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

Handouts for the Course,* Veterinary Public Health-II (Vetm-4172), *Second Semester (2019/2020)
Instructor: Professor Ashwani Kumar (75% share)
Dr. Gezahegne Mamo (25%)

Zoonosis (plu. Zoonses)

- 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, Bubonic Plague (*Yersinia pestis*), brucellosis, Bovine tuberculosis, Leptospirosis, Salmonellosis, spotted fever caused by Rickettsia, Rabies, Arthropod borne viral infections (arboviral infection-RVF, West Nile fever), Parasitic zoonotic diseases: especially cysticercosis, hydatid disease, trypanosomiasis and toxoplasmosis , Emerging zoonotic diseases-Ebola, SARS, Avian flu, MERS, Zika and recent COVID19

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

- the etiological agents
- the mode of transmission
- the reservoir host

Zoonoses classes based on Etiology

- **Bacterial zoonoses** e.g. anthrax, brucellosis, bubonic plague, leptospirosis, salmonellosis, lyme disease
- **Viral zoonoses** e.g. rabies, arbovirus infections, yellow fever, influenza, Ebola, zika, MERS-CoV, COVID19
- **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 zoonoses** e.g. scabies, myiasis

Classification of zoonoses 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., Bubonic 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 zoonoses according to the reservoir host:

- **Anthropozoonoses**: Infections transmitted to man from lower vertebrate animals e.g. rabies, leptospirosis, bubonic 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
- Microbes can be transported directly among hosts, indirectly through food and water, or through vectors such as mosquitoes and ticks, and they may survive as environmental contaminants or microbial populations where they are maintained in nature outside of living hosts
- The result has been the creation of a new era of emerging and reemerging diseases that has been characterized especially by new zoonotic diseases.

- Over the last 3 decades, approximately 75% of new emerging human diseases have been zoonotic and many have come from and/or through wildlife

Human population growth with its global effects on the ecosystem over the past million years has resulted in the emergence of infectious diseases. Some of the questions we need to ask are: What were the sources of our major infectious diseases, including these 'new' ones? Why do so many animal pathogens, including virulent viruses like Ebola and Marburg, periodically infect human hosts but then fail to establish themselves in human populations?

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:** microbe as a result of mutation may create a new variant which can adapt and become pathogenic to the new host eg. Influenza viruses

- **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

- **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**

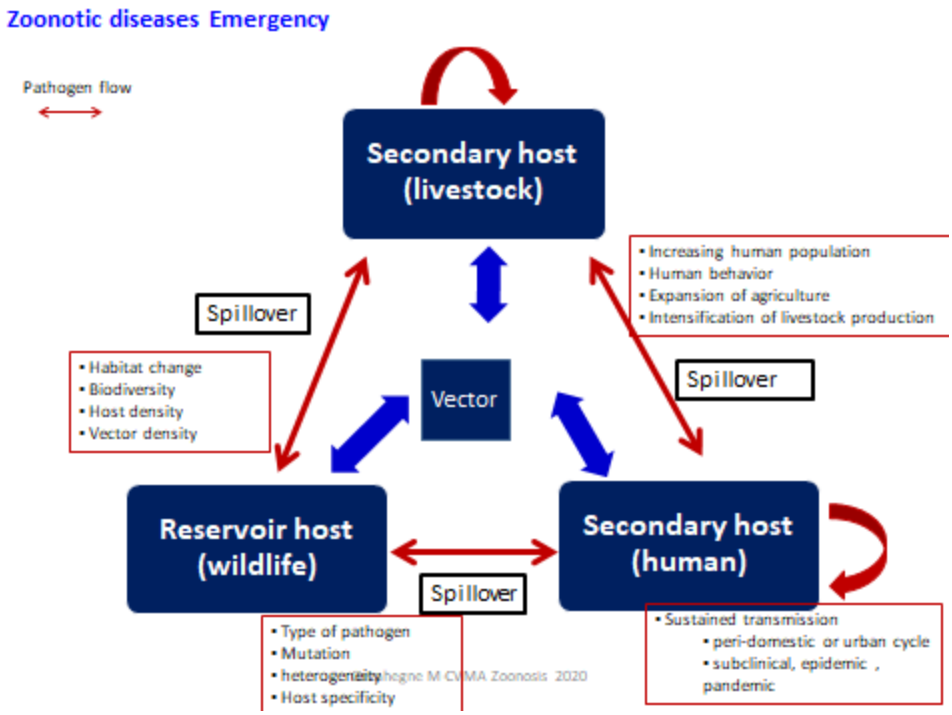
Animals may carry potential risk of increased frequency of zoonotic agents in man e.g. dermatophytosis, tuberculosis, brucellosis etc.

- **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



Bacterial Zoonosis:

Bacterial zoonotic diseases may be acquired or spread in a variety of ways:

- through the air (aerosol)
- by direct contact
- by contact with an inanimate object that harbors the disease (fomite transmission)
- by oral ingestion
- by insect transmission

Some of the bacterial zoonotic diseases include: Anthrax, Bovine tuberculosis, Brucellosis , Camplyobacteriosis, Leptospirosis, Salmonellosis, *Escherichia coli* O157:H7, Listeriosis

Anthrax

- ❑ Anthrax is a serious zoonotic disease that can affect most mammals, but is particularly important in herbivores.
- ❑ The word anthrax is derived from a Greek word meaning charcoal or carbuncle.
- ❑ Common names include: Malignant Pustule, Malignant Edema, Woolsorters' Disease, and Maladi Charbon
- ❑ Anthrax, the disease, likely originated 6,000 to 7,000 years ago in Mesopotamia and Egypt, where agricultural civilization was first recorded

Anthrax: Etiology and Ecology

- ❑ Anthrax is caused by member of Genus Bacilus, *Bacillus anthracis.*

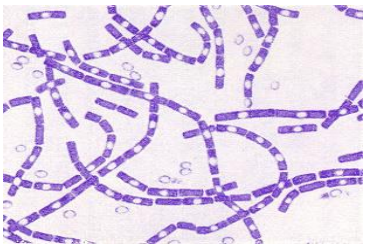


Fig. Bacillus anthracis (Gram stain): The cells have characteristic squared ends. The endospores are ellipsoidal shaped and located centrally in the sporangium. The spores are highly refractive to light and resistant to staining

- ***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* grows 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^{0} C X 30 minutes
  - *B. anthracis* is dependent on sporulation for species survival making it an obligate pathogen

***Bacillus anthracis* spore**

- When conditions are not conducive to growth and multiplication of vegetative bacilli, *B. anthracis* tends to form spores
- Spore is the resting stage of the organism
- Spores are not produced in the unopened carcass (within the anaerobic environment of an infected host the organism is in the vegetative form).
- Sporulation requires a nutrient poor environment and the presence of free oxygen.

- The spore form is the predominant phase in the environment, can survive for decades in soil, and it is through the uptake of spores that anthrax is contracted.
- Anthrax is a seasonal disease. heavy rains, alternating with dry periods, may concentrate the spores and result in outbreaks among grazing animals.

Cycle of infection in Anthrax

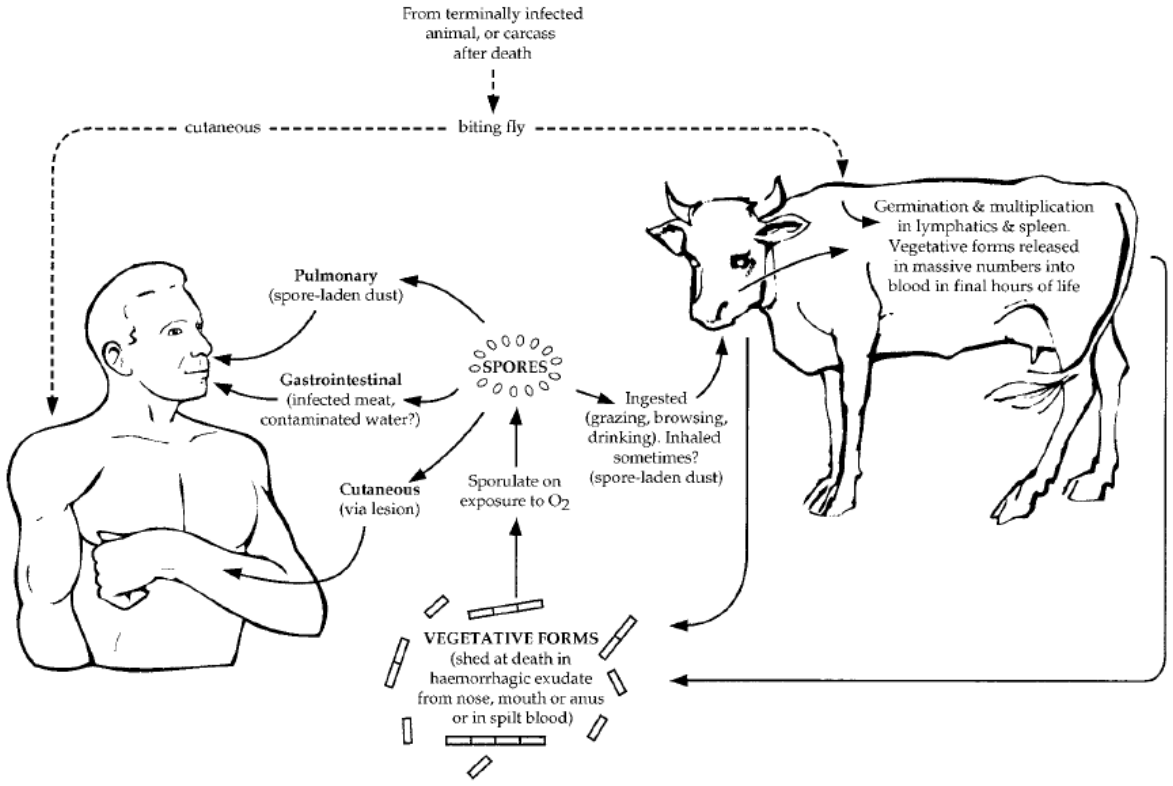
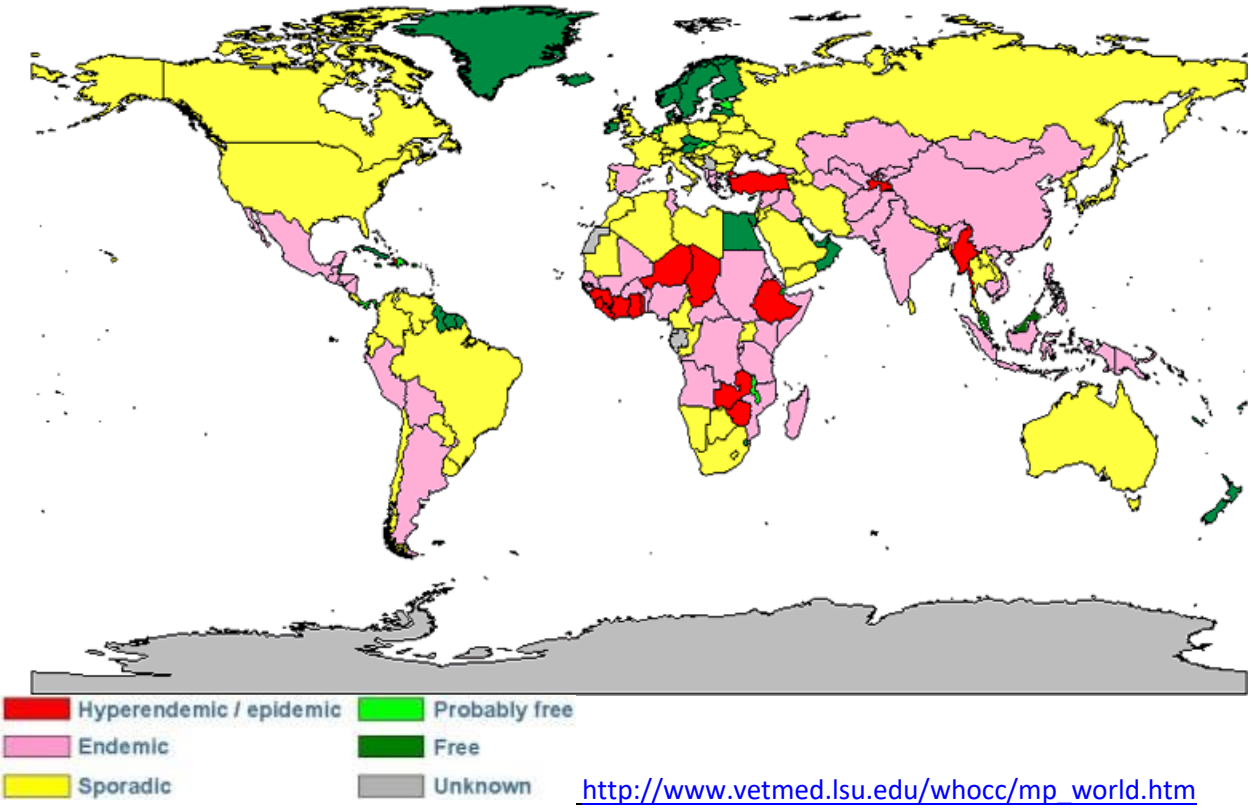


Fig. 1 Cycle of infection in anthrax. The spore is central to the cyde, although vegetative forms may also play a role in establishing infection when, for example, humans or carnivores eat meat from an animal that died of anthrax or when biting flies transmit the disease. The infectivity of vegetative 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

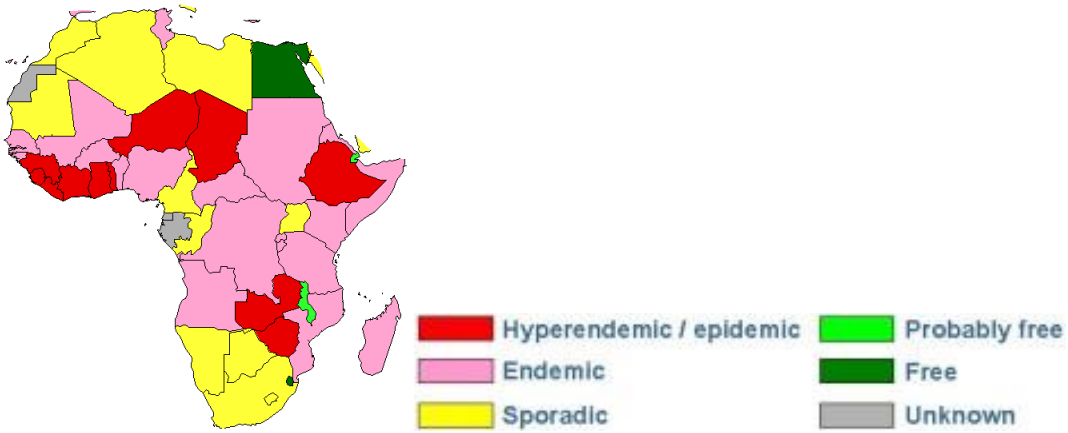
- *B. anthracis* appear to be one of the most monomorphic species known, i.e. isolates from whatever type of source or geographical location are almost identical phenotypically and genotypically
- Anthrax remains enzootic in many regions of the world, and cases of anthrax among humans are frequently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



http://www.vetmed.lsu.edu/whocc/mp_world.htm

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



- In Ethiopia, very few studies on anthrax at a national level

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.

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. PMID: 11913590

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

Bacillus species	Susceptible animals	Clinical manifestations
B. anthracis	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
- 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 parentral 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 <u>Do not open the carcass</u>**!
- 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 is important in endemic areas like Ethiopia- non-encapsulated toxigenic strain (Sterne Strain) has been used effectively in livestock.

Disinfection of environment

- **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/m^2 exposed for at least 2 hours
  - 10% formaldehyde
  - 4% glutaraldehyde (pH 8.0-8.5)
  - 3% hydrogen peroxide
  - 1% peracetic acid

<u>Anthrax in Human (Zoonotic Anthrax)</u>

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**

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

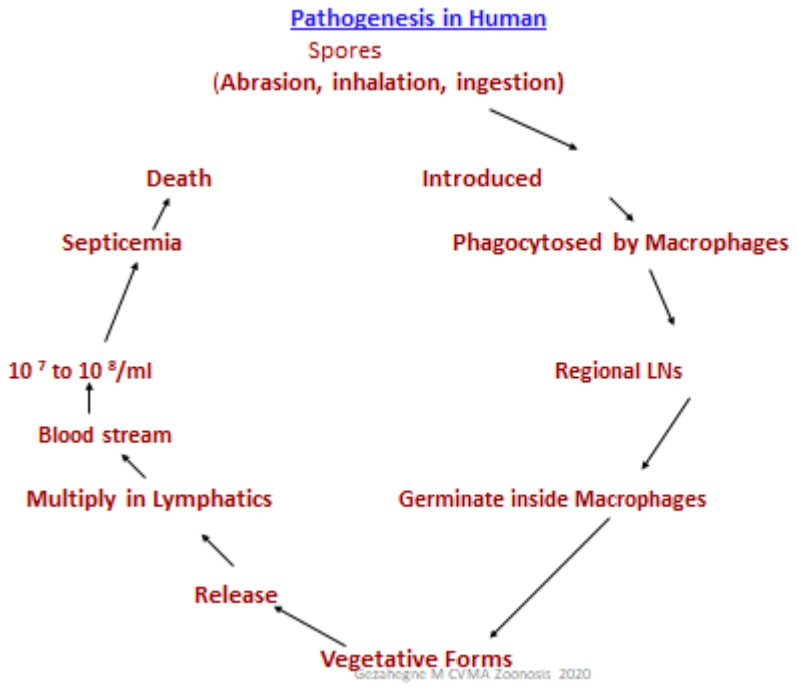
Inhalation Anthrax

- 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%

Intestinal Anthrax

- Due to in ingestion of infected carcasses
- Mucosal lesion to the lymphatic system
- Common in Ethiopia caused by ingestion of raw meat from slaughtered sick animal
- Extremely high mortality rate

Pathogenesis in Human

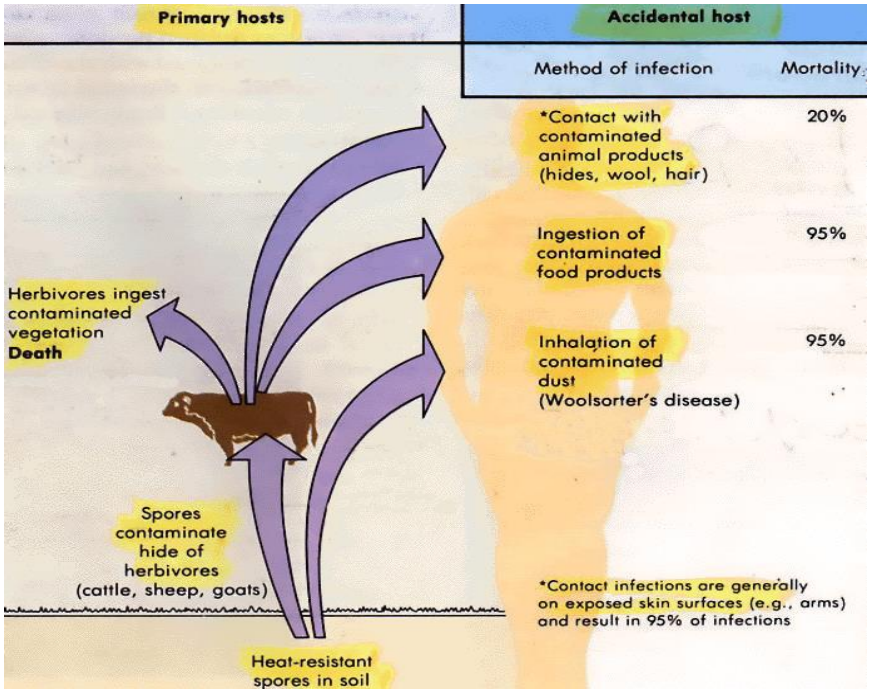


Anthrax in Human

- Three well-defined cycles
  - **Survival of spores in the soil**
  - **Animal infection**
  - **Infection in humans**

Human Cases

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



Zoonotic Anthrax in Ethiopia

- In Ethiopia, few anthrax cases have been reported in humans

1. **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.

