis Humans/intermittent fever, systemic disease
<i>B. melitensis</i>	Goats, sheep/abortion, orchitis, arthritis	Cattle/sporadic abortion, brucellae in milk Humans/Malta fever, severe systemic disease
<i>B. suis</i>	Pigs/abortion, orchitis, arthritis, spondylitis, infertility	Humans/intermittent fever, systemic disease
<i>B. ovis</i>	Sheep/epididymitis in rams, sporadic abortion in ewes	
<i>B. canis</i>	Dogs/abortion, epididymitis, discospondylitis, sterility in male dogs	Humans/mild systemic disease
<i>B. neotomae</i>	Desert wood rat/not isolated from domestic animals	

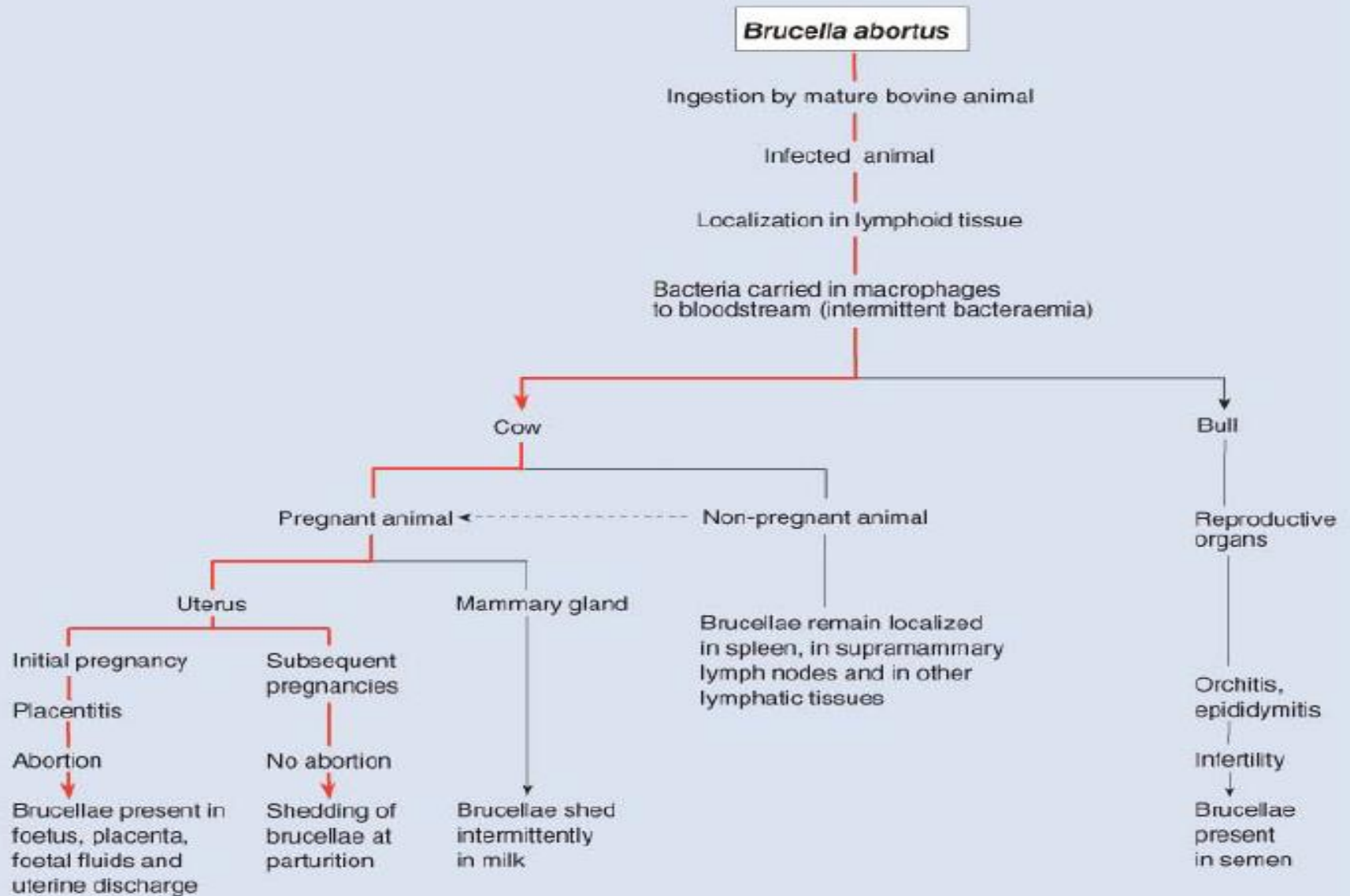
Pathogenesis of Brucellosis

- The **establishment and outcome of infection with brucellae** depend on the **number of infecting organisms** and **their virulence** and **host susceptibility**
- Brucellae which have **major outer-membrane lipopolysaccharide (LPS)** are **virulent and produce smooth colony- *B. abortus*, *B. mellitensis*, *B. suis***
- Brucellae which **lack LPS** are **less virulent and produce rough colony- *B. ovis*, *B. canis***
- Brucellae organism when engulfed by phagocytes on mucous membrane are transported to regional lymph nodes. Brucellae persist within macrophages but not in neutrophils
- **Inhibition of phagosome-lysosome fusion** is the major mechanism for intracellular survival. Other mechanism used by brucellae included the **presence of stress protein, production of catalase and superoxide dismutase** help the bacteria to resist oxidative killing

Pathogenesis of Brucellosis

- Intermittent bacteraemia results in spread and **localization in the reproductive organs** and associated glands **in sexually mature animals**.
- **Erythritol**, a polyhydric alcohol which **acts as a growth factor for brucellae** is present in **high concentrations in the placentae** of cattle, sheep, goats, and pigs. Erythritol also found in **mammary gland and epididymis** which are targets of brucellae. In **chronic brucellosis**, organisms may localize in **joints or intervertebral discs**

Pathogenesis



Diagnosis of Brucellosis

- The diagnosis of brucellosis depends on **serological testing and on the isolation and identification of *Brucella* species**
- **Care should be taken during sample collection, transportation and handling the specimen which should be processed in a biohazard cabinet (require Biosafety Level 3 facility)**
- MZN-staining smears from **specimens, particularly cotyledons, foetal abomasal contents and uterine discharges, often reveals characteristics MZN-positive coccobacilli**
- PCR can be used to detect the brucellae in tissue
- A nutritious medium (Brucella agar, Columbia agar supplemented with 5% horse serum and appropriate antimicrobial agent is used for isolation. Plates are incubated at 37°C in 5-10% CO₂ for up to 5days. Majority of brucellae are capnophilic (aerobic bacteria which require CO₂ for their growth)

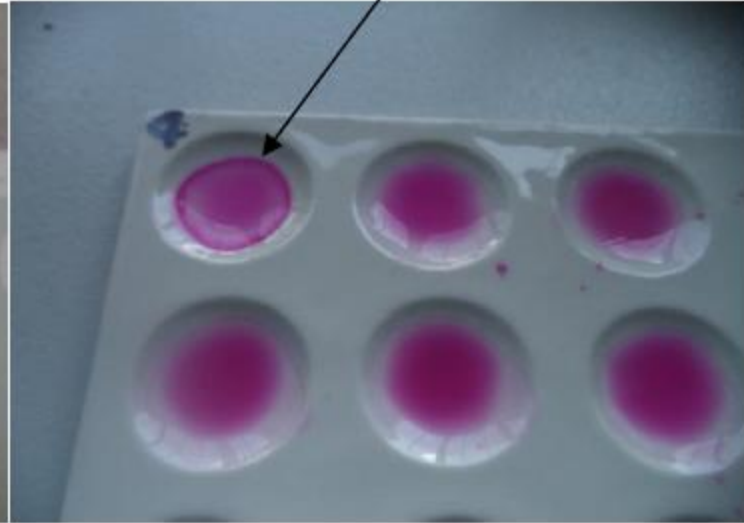
Diagnosis of Brucellosis

- Serological testing is used for screening for international trade and for identifying infected herds or flocks and individual animals in national eradication schemes: **Rose Bengal test (RBT), Complement fixation test, ELISA, Serum agglutination test**
- Brucellae shares antigens with other Gram-negative bacteria such as *Yersinia enterocolitica* serotype O:9 and hence cross-reactions can occur in agglutination tests
- **RBT is simple, rapid test which detects early infection and can be used as an initial screening test.** False positive reactions are due to residual antibody activity from vaccination, colostral antibody in calves, cross reaction with certain bacteria and laboratory error. False negative reactions are observed during early incubation of disease and immediately after abortion however, the Rose Bengal test is an excellent test for the large scale screening of sera
- **Complement fixation test:** due to **its high accuracy CFT is used as confirmatory test for *B. abortus*, *B. melitensis*, and *B. ovis* infections and it is the reference test recommended by the OIE for international transit of animals** . However, this method has some disadvantages such as high cost, complexity for execution, and requirement for special equipment and trained laboratory personnel

