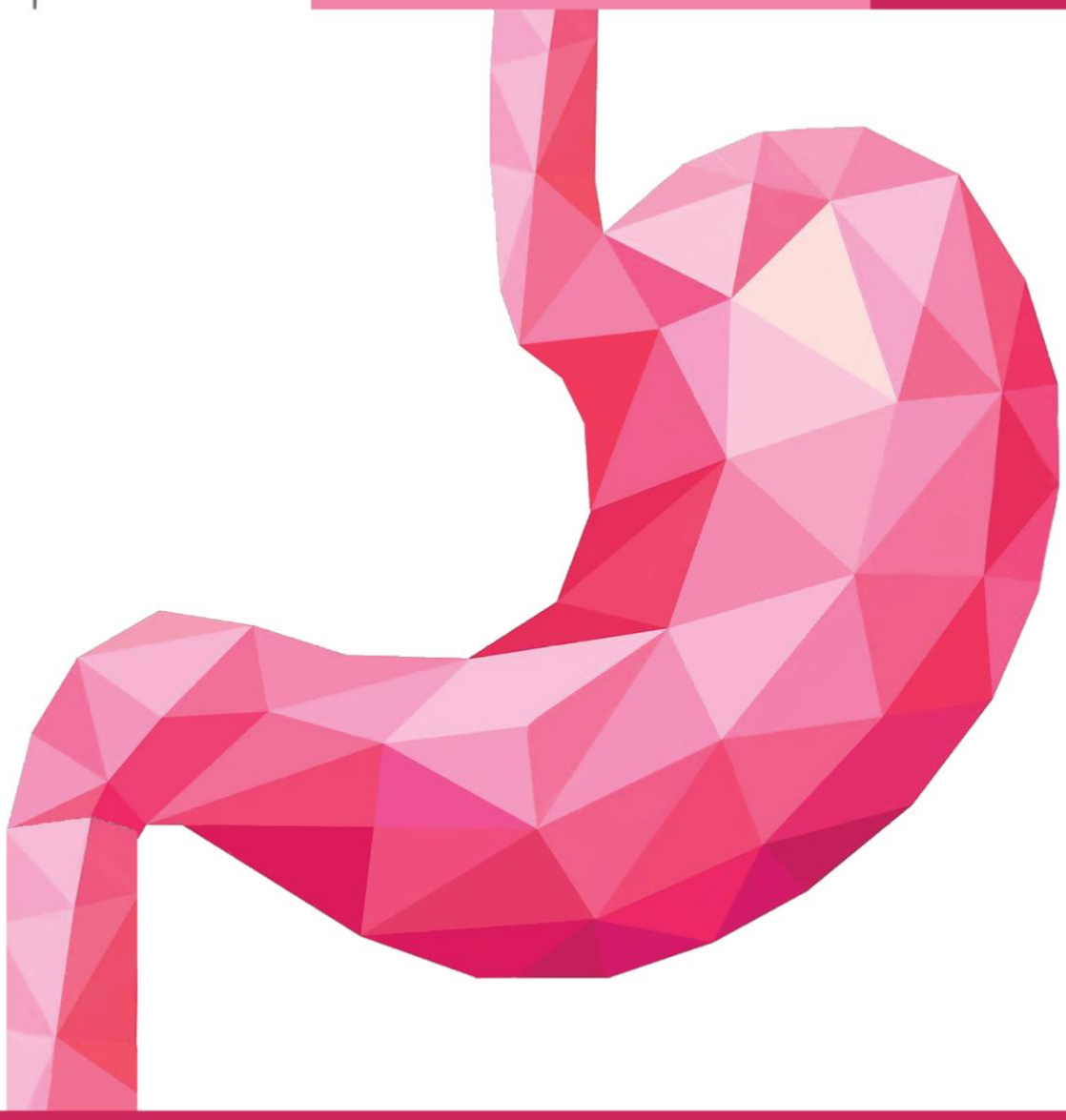
GIS 7

MICROBIOLOGY



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In this lecture we will continue talking about bacterial infections of the gastrointestinal tract, particularly about the following:

1- **Brucella:** the causative agent of **brucellosis**
2- **Leptospira interrogans:** the causative agent of **leptospirosis**.
3- **Mycobacterial tuberculosis:** mainly **abdominal tuberculosis.**

Now let's get into details ☺

Note: the underlined information is mentioned in the slides but not mentioned by the professor.