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

Immunization Newsbriefs (c) Copyright Information Inc., Bethesda, MD. Brought to you by the National Network for Immunization Information (NNii). Visit NNii's new website at http://www.immunizationinfo.org.
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.

3. **ETHIOPIA: Suspected anthrax epidemic in Afar Region**

World Health Organization

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

Biological Terrorism and Anthrax:

History

Sverdlovsk, Russia, 1979:

- 94 people sick – 64 died
- Outbreak was related to military facility

South Africa, 1978-1980

- Anthrax used by Rhodesian and South African apartheid forces on black tribal land
- Thousands of cattle died, 10,738 human cases, 182 known deaths

Tokyo, 1993

- Aum Shinrikyo – Japanese religious cult – "Supreme truth"
- Attempt at biological terrorism
- Released anthrax from office building
- Vaccine strain used, No human injuries

U.S., 2001

- Using anthrax-contaminated letters
- 22 cases – 11 cutaneous and 11 inhalational, 5 deaths
- Postal workers affected -inhalation anthrax (40% mortality)

Previous acts of biological terrorism have been small in scale, however, anthrax unless strict global biosecurity is enforced, anthrax is a potential bioterrorism weapon.

<u>Brucellosis: Zoonotic brucellosis</u>

- Brucellosis is caused by Gram negative bacteria under the genus *Brucella*
- *Brucella species are small (0.6 x 0.6 to 1.5 µm), nonmotile*, coccobacillary, Gram-negative bacteria
- *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),

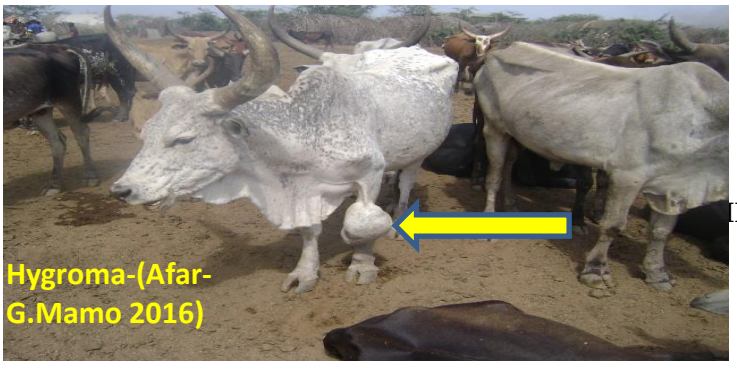
- ***B. canis*** (dogs)
- ***B. neotomae*** (desert wood rats).
- ***B. ceti*** **(**dolphins/porpoises), **B. *pinnipedialis*** (seals), ***B. microti*** (voles) and ***B. inopinata*** (reservoir undetermined)- New members included recently

***Brucella* species and their host/clinical significance**

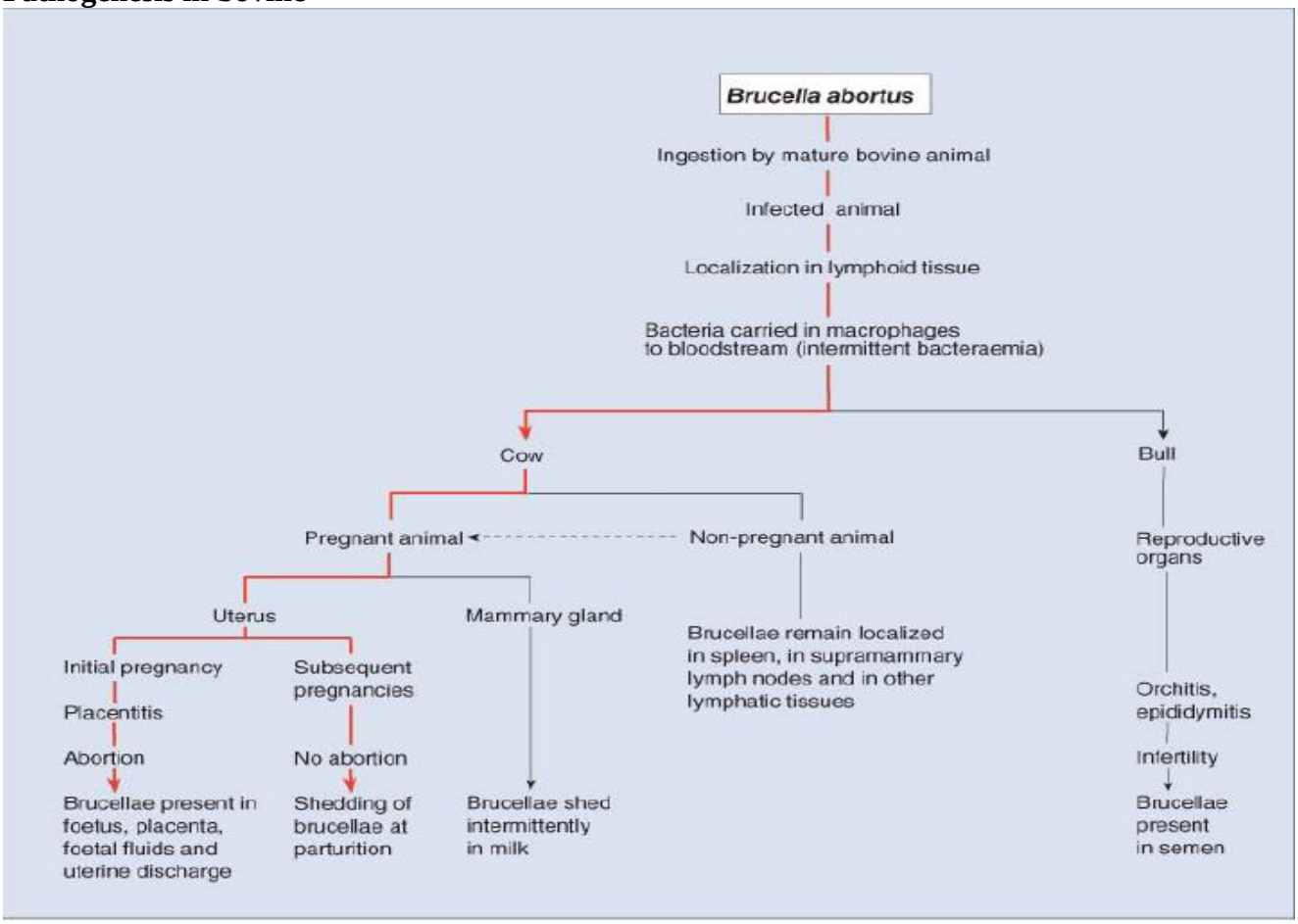
Brucella species	Usual host/clinical significance	Species occasionally infected/ clinical significance
B. abortus	Cattle/abortion, orchitis	Sheep, goats, pigs/sporadic abortion Horses/bursitis Humans/intermittent fever, systemic disease
B. melitensis	Goats, sheep/ abortion, orchitis, arthritis	Cattle/sporadic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

Pathogenesis of Brucellosis

- Brucella infection mostly localizes 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 in bovine



Quinn *et al*., 2011

Epidemiology of Brucellosis

- Brucellosis affects a wide range of animals including ruminants in which it is characterized by abortion , still births or weak calves
- It remains a **major zoonotic disease with a worldwide distribution** but some countries such as the United States of America, United Kingdom, Australia and Japan have eradicated it in livestock rendering the human populations free from the disease
- The mode of transmission among animals is through **exposure of mucous membranes, inhalation of aerosols or direct contact with infected materials**

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.

- 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.

Epidemiology of Zoonotic Brucellosis

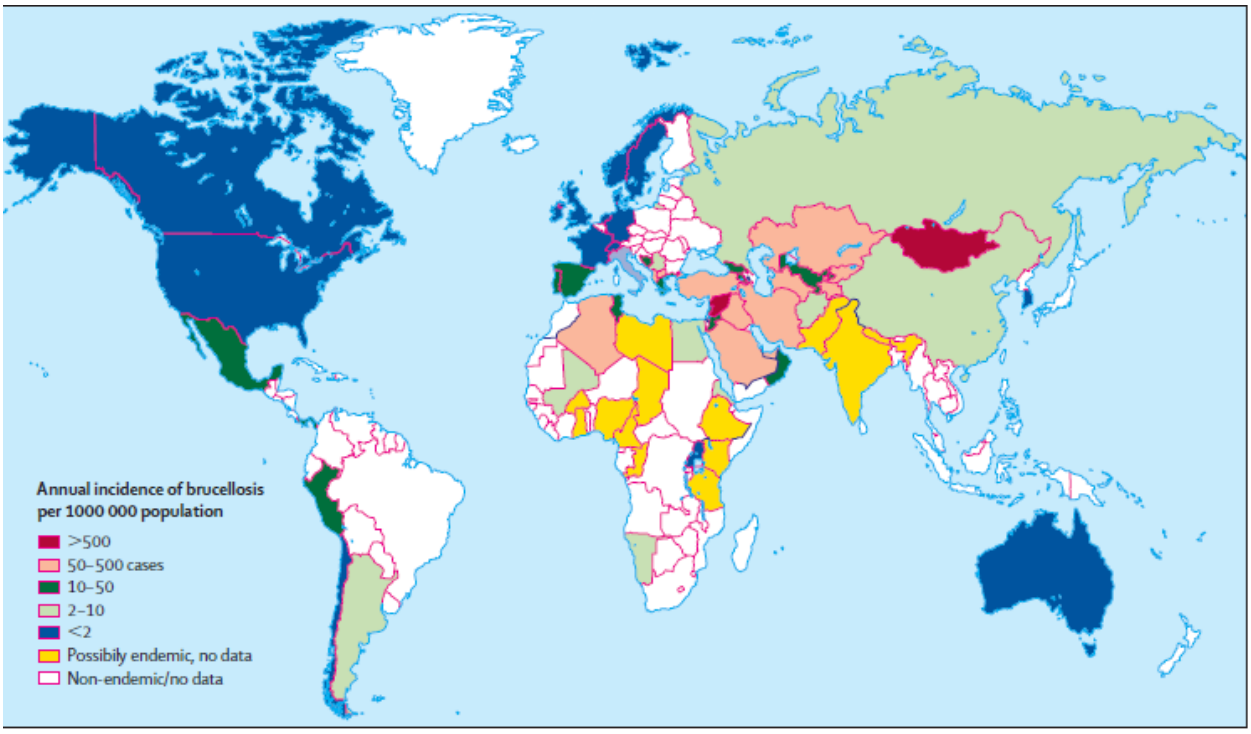


Figure 1: Worldwide incidence of human brucellosis

Source: Pappis *et al.*, *2006*

Brucella as biologic weapon

- Airborne transmission of brucellosis has been studied in the context of using brucella as a biologic weapon.
- *B. suis* was the first agent contemplated by the U.S. Army as a potential biologic weapon and is still considered in that category
- In a **hypothetical attack scenario**, it was estimated that release of an **aerosolized form of brucella** under optimal circumstances for dispersion **would cause 82,500 cases of brucellosis and 413 fatalities**
- Cases of laboratory-acquired brucellosis are the perfect examples of airborne spreading of the disease

Zoonotic 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)***

PLOS | NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

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

Biruk Zerfu[1], Girmay Medhin[2], Gezahegne Mamo[3], Gezahegn Getahun[4], 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 ≥ 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 ≥ 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

PLOS | ONE

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 Medhin[1], Mekonnen Bayissa[2], Gezahegne Mamo[3]

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

* mengistu.legesse@aau.edu.et

Abstract

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 participantsalso 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 (μg/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 wk	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

Pappas *et al, 2005 N Eng J Med 352*

Epidemiology of livestock Brucellosis in Ethiopia

- In **Ethiopia, brucellosis is known to be endemic** since first reported in 1970s (Domenech, 1977; Meyer, 1980) and is **still a major disease of both socio-economic and public health importance**
- Brucellosis has been reported in animals and humans in different localities of the country

Bovine brucellosis

- Extensive seroprevalence has been done
  - Intensive production system: **ranges from 0 to 50%**
  - Extensive production system including pastoral area: **ranges from 0.77% to 18.6%**
  - ***In 2016, first published report of B. abortus***

World's Veterinary Journal
World Vet J, 6(2): 80-88, June 25, 2016
ISSN 2322-4568

Isolation and Identification of *Brucella* Species from Dairy Cattle by Biochemical Tests: The First Report from Ethiopia

Minda Asfaw Geresu[1]*, Gobena Ameni[2], Alehegne Wubete[3], Angela M Arenas-Gamboa[4] and Gezahegne Mamo Kassa[5]
[1]*School of Agriculture, Animal and Range Sciences Course Team, Madda Walabu University, Bale-Robe, Ethiopia*
[2]*Aklilu Lemma Institute of Pathobiology, Addis Ababa University, Addis Ababa, Ethiopia*
[3]*National Animal Health Diagnostic and Investigation Center, Sebeta, Ethiopia*
[4]*College of Medicine, Texas A and M University, College Station, TX 77845, U.S.A.*
[5]*College of Veterinary Medicine and Agriculture, Addis Ababa University, Bishoftu, Ethiopia*
*Corresponding author's Email: minda.asfaw@gmail.com

ABSTRACT

Isolation of *Brucella* organism is considered as the gold standard diagnostic method for brucellosis since it is specific and allows biotyping of the isolate, which is relevant for control of brucellosis using vaccination. Serological studies revealed that brucellosis is endemic in bovines in Ethiopia. Even though seroprevalence of brucellosis is established in different species of animals, so far there was no successful attempt to isolate and identify *Brucella* spp. in dairy cattle at farm level in the country. Therefore, the endeavor of the present study was to isolate *Brucella* spp. from seropositive cattle with a history of abortion. A total of 570 dairy cattle from 35 herds were screened serologically by Rose Bengal plate test based on the history of abortion in the farm. Among the tested samples 13 (2.28%) were found positive by Rose Bengal plate test screening while 33 samples were found sero negative upon serological screening test but were collected from the cattle with history of recent abortion. Forty six clinical samples were cultured which were both from *Brucella* seropositive and seronegative (dairy cattle with history of abortion) upon Rose Bengal plate test screening. Three (6.52%) samples were *Brucella* culture positive and further characterization of all the three isolates based on biochemical tests result confirmed that the pathogen was *Brucella abortus*. *Brucella abortus* was isolated from placental cotyledon 1/9 (11.1%) and vaginal swab 2/23 (8.69%) while no isolate was obtained from milk and fetal abomasal contents (abomasal aspirate) of aborted fetus. Our finding revealed the occurrence of *B. abortus* in dairy cattle of Ethiopia through isolation of the organism for the first time from seropositive dairy cattle with a history of abortion. The organisms were isolated from placental cotyledon (one isolate) and vaginal swab (two isolates) while no isolate was obtained from milk and fetal abomasal contents (abomasal aspirate) of the aborted fetus. Hence, the bacteriological isolation and identification of *Brucella abortus* from dairy cattle indicates the importance of brucellosis in dairy cattle industry of the area and potential public health implication for human population in the study areas.

Key words: Isolation, Dairy cattle, *Brucella abortus*, Biochemical test, Ethiopia

ORIGINAL ARTICLE
pii: S232245681600012-6
Received: 111 May 2016
Accepted: 10 June 2016

Caprine and ovine brucellosis

- seroprevalence ranging from 1.6% to 9.4% in lowland pastoral regions of Ethiopia and highland ranges from 1.6 to 4.9%
    - **Only one isolation of the *Brucella species published* reported (Sintayehu et al 2015)**
      Recently MSc graduate in Vet. Microbiology –isolated 8 *M. meletinsis from aborted cases of goat from Afar-CVMA-ALIPB- AAU Brucella project – 2017- 4 isolates confirmed with PCR*

Tekle et al. BMC Microbiology (2019) 19:108
https://doi.org/10.1186/s12866-019-1474-y

BMC Microbiology

RESEARCH ARTICLE Open Access

Isolation and identification of *Brucella melitensis* using bacteriological and molecular tools from aborted goats in the Afar region of north-eastern Ethiopia

Muluken Tekle[1], Mengistu Legesse[2], Bedaso Mammo Edao[1], Gobena Ameni[2] and Gezahegne Mamo[1*]

Abstract

Background: Infection with *Brucella melitensis* (*B. melitensis*) is one of the most important causes of abortion in goats and sheep, and also causes severe systemic disease in exposed humans. In Ethiopia, based on seroepidemiological studies, brucellosis is known to be endemic. However, there is little information on the isolation and molecular detection of *Brucella* species in small ruminants. Therefore, the present study was conducted in the Amibara district of Afar Region of Ethiopia to isolate and molecularly detect Brucella infection in small ruminants.

Results: Out of the total 64 samples cultured, eight samples (five vaginal swabs and three milk) were positive for *Brucella* species based on colony morphology, growth characteristics, modified acid fast staining and biochemical tests results. Further identification using Brucella- ladder PCR method showed that four of the isolates (three from vaginal swabs and one from milk) from goats amplified fragments of 1071 bp, 794 bp, 587 bp, 450 bp and 152 bp in band size. The molecular result combined with the microbiological and biochemical characteristics of the isolates indicated that the isolates were strains of *B. melitensis*.

Conclusion: The finding of this study could suggest economic and zoonotic significance of *B. melitensis* and warrants for the need for control strategies in livestock and creation of awareness in the pastoral communities on the safe consumption of foods of animal origin and avoidance of physical contact with aborted materials.

Keywords: Abortion, *B. melitensis*, Goats, Isolation, Molecular detection, Afar region, Ethiopia

Table 1 Types of samples and *Brucella* isolates

Sample Type	Animal Species		Sample cultured	Isolates	Percentages (%)
	Sheep	Goat			
Milk	2	26	28	3	10.71
Vaginal swab	–	27	27	5	18.51
Fetal abomasal content	–	2	2	0	0
Fetal membrane	2	5	7	0	0
Total	4	60	64	8	12.5

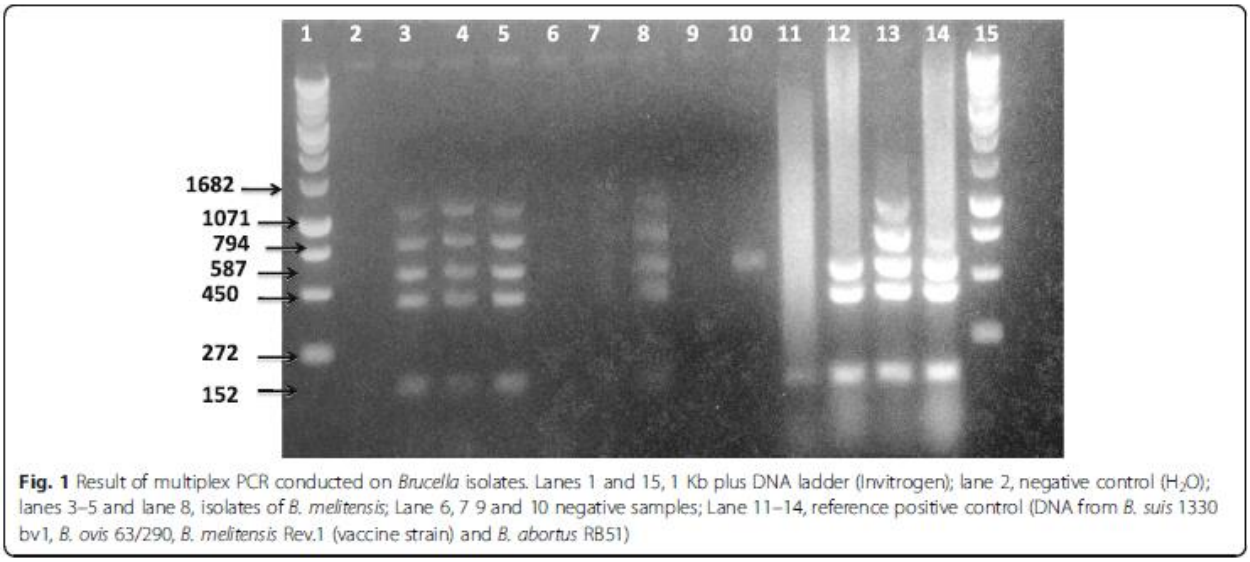


Fig. 1 Result of multiplex PCR conducted on *Brucella* isolates. Lanes 1 and 15, 1 Kb plus DNA ladder (Invitrogen); lane 2, negative control (H_2O); lanes 3–5 and lane 8, isolates of *B. melitensis*; Lane 6, 7 9 and 10 negative samples; Lane 11–14, reference positive control (DNA from *B. suis* 1330 bv1, *B. ovis* 63/290, *B. melitensis* Rev.1 (vaccine strain) and *B. abortus* RB51)

Camel brucellosis

- Brucellosis in camel is largely understudied.
- Few studies carried out so far indicated that the prevalence ranges from **1.8% to 5.7%** in camels of Borena, Somali and Afar lowland areas of Ethiopia
- **No isolation of the organism**

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**
  - **RB51 vaccine**
- In small ruminants,
  - **Modified live *B. melitensis Rev. 1* strain-** adminstered by subcutaneous or conjunctival routes is used to vaccinate kids and lambs up to 6months of age

Effort is underway to design Ethiopia-Brucellosis control Strategy that may have a significant impact on the prevention and control of brucellosis in livestock and human.