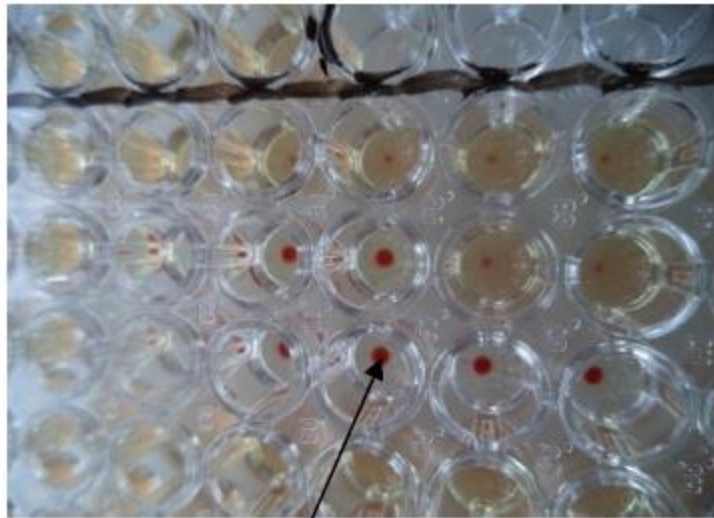
1.Serological test result



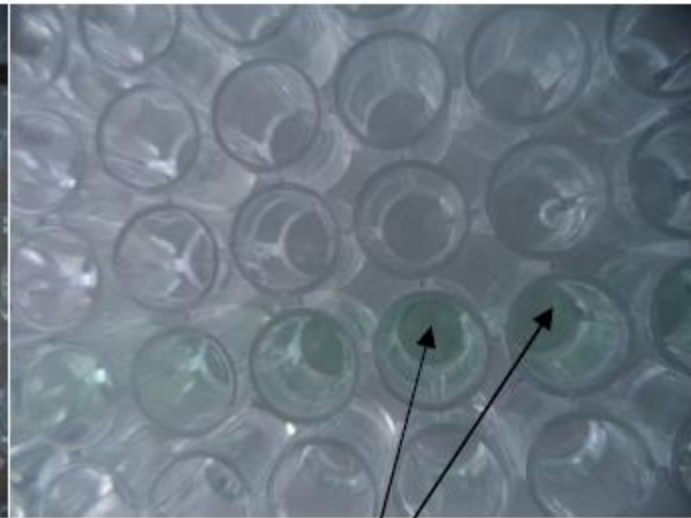
A.Card test



B.RBPT



C. CFT



D. iELISA

Zoonotic brucellosis

- Brucellosis is an **occupational diseases** in shepherds, abattoir workers, veterinarians, dairy-industry professionals, and personnel in microbiologic laboratories
- **Consumption of unpasteurized dairy products**-especially raw milk, soft cheese, butter, and ice cream- is the most common means of transmission
- **Hard cheese, yogurt, and sour milk are less hazardous**, since both propionic and lactic fermentation takes place.
- **Bacterial load in animal muscle tissues is low**, but consumption of undercooked liver and spleen has been implicated in human infection.

Zoonotic brucellosis

- Human brucellosis is the most common zoonosis in the world accounting for more than **500 000 reported cases annually**
- Humans contract brucellosis from animals through
 - **ingestion of contaminated, unboiled or unpasteurized milk**
 - **direct contact with infected animals parts, animal carcasses, aborted materials and laboratory contamination** through the inhalation of infected aerosolized particles
- Human-to-human transmission by tissue transplantation or sexual contact has been reported.

Epidemiology of Brucellosis in Ethiopia

Human brucellosis

- In Ethiopia, few studies carried out on exposed individuals revealed a prevalence ranging from 3% to 34.1% with the highest prevalence being recorded in pastoralist communities of Borena, Somali and Afar Region due to the high rate of exposures to infection particularly the cultural habit consumption of raw milk and other animal products.
- No record of isolation of the organism human cases in Ethiopia so far except one report indicating *B. melitensis* Biovar 1 isolated in UK from human (Ethiopian origin) (Adrian *et al.*, 2006, *J Clin Microbiol*, 44: 1982-93)

RESEARCH ARTICLE

Community-based prevalence of typhoid fever, typhus, brucellosis and malaria among symptomatic individuals in Afar Region, Ethiopia

Biruk Zerfu¹, Girmay Medhin², Gezahegne Mamo³, Gezahegn Getahun⁴, Rea Tschopp^{5,6}, Mengistu Legesse^{2*}

Results

Out of 630 sera screened by DCAT, 83 (13.2%) were reactive to H and/or O antigens for *S. Typhi* infection. Among these, 46 (55.4%) were reactive by the titration test at the cut off value $\geq 1:80$. The combined sero-prevalence for *S. Typhi* by the two tests was 7.3% (46/630). The seroprevalence for *Rickettsia* infection was 26.2% (165/630) by DCAT and 53.3% (88/165) by the titration test at the cut off value $\geq 1:80$. The combined sero-prevalence for *Rickettsia* infection by the two tests was 14.0% (88/630). The sero-prevalence for *Brucella* infection was 12.7% (80/630) by RBPT, of which 28/80 (35%) were positive by CFT. The combined sero-prevalence for *Brucella* infection by the two tests was 4.4% (28/630). Out 650 suspected individuals for malaria, 16 (2.5%) were found positive for *P. falciparum* infection.

RESEARCH ARTICLE

Knowledge and perception of pastoral community members about brucellosis as a cause of abortion in animals and its zoonotic importance in Amibara district, Afar Region, Ethiopia

Mengistu Legesse^{1*}, Girmay Medhin¹, Mekonnen Bayissa², Gezahegne Mamo³

1 Adilu Lemma Institute of Pathobiology, Addis Ababa University, Addis Ababa, Ethiopia, **2** Amibara District Pastoral Office, Afar Region, Melka were, Ethiopia, **3** College of Veterinary Medicine and Agriculture, Addis Ababa University, Bishoftu, Ethiopia

* mengistu.legessed@aau.edu.et



Sero-epidemiological studies of brucellosis in the Afar Region showed that the disease is prevalent in livestock. However, there is little information regarding the pastoral community members' awareness about brucellosis as a cause of abortion in animals and its zoonotic importance. In this study, we assessed knowledge and perception of pastoral community members about brucellosis as a cause of abortion in animals and its zoonotic importance in Amibara district, Afar Region, Ethiopia. Between October and December 2016, a total of 475 study participants (age range 18–80 years, mean age 35.9 years) were interviewed about abortion in their animals, its causes, and diseases that can be transmitted to humans through consumption of raw milk. Almost all (97.7%) of the study participants reported that abortion in animals, especially in goats, is a major problem in the area, and they mentioned that disease (44.6%), drought (58.4%) and fly bites (29.5%) as the main causes of abortion. The study participants also thought that malaria (42.9%) and bovine tuberculosis (19.3%) can be transmitted to humans through consuming raw milk. Five respondents (4.2%) mentioned brucellosis (locally known as "hahayita") as a disease that can be transmitted through frequent consumption of raw milk. The majority (91.9%) mentioned malaria as a cause of febrile illness in humans and 16 (4.4%) participants mentioned brucellosis as a cause of febrile illness. Some participants also mentioned brucellosis as a cause of joint swelling (hygroma) in cattle. In conclusion, the pastoral community members in the present study area lack clear understanding about brucellosis as one of the diseases that cause abortion in their animals and its zoonotic importance. There is a need to create awareness about the zoonotic and animal health importance of brucellosis through various means such as community health extension/veterinary workers and community leaders.