Brucellae

• Brucellae genus has multiple species (*some DNA related studies have shown that there is actually only **one** member in the genus, B. melitensis, with **multiple** biovars (reservoirs))*

1- **Brucella melitensis** (the **most common** cause of human Brucellosis) and the reservoir is **sheep and goats**. 
2- **Brucella canis** and the reservoir is **dogs**. 
3- **Brucella abortus** and the reservoir is **cattle** (it is named abortus because it causes abortion in cattle but not in humans) 
4- **Brucella suis** and the reservoir is **swine**. 

⇒ **Note: other "species" of Brucella are only found in animals.**

• Although each species of Brucella has a preferred host, all can infect a wide range of animals, including humans.

• Brucellae cause the human disease brucellosis (undulant fever (الحمى المتموجة) or Malta fever or Mediterranean fever) and is characterized by an acute bacteremic phase followed by a chronic stage that may extend over many years and may involve many tissues.

▪ Morphology and Identification ▪

• They are **gram negative** but often stain irregularly, **unencapsulated**, **aerobic**, **nonmotile**, **non-spore forming** and are adapted to an **intracellular** habitat.

• The appearance in young cultures varies from cocci to rods 1.2 µm in length, with short coccobacillary forms predominating.

• Their nutritional requirements are complex.

• They are **microaerophilic**; they grow under reduced pressure of oxygen (5%) as well as 10% of CO2. *(Slides: Whereas B. abortus requires 5–10% CO2 for growth, the other three species grow in air).*

• They are killed by boiling and pasteurization but are resistant to freezing and drying

• Characteristics used to differentiate them from Enterobacteriaceae and other gram-negative bacteria:

Catalase and oxidase positive (the 4 species that infect humans)

Do not ferment any carbohydrates (They are relatively inactive metabolically)

They do not produce neither acid nor gas in sufficient amounts for classification.

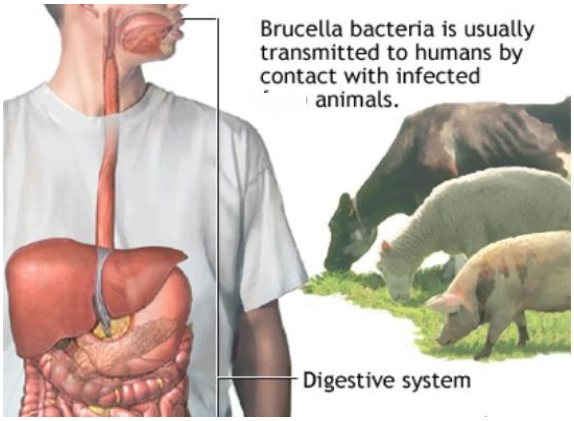
▪ Epidemiology ▪

• Brucellosis is a zoonotic (animal) disease with the main reservoir being wild and domestic animals. Humans become accidentally infected after being exposed to Brucellae species found in **animals or their products**.

⇒ The common sources of infection for humans are:

1- Unpasteurized milk and milk products like cheese (*most commonly cheese made from unpasteurized goat milk*)

2- Occupational contact (e.g. farmers, veterinarians, laboratory workers and slaughterhouse/abattoir workers) with infected animals, their feces, urine or tissues.



• Brucellosis may be acquired **by ingestion (via GIT), inhalation, mucosal (via droplets) or percutaneous exposure.** **Percutaneous exposure** is the most common route and it includes needle sticks, abrasions or skin cuts which allow the entry of Brucellae.

• Due to close contacts with animals, Brucellosis used to be very endemic in our region which is why it is commonly known as **Mediterranean fever**. It was very common to the point that anyone who presents with **undulant fever** (temperature keeps going high and low) and an abnormal **gait** (walking pattern) was diagnosed with brucellosis until proven otherwise.

• In general, microorganisms are classified into **4 biosafety levels** (*1 is the simplest and least pathogenic to humans while 4 is the severest*). Brucellae is considered as **biosafety level 3** which means you can't undergo studies with it in a standard laboratory unless using **biosafety level 3 cabinet.**

• B. melitensis and B. suis have historically been developed as biological weapons by several countries and could be exploited for bioterrorism.
• Accidental injection of the live vaccine strains of B. abortus (S19 and RB51) and B. melitensis (Rev 1) can cause disease.