National Brucellosis Prevention Control and Elimination Strategic Plan (2020-2029): a one-health approach based joint strategy plan for Ministry of Health (MoH), Ministry of Agriculture (MoA), Environment, Forest and Climate change Commission (EFCCC)

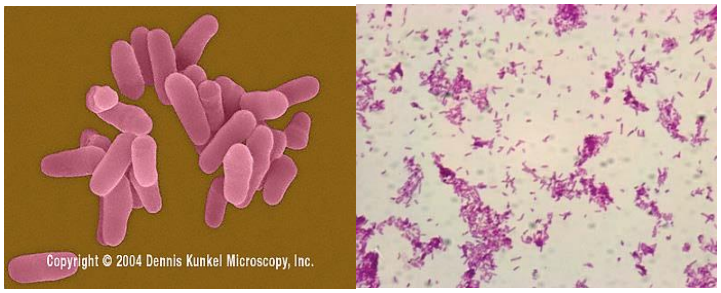
Zoonotic Tuberculosis

Tuberculosis (TB)

- Chronic disease of humans and animals characterized by granulomatous lesions
- *Mycobacterium tuberculosis* complex: mainly *M .tuberculosis, M. bovis, M. africanum*
- Major public health problems that cause global morbidity and mortality

***Mycobacterium* species**

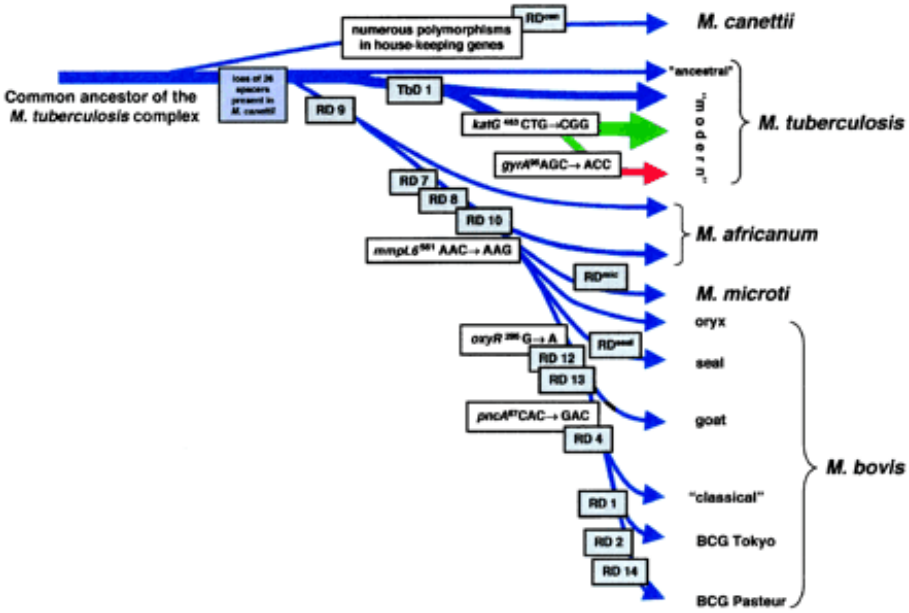
- **Thin rod shaped, non-motile, non-sporing, do not form capsule, strict aerobic bacilli, acid-fast bacilli**



Scanning Electromicroscopy photo of *Mtb* ***AFB staining of Mtb***

Mycobacterium tuberculosis complex

- Tuberculosis caused by members of MTC, clinically are indistinguishable and requires isolation and molecular characterization of the isolates
- ***Mtb* complex species level diagnosis : RD9, RD4, RD10**



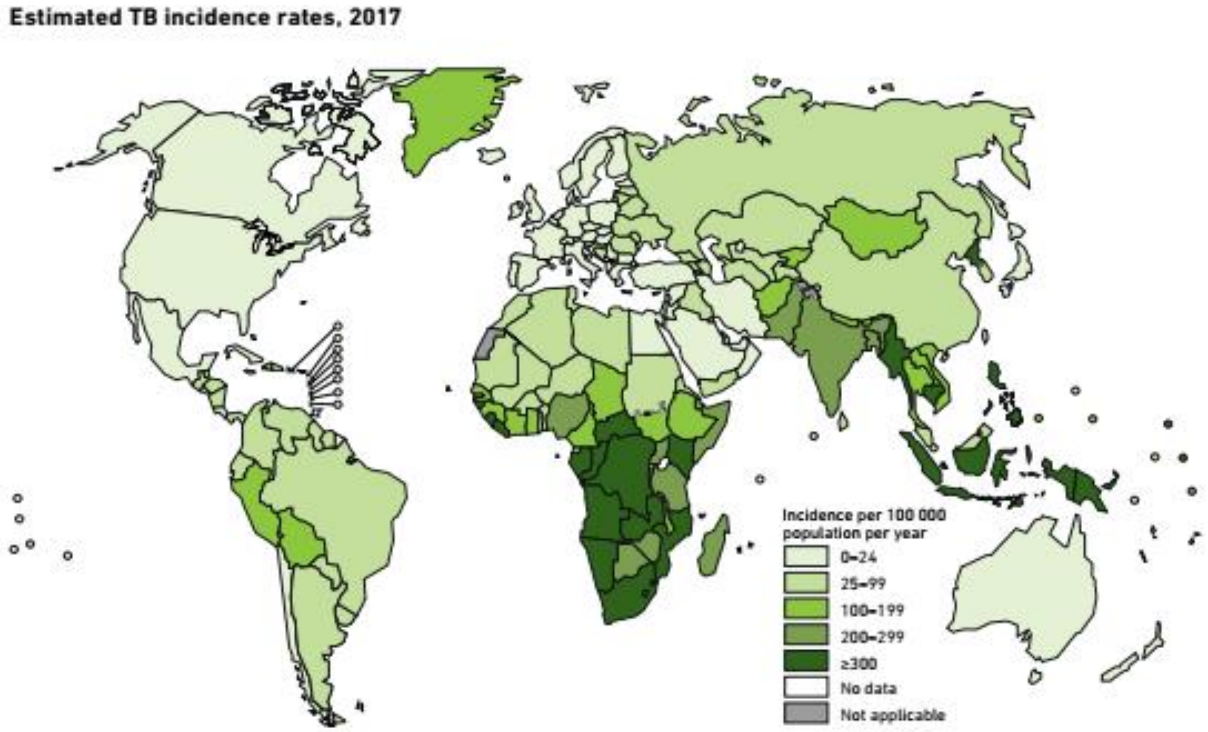
Brosch et al., 2002, PNAS 99:3684-9

- *M. bovis and M. tuberculosis* arise from Common Ancestor

- Hence, the assumption of animals as the origin of human TB found to be wrong based on molecular analysis

Human TB

- 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)



WHO, 2018

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
- Previously it had been speculated that *M. tuberculosis* evolved from *M. bovis* by specific adaptation of an animal pathogen to the human host

- Genomic study of both species has showed that members of *M. tb* complex arise from common ancestor, and *M. tuberculosis* does not evolved from *M. bovis*

Tuberculosis Caused by *M. bovis*

- *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

Human TB and *M. bovis* role

- 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

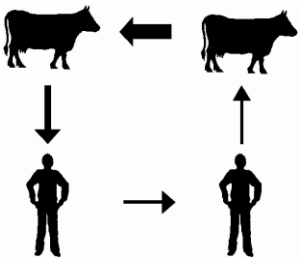
Zoonotic tuberculosis in Africa

- In Africa, nearly 85% of livestock and 82% of the human population live in areas where the disease is endemic or partially controlled (Cosivi *et al.,* 1998. *Emerg. Infec. Dis)*
- 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 as other works by Gumi *et al*., 2012- 1.73%- Borna from PTB, Mamo , 2014 - 1.3%-Afar from PTB cases, Firdessa *et al., 2013- No M. bovis from 1000 FNA samples*

Zoonotic Transmission

- Foodborne: ingestion of contaminated unpasteurized dairy products

- Airborne: Inhalation of aerosol droplets
- Direct inoculation (cutaneous)
  - Butcher's wart in Hunters



Cycle of *Mycobacterium bovis* transmission between cattle and humans. Adapted from *Collins and Grange* (1987)

- 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 a work (Firdessa *et al., 2013)* 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*

Epidemiology of BTB in Ethiopia (2000-2016)

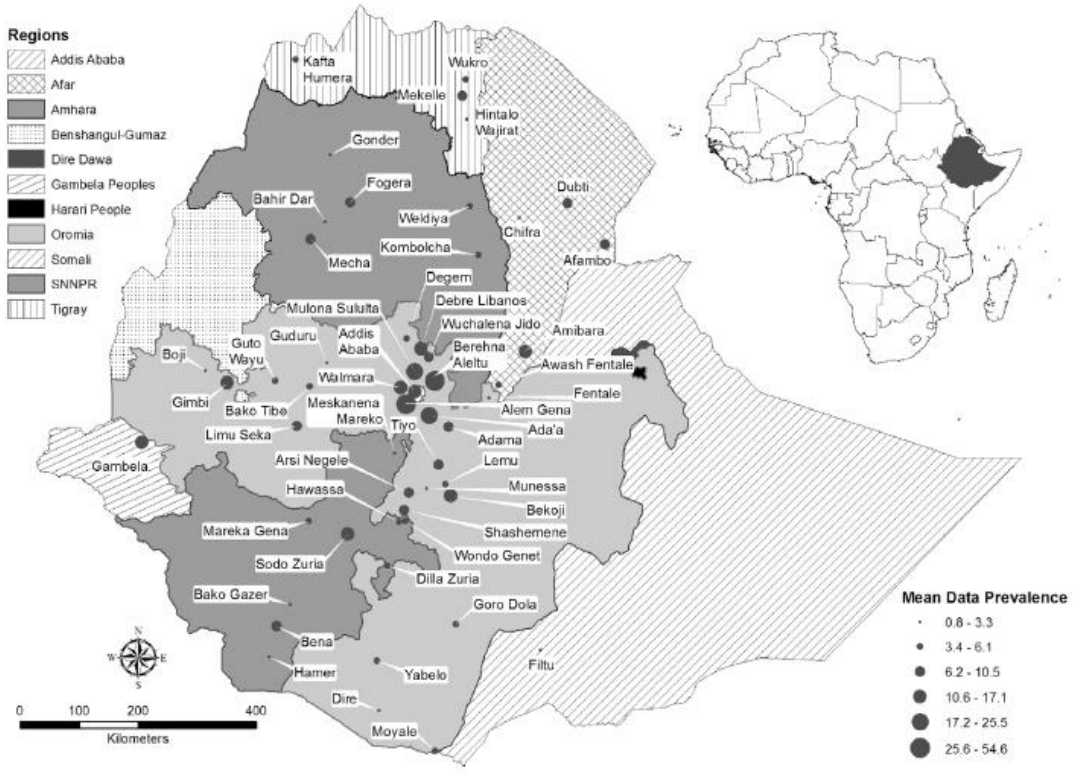


Fig. 4. Distribution and mean prevalence of bovine tuberculosis in districts of Ethiopia. Most of the studies were conducted in Addis Ababa, Amhara, Oromia and Southern Nations and Nationalities Peoples regions while no valid published study was obtained from Benishangul-Gumuz, Harari and Dire Dawa regions. 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.

Molecular Epidemiology of BTB in Ethiopia

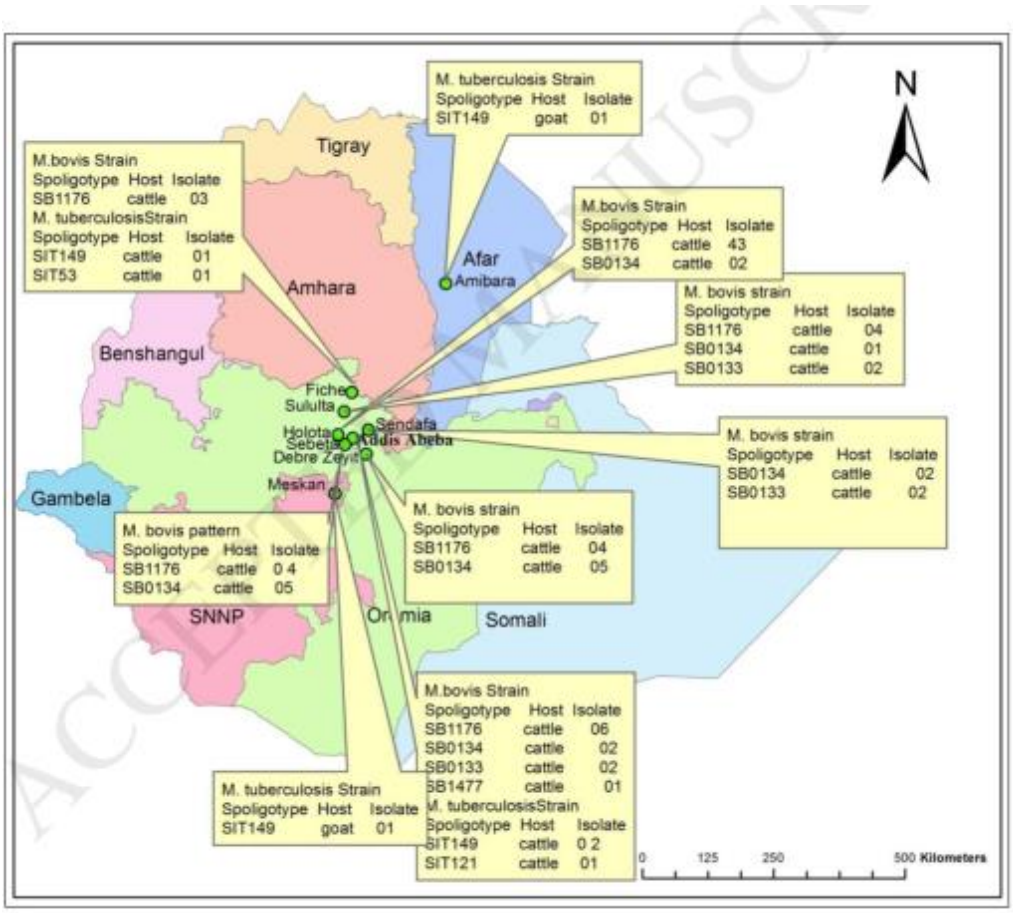
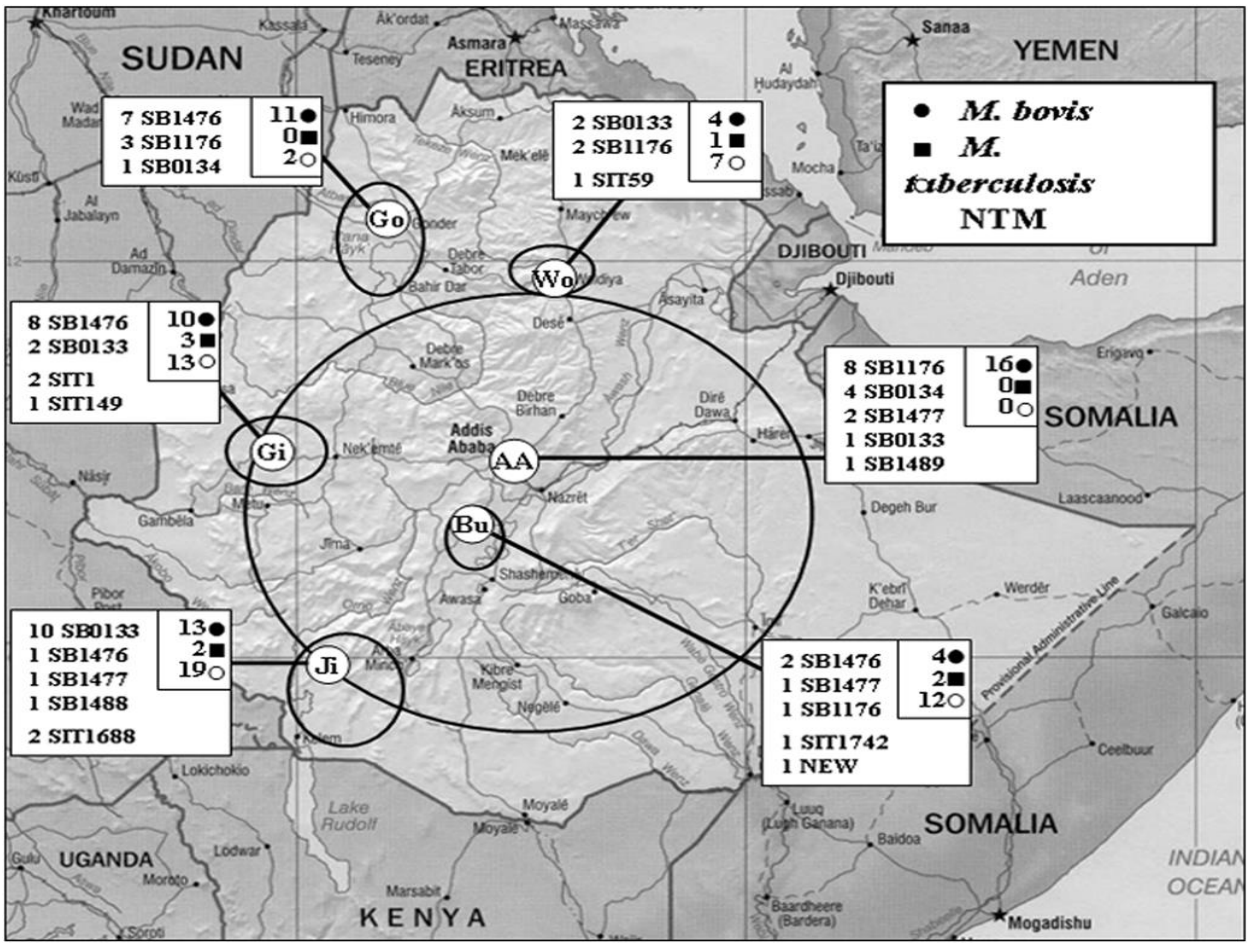


Fig. 2. Geospatial distribution of of *M. bovis* and *M. tuberculosis* spoligotype isolated from tissues of PPD reactor animals, Ethiopia

Romha et al., 2017 (Prev. Vet Medicine 147)



Berg et al., 2009- PLoS One 4 (4): e5068

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. Recent studies in Ethiopia showed the need for further detail study on the role of *M. bovis* in the epidemiology of EPTB and its link with raw milk consumption.