Treatment of Brucellosis in Humans

Table 4. Antibiotics Used in the Treatment of Brucellosis in Humans.

Antibiotic	Minimum Inhibitory Concentration ($\mu\text{g/ml}$)	Dose	Combinations
Doxycycline	0.06–1	100 mg twice daily for 6 wk	Doxycycline combined with streptomycin, with rifampin, with gentamicin, or with ciprofloxacin; doxycycline and streptomycin combined with rifampin or trimethoprim–sulfamethoxazole; doxycycline combined with rifampin and trimethoprim–sulfamethoxazole
Streptomycin	0.25–16	15 mg/kg of body weight intramuscularly for 2–3 w <small>N Engl J Med 2005;352:2325-36.</small>	Streptomycin and doxycycline; streptomycin and doxycycline combined with rifampin or trimethoprim–sulfamethoxazole
Rifampin	0.1–2	600–1200 mg/day for 6 wk	Rifampin and doxycycline; rifampin and doxycycline combined with streptomycin or trimethoprim–sulfamethoxazole; rifampin and ofloxacin; rifampin and ciprofloxacin
Gentamicin	0.25–2	5 mg/kg/day in 3 divided intravenous doses for 5–7 days	Gentamicin and doxycycline
Trimethoprim–sulfamethoxazole	0.38–8	960 mg twice daily for 6 wk	Trimethoprim–sulfamethoxazole combined with doxycycline, with rifampin, or with streptomycin; trimethoprim–sulfamethoxazole and doxycycline combined with streptomycin or with rifampin
Ofloxacin	0.1–2	400 mg twice daily for 6 wk	Ofloxacin and rifampin
Ciprofloxacin	0.25–1	500 mg twice daily for 6 wk	Ciprofloxacin with doxycycline or rifampin

Control of Brucellosis

- Treatment of animals with brucellosis is not practical
- National eradication schemes are based on the detection and slaughter of infected animals- if the economy allows
- Vaccination of young animals
- In cattle three types of vaccines : **attenuated strain 19(S19), adjuvanted 45/20 vaccine and RB51 vaccine**
- In small ruminants, the **modified live *B. melitensis* Rev. 1 strain-** administered by subcutaneous or conjunctival routes is used to vaccinate kids and lambs up to 6months of age
- **In Human,** antimicrobial therapy such as tetracycline. cephalosporins should be administered early in infection for longer period

Anthrax

- **Anthrax is a serious zoonotic disease that can affect most mammals, but is particularly important in herbivores.**
- **Common names include: Malignant Pustule, Malignant Edema, Woolsorters' Disease, Ragpickers' Disease,**
- **Anthrax, the disease, likely originated 6,000 to 7,000 years ago in Mesopotamia and Egypt, where agricultural civilization was first recorded**
- **Anthrax is caused by member of Genus Bacillus, *Bacillus anthracis*.**
- ***B. anthracis* which causes anthrax and its endospores can survive in soil for up to 50 years and present in endemic areas**



***Bacillus anthracis* (Gram stain) The cells have characteristic squared ends. The endospores are ellipsoidal shaped and located centrally in the sporangium. The spores are highly refractile to light and resistant to staining**

Anthrax: Etiology and Ecology

• *Bacillus anthracis* exists in two forms:

• **Vegetative form** and **Spore form**

- Within an infected host, spores germinate to produce the vegetative forms which eventually kill the host. These bacilli are released by the dying or dead animal into the environment (usually soil under the carcass).
- Within the host the organism is exclusively in vegetative form
- Vegetative forms of *B. anthracis* grow and multiply readily in normal laboratory nutrient agars or broths,
- They are more "fragile" than the vegetative forms of other *Bacillus* species, dying in simple environments such as water or milk. Moist heat kills the Vegetative cells 60 °C X 30 minutes
- *B. anthracis* is dependent on sporulation for species survival making it an obligate pathogen

Sporulation and survival

- The rate and extent of sporulation by vegetative cells is affected by complex environmental factors
 - Temperature : 8- 45^o
 - Humidity : greater than 96%
 - Water activity (available water within the microenvironment)
 - PH: 5-9
 - Oxygen availability
 - Presence of certain cations particularly Mn⁺⁺
- The spore form of anthrax is markedly resistant to biological extremes of heat, cold, pH, desiccation, and chemicals .
 - Spores can be killed with 100^o C X 10 minutes, 4% Formaldehyde kills spores, 4% KMnO₄ kills spores, Hypochlorite (0.5%) commercially available kills spores
- It is estimated that 2,500 to 55,000 spores represent the lethal inhalation dose for humans.