▪ Pathogenesis ▪

• The Brucellae are obligate parasites of animals and humans and are characteristically located intracellularly.
• Despite being Gram-negative bacteria, Brucellae endotoxin activity is **less** severe than other gram-negative bacteria. They do not produce any exotoxin either.
• Brucellae targets the **reticuloendothelial system** starting from macrophages and lymph nodes reaching the liver, spleen and bone marrow.
• From the portal of entry, Brucellae pass the epithelial barrier and innate immune cells reaching the **macrophages** (antigen-presenting cells) → They multiply inside the macrophages and they get carried by these infected cells to parenchymatous organs especially parts of **reticuloendothelial system (spleen, liver, bone marrow)**

⇒ the traveling of the bacteria occurs via lymphatics and blood stream

• The Hallmark of brucellae pathology is the formation of **granulomas** in lymphatic tissue, liver, spleen, bone marrow, and other parts of the reticuloendothelial system. These granulomatous nodules consist of epithelioid and giant cells, with central necrosis and at an advanced stage peripheral fibrosis. In such lesions, the Brucellae are principally **intracellular**.

• The main histologic reaction in brucellosis consists of proliferation of mononuclear cells, exudation of fibrin, coagulation necrosis, and fibrosis.

▪ Clinical Findings ▪

• The incubation period ranges from **1–4 weeks**. The onset is insidious, with malaise, fever, weakness, aches, night sweats and musculoskeletal symptoms.

• Possible presentations of brucellosis:

1- High grade undulant fever which is similar to typhoid fever but less severe. Fever usually rises in the afternoon and falls during the night which is usually accompanied by drenching sweat.
2- For younger people → fever + monoarthritis (*inflammation of one of the joints*) usually of the hip or knee joint. This is what affects the gait.
3- For older people → fever + low back pain because of the involvement of the vertebral column.

• There may be gastrointestinal and nervous symptoms, lymph nodes enlargement, palpable spleen, meningitis, cholecystitis, hepatitis with jaundice.

• Deep pain and disturbances of motion, particularly in vertebral bodies, suggest osteomyelitis. These symptoms of generalized Brucella infection generally subside in weeks or months, although localized lesions and symptoms may continue.

• After the initial infection, a chronic stage may develop, characterized by weakness, aches and pains, low-grade fever, nervousness, and other nonspecific manifestations compatible with psychoneurotic symptoms.

▪ Diagnostic Laboratory Tests ▪

Definitive diagnosis of Brucellosis requires isolation by taking and culturing specimens

A. Specimens

Blood or biopsy material (lymph nodes, bone and bone marrow, spleen and so on) should be taken for *culture*, and serum for *serologic tests*.

B. Culture

Brucella agar is used; a selective agar plate → then incubate in **microaerophilic** conditions.

The medium is highly enriched and —in reduced form— is used primarily in cultures for anaerobic bacteria. Brucella species bacteria grow on commonly used media, including trypticase-soy medium with or without 5% sheep blood, brain–heart infusion medium, and chocolate agar.

Note: The typical virulent organism forms a smooth and transparent colony upon culture

• **Problems with Brucellae culture that may make serological tests preferable** ***(next page)***

- The bacteria are hard to isolate due to their **intracellular** characteristics
- The bacteria take a **long time** to grow needing an incubation period of at least 8 weeks.

C. Serology

- **IgM** antibody levels start rising **in 3 weeks** of acute illness (slides: in the first week) and **peak at 3 months**.
- **IgG and IgA** antibody levels rise about **3 weeks** after onset of acute disease and peak **at 6-8 weeks**, and remain high during chronic disease. (peak earlier during the infection)

The serology criteria should include agglutination and non-agglutination tests:

1. **Serum agglutination test**: **IgG agglutinin titers above 1:80** OR a **four-fold** rise in titer on a repeat specimen is presumptive diagnosis (indication for active infection).

Note: usually when we test for acute infection, we look for IgM antibodies, but with Brucellae infection we look for IgG antibodies as they rise and peak earlier.

Problems with agglutination test:

Cross reaction with other bacteria or antigens such as individuals infected with Cholera or injected with Cholera vaccine. This may affect Brucella agglutination titers.