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 cannot 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. Holeta Bull breeding center have established BTB free farm based on Test and slaughter method

Zoonotic TB in Pastoral Regions of Ethiopia

TB is of public health importance in pastoral settings

- Gumi *et al.*, 2012- confirmed 3 (1.7%) *M. bovis isolates* using spoligotyping from sputum samples of PTB human cases in South-East Ethiopia
- Mamo , 2014 (PhD research)- confirmed 2 *M. bovis* were confirmed using spoligotyping and MIRU-VNTR from PTB human cases in Afar Region
- These results indicate
    - the zoonotic importance of *M. bovis* in the pastoralist communities
    - Aerosol transmission is more important than ingestion- which need further investigation

Pastoral setting and potential risk factors bovine tuberculosis

- **Large herd size , mixing of different species of Livestock**
- **Close contact with livestock**
- **Common habit of consumption raw milk/ animal products by pastoralists communities**

Reverse Zoonosis (Anthroponosis) of TB in Ethiopia

- Reverse zoonotic TB is the transmission of *M. tuberculosis from human to animals.*

The Veterinary Journal xxx (2010) xxx xxx

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Short Communication

Mycobacterium tuberculosis infection in grazing cattle in central Ethiopia

Gobena Ameni [a,c,*], Martin Vordermeier [b], Rebuma Firdessa [c], Abraham Aseffa [c], Glyn Hewinson [b], Stephen V. Gordon [b,d,e], Stefan Berg [b]

[a] Alchlu Lemma Institute of Pathobiology, Addis Ababa University, PO Box 1176, Addis Ababa, Ethiopia
[b] TB Research Group, Veterinary Laboratories Agency, Weybridge, New Haw, Addlestone, Surrey KT15 3NB, UK
[c] Armauer Hansen Research Institute, PO Box 1005, Addis Ababa, Ethiopia
[d] College of Life Sciences, University College Dublin, Belfield, Dublin 4, Ireland
[e] UCD Conway Institute, University College Dublin, Belfield, Dublin 4, Ireland

ARTICLE INFO

Article history:
Accepted 7 May 2010
Available online xxxx

Keywords:
Mycobacterium tuberculosis
Tuberculosis

ABSTRACT

A preliminary study to characterise mycobacteria infecting tuberculous cattle from two different management systems in central Ethiopia was carried out. Approximately 27% of isolates from grazing cattle were *Mycobacterium tuberculosis*, while cattle in a more intensive-production system were exclusively infected with *M. bovis*. The practice of local farmers discharging chewed tobacco directly into the mouths of pastured cattle was identified as a potential route of human-to-cattle transmission of *M. tuberculosis*.

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.

Hindawi Publishing Corporation
Veterinary Medicine International
Volume 2012, Article ID 869146, 8 pages
doi:10.1155/2012/869146

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] Yalelet Worku,[2,4] Mengistu Legesse,[1,3] Girmay Medhin,[1] Gunnar Bjune,[3] and Gobena Ameni[1]

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 0.5% (95% CI: 0.2%–0.7%) at ≥4 mm and 3.8% (95% CI: 3%–4.7%) at cutoff ≥2 mm. The herd prevalence was 20% (95% CI: 12–28%) and 47% (95% CI: 37–56%) at ≥4 mm and ≥2 mm cut-off points, respectively. The overall animal prevalence of Mycobacterium avium complex infection was 2.8% (95% CI: 2.1–3.5%) and 6.8% (95% CI: 5.8–7.9%) at ≥4 mm and ≥2 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* (SIT149) 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.

Campylobacteriosis

- Campylobacteriosis is the leading cause of human gastrointestinal illness from a zoonotic source with 2.45 million people a year suffering from *Campylobacter*-causing illness, of which 80% of the cases are foodborne
- Campylobacter are a group of tiny strictly micro-aerophilic curved or spiral Gram negative rods
- *Campylobacter jejuni* and *Campylobacter coli* cause food poisoning and are associated with acute enterocolitis in man.
- *Campylobacter jejuni* occur in large numbers in cattle feces, and poultry as normal flora.
- *Campylobacter coli* are commonly associated with human diarrhoea, and enteritis in pigs

Campylobacteriosis in human

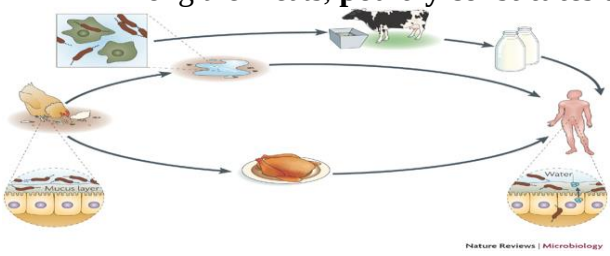
- *Campylobacter jejuni* and *C. coli* cause illness characterized by diarrhoea, abdominal pain, fever, nausea, vomiting, and abdominal complaints.
- The jejunum, ileum and colon are primarily affected resulting in acute inflammation and occasionally, abscess formation.
- The disease is self-limiting.

Clinical signs

- Incubation period ranges between 2-11 days with an average of 3-5 days.
- It is preceded by fever, followed by foul smelling and watery diarrhea, which runs for 3-4 days. The diarrhea may sometimes contain blood and mucus in feces.
- Abdominal pain is associated with backache, and a high mortality.
- The condition is self-limiting but may last for up to 10 days**.**

Mode of infection

- Infection occurs by ingestion of campylobacter organisms in contaminated food stuffs.
- Foods involved include meat from infected animals, unpasteurized milk and possibly cross-contamination from these sources to foods eaten uncooked or unrefrigerated.
- Among the meats, **poultry constitutes the greatest potential source of infection to humans.**



Basic transmission routes of *Campylobacter*

- Microorganisms are present in poultry gut and feces up to 1,000,000 organisms/g of feces.
- Carelessness in the kitchen e.g. cutting chickens with the same knife used to cut other foods without proper cleaning prior to use.
- Pork is a major source of *Campylobacter coli.* Contamination of pork occurs during slaughter.

Epidemiology of Camplyobacteriosis

- In developed countries, including the U.S., the UK, the Netherlands, France, Sweden and Australia, Campylobacter is one of the most frequently reported causes of acute infectious diarrhea
- In the United States, it is estimated that 2.5 million cases of Campylobacteriosis occur annually

In Ethiopia, few published reports are available regarding the occurrence of campylobacter in food animals (cattle, sheep, goats and chickens) and their products. Reports showed around 10% prevalence of camplobacteriosis

- In Ethiopia, the major *Campylobacter spp* isolated from caracasses include *C. jejuni and C. coli*

Table 1: **Prevalence of thermophilic *Campylobacter* species in sheep and goat carcasses in an abattoir in Debre Zeit, Ethiopia (2007-2008)**

	Proportion of positive no. (%)			
	Before evisceration	After evisceration	After washing	Total
Sheep carcass (n=218)	0 (0)	13 (6.0)	10 (4.5)	23 (10.6)
Goat carcass (n=180)	3 (1.7)	13 (7.2)	1 (0.6)	17 (9.4)
Total (n=398)	3 (0.8)	26 (6.5)	12 (2.8)	40 (10.0)

Table 3: ***Campylobacter* species distribution in sheep and goat carcasses examined in an abattoir in Debre Zeit, Ethiopia (2007-2008)**

	Campylobacter species		
	C. jejuni No. (%)	*C. coli* No (%)	Total No (%)
Sheep carcass (n=218)	17 (7.0)	6 (2.8)	23 (10.6)
Goat carcass (n=180)	12 (6.7)	5 (2.8)	17 (9.4)
Total (n=398)	29 (7.3)	11 (2.7)	40 (10.1)

Woldemariam *et al*., 2004

Preventive measures

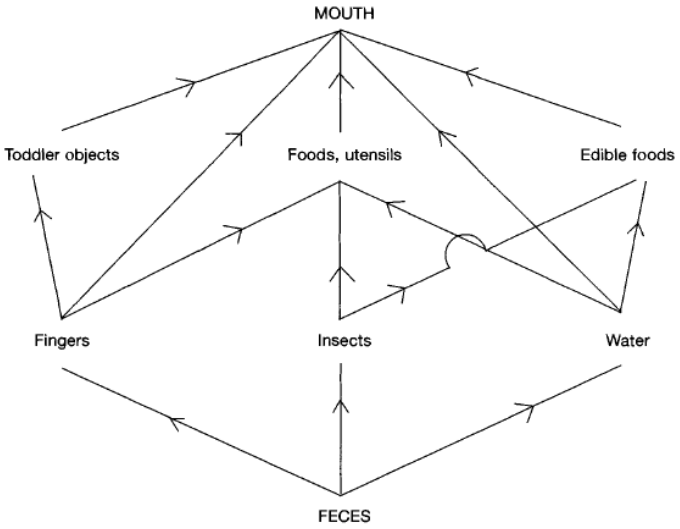
- Thorough cooking of all foodstuffs derived from animal sources.
- Prevention of re-contamination after cooking.
- Proper refrigeration of foods.
- Recognition, control and prevention of campylobacter infections in animals, and
- Maintenance of high standard of hygiene.

Zoonotic Salmonellosis

- Salmonellosis is one of the major zoonotic diseases all over the world caused by *Salmonella* spp. (approximately 2000 serotypes)
- Salmonellosis in human is estimated to be a cause for about 22 million cases and 200,000 deaths due to typhoid fever and 93.8 million cases of gastroenteritis

- Globally, around 155 000 deaths in human occur due to **non-typhoidal Salmonellae (NTS**) –that are caused mainly by S. Typhimurium and S. Enteritidis
- Most of the infections are subclinical. Usual clinical signs are diarrhea, vomiting, low-grade fever. Sometimes progresses to dehydration and death especially in very young or very old individuals
- Incubation period: 6-72 hours, usually 12-36hr
- Case-fatality rate: 1-2% with most serotypes, particular risk in very young, aged and debilitated
- In infected individuals up to 5% of patients may become carriers of Samonella organisms upon recovery from salmonellosis
- Transmission: Ingestion usually through the fecal-oral cycle

Fecal-Oral routes of transmission



- Humans usually acquire infection through the consumption of contaminated products (raw milk, meat, other animal food products) or contact with infected animals
- The minimum number of bacteria necessary for salmonellosis gastroenteritis ranges between 10^5 to 10^{10} /g depending on the serotype

Salmonella outbreaks occur in different forms:

- **Sporadic cases** involving only one or two persons in a household
- **Family outbreaks** in which several members of the family are affected
- **Large outbreaks** caused by a widely distributed infective food item
- **Institutional outbreaks** which may be caused by a contaminated single food item.

Factors associated with Salmonella outbreaks

- Consumption of inadequately cooked or thawed meat or poultry,
- Cross-contamination of food from infected food handlers.

- Presence of flies, cockroaches, rats, in the food environment that acts as vectors of the disease.

Epidemiology of Salmonellosis in Ethiopia

- All food animals are considerable reservoirs of Salmonella and pose a significant risk to public health
- In Ethiopia, the prevalence of Salmonella in slaughtered animals were: Cattle (7.07%), sheep (8.41%), goats (9.01%) and pigs (43.81%)
- A number of serotypes of Salmonella has been isolated in food animals (see table)

Table 4 Frequencies (%) of dominant serotypes

Host, n, Authors	Serotype	Number (%)
Cattle, (n = 69), [29-31][‡]	S. Mishmarhaemek	14 (20.3)
	S. Typhimurium	12 (17.4)
	S. Newport	9 (13)
	S. Eastbourne	6 (8.7)
	S. Infantis	5 (7.3)
	S. Anatum	4 (5.8)
	Others	19 (27.5)
Small ruminants, (n = 55), [33,34]	S. Infantis	15 (27.3)
	S. Typhimurium	10 (18.2)
	S. Butantan	8 (14.6)
	S. Heidelberg	4 (7.3)
	Others	18 (32.7)
Pigs, (n = 267), [36,37]	S. Hadar	85 (31.8)
	S. Eastbourne	40 (15)
	S. Saintpaul	37 (13.9)
	S. Kentucky	20 (7.5)
	S. Typhimurium	15 (5.6)
	Others	70 (26.2)
Camels, (n = 116), [38]	S. Saintpaul	45 (38.8)
	S. Braenderup	26 (22.4)
	S. Muenchen	10 (8.6)
	S. Kottbus	7 (6)
	S. Havana	6 (5.2)
	Others	22 (19)

[‡]The data excludes isolates from holding pens and hand swabs [30].

- Human Salmonellosis is one of the major diseases in Ethiopia
- Several risk factors including under and mal-nutrition, HIV-AIDS, consumption of contaminated animal products may substantially contribute to its occurrence
- Prevalence estimates of Salmonella in stool samples of diarrheic children, diarrheic adults and carriers were 8.72%, 5.68%, and 1.08% respectively.

- **Non-typhoidal (NTS)** isolates are the predominate causes of Salmonellosis in Ethiopian patients **indicating the role of food animals in the zoonotic salmonellosis**

Major serotype of human Salmonellosis in Ethiopia

Table 6 Frequencies (%) of serotypes isolated from samples taken from patients

Serotypes	Number of isolates		
	[47]	[46]	Total (%)
S. Concord	27 (12.5)	85 (75.2)	112 (34)
S. Typhi	105 (48.6)	2 (1.8)	107 (32.5)
S. Typhimurium	24 (11.1)	7 (6.2)	31 (9.4)
S. Paratyphi	18 (8.3)	2 (1.8)	20(6.1)
Others	42 (19.4)	17 (15.0)	59(17.9)
Total	216	113	329 (100)

Tadesse and Sisay, 2014

Control measures of zoonotic Salmonellosis

- Efficient refrigeration and hygienic handling of food.
- Consumption of properly cooked meat,
- Complete thawing of frozen meats and adequate cooking.
- Heat processing of meat, milk , fish and poultry to destroy salmonella organisms in food

Reading Assignment

- *Escherichia coli* serotype O157:H7 its public health significance in Ethiopia
- Listeriosis in Ethiopia and its zoonotic significance
- Leptospirosis in Ethiopia and its public health significance
- Erysipelas its public health significance and status in Ethiopia

Contact: Gezahegne Mamo (PhD, Associate Prof.); Email gezahegne.mamo@aau.edu.et

PART-I, MILK HYGIENE

Introduction

Milk Industry

1. In the last three decades, world milk production has increased by more than 58 percent, from 522 million tonnes in 1987 to 828 million tonnes in 2017.

2.Global cow milk production 2015 to **2019**

In 2015, 497 million metric tons of cow **milk** was **produced worldwide**; by **2019** that figure had risen to around 522 million metric tons. Liquid **milk** made up the largest share of the **dairy** market in terms of market value.

3.World trade in dairy products expanded to 75 million tonnes (in milk equivalents), an increase of 2.1 million tonnes, or 2.9 percent from 2017.

4.Global butter exports expanded by 7.5 percent to 917 920 tonnes in 2018, mainly contributed by NewZealand, the United States of America and India,

World cheese exports increased to 2.57 million tonnes in 2018,

World **SMP(Skimmed milk powder)** exports expanded by 8.6 percent to 2.6 million tonnes, in 2018.

World **WMP(whole milk powder)** exports reached 2.46 million tonnes in 2018.

In Ethiopia, 11.4 million milking cows are currently producing 3,044,977 tons of milk every year. Ethiopian dairy cattle population is distributed over all areas of the country, but the four regions with the highest number of milking cows are Oromia (44%), Amhara (17%), SNNP (22%) and Tigray (9%).

Milk hygiene is a study of all the methods necessary to ensure the production, handling, and final delivery to the consumer of clean, wholesome, unadulterated milk or milk products in such a way it is safe and is having prolonged shelf life.

From economic point as well as public health point of view, Milk Hygiene is important.

Milk hygiene measures start from the dairy farm and continues till it reaches to the consumers and finally consumed by public.

Emphasis is given on different measures to be taken and to evaluate the quality of milk and milk products by different quality control tests.

Types of milk Whole milk: Milk as it is from a dairy animal

Butter milk: Buttermilk is the liquid left behind after churning butter out of cream. The butter milk contains carbohydrates (4.8g) ; (0.9g) fat and (3.3g) protein and calcium per 100 ml.

Skim milk: Skimmed milk is made when all the *milk-fat* is removed from whole milk.

Whey: Whey or milk serum is the liquid remaining after milk has been curdled and strained. Generally 100 L of milk produces about 12 kg of cheese or about 3 kg of casein. In either case, about 87 L of whey is made as a by-product.Whey is also a great way to add sweetness to a product without having to list sugar as an ingredient as whey contains up to 75% lactose.

Powdered milk (or milk powder): produced by removing the water from milk. It may be prepared from skim milk or butter milk or whole milk.

Major Dairy Products

1. **Cream:** It is a fat rich product in which fat is concentrated to not less than 25% by centrifugation.
2. **Butter:** It has minimum 80% fat, 16%water, 1.5-2%SNF, 2.5-3% salt.
3. **Cheese:** Milk fat and casein are concentrated 6-10 times to prepare cheese by dehydration process

Un-organised Dairy industry

- Sales directly from producers to consumers.
- Indirectly through intermediary traders.
- Prices are usually not controlled.
- Prices tend to be higher than those in the formal system.
- Quality, (i.e. keeping quality, safety, wholesomeness) of product not known to the consumers.
- Producers may face economic loss if milk is not sold and spoiled.

Organized Dairy Industry

- Milk Production at Dairy Farms
- Collection Centers
- Dairy Plant
- Distribution

Milk Hygiene Practices

- At Dairy Farm
- At Collection centers
- At Dairy Processing Plant
- During marketing/distribution

2. Nutritional Value and Milk Characteristics

Nutritional Value of milk

Milk is a rich source of protein. Insoluble milk proteins are called casein, whereas soluble proteins are known as whey proteins. One important property of casein is its ability to increase the absorption of minerals, such as calcium and phosphorus.It may also promote lower blood pressure. Whey proteins have been associated with many beneficial health effects such as decreased blood pressure and improved mood during periods of stress.Whey protein is excellent for growing and maintaining muscles.

Milk fat: Whole milk is very high in saturated fats, which make up about 70% of its fatty acid content.Polyunsaturated fats are present in minimal amounts, making up around 2.3% of the total fat content.Monounsaturated fats make up the rest — about 28% of the total fat content.In addition, trans fats are naturally found in dairy products.

Carbohydrates in milk are mainly in the form of the simple sugar lactose, which makes up around 5% of milk. In digestive system, lactose breaks down into glucose and galactose. These are absorbed into bloodstream, at which point liver converts galactose into glucose.

Milk contains all the vitamins and minerals necessary to sustain growth and development in a young calf during its first months of life. The following vitamins and minerals are found in particularly large amounts in milk:**Vitamin B12, Calcium,Riboflavin,Phosphorus.**

More than 50 different harmones are naturally present in cow's milk, which are important for the development of the newborn calf .With the exception of insulin-like growth factor-1 (IGF-1), cow milk hormones have no known effects in humans.

Possible adverse effects

Lactose intolerance: Lactose, or milk sugar, is the main carbohydrate found in milk.It's broken down into its subunits — glucose and galactose — in digestive system.However, some people lose the ability to fully digest lactose after childhood — a condition known as lactose intolerance.Lactose intolerance is associated with many unpleasant symptoms, including gas, bloating, abdominal cramps, diarrhea, nausea, and vomiting.

Milk allergy: It is rare in adults but more frequent in young children.Most often, allergic symptoms are caused by whey proteins called alpha-lactoglobulin and beta-lactoglobulin, but they can also be due to caseins. The main symptoms of milk allergy are skin rash, swelling, breathing problems, vomiting, diarrhea, and blood in stools

Acne: Milk consumption has been associated with acne — a common skin disease characterized by pimples, especially on the face, chest, and back .High milk consumption is known to increase levels of insulin-like growth factor-1 (IGF-1), a hormone thought to be involved in the appearance of acne

Milk Charecterisitics

Chemical composition

Milk of cows and buffalo is consumed by humans worldwide. However, goats, sheep and camel are other animal species producing milk for human use. The normal constituents of milk are water, fats, proteins, lactose and ash. Though these constituents are common in milk of all species of animals but their quantities vary as given in Table 1. The factors like breed of the milch animal, species, stage of lactation, feed, season, disease conditions of udder etc. bring variation in milk composition. Colostrum has high contents of fat and proteins and low concentration of lactose. However, immunoglobulins are exceptionally in higher proportion.