Cycle of infection in Anthrax

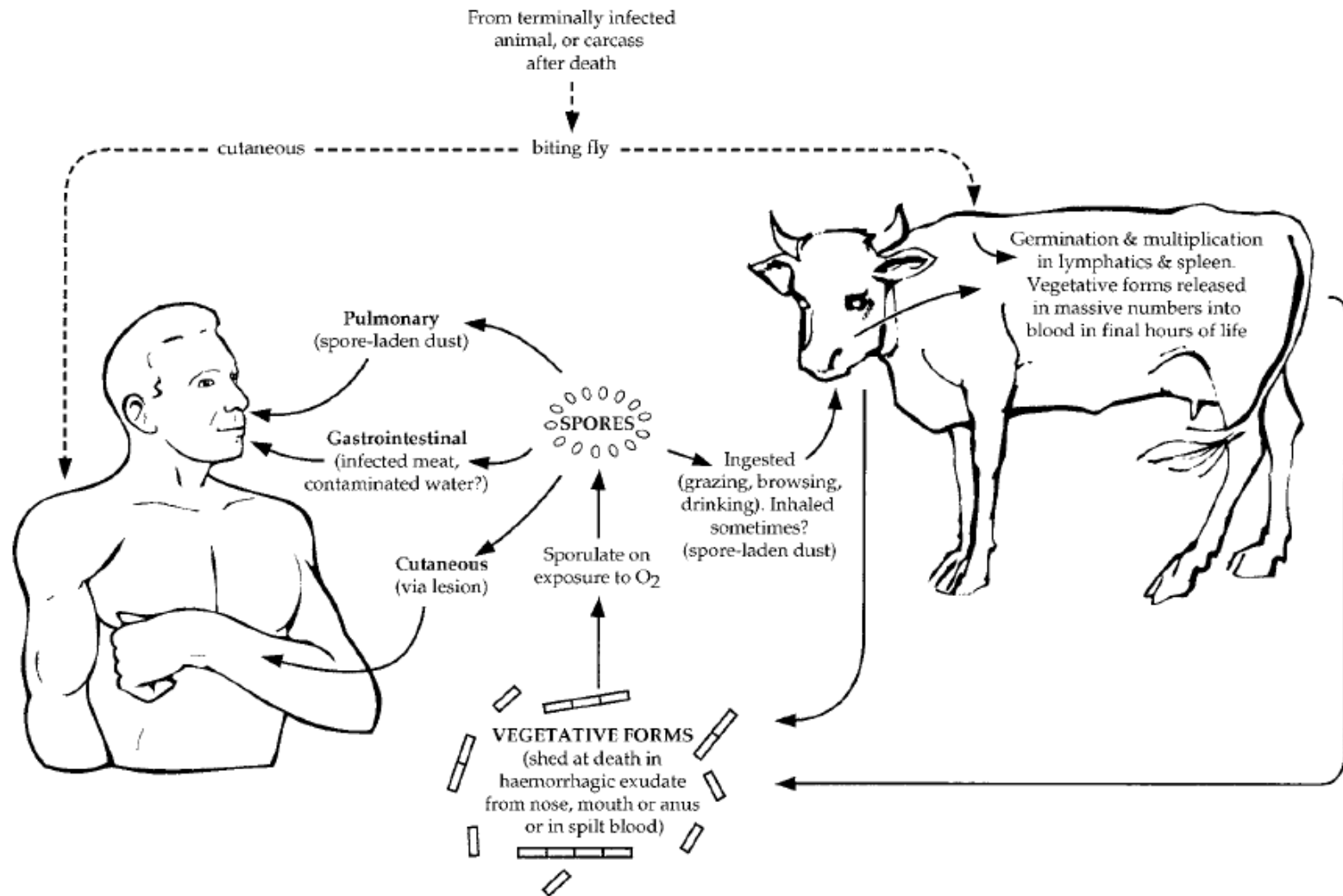
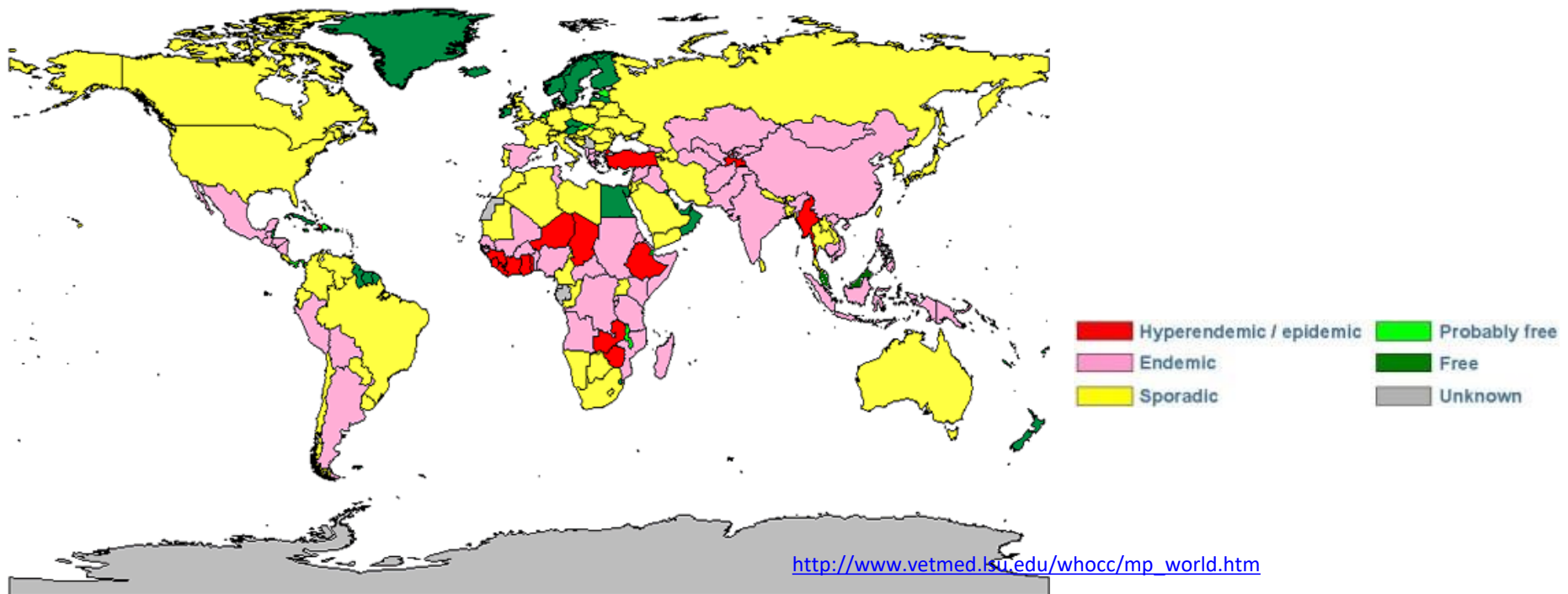


Fig. 1 Cycle of infection in anthrax. The spore is central to the cycle, although vegetative forms may also play a role in establishing infection when, for example, humans or carnivores eat meat from an animal that died of anthrax or when biting flies transmit the disease. The infectivity of vegetative forms is difficult to establish since it is close to impossible to prepare truly spore-free vegetative cell suspensions in the laboratory.