Prozone phenomena: serum agglutination test of Brucellae infected individuals may give a false negative (*no agglutination*) at **lower** dilutions (*higher concentrations of antibodies*) but agglutination may appear at **higher** dilutions (*lower antibody concentration*)! The reason for that is because IgA antibodies act as blocking antibodies and interfere with IgG agglutination. To overcome this problem, we do **Coombs test** by adding Anti-Human Anti-Globulins to isolate the effect of IgA and prevent it from interfering with the agglutinating IgG.

2. **Non-agglutination tests** such as ELISA. IgG, IgA, and IgM antibodies concentrations may be detected using enzyme-linked immunosorbent assay (ELISA), which use cytoplasmic proteins as antigens. These assays tend to be more sensitive and specific than the agglutination tests especially in the setting of chronic disease.

▪ Treatment & Immunity ▪

Tetracyclines e.g. doxycycline (200mg/day) + Rifampin(1g/day) for **45 days.**

Even if symptomatic relief may occur within a few days after treatment, **long** duration of treatment is required due to slow and incomplete eradication of the organisms from the host due considering their intracellular nature.

• Brucellae may also be susceptible to streptomycin, TMP/SMX, aminoglycosides, and some quinolones.

▪ Prevention, and Control ▪

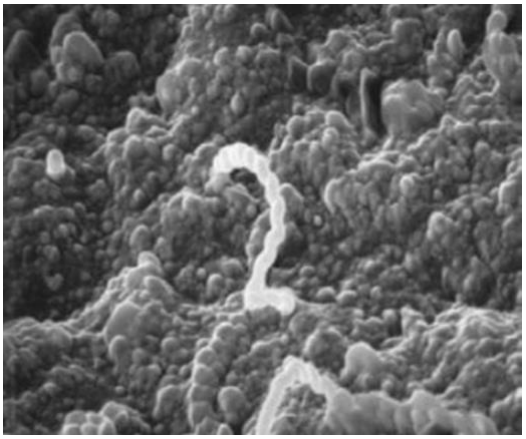
• Because brucellosis is a zoonotic disease, the prevention and control of the disease depends mainly on limitation of spread and eradication of animal infection, pasteurization of milk and milk products, and reduction of occupational hazards wherever possible

• Eradication of brucellosis in cattle can be attempted by **test and slaughter**, active immunization of heifers with avirulent live strain 19, or combined testing, segregation, and immunization. Cattle are examined by means of agglutination tests.

• Vaccines and immunization against Brucella are available for animals but not humans (however, there are some experiments)

Leptospira

• The genus Leptospira comprises two species: the pathogenic spirochete L. interrogans and the free-living saprophytic spirochete L. biflexa, now designated L. interrogans sensu lato and L. biflexa sensu lato, respectively.



• Leptospirae are tightly coiled, thin, flexible **spirochetes** (spirally shaped) 5–15 µm long, with very fine spirals 0.1–0.2 µm wide; one end is often bent, forming a hook.
• They are actively **motile**, which is best seen using a **dark-field microscope.**
• The bacteria can also be seen using transmission electron microscope as in this image. It's spiral with **two periplasmic flagellae** making it look like a question mark symbol.

• Leptospirae derive energy from oxidation of long-chain fatty acids and cannot use amino acids or carbohydrates as major energy sources. Ammonium salts are a main source of nitrogen.
• Leptospirae can survive for weeks in water, particularly at alkaline pH.
• The disease caused by pathogenic Leptospira species is called **leptospirosis**.

▪ Epidemiology ▪

• Leptospirosis is mainly a zoonotic disease but can also affect human beings accidentally.
• **Rodents** like mice and rats are the main animal reservoirs. Kidney involvement in many *animal* species is chronic and results in the shedding of large numbers of leptospirae in the **urine**; which is probably the main source of environmental contamination resulting in infection of humans. Human urine may also contain these spirochetes in the second and third weeks of disease.

• Leptospirosis has a worldwide distribution but occurs most commonly in the tropics and subtropics because the climate and occasionally poor hygienic conditions favor the pathogen's survival and distribution.
• Current information on global human leptospirosis varies but indicates that approximately 1 million severe cases occur per year, with a mean case–fatality rate of nearly 10%.