Species	Water	Fat	Protein	Lactose	Ash
Cow	86.6	4.6	3.4	4.9	0.7
Buffalo	84.2	6.6	3.9	5.2	0.8
Goat	86.5	4.5	3.5	4.7	0.8
Sheep	79.4	8.6	6.7	4.3	1.0
Camel	85.6	5.5	4.5	3.4	0.9
Human	87.7	3.6	1.8	6.8	0.1

Physico-chemical properties of milk

Property	Cow milk	Buffalo milk	Goat milk
Acidity	0.13-0.14%	0.14-0.15%	0.14–0.23
pH	6.4-6.6	6.7-6.8	6.5-6.9
Specific gravity	1.028-1.030	1.030-1.032	1.029–1.039
Freezing point	-0.547^{o}C	-0.549^{o}C	-0.540–0.573^{o} C
Color	Yellowish creamy white	Creamy white	White

Flavor (odor and taste)	Sweat taste of lactose and salty taste of minerals	Sweat taste of lactose and salty taste of minerals	Slightly sweet and sometimes salty

Property	**Sheep milk**	**Camel milk**	**Human milk**
Acidity	0.22-0.25	0.03-0.14	0.06
pH	6.5-6.8	6.5-6.7	6.90
Specific gravity	1.0347-1.0384	1.014-1.017	1.029
Freezing point	-0.57°C	-0.57-0.66^{o}C	-0.561- 0.570 ^{o} C
Color	White	White	Bluish white color
Flavor (odor and taste)	Sweet but not as salty as goat milk	Sweet and sharp taste, sometimes salty	Mild, slightly sweet scent

M**icrobial characteristic of normal milk**

Typically, unless there is an intramammary infection or an animal has a systemic disease, milk in the mammary gland at the site of its production either does not contain bacteria or few organisms varying between 500 and 1000/ml. These organisms are acquired from the teat skin or from the epithelial lining of the teat canal or the duct that conveys the milk from the mammary gland to the teat orifice. Some may also acquire from the environment or due to the use of contaminated intramammary preparations which can subsequently be washed out if first few streams of milk are discarded before collection of milk. Some of the common normal micro flora found in raw milk of healthy animal is constituted by the species of genera *Bacillus, Lactobacillus, Leuconostoc, Micrococcus, Pediococcus and Streptococcus.*

Antimicrobial activity of milk: Raw milk is protected from spoilage by inherent natural antibacterial substances that inhibit the growth of spoilage bacteria. However, if the milk is not cooled, these antibacterial substances breakdown causing bacteria to multiply rapidly. Fresh raw milk has an antimicrobial system called as Lactoperioxdase system which can keep the raw milk safe for few hours. This system is constituted by a protein called as lactoferrin, enzymes as lysozyme and lactoperoxidase and immunoglobulins. Lectoferrin an iron-binding protein has numerous beneficial properties including improved absorption and assimilation of iron, anti-cancer properties and anti-microbial action against several species of bacteria. Recent studies also reveal that it has powerful antiviral properties as well. Lysozyme can actually break apart cell walls of certain undesirable bacteria, while lactoperoxidase teams up with other substances to help knock out unwanted microbes too. The immunoglobulins, an extremely complex class of milk proteins also known as antibodies, provide resistance to many viruses, bacteria and bacterial toxins. Studies have shown significant loss of these important disease fighters when milk is heated to normal processing temperatures.

2.Sources and types of microbial- flora of milk

Psychrotrophes: Those organisms that have optimum and maximum growth temperatures above 20C, but which can grow at refrigerator temperature.

Psychrophyles: Those organisms who are capable of growing at temperatures between 0-7^{o}C and whose optimum and maximum temperatures of growth are about 15 and 20^{o}C respectively. Most important genera under this category are Alcaligenese, Pseudomonas and Streptococcus.Others are Flavobacterium, Micrococcus, Pseudomonas, Lactobacillus etc.

Mesophiles: Those organisms that grow well between 20 and 45^{o}C with optimum between 30-40^{o}C. All pathogens fall under this category.

Thermodurics: Those organisms that can survive heat treatment at relatively high temeperatures but do not necessarily grow at these temperatures. e.g. Streptococci. Lactobacilli.

Thermophiles: They may be defined as those that not only survive relatively high temperature but require high temperature for their growth and metabolic activities.
They may also be defined as organisms with a minimum growth temperatures as 30^{o}C, an optimum between 50-60^{o}C, and maximum growth temperatures between 70-80^{o}C. e.g. species of genera Bacillus and Clostridium

Spore flora: Raw milk generally contains a considerable number of bacterial spores, although this number may be low in comparison with total bacterial count. Spores concentration is generally some hundreds per ml of milk. Bacterial spores enter the milk mainly by contamination with particles of dust, soil and manure. Their number does not increase between production and delivery at dairy. The number of spores in the raw milk is, therefore, chiefly governed by the cleanliness.Two important genera of spore forming bacteria are *Bacillus* and *Clostridium* and different species of such bacteria grow under mesophillic and thermophilic range. Spores of *B. subtilis* are most thermo resistant among mesophilic spores while spores of *B. stearothermophilus* most heat resistant spores.
Lactic acid bacteria: They are the fermentative bacteria which produce lactic acid from hexoses and consist four genera, viz. Lactobacillus, Leuconostoc, Pediococcus, and Streptococcus.
Microflora of raw milk
Interior of udder: Milk from an apparently healthy cow contains microorganisms which are generally acquired from the walls of the ducts along the teat canal, including those which normally exist in the udder. Some microbes may be introduced in the milk through the teat during treatment or from the environment of the animal. They are subsequently washed out in the first few streams of milk withdrawn from the udder.. Usually bacterial count of milk due to this source varies between 500 and 1000 per ml. A few streams of milk should therefore always be discharged before collection of milk.
Environment: The microorganisms found in soil, animal discharges, straw, dust etc. accumulate on the surface of body, get dislodged during the milking process, and enter the pail contributing a load of 10,000 bacteria or more per ml of milk.
Milkers or Milk handlers: Milkers and handlers suffering from disease conditions contribute to the transmission of pathogens to susceptible individuals by contaminating milk or milk products.
Utensils: A milk can or bucket improperly washes, inadequately sanitized or dried or dirty milking machines contaminate milk. Apart from these, disease causing bacteria such those of typhoid fever may find their way into milk from utensils washed with contaminated water.

Wholesalers, retailers and vendors: The main sources of contamination from these outlets are the milk cans and buckets used for transport of milk as well as dippers used to draw milk from the cans. These containers may add pathogens through contaminated water supplies, carrier individuals handling milk and faecal contamination.

Improperly washing and cleaning of the cans/containers can cause a build up of milk residues that facilitate the growth of microorganisms like *Staphylococcus aureus, Bacillus cereus* and fungi. Spoilage organisms such as *Bacillus species* as well as yeasts and molds may be added.

Microflora of pasteurized milk

Thermoduric and thermophillic bacteria may be present. Thermoduric bacteria may be spore formers like Bacillus spp., but largely non-spore forming spp. growing on the surfaces of improperly washed/sanitized utensils, preheating equipment in the processing plants and the pasteurizing equipment. Thermophillic spp are spore forming, heat loving microorganisms e.g. *Bacillus spp* and Lactobacillus spp. and their presence in milk in large numbers indicates unduly long exposure to pasteurization temperature and in some instances to other insanitary practices.

Contamination after pasteurization: Contamination of pasteurized milk with psychrophilic bacteria, e.g. *Pseudomonas spp.* and *Achromobacter spp.* may occur due to polluted water supplies that are used for rinsing equipment, accumulated residues and poorly sanitized equipment and containers.

Contamination during cooling of pasteurized milk: The pasteurization or sterilization is immediately followed by cooling of the heated product wherein cold raw milk is used to cool the outgoing hot milk. Contamination of milk can occur at this stage if care is not taken to use leak proof equipment.

*Contamination during packaging of pasteurized milk:*If filling equipments, measuring devices, valves, packaging material, sealing equipment etc. are not properly cleaned and sanitized, they can contaminate the milk.

Microflora of sterilised or ultra high temperature treated milk (Spore-flora of milk: Such milk is generally sterile. The presence of thermoduric organisms is rare, whereas the only organisms to survive temperatures of upto 135-150^{o}C are spores of thermophilic bacilli, e.g. those of Bacillus stearothermophilus and sometimes mesophilic bacilli and clostridia. The psychrotrophes may get entry in such milk due to faults in filling and scanning of packs during packaging and may produce off flavor.

3.Hygienic Practices at the Dairy- Farm for the Production of High-Quality Milk

Objectives: Clean milk is defined as milk that is drawn from the udder of healthy animals, collected in clean dry utensils, free from extraneous matter like dust, dirt, flies, manure, etc., has normal composition and posses natural milk flavour, low bacterial count and completely safe for human consumption. It is an excellent medium for the growth of microorganisms. If it is produced unhygienically and handled carelessly, it gets contaminated very easily leading to its early spoilage. To prevent this, hygienic production of milk is emphasized.

Hygienic practices at the dairy farm should be focused on the following points:

1. Dairy Farm building
2. Health care of animals at farm
3. Pest control in Dairy farm
4. Water supply at Dairy farm
5. Handling of milk at dairy farm
    - Udder washing
    - Milking of animals
    - Straining of milk
    - Cooling of milk
6. Hygienic control of dairy equipments
7. Health control of Dairy workers
8. Quality Control Tests at Dairy farm

1. **Dairy Farm Building**

1. House Buildings:

1.1.Cow house is a specialized building which should be carefully designed and constructed so as to provide comfortable and healthy housing for cows and at the same to enable them to be milked in clean condition.

1.2.Good ventilation and Lighting

1.3.Pest control in Dairy farm building: Hygienic problems at dairy farm are caused by different types of flies, ticks, mosquitoes, lice, cockroaches etc. which can be controlled by sanitation (concrete floor, good drainage, proper disposal of waste and unused manure), using of screens and electric grids and application of insecticides.

Check the breeding of these flies regularly by removing of waste, dung etc.

Use of some safe insecticides in the form of solution in deodorized kerosene or wetable powder in water and sprayed to all surfaces where flies settle.

i) Spray at monthly interval with a suspension of 7.5 pounds of 5% Rotenone/0.5% Toxaphane powder in 100 US gallon of water. Mixture may be rubbed as dry 55 dust into the hairs of back.
ii) Mist spray of 3-5% of an organic thiocyanate as an oil solution
iii) Dip cattle in arsenic solution (0.175% -0.19%As_2O_3) or 0.5% Toxaphane or 0.5% DDT plus 0.03% gamma BHC
iv) Spray with 0.5% of malathione/0.55 Gamma BHC/0.5% Toxaphane or methoxychlor.

2. Healthy Environment at the Farm

Water supply at dairy farm should be adequate and of suitable chemical and good bacteriological quality.

Adequate;

- For drinking up to 150 liters per cow per day
- For cleansing about 50-75 liters/day per cow
- For cooling the milk whose quantity depends upon the quantity of milk to be cooled and the temperature of the milk to be cooled

3. Handling of Milk at Dairy Farm

A. Udder washing: Wash the udder by water, preferably luke warm, followed by drying by paper towel or clean cloth. Disinfectants like sodium hypochlorite at the rate of 500 ppm may be added in water which will safeguard against infection causing no-apparent mastitis. Frank case of mastitis should be milked separately and in the last.

***Strip Cup Test*:** It is the test at the time milking which detects early stage of mastitis. It prevents abnormal milk from getting into milk.

B. Milking of animals:

First few streams should not be added in milk bucket as it has number of microorganisms from udder and may be contaminants from teat or udder. It should be collected separately and should not be thrown on the floor otherwise it may attract flies and microorganisms.

- **Hand Milking:** Personal hygiene by milkers like clean clothing, trimming of nails, free of diseases etc.
- **Milking machines:** Milking cups should be dipped in 200 ppm of sodium hypochlorite (any other disinfectants) for 15 minutes.
- **Milking pails;** Should be half closed to minimize contamination and should be washed and cleaned after every milking.

C.Straining of milk:

It is done by passing the milk through a thin cloth/metal strainer and indicates how much dust is getting into the milk. The step is not essential when udder has been washed and milking is by machines. Strainer should be cleaned and disinfected. Straining of milk causes little or no improvement in its bacteriological quality. From aesthetic point of view the quality is improved.

D.Cooling of milk:

It is done to keep the milk in a bacteriological stable state, i.e. not allowing the organisms present in milk to grow. It should be done as soon as possible after milking because bacteriostatic effect of milk lasts upto 3-5 hours. It should be cooled to 10^{o}C or below. Cooling devices should be simple and easy to clean.

Methods:

i. In-can immersion cooling: The milk cans are immersed in insulated water tank upto the level of the milk. The water is insulated tank should be chilled. Cooling in such tanks is rapid if the milk is continuously agitated. A can of 10 gallons take approximately 3 hours to bring temperature from 90^{o}F(32.3^{o}C) to 50^{o} F(10^{o}C).

ii. Surface Cooling: In this method, cold water or a referigerant is allowed to pass into seamless straight or corrugated tubes. Milk is allowed to fall on these tubes and cold water/referigerant takes heat of milk. One example of heat exchanger is 20% calcium chloride.

4. Hygienic Control of Dairy Equipments: It is done by cleaning and sterilization and the aim is to leave the surface as free as possible from milk residues and from milk-souring bacteria. Cleaning and sterilization are complementary processes.

Steps of cleaning:

- Pre-rinse with water which removes soil and wet the surface.
- Washing with detergents which breakdown proteins, fats and dissolve precipitates due to hardness of water.
- Removal of used detergent solution together with the suspended and dissolved soil.
- Final rinsing to remove the last trace of detergents.

Some the detergents which are use in dairy farm are phosphoric acid, acetic acid, citric acid, tartaric acid, gluconic acid which are acidic detergents where as washing soda(sodium carbonate), causting soda(Sodium hydroxide), trisodium phosphate, sodium metasilicate as basic detergents. Detergent solution should be used at a temperature of about 40^{o}C(115^{o}F)

Sterilization: It can be done by heat or by chemical like hypochlorites of sodium and calcium:300 ppm for 2 minutes

Iodine compounds: These are excellent bacteriocidal but corrosive, toxic and less soluble. To modify these undesirable properties of iodine, iodine is loosely combined with non-toxic wetting agent like phosphoric acid, which acts as carrier. These are non corrosive, do not stain the material, minimum iodine odour and are not affected by hardness of water.The germicidal properties are more and prevents the formastion of milk stones on the utensils They are well suitable for udder washing and inpreparation of mastitis and have much more lasting effect on the udder skin.

Combined use of detergents and hypochlorites like 0.25% detergent+ 300 ppm hypochlorites are becoming popular as it has many advantages. These include minimum contact of 2 minutes for adequate sterility, increasing penetration power and thus increased germicidal activity and reduced corrosive action.

Care and treatment of rubber parts of the equipments like milking machines:

Rubber can absorb 30% of its own weight of fat and thus become soft and swollen. Absorbed fat penetrate in between hydrocarbon chains of the rubber, lubricating them and causing it to become elastic and to loosen them.

Store rubber- parts in dark and exclude air from it and keep in solution that will extract and saponify any absorbed fat. This can be done by dipping in 1% detergent solution at 180°C(82°C) for 30 minutes which form soap with fat and the soap can be washed away with warm water.

5. Health Control of Dairy Workers:

- Regular medical examination of milk handlers for diseases especially of gastrointestinal tract like typhoid, bacillary dysentery, amoebiasis, diphtheria, sore throat, scarlet fever, tuberculosis, staphylococcal infection etc.
- Personal Hygiene
- Reporting of illness by workers
- Health education to workers

6. Quality Control Tests at Dairy Farm

- Test for mastitis: Strip cup Test
- Compositional Tests: Fats, Total solids to detect substandard supply or as basis of payment
- Sedimentation Test
- Acidity test, Alcohol test: to find probable cause of unduly high bacterial population.

7. Routine Quality Control Tests Performed on Milk Supply from a Dairy Farm

1. Viable Count by SPC
2. Direct Microscopic Count
3. One hour Resazurine/Methylene Blue Reduction Test
4. Coilform Count
5. Tests for mastitis:
    - Leucocyte Test
    - White side Test
    - California Mastitis Test
6. Tests for Antibiotics:

- Agar Diffusion Method
- 2,3,5 Triphenyltetrazolium- Chloride Test

7. Compositional Tests:
    - Butter Fat Test,
    - Solid not Fat Test

4.Hygienic collection and transportation of milk

Functions and Construction of Collection Centre

The function of collection centre is to collect milk of good quality from individual producers, cool it, store and transport it to a dairy plant. The milk which is received at collection centre is in raw form and it may take several hours to transport it to dairy plant for processing. During the first hours after milking the multiplication of bacteria is slow, this is lag phase. This period of relative stability is succeeded by log phase during which the bacteria multiply very rapidly, causing an irremediable deterioration in milk quality. Duration of lag phase varies and it may be 10-15 hours for very clean milk having 1000 bacteria per ml and held at 20°C. This period may be 2 to 3 hours or even less having several thousands bacteria per ml under same conditions. It is during the two hours after milking that means of arresting bacterial growth must be applied. Since bacterial growth depends upon temperature, cooling of milk is a simplest method to check it.

1. Construction of Collection Centre:

Site: Collection centre should be as simple as possible, should be located centrally so that farmers/producers in an area of 2-3 Km. can bring milk to Collection centre within 2-3 hours. It should be located in relation to roads, water supply and drainage.

Construction: Generally there are two rooms, viz. receiving and weighing room and refrigeration room.

Rooms should be kept extremely clean to reduce the minimum risk of contamination.

The floors and walls up to a certain height should be freely washable so that this may be done after the reception of each milking.

Due to cooling, there is lot of humidity in collection center and fungus grows very easily. It is desirable to incorporate an antifungal product such as organoborate in the ceiling and the upper, unwashable parts of the walls. The rooms should be well lit and ventilated.

2. Collection of milk at Collection Centre

- Collection in cans: Milk remains in cans, cooled and send to processing centre.
- Bulk collection in tankers: After reception, milk is weighed, pooled and cooled to 4^{o}C within few minutes, store in insulated tanks and transported.

Cooling of milk:

- By immersion of the can in a tank of cold water or chilled water
- Spray of cold water or chilled water on the outer surface of the cans
- By passing the milk over a surface cooler using a liquid refrigerant. Cooling is rapid and may occur in a few minutes.

3. Quality Control Tests at Collection Centre

- Observation for appearance and odour
- Tests for bacteriological stability like Alcohol Test, Total Acidity Test, 10 minute Resazurin Test
- Tests for viable counts: Dye reduction test
- Tests for mastitis
- Test for fat % which is performed only if payment is to be made at collection centre.

5.Transportation of Milk from Collection Centre to Dairy Plant.

If efficient cooling facilities are available at the collection centre, cans may be transported without the use of insulated vehicles. However, the cans should be protected from the sun and the journey should not take more than about 3 hours. If cooling facilities are not there, use of insulation tank is necessary to prevent a rapid rise in milk temperature. Insulating tanks are used in warm countries which are double walled and the intervening space is filled with cork, foam plastic or a similar insulating material. Insulated tankers should be used to transport cold milk in bulk. If the tankers are not insulated, the conditions of transport should be such that the temperature of milk is not greater than 10^{o}C when it reaches the destination.

6.Hygienic handling and processing of milk at milk proceesing plant (dairy plant)

Receiving of Raw Milk for Processing

The first step at the dairy plant is to receive the milk from collection centres.This is the key point in relation to the quality of milk or of the products made there from. At this point, quality control tests are performed to determine quickly the suitability of raw milk for heat treatment. It is also tested to detect substandard supplies viz. determination of fat percentage and solid not fat etc. These tests are necessarily rapid tests which can serve as a basis for acceptance or rejection of milk and are called **Platform Tests which** are given below:

1. Taste and Smell
2. Temperature:

- 10^{o}C- stability for 5 hours
- 4^{o}C- stability for 48 hours
- Higher than 10^{o}C- Bacteriologically unstable.