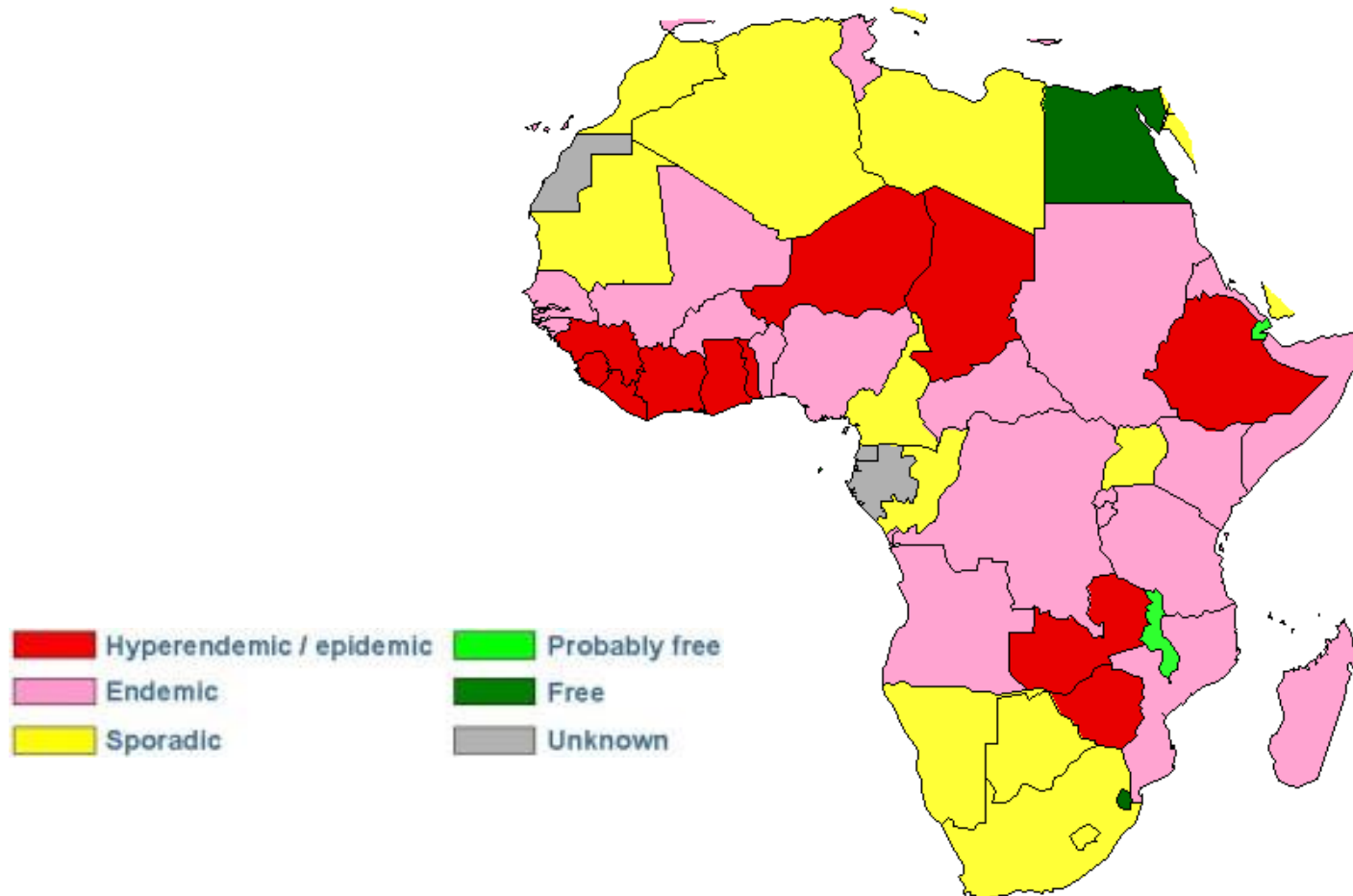
Epidemiology of Anthrax

- *B. anthracis* appear to be one of the most monomorphic species known, i.e. isolates from whatever type of source or geographical location are almost identical phenotypically and genotypically
- Anthrax remains enzootic in many regions of the world, and cases of anthrax among humans are frequently reported
- The disease is common in some Mediterranean countries, several sub-Saharan African countries, central and south western Asia, some South America countries



Anthrax in Africa

- In Africa anthrax is wide spread and reported in most countries
- In Ethiopia, anthrax is hyperendemic/epidemic in most species of domestic animals and also cases have been reported in humans



Anthrax in Ethiopian livestock

- In Ethiopia, anthrax is hyperendemic/epidemic in most species of domestic animals and also cases have been commonly reported in humans
- But **very few studies** at a national level

Vet Rec. 2002 Mar 9;150(10):318-20.

[Anthrax outbreak in Mago National Park, southern Ethiopia.](#)

Shiferaw F, Abditcho S, Gopilo A, Laurenson MK.

Source

Ethiopian Wildlife Conservation Organisation, Ministry of Agriculture, Addis Ababa.

PMID: 11913590

[PubMed - indexed for MEDLINE]

Rev. sci. tech. Off. int. Epiz., 2004, 23 (3), 951-956

Anthrax in Wabessa village in the Dessie Zuria district of Ethiopia

G. Shiferaw

Department of Microbiology, Kombolcha Regional Veterinary Laboratory, P.O. Box 9, Kombolcha, Ethiopia

Submitted for publication: 26 February 2004

Accepted for publication: 7 September 2004

Summary

In 2002 an investigation of sudden death in a goat in Wabessa village in the Dessie Zuria district of Ethiopia was undertaken using fresh blood brought to the Kombolcha Regional Veterinary Laboratory. The sample was examined using standard bacteriological techniques and animal pathogenicity tests were also performed. The laboratory investigation revealed *Bacillus anthracis* as the cause of sudden death. Information gathered from stockowners in the same village revealed other similar recent cases and deaths, both in animals and humans, with farmers clearly describing the clinical signs and necropsy findings of anthrax. The disease occurs annually in this area in May and June, and in the 2002 outbreak mortality rates of 7.7%, 32.7% and 47.1% were observed in cattle, goats and donkeys, respectively. This study indicates that the community of this particular village neither knows of, nor practises, any of the conventional methods for anthrax control. The cutaneous form of the disease in humans and the environmental contamination associated with the practise of opening cadavers are briefly described and the findings are discussed with reference to the epidemiology of anthrax in both Ethiopia and elsewhere. Control strategies are also recommended.

Keywords

Anthrax – *Bacillus anthracis* – Ethiopia – Goat – Outbreak.

[Tropical and Geographical Medicine](#) [1989, 41(2):108-112]

[Seboxa T, Goldhagen J](#)

Gondar College of Medical Sciences, Department of Internal Medicine, Ethiopia.

[Anthrax in Ethiopia.](#) PMID:2763354)

Abstract

Twenty-seven patients with cutaneous anthrax were identified over a three-year period at Gondar College of Medical Sciences in North Central Ethiopia. Nine patients who delayed seeking medical care presented with severe symptoms and three patients died. Eighteen patients were clustered within four families in which an attack rate of 32% occurred. Ninety-three percent of patients could trace their disease to exposure to the products of a specific diseased animal. Characteristics of anthrax in Ethiopia include a known exposure to diseased animals, occurrence within families, frequent treatment by local healers, and high morbidity and mortality.

Anthrax in animal

Host range

- Anthrax is primarily a disease of herbivores. Most domestic and wild animals including carnivores, birds, primates and humans can be affected

<i>Bacillus species</i>	Susceptible animals	Clinical manifestations
<i>B. anthracis</i>	Cattle, sheep	Fatal peracute or acute septicaemic anthrax
	Pigs	Subacute anthrax with oedematous swelling in pharyngeal region; an intestinal form with higher mortality is less common
	Horses	Subacute anthrax with localized oedema; septicaemia with colic and enteritis sometimes occurs
	Humans	Skin, pulmonary and intestinal forms of anthrax are recorded in man periodically

Animal Transmission

- Grazing animals become infected through ingestion of spores in the soil (most common) and possibly inhalation of spore
- Carnivores ingestion of contaminated meat
- Inhalation
- Animal to animal or human to human transmission is rare (not contagious)
- Bacteria present in hemorrhagic exudate from mouth, nose, anus contaminate the environment
- Other source of contaminations
 - Biting flies
 - Vultures
 - Contaminated surface water pool
- Infective lethal dose ranges from dose 2,500 to 55,000 spores
- Experimental parental administration need <10 spores in susceptible herbivores to $>10^7$ spores in more resistance species

Diagnosis of Anthrax

- Clinical Signs in ruminants
 - Peracute – Sudden death
 - Acute – Tremors, dyspnea – Bloody discharge from body orifices
 - Chronic (rare) – Pharyngeal and lingual edema –Death from asphyxiation
- Necropsy not advised and Do not open the carcass!
- Samples of peripheral blood needed
 - Cover collection site with disinfectant (70% alcohol) soaked bandage to prevent leakage
 - Thin smears of blood or fluid, stained with polychrome methylene blue, reveal chains of square-ended, blue-staining rods surrounded by pink capsules
- Serological assay
 - ELISA: based on anthrax toxin (Protective antigens-PA, Lethal factor-LF and Edema Factor-EF) for routine confirmation and vaccine response)
- Molecular techniques: - PCR Fingerprinting
 - RFLP
- Animal Inoculation: Guinea pig and mice inoculation



Prevention and Control

- Anthrax is a notifiable disease-report to authorities
- Quarantine the area
- Do not open carcass
- Minimize contact
- Wear protective clothing
- Carcass disposal options
 - Incineration
 - Deep burial
- Decontaminate soil
- Remove organic material and disinfect structures
- Discourage scavengers
- Use insect control or repellants
- Isolate sick animals
- Prophylactic antibiotics- Penicillins, tetracyclins
- Vaccination important in endemic areas- nonencapsulated toxigenic strain (Sterne Strain) has been used effectively in livestock.