▪ Pathogenesis ▪

• Transmission
Directly → most commonly through **cuts, abraded skin**, but also can occur through mucous membranes, especially the conjunctival and oral mucosa
Indirectly → (Less commonly than the direct route) through contaminated **water and food.**

The disease is biphasic:

Leptospiremic phase: After entry, and an incubation period of 1–2 weeks the organisms proliferate, cross tissue barriers, and disseminate hematogenously
⇒ This is followed by a window period of a feeling of comfort and resolution

The immune phase: develops after initial improvement and when the **IgM antibody titer rises.** It manifests itself often as "aseptic meningitis" with an intense headache, stiff neck, and pleocytosis of the CSF. Due to the appearance of antibodies, the bacteria leave the blood and establish themselves in the **solid parenchymatous organs** (particularly liver, kidneys and lungs) producing hemorrhage and necrosis of tissue and may result in dysfunction of those organs (liver failure which results in jaundice, pulmonary hemorrhage, nitrogen retention/acute renal failure).
• Nephritis and hepatitis may also recur, and there may be skin, muscle, and eye lesions.

• The degree and distribution of organ involvement vary in the different diseases produced by different leptospirae in various parts of the world

▪ Clinical Findings ▪

• Leptospirosis is characterized by a broad spectrum of clinical manifestations, varying from asymptomatic, self-limited mild flu-like infection to fulminant, fatal disease (**Weil's Syndrome**) which is present in just about 1% of cases.

• In the **leptospiremic phase**: **systemic symptoms** appear on the patient like fever, chills, rigors, nausea, vomiting, abdominal pain, musculoskeletal symptoms and night sweats. When the **second phase** starts, the hallmark would be the pathology of the affected organ as previously mentioned (e.g. liver failure).

• Remarkable redness of the eye occurs if the infection was through the conjunctiva.

▪ Diagnostic Laboratory Tests

A. Specimens	B. Microscopic Examination	C. Culture	D. Serology
Specimens consist of blood, CSF, urine and tissues for *microscopic examination* and *culture.*	**Dark-field examination or thick smears stained by the Giemsa technique or silver impregnation**	**Fresh blood, CSF, urine or crushed tissue can be cultured. Leptospirae grow best under aerobic conditions at 28–30 C in selective semisolid medium (e.g. Ellinghausen-McCullough-Johnson- Harris EMJH) in 10 mL test tubes with 0.1% agar and 5-fluorouracil.** **• Growth is slow, and cultures should be kept for at least 8 weeks before announcing that the culture is negative.**	**The diagnosis of leptospirosis in most cases is confirmed serologically with microscopic agglutination test (MAT) and ELISA to confirm the presence of leptospirae antigens and antibodies.**

▪ Treatment and Immunity ▪

• May be self-limited → **symptomatic** treatment
• Treatment of **mild** leptospirosis should be with **oral doxycycline**, ampicillin, or amoxicillin.
• **Severe** leptospirosis should be treated with **IV penicillin** as soon as the diagnosis is made to prevent the development of Weil's syndrome and organ failure.
• Serovar(serotype)-specific immunity follows infection, but reinfection with different serovars may occur.

▪ Prevention, and Control ▪

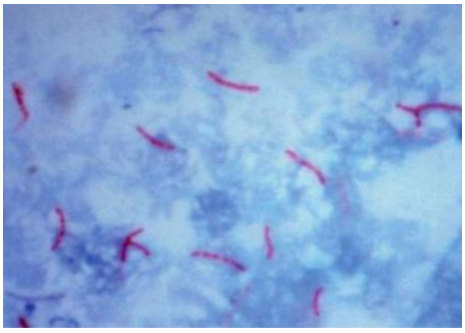
• Leptospirae is excreted in **urine** both during the active illness and during the asymptomatic carrier state.

• Leptospirae remain viable in stagnant water for several weeks; drinking, swimming, bathing, or food contamination may lead to human infection. Thus, people most likely to come in contact with water contaminated by rats (*e.g. miners, sewer workers, farmers, and fishermen*) run the greatest risk of infection ⇒ they should avoid exposure to urine and tissues from infected animals through **proper eyewear, footwear, and other protective equipment**. Targeted rodent control strategies could also be considered.