3. Fat %: If payment is to be made.
4. Sedimentation Test
5. Acidity Test: Carried out by titrating a definite volume of milk with standard alkali solution.

Apparent acidity: acidity naturally present in milk which is due to SNF, casein, acid phosphates, carbon dioxide and citrates.

True acidity: produced due to multiplication of microorganisms which utilize lactose and produce lactic acid.

Whenever acidity of milk is mentioned in any context, it means both types of acidity. Apparent acidity varies from 0.08 to 0.1%. In some breeds it is as high as 0.23%.

True acidity may go as high as 10%.

- At acidity about 0.3% milk curdles on boiling
- At 0.6-0.86% coagulate spontaneously
- At 0.8-1%, lactic acid bacteria stops working and other groups start multiplying.

6. Alcohol Test: It is mainly used with milk for manufacturing purposes particularly for condensing and sterilization purposes.
7. Clot on Boiling Test (COB): Determine the acidity and suitability of milk for processing. Positive test is observed when milk is having acidity above 0.17% and is thus not suitable for distribution or for processing.
8. Alizarin-Alcohol Test: Similar to alcohol test and helps to indicate the approximate percentage of acidity.
9. Ten minute Resazurin test: Provides a rapid measure of the sanitary condition and keeping quality of milk.
10. Watering Test: Specific gravity of normal milk: 1.032. If it is appreciably lower, the milk should be rejected for processing

Routine Quality Control Tests which are being performed at Dairy Plant:

Platform tests are quick tests and are crude too. As we may be wrong on our judgment on the basis of platform tests, some other routine quality control tests are being performed to see the quality of milk.

1. Viable Count: by Standard Plate Count (SPC) method.
2. Direct Microscopic Count:
3. Methylene blue dye reduction test
4. Resazurin Dye Reduction Test

Tests for preservatives

- Turmeric Test for Boric acid or borax
- Hehner test for formaldehyde
- Test for Benzoic acid
- Ferric Chloride Test for salicylic acid
- Test for hydrogen peroxide
- Test for hypochlorites

5. Test for antibiotics
   - Agar Diffusion method
   - Triphenyl -teterazolium chloride reduction test
6. Tests for mastitis
   - Leucocyte count
   - Hotis test

- White side test
- Silver nitrate test
- Chloride test
- Bromothymol Blue test

Pasteurization of Milk

Definition of Pasteurization: It may be defined as the heating of milk to such temperatures and for such periods of time as are required to destroy any pathogen that may be present, while causing minimal changes in the composition, flavour and nutritive value.

Pasteurization does not necessarily mean the destruction of all the microorganisms but must accomplish destruction of any pathogens which fortunately are all sensitive to heat.

Why we prefer pasteurization and not boiling of milk on large scale?

i) Boiling causes changes in flavor that are disliked by many consumers, caused pronounced physical changes and also some loss of nutritional value. Also destroys the cream line(the amount of fat that has risen to the top of a milk bottle).

ii) Technically difficult on large scale, commercially uneconomical

Outline of Methods of Pasteurization:

Relationship between time and temperature for killing any microorganism is not a simple straight line but follows a curve in which as the temperature rises, the time needed for destruction falls much more rapidly. Thus to kill the more heat resistant pathogens in milk, combinations like60^{o}C for at least 30 minutes, 71^{o}C about 10 seconds, 75^{o}C less than 2 seconds can be seen, which means as the temperature increase the time reduces from 30 minutes to few seconds and may be a fraction of second at 78^{o}C.

i) The **"Batch holder" process**_____ 60 ^{o}C to 65.5^{o}C for atleast 30 minutes.

ii) **The High temperature short time(HTST) continuous process**- milk is rapidly brought to 71-72^{o}C and is held for not less than 15 seconds and is then cooled rapidly to 10^{o}C or below.

iii) **The ultra-high temperature (UHT) continuous process**___ 135 -150 ^{o}C for few seconds.

Milk Contamination

Why doesn't pasteurization make our milk completely safe? Pasteurized milk still causes outbreaks of foodborne illness. In this section, we'll look at the many ways milk can become contaminated on its journey from the cow to the table.

- Pasteurization: We know that pasteurization doesn't kill all the bacteria in milk, but it won't even kill the ones it's supposed to if the guidelines for time and temperature aren't met. One way the dairy industry checks milk to make sure it has been properly pasteurized is by testing for alkaline phosphatase. This enzyme has the same D-value as the tuberculosis bacterium, so if it's found in pasteurized milk, that means that time and temperature requirements were *not* met
- Equipment: Post pasteurization contamination (PPC) because of flaws in equipment or poor sanitation practices is the most common reason for pasteurization failures Equipment has to be properly maintained and tested, and cleaned and sterilized between uses.
- Storage and Transfer after Pasteurization:

Keeping Quality of Pasteurized milk: Adequately pasteurized milk in its original container is a hygienic product ready for direct consumption. Pasteurized milk when of good quality prior to pasteurization remains wholesome for as long as 24 hours at a temperature of 18^{o}C and for longer period at lower temperature. It is

preferable to keep milk under refrigeration in the home and it should be kept in original container until the time of consumption. If the milk is partially consumed, the original casp should be replaced, if not soiled.
Sterilized milk does not require refrigeration until opened but should not be exposed to high temperature. After the bottle has been opened, it should be consumed immediately or refrigerated. If it is not possible, it is advisable to boil.

Quality Control Test on Pasteurized milk

i) **Phophatase Test:** The test is done to test the adequacy of pasteurization, i.e. whether the milk has been pasteurized or not. Phosphatase is an enzyme which is present in the raw milk. By pasteurization, this enzyme is inactivated and absence of this enzyme is detected in this test. Usually phosphatase test is done along with coli form count because later tell about postpasteurization contamination.

Interpretation:

a)Phosphatase positive and Coliform positive: Milk is either inadequately pasteurized or Pasteurization is adequate but has been adulterated by raw milk after pasteurization (This raw milk adds phosphatase as well as coliforms).

b)Phosphatase negative and coliform positive: Pasteurization adequate but there is postpasteurization contamination with water which is the source of coliforms.

ii) **Thermoduric Count (Laboratory Pasteurization Count)**: If SPC of pasteurized milk is high, the cause of the count should be determined. Check SPC of raw milk supply. If SPC of pasteurized milk is also high and near to SPC of raw milk, it means contamination of raw milk with thermodurics which may be due to poor sanitary conditions on farm, improper cleaning and sanitizing milking equipments. On the other hand if the SPC of pasteurized milk is higher than that of raw milk, source of thermodurics and thermophiles are within pasteurization plant i.e. due to improper cleaning and sanitization of equipment, faulty pipelines, accumulation of foam in pasteurizers. Proper cleaning and sanitizing practice will eliminate the problem.

iii) **Thermophiles Count:** Can be done by agar plate method with incubation at 55^{o}C(131^{o}F). A microscopic examination of films prepared from pasteurized milk can be employed. The presence of large rod shaped bacteria, which retain the blue stain, strongly suggest that thermophiles are present.

iv) Coliform count

v) Psychrophiles Count

Effect of Pasteurization on Nutritive Value of Milk:

Milk Component	Normal value	Altered value
Lactose	4.75%	No change
Fat	3.75%	Chemically no change but 10-30%reduction
Protein:	3.4%	No effect on caseinogen and lactglobulin but 5% of lactalbumin

Caseinogen(major protein) Lactalbumin Lactglobulin		may be coagulated
Calcium as in soluble and insoluble forms	-	Total quantity remains same but 6% of soluble becomes insoluble
Phosphorus	-	5% reduction
Iron, copper and manganese	-	No change
Iodine	-	20% reduction
Vitamin A, D, E, riboflavin, nicotinic acid	-	No change
Vitamin B1 and C	-	10-20% reduction

7.Milk Adulterants

Adulteration means all non-accidental, preventable changes to dairy or dairy processes that reduce quality or create avoidable risks.

Milk adulteration refers to marketing a product as "milk", while the product does not comply with the legal definition of milk.

Adulterants are mainly added to increase the shelf life of milk. Some of the preservatives like acid and formalin are added to the milk as adulterants, thereby increasing the storage period of milk. Generally, water is added to the milk to increase the volume content of the milk. Some of the common adulterants found in milk and their detection are discussed below:

1. Water
2. Formalin
3. Sugar
4. Starch,
5. Acids
6. Soap
7. Ammonium sulfate.
8. Urea
9. Salicylic acid
10. Hydrogen peroxide
11. Boric acid or borax
12. Neutralizers like hydrated lime, sodium hydroxide, sodium carbonate or sodium bicarbonate

Synthetic milk: It is an artificially manufactured substance that has the appearance of natural milk but does not possess milk's nutritional components and taste. Synthetic milk is produced by chemically combining different low-grade substances. The common practice is to mix synthetic milk with natural milk. Such mixed milk is

usually referred to as adulterated milk. Synthetic milk can also be added to other milk-based products. It is prepared by mixing urea, caustic soda, refined oil (cheap cooking oil) and common detergents. Detergents are added to emulsify and dissolve the oil in water giving the frothy solution, the characteristic white colour of milk. Refined oil is used as a substitute for milk fat. Caustic soda is added to the blended milk to neutralize the acidity, thereby preventing it from turning sour during transport. Urea/ sugar are added for solid-not-fat (SNF). The above prepared synthetic milk looks like natural milk, except in taste and nutritional qualities.

The use of synthetic milk has been found to have cancerous effects on human beings. Urea and caustic soda are very harmful to heart, liver and kidneys. Urea is an additional burden for kidneys as they have to do more work to remove urea from the body. Caustic soda which contains sodium acts as slow poison for those suffering from hypertension and heart ailments. Caustic soda also deprives the body from utilizing lysine, an essential amino acid in milk, which is required by growing babies. Such artificial milk is harmful for all, but is more dangerous for pregnant women, fetus and persons who are already having heart and kidney problems.

8.USE OF PRESERVATIVES IN MILK

Methods to prolong the keeping quality of milk:

- Artificial cooling on the farm as well as during transport: Cooling is an ideal but costly method. Many developing tropical countries may not afford.
- Pasteurization or sterilization: Pasteurization /sterilization are practicable only if milk plant exists, which is not the case in many developing or underdeveloped countries.
- Use of some preservatives:

1. It is an alternative in absence of facilities for cooling or pasteurization /sterilization.

Definition of Preservative:

It is any chemical compound/or process which when applied to milk, retards alterations caused by the growth of microorganisms, or enables the physical properties, chemical composition and original nutritional value to remain unaffected by microbial spoilage.

Differentiation of preservative, stabilizer and food additive

- Preservatives prevent or inhibit spoilage of food due to fingi, bacteria and other microorganisms.
- Stabilizers are often confused with preservatives. Stabilisers maintain the physical and textural characteristics of a product whereas preservatives help to prevent food spoilage by microbes.
- Examples: Chocomilk, contain cocoa powder that can form a sediment when the drink is on the supermarket shelf. Stabilisers help to prevent this.
- Ice cream: Stabilizer prevents the appearance of largem grainy ice crystals or lumps that would make the ice cream feel grity.They help to maintain a firm texture, smooth taste and good stoarage qualities.
- Food additives are substances added to food to preserve flavor or enhance its taste and appearance. Some additives have been used for centuries; for example, preserving food by pickling (with vinegar), salting as with bacon, preserving sweets or using sulfur dioxide as in some wines.

Chemicals as milk preservative:

- Antibiotics (like Penicillin, Streptomycin, Aureomycin etc.).
- Quarternary ammonium compounds (like cetyl- trimethyl ammonium bromide).
- Menadione

- Chloropicrin
- Bromine compounds of acetic acid
- Mercaptopropionic acid or its compounds
- Formaldehyde
- Extracts of plants
- Peroxides
- Oxygen under pressure

None of the above chemicals fulfills all the properties of a good preservative. Peroxides are considered to be best.

Peroxides as Milk Preservatives:

- Hydrogen peroxide (H_2O_2)
- Calcium peroxide(CaO_2)
- Magnesium peroxides(MgO_2)

Advantage of use of H_2O_2 as milk preservative

- Most acceptable preservative available at present.
- Can be destroyed easily, quickly and completely by catalase. After enzymatic treatment, the breakdown products are water and oxygen which are undetectable in milk. No toxic residues remain once hydrogen peroxide has been destroyed.

  $2H_2O_2$ _____ $2H_2O + O_2$

- Strong oxidizing, bleaching and germicidal agent.
- The concentration used in diary industry appear to have very little influence on the constituents of milk especially native proteins.
- The quantity of hydrogen peroxide required for significant inhibition of bacterial growth is small and does not constitute any appreciable dilution of milk.
- Most of hydrogen peroxide added is decomposed by catalase of microorganisms and leucocytes of milk, while heat treatment can destroy hydrogen peroxide to a certain extent.

Methods of use of H_2O_2

- *Short time treatment in place of pasteurization to reduce the total bacterial count*.

  0.08- 0.1% by weight of H_2O_2 for 20-40 minutes at 49°-54°C. At this temperature peroxide should not remain in milk for more than one hour. After the milk is cooled to about 38°C, catalase is added. Such method is used for the manufacture of milk products like cheese.

- *As a preservative to maintain the keeping quality of milk for a longer period.*

1. Addition of relatively small amount **(0.01 to 0.08%)** of H_2O_2 to improve the keeping quality between milking and arrival at pasteurization plant

2. Addition of a relatively high amount of H_2O_2 to improve the keeping quality and to render pasteurization unnecessary. Concentrations used are:

- *0.1% - 0.24% by weight at 13°C for 8 hours contact.*
- *0.4% by weight at 5°C for 24-35 days.*
- *0.8% by weight at 5°C for 32-40 days.*
- *1.0% at 28°C upto39 days*
- *1.2% at 5°C for 100-110 days.*

However the concentrations higher than 0.1% by weight of H_2O_2 may unfavorably influence the constituents of milk.

Quality Control Test to detect Hydrogen peroxide in milk

Potassium iodide starch paper strip Test:

It is test for the presence of H_2O_2 in milk. It is as sensitive as to detect less than 0.001% by weight of H_2O_2.

- Equal volumes of milk and concentrated hydrochloric acid are mixed.
- One drop of weak formaline solution is added.
- Warm the mixture to 60°C.
- In presence of H_2O_2, the paper becomes blue or violet.

9. Milk associated health problems

I. Milk borne infectious diseases

A. Infections of Animals that can be transmitted from Milk to Man

a) Diseases of principal concern:Brucellosis,Tuberculosis,Streptococcal infections,Staphylococcal enterotoxin poisoning,Salmonellosis,Q-fever,

b) Diseases of lesser importance: These are usually transmitted to milkers through contact (i.e. act of milking) rather than through ingestion.Cow pox,Vaccinia virus,Pseudo-cow pox (milker's nodules)

B.Infections primarily of man that can be transmitted through milk: Streptococcal infections, Staphylococcal food poisoning, Diphtheria,Tuberculosis

II. Milk borne non-infectious disease conditions (Toxicological problems)

- Residues of antibiotics
- Residues of disinfectants
- Residues of insecticides
- Mycotoxins
- Radionuclides
- Plant toxins
- Bacterial enzymes

Brucellosis: It is a classical example of milk borne diseases. All the three principal species (*Brucella melitensis, Brucella abortus, Brucella suis*) can infect man and are excreted in the milk of dairy animals in considerable numbers even in the absence of noticeable udder changes.*Br. melitensis*, most virulent for man, is generally associated with goats and sheep, *Br.suis* next in order of virulence for man followed by *Br.abortus* in cattle. Infection to man occurs by ingestion of unpasteurised milk and dairy products, through broken skin when the animal is milked or by inhalations of aerosols. It causes intermittent (undulant) high fever of long duration in humans, general malaise, swelling around the joints etc..

In animals it causes late abortions and the organisms are excreted whose number varies from day today and is generally highest during the first few days after calving or abortion. This number may be as high as 200, 000 per ml which reduces to 10,000-20,000 per ml. Excretion in cattle and buffaloes is observed up to 1 year while in goats and sheep excretion is up to 6 months. The excretion of the organisms is intermittent. That is why, in a herd of dairy cows, the proportion of animals excreting the organisms at any one time is 15-35% which may be 50-60% in case of infected goats.

There is no sign of mastitis in aborted animals and the organisms get lodged in udder and suprammary lymph nodes which are the site of predilection for Brucella in non pregnant cows. There is no change either in the appearance and taste of milk or its chloride and catalase content. However, in infected goats, udder may show mastitic changes and milk may show clots and discoloration. Sometimes no change in color.

Brucellae in raw milk are conveyed into milk products (cream, butter) made from unheated milk. Souring of milk inhibits but may not eliminate organisms completely for several days. *Brucella malintensis* can remain viable for 15 days at 11-14^{o}C, pH 4-5. Can multiply in milk at 20-38^{o}C and remain viable for about 3 days.

Control of milk borne brucellosis is possible by control, prevention and eradication of the disease from dairy animals and by adequate heat treatment of milk.

Tuberculosis

Milk borne tuberculosis is of non pulmonary type. Significant features are:

1. Cattle and goats infected with any of three types of tubercle bacilli may excrete the organisms in milk even though their udders may be clinically normal. Number of organisms excreted is quite high($5x10^6$ per ml) and therefore single excretor in a herd of 100-200 animals will be able to cause milk borne tuberculosis in consumers. The excretion is intermittent.
2. The human and bovine strains of the tubercle bacilli may be transmitted between animals and human beings who are in direct contact with them, not only through the ingestion of infected material but also through respiratory tract.
3. Tubercle bacilli in milk may be derived from:
    - Affected udder is the prime source.

- contaminated environment (manure, dust)
- Blood to milk of infected animals.
- Splashing of uterine discharges into the milk during milking operations
- Through milkers hands and clothes.

4. Human type tubercle bacilli may gain direct access to milk from milkers and other milk handlers.
5. Avian type bacilli also cause natural infection in cattle and may be excreted in milk. Human infection with avian type is very rare.

Control of milk borne tubercullosis is possible by control, prevention and eradication of the disease from dairy animals and by adequate heat treatment of milk.

Staphylococcal enterotoxic gastroenteritis

Some of the strains of *Staphylococcus aureus* produce heat stable toxins which cause gastroenteritis which is intoxication (one which is due to preformed toxin in the milk) in human beings. *Staphylococcus aureus* that produce it are widely distributed in milch animals (lesions on the teats and skin of udder) and apparently healthy food handlers. Being heat stable enterotoxins, the toxicity may occur even where milk is pasteurized and produced under hygienic conditions.

Control measures include:

- Milk should be cooled as soon as possible to 10^{o}C or lower so that multiplication does not take place and toxin is not produced.
- Avoid contamination of milk after post heating process.
- Infected workers should not handle milk and milk products.

Q fever

The principal reservoirs of *Coxielle burnetii*, the cause of Q-fever, are cattle, sheep and goats. Most human infections takes place by inhalation of dust previously contaminated with amniotic fluid and fetal membrane of infected animals(sheep, goat, cattle)), but oral infection through drinking raw infected milk also occurs. Infected cattle excrete *C.burnetii in* their milk for long periods (more than 200 days).The milk and udder however, do not show any abnormality. Animals generally remain asymptomatic carriers therby causing public health problems. *C.burnetii* in raw milk is transferred to milk products made from it.

Control measures include:

Although adequate heating of the milk destroys the organisms, it may escape destruction at the lowest temperature accepted for pasteurization (61.5^{o}C for 30 minutes), thus leading to potential hazard. Treatment at 63^{o}C for 30 minutes or at 72^{o}C for 15 seconds (an increase of 3^{o}C/5^{o}F in temperature of pasteurization) has been recommended for inactivation of *C.burnetii* in milk. In areas where milk hygiene programmes are not well developed, pasteurization at 66^{o}C by holding method or at 75^{o}C for 15 seconds provides an additional margin of safety.