Disinfection

- Preliminary disinfection
 - 10% formaldehyde
 - 4% glutaraldehyde (pH 8.0-8.5)
- Cleaning – Hot water, scrubbing, protective clothing
- Final disinfection: one of the following disinfectants at a rate of 0.4 lt/m² exposed for at least 2 hours
 - 10% formaldehyde
 - 4% glutaraldehyde (pH 8.0-8.5)
 - 3% hydrogen peroxide
 - 1% peracetic acid

Anthrax in Human

Based on the source of infection, human Anthrax cases can be classified into

- Agricultural /Non Industrial anthrax (through infected animals):
 - Cutaneous anthrax
 - Rarely Gastrointestinal anthrax
- Industrial Anthrax (Through animal products):
 - Mostly through animal products(wools, hair, hides, bones)
 - Likely to develop Cutaneous and pulmonary anthrax (inhalation)

Clinically three forms of Human anthrax occur

- Cutaneous anthrax
- Pulmonary anthrax
- Gastrointestinal anthrax



The skin (cutaneous) form of anthrax in human

Cutaneous Anthrax

- Mainly in professionals(Veterinarian, butcher, Zoo keeper, lab workers)
- Spores infect skin- a characteristic gelatinous edema develops at the site (Papule- Vesicle-Malignant Pustule- Necrotic ulcer)
- 80-90% heal spontaneously (2-6wks)
- 0-20% progressive disease – develop septicemia
- 95-99% of all human anthrax occur as cutaneous anthrax

Intestinal Anthrax

- Due to ingestion of infected carcasses
- Mucosal lesion to the lymphatic system
- Common in Ethiopia
- **Extremely high mortality rate**

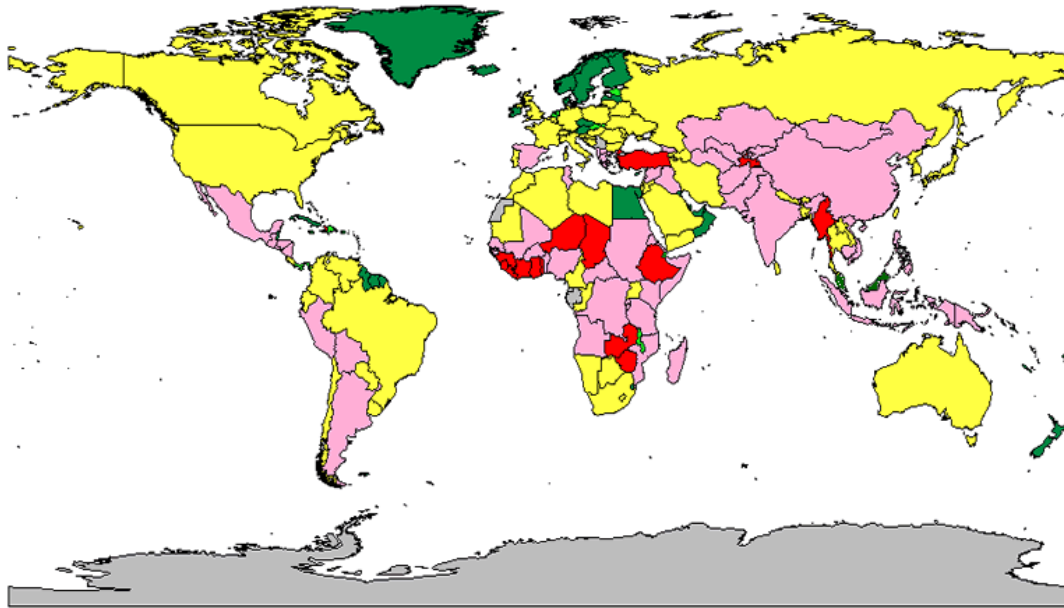
Inhalation Anthrax

- Require very high infective dose (> 10,000 spores)
- Acquired through inhalation of spores (Bioterrorism - aerosol)
- Present with symptoms of severe respiratory infection(High fever & Chest pain)
- Progress to septicemia very rapidly
- Mortality rate is very high > 95%

Anthrax in Human

Human Cases

- *Bacillus anthracis* is found worldwide with an estimated 20,000-100,000 human cases each year
-



Zoonotic Anthrax in Ethiopia

- In Ethiopia, anthrax is hyperendemic/epidemic in most species of domestic animals and also cases have been reported in humans

News Report: "Ethiopia: Anthrax Infects 10 People in Oromiya Region"

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July 15, 2002

INTERNATIONAL IMMUNIZATION NEWS

"Ethiopia: Anthrax Infects 10 People in Oromiya Region"
Africa News Service (www.allafrica.com) (07/12/02)

An outbreak of anthrax has sickened at least 10 people in the eastern Ethiopia. The disease first infected and killed 15 head of cattle in the Fentale district of Oromiya Region. Dr. Yilma Jobre of the International Livestock Research Institute in Addis Ababa, noted that although it is transmittable to humans, it is still rare for humans to contract anthrax from cattle. He suggested that farmers burn or bury the carcasses of diseased cattle rather than slaughtering them, adding that anthrax spores are found naturally in soil and can be released into the atmosphere by rainfall.

ETHIOPIA: Suspected anthrax epidemic in Afar Region



2000 - Anthrax in Ethiopia

WHO has received reports of clusters of cases of suspected anthrax in the Afar region of Ethiopia. This area is inhabited by pastoralists who depend on livestock and cases of anthrax are known to occur. Reports from organizations (e.g. United Nations Development Programme, Médecins Sans Frontières) working in the area indicate clusters of cases and increased numbers of cases of a clinical syndrome consistent with anthrax. No systematic epidemiological investigation has been carried out thus far.

Prevention and control in humans

- Humans protected by preventing disease in animals
 - Veterinary supervision (surveillance and monitoring in livestock)
 - Trade restrictions
- Improved industry standards
- Safety practices in laboratories
- Post-exposure antibiotic prophylaxis (Penicillin, Ciprofloxacin , Doxycycline)- for about 60days in case of inhalation anthrax to kill the germinating spores
- Vaccination risk groups: veterinarians , lab workers, livestock handlers, military personnel
 - Immunization series
 - Five IM injections over 18-week period – Annual booster

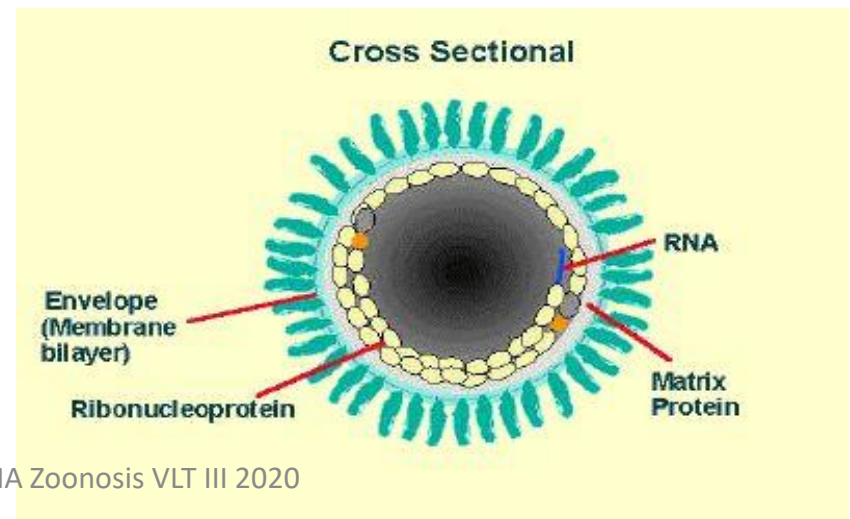
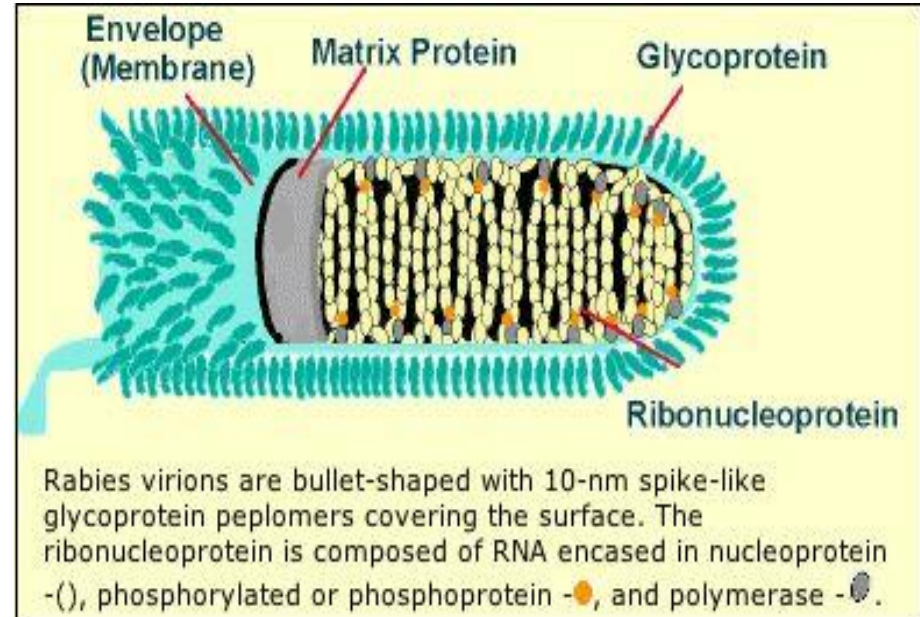
Rabies