• Vaccines for **agricultural and companion animals** are generally available, and their use should be encouraged. **No vaccines for humans.**

Mycobacterium Tuberculosis (M. tb)

• The genus mycobacterium includes 3 members:
Mycobacterium Tuberculosis the causative agent of tuberculosis (السل، التدرن الرئوي)
Mycobacterium Leprae the causative agent of leprosy (الجذام)
Non tuberculous mycobacterium (NTM) also known as environmental Mycobacteria which affect immunocompromised patients mainly (a major cause of death in these patients, in addition to the ordinary tuberculosis)
⇒ Example on NTM: Mycobacterium avium complex (MAC)



• It was not until the 19th century, when Robert Koch utilized new staining method (ZN stain) and applied it to sputum from patients, that he discovered the causal agent of the disease Tuberculosis (TB): **Mycobacterium tuberculosis or Koch bacillus.**

• The family mycobacterium tuberculosis complex (MTC) can cause Tuberculosis (TB) in humans and other livings. It includes:M. tuberculosis (Mtb) (prototype), Mycobacterium africanum, Mycobacterium bovis, Mycobacterium microti, Mycobacterium caprae, Mycobacterium pinnipedii, Mycobacterium suricatte, Mycobacterium mungi, Mycobacterium dassie, Mycobacterium oryx and Mycobacterium Canetti.
⇒ TB is considered an **airborne** infectious disease although M. tuberculosis complex organisms can be spread through un-pasteurized milk, direct inoculation and other means.

• Mycobacterium tuberculosis is a **non-spore forming, non-motile slow growing** (divides every 24 hours), **obligate aerobe, facultative intra-cellular** bacillus that can live within macrophages which are professional phagocytes and antigen presenting cell. The bacterium inhibits the phagosome-lysosome fusion in these cells. It can live in macrophages for years with no symptoms (**latent mycobacterium**)

• May be weakly **gram positive** but gram stain is not applicable so **acid fast stain (ZN stain)** is used due to the complexity of the cell wall which is composed mainly of lipids: **wax D, mycolic acid, cord factor (trehalose dimycolate)** → main virulence factors.

⇒ NOTE: The marked pathology of tuberculosis is **granuloma formation**

▪ Epidemiology ▪

• Not everyone infected with mycobacterium develops the disease, only a small number do based on the **10:3:1 formula** ⇒ For every 10 people exposed to the bacteria, 3 will develop latent tuberculosis infection (LTBI) and 1 will develop active tuberculosis (TB disease), 6 will clear the infection.

People who have latent TB infection, which are **one third** of the world's population, do not feel sick, do not have any symptoms, and cannot spread TB to other. But if they become immunopromised for any reason, they might develop **secondary tuberculosis (reactivation)**

• TB remains a leading cause of infectious diseases morbidity and mortality. In 2015, an estimated 10.4 million new TB cases were seen worldwide. However, in Jordan, the number of TB cases has dropped in the last 2 decades to 25 cases per 100,000 individuals now.

Tuberculosis

☆ **Pulmonary tuberculosis makes up 80-90% of TB cases**: The primary site of TB is usually the lungs, from which it can get disseminated into other parts of the body.

⇒ Other routes of spread include contiguous involvement from adjacent tuberculous lymphadenopathy or primary involvement of extra-pulmonary organ.

☆ **Extra-pulmonary tuberculosis makes up 10-20% of TB cases**: bacteria can attack any part of the body such as the pleura, lymph nodes (*Scrofula*), pericardium, kidney, spine (*Pott disease*), brain, meninges (*tuberculous meningitis especially in children*) and abdomen (*abdominal tuberculosis*)

➔ Abdominal tuberculosis comprises **5%** of all cases of TB:

▪ Abdominal TB, can be a source of significant morbidity and mortality and is usually diagnosed late due to its nonspecific clinical presentation.

▪ Abdominal TB usually occurs in four forms: tuberculous lymphadenopathy, peritoneal tuberculosis, **gastrointestinal (GI) tuberculosis** and/or visceral tuberculosis involving the solid organs