Non Infectious Diseases (Toxicological problems)

Residues of antibiotics

Antibiotics are excreted in milk after intramammary as well as after systemic treatment or intrauterine applications. Highest levels in milk are, however, obtained when the drugs have been introduced directly into the mammary glands. Part of the dose introduced are absorbed into the blood stream or inactivated, but main part is eliminated with the milk on subsequent milkings. For example, milk from cows which have received about 1000 units of penicillin per Kg. body weight may contain 0.1-1.0 unit of penicillin per ml 12 hours after treatment and 0.01-0.02unit after 24 hrs.In cases with low yield but treated with higher doses, higher concentration of antibiotics will be found in milk.

Stability of antibiotics in milk: The stability of penicillin is known to be about maximal at the usual pH of milk and it does not suffer any appreciable loss of activity through pasteurization. Momentary heating of milk at 80oC does not affect the

biological activity of tetracycline, streptomycin, chloremphenicols etc.Antibiotics have also been found in active state in milk products like cheese, butter, powdered milk etc.Antibiotic residues are of concern in two respects.
Starter failure and
Sensitivity reactions in sensitized human beings.

***Starter Failure*:** Starter culture like Streptococcus therophilus, *Streptocoocus lactis, Lactobacillus acidophilus, Lactobacillus bulgaricus, Leuconostoc citrovorum* are used in preparing various milk products. The residues of antibiotics have inhibitory action on starter cultures with the result that acid production in the milk is delayed. The pH of the cultured milk remain high and favors vigorous growth of gas producing coliforms and other bacteria. Which will ultimately spoil the milk product. Off flavors develop, faulty texture with large irregular holes are found.

Ingestion of antibiotics contaminated milk may cause a reaction in human whos is sensitive to a particular antibiotic.

Preventive measures:

1. Milk of treated animals must be detained on the farm and not delivered to any dairy plant until mammary excretion of drugs has been ceased. In USA minimum detention period of milk of treated animals is 72 hours while in Denmark it is 96 hours.
2. Manufacturers of antibiotics should clearly indicate on packages the length of time during which the antibiotic continues to be excreted in the milk after the last day of administration.
3. Rapid and precise tests for detecting antibiotics in milk should be developed.
4. Distribution of antibiotics should be controlled.
5. A regular control programme should be instituted together with the penalties for misuse of antibiotics.

***Reactions in sensitized human beings*:** Ingestion of antibiotics contaminated milk may cause a reaction in humans already sensitized to the contaminants. Penicillin in milk produces eczematous eruptions in sensitized persons who drink such milk. Heat treatment has little effect on its penicillin contents. Boiling for 1 hour and autoclaving leaves some of it intact.

Residues of Disinfectants

Important disinfectants used in milk industry are:

- Chlorine compounds (Hypochlorite, chloramines): They do not present a serious toxicological hazard because they are unstable in milk and are rapidly decomposed into inactive ions. However 2% off flavor results if more than 2% of chlorine is added and at 10% it proves to be bacteriostatic.
- Iodine compounds: Residues form complexes with casein of milk and at high levels these may be significantly disturb human physiological processes.
- Quaternary ammonium compounds: They are markedly stable in milk and level as low as 0.00001-00005%

Adverse effects of residues of disinfectants in milk:

- Inhibitory effect on starter cultures.
- Residues of disinfectants interfere with grading of milk by means of MBRT test.

Residues of Insecticides

Important Insecticides used in milk industry are:

- Chlorinated hydrocarbons (DDT, aldrin, dieldrin, benzene-hexachloride, methoxychlor)
- Organic phosphates (melathion, parathion)
- Carbamate compounds (Carbaryl)

Sources of milk contamination by insecticides:

- Insecticides used on the cows and in barn for control of flies, lice and ticks.
- Spray on forage crops and grains to protect from pests.

Because of affinity of these insecticides for fats, they become attached to milk fat with the result that the butter made from contaminated milk contains considerable higher proportion of these. Also cream and cheese from contaminated milk have these residues in high concentration. Pasteurization has little effect on residues of disinfectants especially DDT.

Prevention: Spray of insecticides should be done in right proportion as per manufacturer's instructions and should be done carefylly so that it does go inside the body through respiratory or digestive routes.
Insecticide treated fodders should not be given to milch animals.

Mycotoxins

These are poisonous substances produced by the fungi which are grown on food and grains Such feeds when ingested by animals, result in residues in milk. Fungi and mycotoxins produced by them are many but the important ones are:

- *Aspergillus flavus, Aspergillus parasiticus* which produce aflatoxins Groudnuts are very prone to Aspergillus and thus aflatoxins are excreted in the milk. The mycotoxin is carcinogenic and may cause liver cancer in poultry and rates. In children it causes liver cirrhosis. It is teratogenic also.
- Fusarium species like *F.roseum* produces Zearlenon which has adverse effect on growth.

Enzymes in milk

Enzymes secreted by mammary glands: Catalases, lipases, proteinases, phosphatases peroxidases, xanthine oxidases.
Foreign enzymes produced by the bacteria: Proteolytic and lipolytic types. These enzymes lead to intermediary products in milk e.g. Tyromine which can be produced by decarboxylation of tyrosine which can lead to increase in blood pressure.Enzymes retain significant activity even after pasteurization.

Radionuclides

The radionuclides likely to be found in milk are ^{137}Cs, ^{131}I, ^{90}Sr and ^{89}Sr which are produced by the fission of uranium or plutonium and which easily pass through the atmosphere-soil-plant-animal-man chain ^{131}I is a potential hazard to the thyroid, particularly in infants of 6 months of age.

Toxic trace elements

Residues of some toxic heavy metals like lead, cadmium, mercury etc. may be found in milk due oral intake by lactating dairy animals and fractions of ingested meat may pass into the milk.The source of such metals are soil or emissions from industry etc.

Residues of some drugs /chemicals

- Milk from cows fed phenothiazine (an anathemantic) will develop a pinkish color in few hours after milking.
- Copper sulphate used as anthelmentic for liver flukes finds its way into the milk and produces oxidized flavor.

As far as possible, milk from such animals receiving theses drugs/chemicals should be withhold for at least 48 hrs and preferably 96 hours following the last administion.

PART-II, ZOONOSES

1.RABIES

Synonyms: Hydrphobia, Lyssa, Rabbia, Tallwut

Bullet shaped RNA containing virus having two antigens: glycoprotein and nucleoprotein

Stability

i. In infected tissues in undiluted neutral glycerol: several weeks at 25C; several months at 4C
ii. Virus susceptible to pH below 7.0
iii. Inactivated at 56C in 30 minutes
iv. Rapidly destroyed by sunlight or UV-irradiation
v. Susceptible to detergents, organic solvents

Hosts:
Man,
dogs, cat, cattle, sheep, goat, pig, horse, camel,
rat, rabbit, squirrel,
deer, fox, jackal, mongoose, raccoons, skunk, wolf, coyote, bat.

Transmission
Through bites of dogs containing rabies virus
Direct contact of fresh open wound, abrasion of mucous membrane with saliva of rabid animal
Airborne transmission occurs in caves where bats roost are present, and also in the laboratory handling rabies virus
Rarely inter-human transmission through transplantation of cornea infected with virus

In nature, rabies is usually present in sylvatic form, and the infection is maintained in the jungle by transmission from one wild animal to the other.This the main reservoir from where a spillover to dog occur

Wild animals: Fox, jackals in India; A wolf in Iran. Fox in Europe, spotted skunk in North America
Once the canine species is infected, the virus is maintained by dog to dog transmission and man and other domestic animals serve as accidental hosts.
Rabid dog is the principal reservoir of rabies. Disease acquired by contact with infective saliva

Common modes of direct infection

Bites or scratches enough to cause a breach in the epidermis, licking over areas where skin is abraded, scratched or cut or licking over mucosa.

Salivary glands of 70-80% of rabid animals are found to be infective.

Saliva can be infected even before the symptoms appear which may be apparent after 3-4 days.

Indirect transmission

i. Handling of virus contaminated material: rooms floors, utensils, fabric in contact with infected saliva must be decontaminated.
ii. Consumption of unboiled milk or meat of rabid animals if improperly cooked.

Rabies in dogs

Incubation period: 3-8 weeks, as short as 10 days or as long as 6 months.

Clinical features in 3 phases:

i. Prodromal
ii. Excitation
iii. Paralytic stage

Prodromal

Lasts 2-3 days

Change in temperament, may become affectionate or turn aggressive, refuses to take food, slight rise in temperature, dilatation of pupils and sluggish corneal reflexes.

Excitation Phase (furious rabies)

i. Increasingly irritable and restless
ii. Excitability and photophobia
iii. tendency to eat unusual objects like sticks, stones or soil.
iv. Tendency to bite any one coming in its way.

Dumb rabies

Shuns people, hide in dark places, characteristic change in its bark due to laryngeal paralysis, pharyngeal spasm and paralysis, drooling of saliva, froths from mouth due heavy, rapid respiration, convulsive seizures and muscular incordination.

Dog may die or pass to the paralytic stage

Paralytic Stage

Muscular incordination precedes. the paralysis of whole body, then to coma and death.
Whole course of disease upto a maximum of 10 days.

Salivary excretion of the virus

Excretion in saliva is in sufficient quantity. 54-90% of rabied dogs are positive for rabies virus in salivary gland. Quantity of virus in these glands varies from trace to very high concentration.

Rabies in Man

Incubation period: 20-60 days but vary 15days to 1 year after exposure

Factors affecting IP: Strain of the virus, personal susceptibility, location of the bite, amount of the virus injected, depth of the wound.

Symptoms

Generally well except for the symptoms related to the wounds. Headache, malaise, fever and anxiety are some of the symptoms.

Prodromal Stage

As in first stage. Complain of pain or parathesis at the bitten site even before appearance of any other symptoms, agitation, excitability, nervousness, apprehension

Acute Neurological Phase

Nervous signs 3-10days after the prodrome. Marked hyperactivity, usually intermittent

Agitation, thrashing, running, bitting and other bizzare behaviors.When attempt to drink water, show painful spasms of larynx and pharynx, leading to the fear of water(hydrophobia).Hallucination, neck rigidity, faciculation of muscles near

bite areas, hypersalivation and focal or general convulsions.May die abruptly or pass on to stage of paralysis and then coma. Signs of encepahilitis in few cases

Acute neurological phase, confusion, deorientation, stupor and coma.Stage lasts for 2-10 days

Coma Stage

Untreated cases:
The patient usually develops respiratory arrest shortly after a fit of coma and expires.
Under aggressive treatment, the patient may remain comatose if the respiratory arrested is prevented by assisted ventilation.
However, fatal complications can develop and death may occur from any of these abruptly.
Diagnosis

Diagnosis of Rabies

1. Clinical symptoms diagnostic. Differentiate from tetanus: Tonic contraction of masseter muscles but the ability to swallow is not impaired in tetanus.

2. Laboratory Diagnosis: Detection of rabies virus antigen or antibody by immunofluorescence technique.

i. Laboratory diagnosis during the period of illness (Ante-mortem)
ii. Laboratory diagnosis after the death (Postmortem)

Ante mortem diagnosis in man

Isolation from saliva, cerebrospinal fluid(CSF) and urine at certain time periods during illness; antigen detection in corneal smears and skin biopsy by IFT; detection of rabies antobodies by neutralization test, indirect IFT, CFT. But for these we need a well equipped laboratory.

Antemortem diagnosis in an animal: Detection of virus in the saliva of the animal during the 10-day observation period after bite. However, collection of saliva from a rabid dog is hazardous and therefore the animal should be allowed to die (if rabid) due to natural course of the disease.

Postmortem diagnosis in man

1. Demonstration of Negri bodies in the brain smears or histopathology of fixed sections of the brain.
2. Isolation of virus on inoculation of laboratory animals(mice)

Postmortem diagnosis in an animal(dog)

1. Confinement and observation
2. Decapitation and dispatch of the animal head to a rabies reference laboratory. There the brain is removed and divides into two halves. One half should be placed in a jar containing 10%solution of formalin in normal physiological saline. This material is used for histopathological processing and examination. Another half in a jar containing 50% neutral glycerol in isotonic saline for preservation

Hippocampus major, cerebrum, cerebellum, mid brain and basal ganglia are used for biological test in laboratory animal and immunofluorescence.

Preparation of smears for Negri body studies

Staining of smears

1. Methylene blue in absolute ethyl alcohol
2. Basic fuchsin in absolute ethyl alcohol
3. 20 ml of distilled water + 3-4 drops of methyne blue + 1 drop of stock 2 to this.

4. Mixing

Remove the smear which has been fixed in absolute alcohol for at-least 10 minutes.

Flood the smear with sufficient amount of the stain. Keep for 3-5 minutes.

Wash the smears in distilled water and allow to dry in air for a few minutes.

Negri bodies are bright red intra-cytoplasmic inclusions. Generally 1.5 microns, may be as big as 15-20 microns. They look round or oval

Control of Rabies in humans

1.Health education

- Menace of the disease
- Its mode of treatment
- Danger from animal bite
- Importance of local wound treatment

2. Local wound treatment

- Prompt and thorough wound care very important. This should be initiated at the earliest moment after a dog bite, preferably within 15 minutes.
- Flush and clean the wound thoroughly with plenty of soap and water to remove as much of the virus as possible. Even washing by plenty of water is good, if soap is not available immediately
- Treat the wound with 1% solution of benzalkonium chloride
- Do not suture the wounds for 24-72 hrs to prevent additional surgical trauma which may help the virus to gain access into deeper tissues.
- Give suitable course of an antibiotic to control super-infection.
- Tetanus prophylaxis must be carried.

3. Immunization with anti-rabies vaccine

Control of rabies in animals

1. Institution of statutory regulations for compulsory vaccination of pet dogs.
2. Licensing of pet dogs.
3. Destruction of unlicensed dogs.
4. If unvaccinated animals has been exposed to rabid animal bite, it should be destroyed.
5. In a properly vaccinated dog, pre-exposure vaccination and restrain for at-least 90 days.
6. Pre-exposure(prophylactic) vaccination of domestic animals.
7. Post exposure vaccination of dogs.

Anti-rabies Vaccines

1. Nervous tissue vaccines

Prepared from the brain of animal (usually sheep) infected with a selected strain of fixed rabies virus and inactivated with either carbolic acid (phenol) or beta-propiolactone. Nervous tissue vaccine consists of a 5% suspension of infected animal nervous tissue which had been inactivated (eg. the Semple vaccine was derived from phenol-inactivated infected rabbit brain). These vaccine preparations are now out of date as they were associated with the complication of demyelinating allergic encephalitis.

2. Egg vaccines

Duck embryo vaccine (DEV) or Chick embryo vaccine (CEV): The CEV is not recommended for primary immunization in man, but gives better results in person already immunized with brain tissue vaccine.

3. Tissue culture vaccines

i. Human diploid cell strain(HDCV)
ii. Purified chick embryo(PCEC): Rabipur
iii. Purified Vero Cell Rabies Vaccine(PVRV): Verorab

HDCV is highly effective, in several studies; antibodies have been demonstrated in 100% of all recipients. Five or 6 doses of the vaccine is normally used by intramuscular route.

Pre-exposure and post-exposure Vaccination Schedules in animals

Vaccine	Live or killed	Animal & doses schedule	Revaccination
33% chick embryo suspension infected with modified virus	L	Dogs: 3ml (single dose) Toy dogs and cats: 1.5ml(single dose)	Every 3 years
20% suspension of sheep brain	K	Dogs:5ml(single dose) Cats: 3 ml(single dose) Other animals Wt. upto 100 kg: 10ml(single dose) Wt. above 100 kg: 20 ml(single dose)	Repeat at 6 months and subsequently every year
5% suspension of sheep brain	K	Dogs and cats upto 15 kg: 2ml daily x 7 days Dogs over 15 kg: 5ml daily x 7 days Cats, dogs, cattle, horses and other animals Under 15 kg: 2ml daily x 7 days 15-100 kg: 5ml daily x 7 days 101-800 kg: 15ml daily x 7 days Over 800 kg: 30ml daily x 7 days	Repeat every year

Note: Above vaccine is recommended in post exposure vaccination of all animals in doses mentioned but 7 injections in previously immunized and 14 injections in non-immunized animals

Pre-exposure and post-exposure Vaccination Schedules in humans

Pre-exposure prophylaxis in man

Persons who are regularly at high risk of exposure, such as vets, laboratory workers, animal handlers and wildlife officers should be considered for pre-exposure prophylaxis by active immunization with the cell culture vaccine. Immunization normally consists of 3 doses of vaccine. Antibody can be demonstrated in the sera of virtually 100% of those vaccinated if the diploid cell culture vaccine is used. Booster doses should be offered to persons at continuing risk every one to three years.

Local treatment of wounds should always be carried out in exposed persons who have been vaccinated previously. The WHO expert committee considers that local infiltration with antiserum is optional and systemic passive immunization contraindicated.

Post exposure prophylaxis in man

In cases of animal bites, dogs and cats in a rabies endemic area should be held for 10 days for observation. If signs develop, they should be killed and their tissue examined in the laboratory. Wild animals are not observed but if captured, the animal should be killed and examined. The essential components of post-exposure prophylaxis are the *(i)local treatment of wounds and (ii) active and(iii) passive immunization.*

Wound treatment - surgical debridement should be carried out. The wound should not be sutured up. Experimentally, the incidence of rabies in animals can be reduced by local treatment alone.

Passive immunization - human rabies immunoglobulin around the area of the wound; to be supplemented with an i.m. dose to confer short term protection. There is convincing evidence that combined treatment with rabies immunoglobulin and active immunization is much more effective than active immunization alone. Equine rabies immunoglobulin (ERIG) is available in many countries and is considerably cheaper than HRIG.

Active immunization - the human diploid cell vaccine is the best preparation available. The vaccine is usually administered into the deltoid region, and 5 doses are usually given.

2. Rift Valley fever

Rift Valley fever (RVF) is a viral zoonosis that primarily affects animals but also has the capacity to infect humans. Infection can cause severe disease in both animals and humans. The disease also results in significant economic losses due to death and abortion among RVF-infected livestock.

RVF virus is a member of the Phlebovirus genus, one of the five genera in the family Bunyaviridae. The virus was first identified in 1931 during an investigation into an epidemic among sheep on a farm in the Rift Valley of Kenya. Since then, outbreaks have been reported in sub-Saharan and North Africa. In 1997-98, a major outbreak occurred in Kenya, Somalia and Tanzania and in September 2000, RVF cases were confirmed in Saudi Arabia and Yemen, marking the first reported occurrence of the disease outside the African continent and raising concerns that it could extend to other parts of Asia and Europe.

RVF in humans

Transmission

1) The vast majority of human infections result from direct or indirect contact with the blood or organs of infected animals.
2) The virus can be transmitted to humans through the handling of animal tissue during slaughtering or butchering, assisting with animal births, conducting veterinary procedures, or from the disposal of carcasses or fetuses. Certain occupational groups such as herders, farmers, slaughterhouse workers and veterinarians are therefore at higher risk of infection.
3) The virus infects humans through inoculation, for example via a wound from an infected knife or through contact with broken skin, or through inhalation of aerosols produced during the slaughter of infected animals. The aerosol mode of transmission has also led to infection in laboratory workers.
4) There is some evidence that humans may also become infected with RVF by ingesting the unpasteurized or uncooked milk of infected animals.
5) Human infections have also resulted from the bites of infected mosquitoes, most commonly the Aedes mosquito.
6) Transmission of RVF virus by hematophagous (blood-feeding) flies is also possible.
7) No human-to-human transmission of RVF has been documented.

Clinical features in humans. The disease in humans may be mild or severe

Mild form of RVF in humans: The incubation period varies from two to six days.Those infected either experience no detectable symptoms or develop a mild form of the disease characterized by a feverish syndrome with sudden onset of flu-like fever, muscle pain, joint pain and headache.Some patients develop neck stiffness, sensitivity to light, loss of appetite and vomiting; in these patients the disease, in its early stages, may be mistaken for meningitis.

The symptoms of RVF usually last from four to seven days, after which time the immune response becomes detectable with the appearance of antibodies and the virus gradually disappears from the blood.