- ❑ Rabies is one of the oldest and most terrifying diseases known to man.
- ❑ Written and pictorial records of rabies date back more than 4000 years
- ❑ It is endemic in more than 150 countries around the world
- ❑ Even though the disease can be prevented, it kills an estimated 59 000 people each year, mostly in the world's poorest and most vulnerable communities
- ❑ About 40% of the victims are children younger than 15 years living in Asia and Africa.
- ❑ A staggering 99% of human cases are acquired via the bite of an infected dog, rather than through exposures to the many and varied wild animals that act as viral reservoirs on different continents.



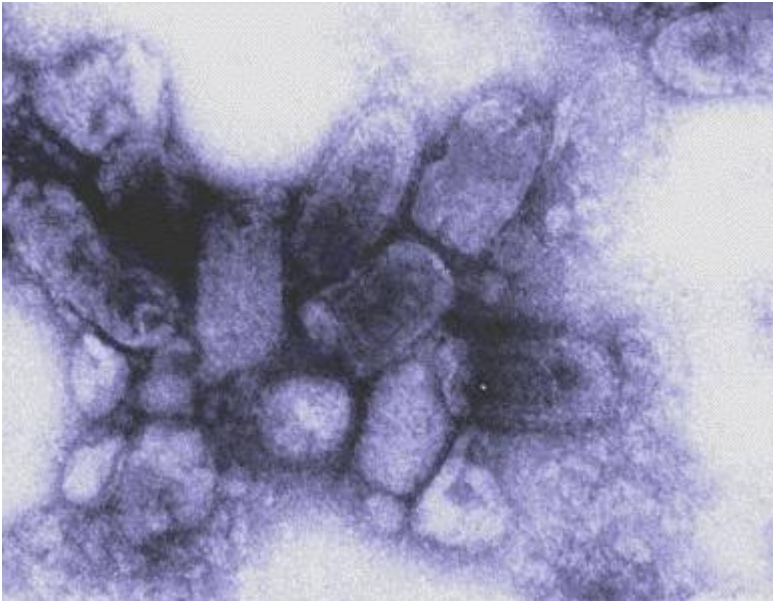
Rabies Virus

- Rabies is caused by “Bullet” shaped- Rhabdovirus (180nm x75nm) with 10nm spike-like glycoprotein covering the surface
- RNA virus with approx. 12kb genome
- 2 major structural components
 - Helical RibonucleoproteinNP core
 - Surrounding envelope



Rabies

- ❑ Rabies is one of the oldest and most terrifying diseases known to man.
- ❑ reservoirs on different continents.

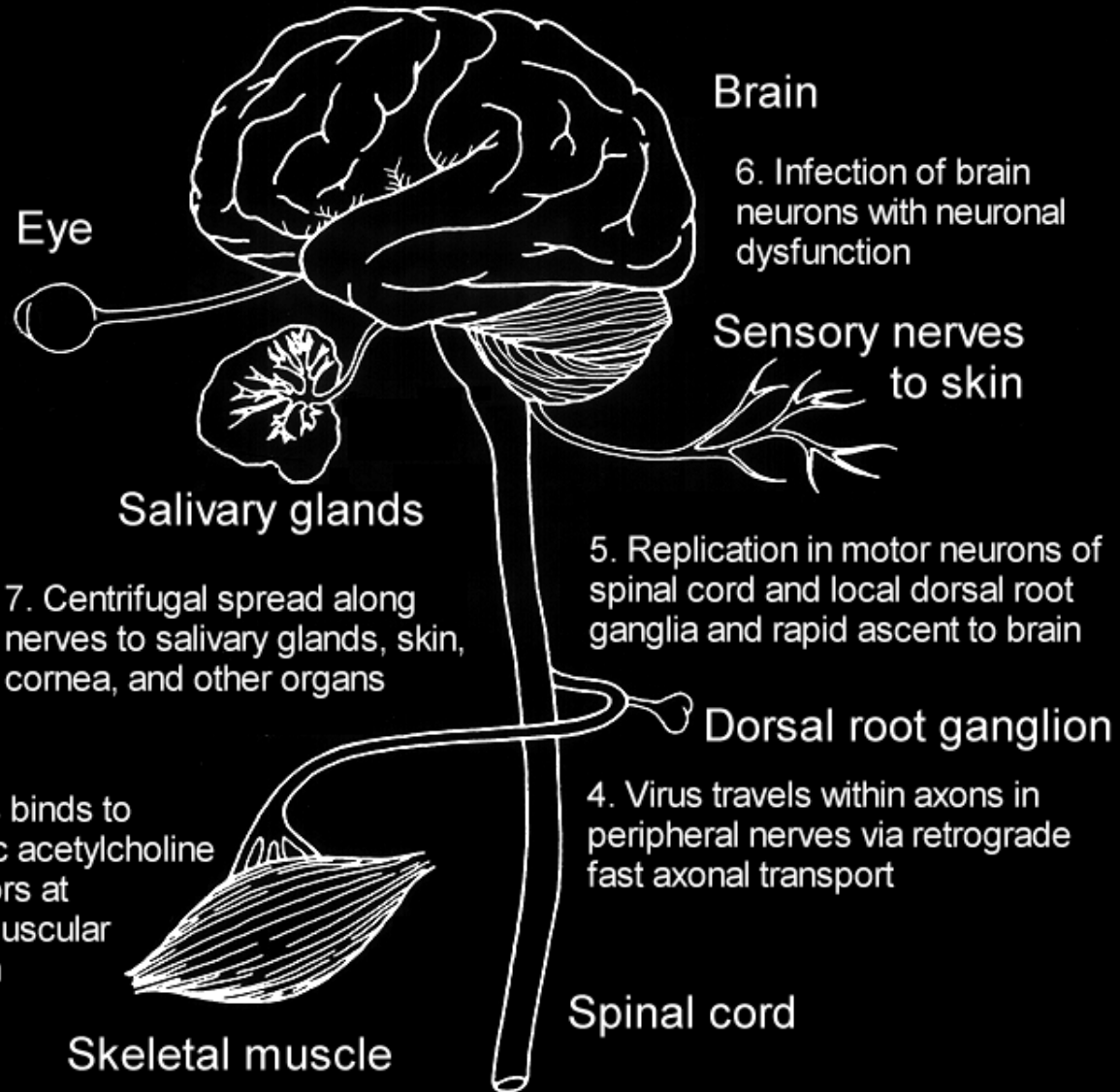
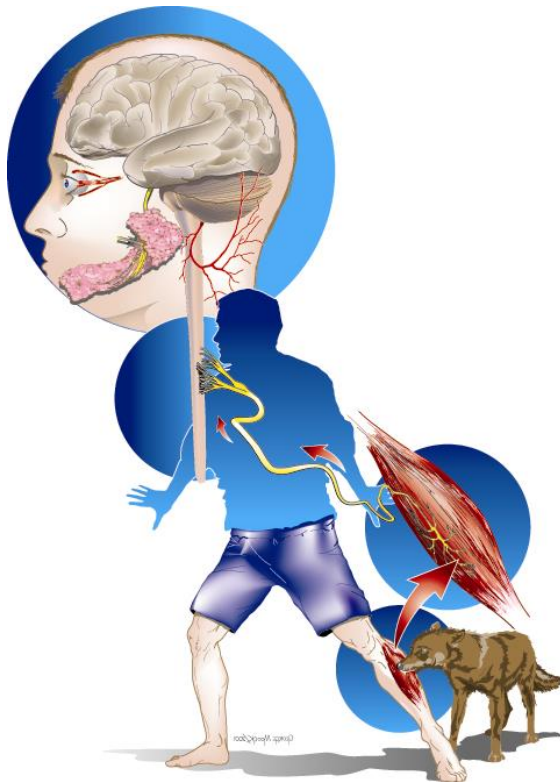