Gastrointestinal (GI) tuberculosis

Pathogenesis	More details about the disease	Clinical findings
Can occur by the following ways: 《1》 **Ingestion of tuberculous mycobacteria** (as with ingestion of sputum or undercooked meat or unpasteurized milk contaminated with M. bovis which lives in cattle) 《2》 **Hematogenous or lymphatic spread** to the GIT from another foci. E.g. In the setting of active pulmonary TB or miliary TB. 《3》 **Direct spread** from a contiguous (adjacent) location. E.g. from the peritoneum (*when there is peritoneal tuberculosis*) or retrogradely from fallopian tubes or from the ovaries 《4》 May occur via **reactivation** of latent TB infection	▪ The mucosal layer of the GI tract can be infected with the bacilli, leading to the formation of epithelioid tubercles in the lymphoid tissue of the submucosa. After 2-4 weeks, caseous necrosis will occur in the tubercles, causing ulceration of the overlaying mucosa which will spread into the deeper layers and adjacent lymph nodes as well as the peritoneum. Rarely, the bacilli can enter into the portal circulation or the hepatic artery thus involving organs such as the liver, pancreas or spleen.	▪ Although any portion of the gastrointestinal tract may be affected, the terminal ileum and the cecum are the sites most commonly involved. ▪ The clinical presentation tends to be non-specific: Abdominal pain (at times similar to that associated with appendicitis), swelling, obstruction, hematochezia, and a palpable mass in the abdomen (***where the granuloma is formed***) are common findings at presentation. • General complaints and systemic symptoms are also common: Fever, chills, consumption (weight loss), anorexia, extreme pallor and night sweats.

▪ Laboratory diagnostic methods ▪

A. Smear microscopy

• Three specimens from each patient with suspected TB should be examined microscopically for Acid Fast Bacilli AFB (using Ziehl-Neelsen stain).
• Mycobacteria can also be examined by yellow fluorescence after staining with **auramin**.

B. Culture

• Both liquid and solid mycobacterial cultures should be performed for every specimen, examples: Lowenstein-Jensen or Middlebrook 7H10, Radiometric broth culture (BACTEC radiometric system), mycobacterial growth indicator tube (MGIT).

• Agar should be incubated for at **least 4-5 weeks** before we start seeing colonies on the agar plate and **up to 8 weeks** before announcing that the culture is negative for M. Tb

• Culture for acid fast bacilli is the most specific test for TB and allows direct identification and determination of susceptibility of the causative organism.

• Screening tests (To test if the patient has been exposed to mycobacterium tuberculosis)

Tuberculin skin tests (TSTs)
Inject intradermally purified bacterial protein derivatives → After 48 hours the patient comes with induration (*erythema and elevation in the skin*) → An induration of **less** than 5 millimeters is considered a negative test result and more than 10 millimeters is considered a positive test result.
⇒ Vaccination, previous exposure to TB, and exposure to NTM may cause a false positive result.

Interferon-gamma release assays (IGRAs)
In which the blood of the suspected patient is incubated with TB antigens from the lab → if the monocytes of the patient have been sensitized to MTB (i.e. the patient is infected with TB) antigens they will produce large amounts of interferon gamma.

• Nucleic acid amplification tests (NAAT) are also used for diagnosis and screening.

▪ Treatment ▪

The course of TB treatment depends on whether the individual is in the latent or active stage, and on his or her probability of risk.

▪ Treatment of TB usually involves a mixture of multiple drugs and 2 phases:

- **An intensive initial 2-month phase**: main anti-tuberculosis chemotherapeutic drugs used are: **isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), and either ethambutol (EMB) or streptomycin (SM)**.
- Followed by **a slower 4 to 6-month continuation phase of INH and RIF**.

⇒ Side effects of these drugs: nephrotoxicity, hepatotoxicity, ototoxicity, body fluid color change (*mainly due to rifampin*).

▪ **Isoniazid preventive therapy IPT for 9 months** is the recommended treatment for **LTBI** but the regimen's main drawback is the duration of therapy
If not treated properly, TB disease can be fatal

▪ Prevention ▪

• The best way to prevent TB is to diagnose and isolate infectious cases rapidly and to administer appropriate treatment until patients are rendered noninfectious (usually 2–4 weeks after the start of proper treatment) and the disease is cured.

• Additional strategies include treatment of persons with LTBI who are at high risk of developing active disease and the **Bacillus Calmette–Guérin (BCG) vaccination** which is a **life attenuated** vaccine derived from M. bovis (*it is the only licensed vaccine against TB*)
⇒ In Jordan, it's the first vaccine given to infants and often leaves a scar on the arm.
⇒ Protection provided by the vaccine ranges from **0-80%**: Most effective against the severest forms of TB: tuberculous meningitis and miliary TB, but against pulmonary TB which is the commonest form of TB, the protection may reach 0%.

GOOD LUCK