Severe form of RVF in humans: While most human cases are relatively mild, a small percentage of patients develop a much more severe form of the disease. This usually appears as one or more of three distinct syndromes: ocular (eye) disease (0.5-2% of patients), meningoencephalitis (less than 1%) or haemorrhagic fever (less than 1%).

Ocular form: In this form of the disease, the usual symptoms associated with the mild form of the disease are accompanied by retinal lesions. The onset of the lesions in the eyes is usually one to three weeks after appearance of the first symptoms. Patients usually report blurred or decreased vision. The disease may resolve itself with no lasting effects within 10 to 12 weeks. However, when the lesions occur in the macula, 50% of patients will experience a permanent loss of vision. Death in patients with only the ocular form of the disease is uncommon.

Meningoencephalitis form: The onset of the meningoencephalitis form of the disease usually occurs one to four weeks after the first symptoms of RVF appear. Clinical features include intense headache, loss of memory, hallucinations, confusion, disorientation, vertigo, convulsions, lethargy and coma. Neurological complications can appear later (> 60 days). The death rate in patients who experience only this form of the disease is low, although residual neurological deficit, which may be severe, is common.

Haemorrhagic fever form: The symptoms of this form of the disease appear two to four days after the onset of illness, and begin with evidence of severe liver impairment, such as jaundice. Subsequently signs of haemorrhage then appear such as vomiting blood, passing blood in the faeces, a purpuric rash or ecchymoses (caused by bleeding in the skin), bleeding from the nose or gums, menorrhagia and bleeding from venepuncture sites. The case-fatality ratio for patients developing the haemorrhagic form of the disease is high at approximately 50%. Death usually occurs three to six days after the onset of symptoms. The virus may be detectable in the blood for up to 10 days, in patients with the hemorrhagic icterus form of RVF.

The overall case fatality has been less than 1% in those documented. Most fatalities occur in patients who develop the haemorrhagic icterus form.

Diagnosis

- Acute RVF can be diagnosed using several different methods. Serological tests such as enzyme-linked immunoassay (the "ELISA" or "EIA" methods) may confirm the presence of specific IgM antibodies to the virus. The virus itself may be detected in blood during the early phase of illness or in post-mortem tissue using a variety of techniques including virus propagation (in cell cultures or inoculated animals), antigen detection tests and RT-PCR.

Treatment and vaccines

- As most human cases of RVF are relatively mild and of short duration, no specific treatment is required for these patients. For the more severe cases, the predominant treatment is general supportive therapy.

- An inactivated vaccine has been developed for human use. However, this vaccine is not licensed and is not commercially available. It has been used experimentally to protect veterinary and laboratory personnel at high risk of exposure to RVF. Other candidate vaccines are under investigation.

RVF in host animals

- RVF is able to infect many species of animals causing severe disease in domesticated animals including cattle, sheep, camels and goats. Sheep appear to be more susceptible than cattle or camels.
- Age has also been shown to be a significant factor in the animal's susceptibility to the severe form of the disease: over 90% of lambs infected with RVF die, whereas mortality among adult sheep can be as low as 10%.
- The rate of abortion among pregnant infected ewes is almost 100%. An outbreak of RVF in animals frequently manifests itself as a wave of unexplained abortions among livestock and may signal the start of an epidemic.

RVF vectors

- Several different species of mosquito are able to act as vectors for transmission of the RVF virus. The dominant vector species varies between different regions and different species can play different roles in sustaining the transmission of the virus.
- Among animals, the RVF virus is spread primarily by the bite of infected mosquitoes, mainly the Aedes species, which can acquire the virus from feeding on infected animals.
- The female mosquito is also capable of transmitting the virus directly to her offspring via eggs leading to new generations of infected mosquitoes hatching from eggs. This accounts for the continued presence of the RVF virus in enzootic foci and provides the virus with a sustainable mechanism of existence as the eggs of these mosquitoes can survive for several years in dry conditions. During periods of heavy rainfall, larval habitats frequently become flooded enabling the eggs to hatch and the mosquito population to rapidly increase, spreading the virus to the animals on which they feed.
- There is also a potential for epizootics and associated human epidemics to spread to areas that were previously unaffected. This has occurred when infected animals have introduced the virus into areas where vectors were present and is a particular concern. When uninfected Aedes and other species of mosquitoes feed on infected animals, a small outbreak can quickly be amplified through the transmission of the virus to other animals on which they subsequently feed.

Prevention and control

- Outbreaks of RVF in animals can be prevented by a sustained programme of animal vaccination. Both modified live attenuated virus and inactivated virus vaccines have been developed for veterinary use. Only one dose of the live vaccine is required to provide long-term immunity but the vaccine that is currently in use may result in spontaneous abortion if given to pregnant animals. The inactivated virus vaccine does not have this side effect, but multiple doses are required in order to provide protection which may prove problematic in endemic areas.
- Animal immunization must be implemented prior to an outbreak if an epizootic is to be prevented. Once an outbreak has occurred animal vaccination should not be implemented because there is a high risk of intensifying the outbreak.
- During mass animal vaccination campaigns, animal health workers may, inadvertently, transmit the virus through the use of multi-dose vials and the re-use of needles and syringes. If some of the animals in the herd are already infected and viraemic (although not yet displaying obvious signs of illness), the virus will be transmitted among the herd, and the outbreak will be amplified.
- Restricting or banning the movement of livestock may be effective in slowing the expansion of the virus from infected to uninfected areas.
- As outbreaks of RVF in animals precede human cases, the establishment of an active animal health surveillance system to detect new cases is essential in providing early warning for veterinary and human public health authorities.

Public health education and risk reduction

- During an outbreak of RVF, close contact with animals, particularly with their body fluids, either directly or via aerosols, has been identified as the most significant risk factor for RVF virus infection.
- In the absence of specific treatment and an effective human vaccine, raising awareness of the risk factors of RVF infection as well as the protective measures individuals can take to prevent mosquito bites, is the only way to reduce human infection and deaths.
- Public health messages for risk reduction should focus on:
    - reducing the risk of animal-to-human transmission as a result of unsafe animal husbandry and slaughtering practices. Gloves and other appropriate protective clothing should be worn and care taken when handling sick animals or their tissues or when slaughtering animals.
    - reducing the risk of animal-to-human transmission arising from the unsafe consumption of fresh blood, raw milk or animal tissue. In the epizootic regions, all animal products (blood, meat and milk) should be thoroughly cooked before eating.
    - the importance of personal and community protection against mosquito bites through the use of impregnated mosquito nets, personal insect repellent if available, by wearing light coloured clothing (long-sleeved shirts and trousers) and by avoiding outdoor activity at peak biting times of the vector species.
    - As noted above, laboratory workers are also at risk. Samples taken from suspected human and animal cases of RVF for diagnosis should be handled by trained staff and processed in suitably equipped laboratories.

Vector control

- Other ways in which to control the spread of RVF involve control of the vector and protection against their bites.
- Larviciding measures at mosquito breeding sites are the most effective form of vector control if breeding sites can be clearly identified and are limited in size and extent. During periods of flooding, however, the number and extent of breeding sites is usually too high for larviciding measures to be feasible.

RVF forecasting and climatic models

Forecasting can predict climatic conditions that are frequently associated with an increased risk of outbreaks, and may improve disease control. In Africa, Saudi Arabia and Yemen RVF outbreaks are closely associated with periods of above-average rainfall.

Within the framework of the new International Health Regulations (2005), the forecasting and early detection of RVF outbreaks, together with a comprehensive assessment of the risk of diffusion to new areas, are essential to enable effective and timely control measures to be implemented.

3. 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 *Teania saginata is Cysticercus bovis while of Teania solium*: *C. cellulosae*

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

- Bovine Cysticercosis
- Porcine Cysticercosis
- Human Cysticercosis
- *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 neurocyticercosis (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

1. Due to adult parasite (Human Taeniasis)
2. 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***

Somatic disease

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

4.Neurocysticercosis:

Cysts in brain:

1. 60% of the patients are having these cysts in brain
2. headaches, nausea, vomiting, lethargy and altered mental status.
3. Psychic symptoms, including epileptic seizures
4. 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 corpophagus bettles may disseminate the eggs.
A person can have mixed infection with T.saginata and T.solium.

Diagnostic procedures in animals

1.Routine meat inspection:

1. Two deep cuts in the external and one deep cut in the internal muscles of mastication.
2. The cut surfaces of the muscle and the tongue are inspected visually.
3. 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.
4. 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.

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

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

<u>Localized infestation</u>: 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, 30ux20u 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.
Prrrraziquintal 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

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

4. 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. oligarthus* 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.
- 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 5-10cm 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 ofen 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:
1. 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.
2. Cysts of moderate size are generally asymptomatic and may cause mild abdominal heaviness.
3. Large cysts, however, cause mechanical obstruction.
4. Physical examination reveals hepatomegaly
5. Jaundice may ensue from pressure on major biliary ducts.
6. Fever, malaise, headache and eosinophilia are generally associated with the clinical disease.
7. In pulmonary form, there is often coughing with or without haemoptysis(spitting of blood) Coughing with membrane from a ruptured cyst.
8. Liver lesions are sometimes painful
9. Cranial cysts may produce nervous symptoms
10. 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 occurenec 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 heir 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:

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 demonstartion of adult *E.granulosus* in the feces of dogs purged with praziquantel or other taenicide.

Prevention and control

1. Heath educational efforts for improving personal hygiene in the handling of dogs as pets and companion
2. The prevention of dogs from gaining access to raw offal and the proper disposal of the offal is an essential control measure.
3. Reducing the dog population (Control of stray dogs).
4. Minimizing dogs' role in the transmission by mass treatment (praziquantel: 5 mg/kg body weight).
5. Control of environmental contamination by dog feces.
6. Awareness of the public especially the farmers in relation to responsible dog ownership
7. 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.

5. 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 Donovon bodies, LD bodies):

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

Types of Leishmanisis

1.Visceral leishmanisis(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. Mucucutaneous leishmaniasis(Espundia)

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

Visceral leishmaniasis
Clinical features:

1. I-period: few months to two years, optimum is 4 months
2. Discomfort below the left costal margin from the enlarged spleen
3. Weak and emaciated appearance but with good diet and clean tongue
4. Temperature of 102F but quite unaware that he has fever

- ✓ Fever is like enteric fever but no toxaemia 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

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

Epidemiology

- Man to man transmission by the bite of sand fly, *Phleobotomous aergentipee(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

1. 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 Leishmaniais

- 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: *Phleobotomus paptasi* and *Phleobotomus sergenti* transmit this type leishmaniasis.

Dogs act booster animal during epidemic

Mucocutaneous Leishmaniais

- *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 leishmaniais.
- 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 leishmaniais is a serious type of condition.
- The primary lesions are similar to cutaneous leishmaniais 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.

7. 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:Stuffiness, 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,chortness 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 Asppergillus, 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:

1. Peanuts, barley, coconut, cotton seeds: invariabily mycotoxins are present.
2. Corn, millet, rice, oat, sorghum, beans, dry fruits: mycotoins have been isolated.
3. Soyabean: very rarely involved. Natural toxin production is rare. Presence of calcium, magnesium, boron in soyabeans inhibit toxin production by fungi.
4. 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.
5. Fortifying the food with peanuts cakes means fortifying the feed with toxin.

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. 20-30oC 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 B_1 is considered the most toxic and is produced by both Aspergillus flavus and Aspergillus parasiticus. Aflatoxin G_1 and G_2 are produced exclusively by *A. parasiticus*. Aflatoxins M_1, M_2 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.

8. Prevention, Control and Eradication of Zoonotic Diseases

Prevention implies all measures taken to exclude a disease from an unaffected (healthy) population.

Measures of prevention

1. Quarantine
2. Approach preventive strategies that may include immunization, environmental hygiene and chemoprophylaxis
3. Education of people about disease prevention
4. Early diagnosis

Control

1. This is strategy that employs all tactics useful for reducing the frequency of illnesses which are already present in population.
2. It aims to reduce the morbidity and mortality.
3. Control reduces the incidence of disease, its duration and effects.

Eradication

1.Eradication of a disease implies termination of all transmission of infection by extermination of the infectious agent. Permanent reduction to zero of the worldwide incidence of infection caused by a specific agent as a result of deliberate efforts. Example: smallpox.

General approaches for prevention and control of Diseases

1. Quarantine
2. Test and slaughter
3. Environmental hygiene
4. Mass immunization
5. Vector control
6. Reservoir control
7. Early diagnosis
8. Epidemiological Diagnosis
9. Treatment
10. Genetic Improvement
11. Health Education

Approaches for prevention and control of Zoonoses

1. Approach for control in animals
2. Prevention and control of zoonoses in man
3. Control of vectors and vehicles
4. Establishment of surveillance

1. Approach for control Zoonoses in animals

1.1. Control of animal populations
1.2. Reduction of susceptible
1.3. Maintain disease free status

1.1.Control in animal populations

1.1.1. Infected/contact animals

- Quarantine
- Treatment
- Destroy

1.1.2. Uncontrolled, owned susceptible animals

- Identification
- Control

1.1.3. Stray susceptible animals

- Capture/euthanize

1.1.4. Wild veretebrate reservoirs

- Hunt, trap, poison, predation, anti-fertility agents

1.1.5. Vectors

- Environmental/ecological control
- Chemical/biological control

- Fertility control

2. Approaches for prevention and control of zoonoses in man

2.1. Prevent infection
2.2. Diagnose infection
2.3. Treat disease

2.1. Prevent infection

2.1.1. Protect high risk groups
- Health education; immunization; chemoprophylaxis, monitoring health status, including occupational health programmes

2.1.2. Prevent spread by man
- Prevent food contamination
- Prevent animal contacts
- Medical intervention
- Prevent environmental contamination

2.1.3. Educate medical/veterinary personnels like
- risk of infection and spread, diagnosis, prophylaxis and treatment.

2.2. Diagnose infection

2.2.1 Improve diagnostic services
2.2.1.1.Clinical diagnosis
2.2.1.2. Laboratory diagnosis

2.3. Treat disease

2.3.1. Establish facilities and therapeutic regimes
- Referral capability
- Monitor treatment outcome
- Feedback to epidemiological services

Vector control

It focuses on utilizing preventative methods to control or eliminate vector populations. Common preventative measures are:
3.1. Chemical Control
3.2. Habitat/Environmental Control
3.3. Reducing Contact
3.4. Biological Control
3.5. Health education
3.6. Genetic control

3.1.Chemical control of vectors

3.1.1 Contact poisons

3.1.1.1. Natural: Pyrethrum, rotenone, mineral oils

3.1.1.2.Synthetic:
- Organochlorine: DDT, Lindane
- Organophosphates: Clorothione, Dichlorovos, parathione
- Crabamates: Carbaryl, dimetalin

3.1.1.3.Stomach poisons:

- Sodium fluoride

3.1.1.4.Fumigants:

- Sulphur dioxide, methyl bromide, hydrogen cyanide

3.1.1.5. Insecticides, larvicides, rodenticides, Lethal ovitraps and repellents can be used to control vectors. For example, larvicides can be used in mosquito breeding zones; insecticides can be applied to house walls or bed nets, and use of personal repellents can reduce incidence of insect bites and thus infection.

3.2.Habitat/Environmental Control

- 3.2.1.Removing or reducing areas where vectors can easily breed can help limit population growth. For example, stagnant water removal, destruction of old tires and cans which serve as mosquito breeding environments and good management of used water can reduce areas of excessive vector incidence.

3.3. Reducing Contact

- Limiting exposure to insects or animals that are known disease vectors can reduce infection risks significantly. For example, bed nets, window screens on homes, or protective clothing can help reduce the likelihood contact with vectors. To be effective this requires education and promotion of methods among the population to raise the awareness of vector threats.

3.4.Biological Control

- The use of natural vector predators, such as bacterial toxins or botanical compounds, can help control vector populations. Using fish that eat mosquito larvae or reducing breeding rates by introducing sterilized male tsetse flies have been shown to control vector populations and reduce infection risks.

Biological control

It makes use of natural predators and parasites to control unwanted species.

Examples

The use of fish which are predators of anipheline

Use of some

- bacteria like *Bacillus thrugiensis*;
- fungi like Coelomomyces and
- protozoa like Thelohania, Vorticella

4. Control of vehicles of transmission of zoonotic diseases

Vehicle-borne transmission

is an indirect transmission of an infectious agent that occurs when a vehicle (or fomite) touches a person's body or is ingested

Common vehicles

- food,
- water,
- drugs,
- blood products, and
- medical devices,

1. Establish food hygiene

- Hygiene in animal production

- Hygiene of slaughter
- Hygiene in handling and processing food stuffs.

2. Ensure safety of other animal products(wool, hides, bones, fat, others)
Hygiene during collection, storage, processing, transport.

3. Ensure safety or use of animal carcasses and washes
Animal carcass disposal

5. Establishment of surveillance

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.

Zoonosis Surveillance

- The interconnected roles of wildlife, domestic animals, the environment, and human populations in zoonotic disease pathogenesis pose distinct challenges for surveillance. The approach to zoonotic disease surveillance involves interdisciplinary strategies.

Objectives of zoonotic disease surveillance

1. Designing systems for early identification of a human and animal health threat;
2. Describing the epidemiological and ecological factors influencing zoonoses;
3. Guiding and evaluating prevention, education, and control measures;
4. Describing the public health burden.
5. Strategies for surveillance of zoonoses
6. **Surveillance and reporting of human infections**: With the exception of rabies, zoonotic diseases are usually first recognized when human illness is reported. Surveillance depends on timely reporting of suspected and confirmed zoonotic infections by healthcare providers and laboratories to public health authorities. Depending on the pathogen and available resources, the animal source may be identified as part of the public health investigation.
7. **Surveillance and reporting of animal diseases:** The veterinarians are required to report certain animal diseases to animal health and agriculture officials. Diseases under surveillance include diseases of livestock and poultry with serious economic implications and suspected foreign animal diseases

Surveillance of zoonotic pathogens in animals

1. **Veterinary surveillance,**
2. **Sentinel surveillance,**
3. **Longitudinal surveillance**
4. **Laboratory-based surveillance.**

1.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.

2.Sentinel surveillance: Monitoring animals for zoonotic pathogens can provide early recognition of human health risks and may allow for control efforts prior to the transmission of disease to humans. Mortality events are particularly important

-
- **3.Longitudinal surveillance: Where** resources are available, meaningful surveillance to elucidate disease patterns in animal reservoirs includes ongoing systematic data collection.
-
- **4.Laboratory-based surveillance:** Effective surveillance for zoonotic pathogens requires diagnostic laboratory capacity for both human and animal specimens. Reporting positive findings for zoonotic pathogens to public health authorities

1. WRITTEN ASSIGNMENT
2. PART-I: MILK HYGIENE
3. PART-II: ZOONOSES

Assignment is to be written under the following headings

1. TITLE
2. ABSTRACT
3. ETIOLOGY
4. MODES OF TRANSMISSION
5. EPIDEMIOLOGY
6. DIAGNOSIS
7. PREVENTION AND CONTROL
8. CONCLUSION
9. REFERENCES
10. REVIEW QUESTIONS (05)

Topics of Assignment

S.N.	Students Group	Topic of Assignment
1		TRICHINOSIS: A ZOONOTIC DISEASE
3		
4	**A**	
5	**B**	ORNITHOSIS: A ZOONOTIC DISEASE
6		
7		
8		
9	**C**	SCHICHSTOSOMIASIS: A ZOONOTIC DISEASE
10		
11		
12		
13	**D**	ZOONOTIC SIGNIFICANCE OF AVIAN INFLUENZA
14		
15		
16		
17	**E**	ORF AS A ZOONOTIC DISEASE
18		
19		
20		
21	**F**	DERMATOPHYTOSIS (RING WORM) AND ITS ZOONOTIC SIGNIFICANCE
22		
25	**G**	EBOLA: AN EMERGING ZOONOSIS
26		
29	**H**	RICKETTSIAL ZOONOSES: Q-FEVER