PATHOGENCITY

- ❑ Rabies causes encephalitis and myelitis
- ❑ Perivascular infiltration throughout entire central nervous system
- ❑ Causes cytoplasmic eosinophilic inclusion bodies (Negri bodies) in neuronal cells
- ❑ Several factors may affect outcome of rabies exposure.
 - Rabies variant
 - Dose
 - Route
 - Location of exposure
 - Individual host factors

Rabies Pathogenesis

- The incubation period is usually 6 to 8 weeks in humans but it may be longer.



2. Viral replication in muscle

Gezahegne M-CVMA Zoonosis VLT III 2020

1. Virus inoculated

Viral Spreading in Animals

- ❑ In cats and dogs, the clinical symptoms usually begin before the virus is found in the saliva.
 - Sometimes virus can be found one to three days before clinical symptoms in cats and three to five days in dogs. Ferrets is something < 10 days.
- ❑ No reliable information regarding the appearance of virus in the saliva is available for other animal species .
 - There is some evidence that certain wildlife species, like skunks and bats, may excrete virus in their saliva for long periods before becoming ill.

Rabies

- ❑ **All mammals are susceptible to rabies including dogs, human, livestock and wild animals**
- ❑ **Rabies is one of the most important zoonotic diseases of globally; endemic in more than 150 countries around the world**

Transmission

- ❑ The virus is typically transmitted by being deposited under the skin, usually through a bite wound.
- ❑ Contamination of a pre-existing wound is possible but unlikely because the local inflammatory reaction which follows a wound, quickly seals the comprised skin and prevents entry of the virus.
- ❑ Transmission across mucous membranes is also possible, but less likely than a bite wound.

Aberrant Routes of Transmission

- ❑ A small number of human rabies cases have been reported following corneal transplants.
 - ❑ This suggests that human rabies cases may be underreported.
- ❑ Aerosol (airborne) transmission of the virus has also been reported. However, this is possible only under unusual circumstances.
 - ❑ Persons have contracted rabies presumably by airborne transmission after visiting bat caves

Survivability of the Rabies Virus

- ❑ **The rabies virus is extremely short lived under most environmental conditions.**
 - **It is easily inactivated by heat and drying.**
 - **Capable of surviving for a few days in cold temperatures.**

- ❑ **Thorough and immediate washing of bite wounds with plenty of soap and water is the best preventative treatment for rabies**

Terrestrial Reservoirs of Rabies

- ❑ Different terrestrial mammalian species are the rabies reservoirs .
 - ❑ skunk, raccoon, fox, coyote, wild carnivores

Rabies

- ❑ **All mammals are susceptible to rabies including dogs, human, livestock and wild animals**
- ❑ **Rabies is one of the most important zoonotic diseases of globally**
- ❑ **It is endemic in more than 150 countries around the world**

Rabies in Ethiopia

The status of rabies in Ethiopia: A retrospective record review

Asefa Deressa¹, Abraham Ali¹, Mekoro Beyene¹, Bethelehem Newaye Selassie¹, Eshetu Yimer², Kedir Hussen¹

Abstract

Background: Rabies, a viral disease that affects all warm-blooded animals, is widespread in many regions of the world. Human rabies, transmitted by dogs is an important public health issue in Ethiopia. To-date, effective rabies control program still remains to be a reality and needs to be strengthened..

Objective: Reviewing of recorded data to generate information on the status of rabies in Ethiopia for the year 2001-2009.

Methods: A retrospective data were used from the Ethiopian Health and Nutrition Research Institute rabies case record book registered between 2001 and 2009.

Results: The fatal human cases during the period of study were 386 humans with annual range of 35 to 58. The over all post exposure treatment for humans was 17,204 within and around Addis Ababa. During the same period, 20,414 suspected rabid animals were clinically examined; nevertheless, only 10%were positive for rabies. Among 3,460 animal brains investigated in the laboratory with FAT, 75% were confirmed as rabies positive. The production and distribution of anti-rabies vaccine reached 130,673 treatment doses for human vaccine and 85,055 doses for animal vaccine respectively in the period of 2001-2009.

Conclusion: The recorded data showed the underestimate of rabies diagnosis, post exposure prophylaxis and fatal human cases, which could be attributed due to the absence of national rabies surveillance system. Therefore, It is of paramount importance to assess and map the national picture of rabies within a given time interval to launch a national rabies control strategy. [*Ethiop. J. Health Dev.* 2010;24(2):127-132]

Table 1: Clinical observation and quarantine of animals suspected of rabies from Addis Ababa and surrounding areas between 2001 and 2009.

Species	Total # under clinical observation & quarantine	Animals free from rabies after 10 days of quarantine No. (%)	% positive for rabies No. (%)
Dog	20113	18102 (90%)	2011 (10%)
Cat	265	125 (47%)	140 (53%)
Others (donkey, monkey, horse, cattle)	36	16 (44%)	20 (55%)
Total	20414	18243 (89%)	2171 (10.63%)

Table 2: The Brain of animals examined for rabies from Addis Ababa and surrounding areas between 2001 and 2009.

Year	Dog		Cat		Others	
	Brain examined	N ^o (%) positive	Brain examined	N ^o (%) positive	Brain examined	N ^o (%) positive
2001	379	274 (72)	15	11 (73)	1	0
2002	1259	919 (73)	20	17 (85)	19	14 (73.68)
2003	400	302 (75)	12	10 (83)	10	5 (50)
2004	115	102 (88.7)	9	5 (55)	5	3 (30)
2005	297	237 (79.8)	17	12 (70)	3	2 (66)
2006	280	193 (68.9)	11	5 (45)	7	5 (71.43)
2007	240	202 (84)	15	10 (66)	7	3 (42.8)
2008	67	46 (68.65)	12	10 (83)	10	7 (42.8)
2009	224	183 (81.7)	15	11 (73)	11	5 (45)
Total	3261	2458 (75.37)	126	91 (72)	73	44 (60)

Table 4: Sex and age specific fatal human rabies cases from Addis Ababa and surrounding areas between 2001 and 2009.

Age Group	Male		Female		Total	
	#	%	#	%	#	%
0-14	110	66.66%	55	33.33%	165	42.74
15-49	86	53.42%	75	46.58%	161	41.71
50+	35	58.33%	25	41.66%	60	15.54
Total	231	59.84	155	40.16	386	100

Control of urban rabies

- ▶ Registration and licensing of all domestic dogs.
- ▶ Immediate destruction of dogs and cats bitten by rabid animals
- ▶ Restraint of dogs in public place
- ▶ Immunization of dogs
- ▶ Health education about prevention of rabies



Elements of Human Rabies Prevention

- **Avoiding exposure** :-
 - Avoiding contact with unknown animals
 - Nursing rabid Human /animal with Care
- **Pre-Exposure Prophylaxis (PrEP)** : –
 - is recommended for anyone who is at continual, frequent or increased risk for exposure to the rabies virus such as laboratory workers, veterinarians and animal handlers.
- **Post-Exposure Prophylaxis (PEP)**:-
 - anti-rabies prophylaxis (Vaccine) administered after an exposure