MCB 402 E-NOTE TEMPLATE

COURSE CODE: MCB 402

COURSE TITLE: Pathogenic Microbiology and Principle of Epidemiology

NUMBER OF UNITS: 3 Units

COURSE DURATION: Three hours per week

COURSE DETAILS:

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Other Lecturers: Dr. Odedara, O. O. and Dr. Shittu, O.B.

COURSE CONTENT:

A detailed coverage of classification and characteristics of bacteria – the morphology, life cycle and biochemical characteristics of bacteria and other eukaryotes to include their isolation and identification. The significant role of bacteria in agriculture, industry, medicine, pharmaceuticals and foods. Bacterial infections and methods in diagnostic bacteriology; aspects of molecular bacteriology.

COURSE REQUIREMENTS:

Departmental course for B.Sc Microbiology

READING LIST:

1. Banor, J.D., Akermann, P.G., Toro, G.: Clinical Laboratory Methods. 8th Edition. The C.V. Mosby Company. Saint Louis, U.S.A. 1974
2. Brooks, G., Balel, J., Morge, S.: In: Jaetz, Melnick and Adelberg (Editor). Medical Microbiology. 21st Ed. Aphenton and Lange. Stanford, California. 1998
3. Davidson, I. and Henry, J.B.: Clinical Diagnosis. 15th Ed. W.B. Saunder Company. 1974

LECTURE NOTES:

STAPHYLOCOCCI

The staphylococci are gram positive cocci. Staphylococci are isolated most frequently from skin, boils, abscesses, etc. In general, under the right conditions they can

infect almost any tissue of the body. Some strains of *Staphylococcus aureus* produce a potent enterotoxin which can cause food poisoning in man.

The pus from boils, abscesses, or other infectious material is streaked over a plate of agar, preferably blood agar, because many of the staphylococci are hemolytic (produce hemolysins which lyse erythrocytes).

Microscopic examination of the isolated organism and a study of the pigmentation of the colony are usually sufficient for identification to species. The colonies of *Staphylococcus aureus* are golden in colour and S100 medium, a selective medium for staphylococci, demonstrates this golden pigment very well. Colonies of *Staphylococcus epidermidis* are white. A final test used in the identification of staphylococci is the coagulase test which differentiates the highly pathogenic *Staphyloccus aureus* from the usually non-pathogenic *Staphylococcus epidermidis. Staphylococcus aureus* is coagulase positive, that is, will cause plasma to clot or coagulate. *Staphylococcus epidermidis* is coagulase negative.

THE STREPTOCOCCI

The streptococci are gram positive cocci which characteristically grow in chain formation and sometimes in pairs. They are non-motile and non-spore forming. Many strains of beta-hemolytic streptococci produce capsules. The capsules are present only in the very young cultures. The streptococci in general are quite fastidious in their cultural requirements and need an enriched medium containing blood or serum.

From the standpoint of medical bacteriology streptococci are considered under the following headings:

1. Hemolytic streptococci (beta): Produce complete hemolysis or clear areas around the bacterial colony.

   Group A – responsible for most of the streptocoecal infections in man (*Streptococcus pyogenes*).

Group B, C, F, G, H, K and L – other – harmless inhabitants of mucous membranes or occasional incitants of human infection.

Group E, M and N – occur only in lower animals.

2. Viridans streptococci (alpha): Produces partial hemolysis or green areas around bacterial colony.

   By biochemical characteristics they are divided into several species. The most common of which are *Streptococcus salivarius* and *Streptococcus mitis* both common throat organisms. Both can give rise to subacute bacterial endocarditis (SBE).

3. Non-hemolytic streptococci (gamma): No hemolysis whatsoever around bacterial colony.

4. Enterococci (Group D): The example of organism in this group is *Streptococcus faecalis*. They may be beta hemolytic, non-hemolytic or produce "greening" of blood agar. *Streptococcus faecalis* is identified by the presence of growth in media containing 0.5% sodium azde and 6.5% NaCl at 45^{o}C. These conditions are lethal to other streptococcus species.

Note: (1) The typical beta hemolytic streptococcus colony is surrounded by a zone of clear hemolysis. Two types of beta hemolysis are released: (a) streptolysis O which is destroyed by atmospheric oxygen is therefore demonstrated only in deep colonies and (b) streptolysis S which is oxygen stable and is responsible for surface colony hemolysis.

(2) Beta hemolysis alone does not mean that organism is group Astreptococci.A simple test to seprate group A streptococci from the other groups is their susceptibility to Bacitracin. A bacitracin disc is placed on a blood agar plate seeded with the unknown beta hemolytic streptococcus. Group A streptococci (*Streptococcus pyogenes*) are sensitive to bacitracin and a zone of growth inhibition will be produced around the disc. Non group A streptococci will fail to show growth inhibition around the bacitracin disc.

Diplococcus pneumonia

The pneumococci in their most typical form are encapsulated, gram positive diplococcic. In pus, body fluid, sputuns and body tissues they may be found in short chain and occasionally as individual cocci. Morphologically the pneumococci may be very hard if not impossible to distinguish from the streptococci.

On blood agar, the encapsulated organisms form round glistening unpigmented and mucoid colonies. The pneumococci colonies are surrounded by a zone of alpha hemolysis (green) and must be differentiated from alpha hemolytic streptococci. The various methods employed in the laboratory to differentiate *Diptococcus pneumonia* from alpha hemolytic streptococci are:

1. Optochin disc – contains ethylhydrocupreine hydrochloride, an antibacterial drug which inhibits the growth of pneumococci but not streptococci. This is the method most commonly used in diagnostic laboratories since it is quite simple and accurate.
2. Bile solubility – The pneumococci are bile soluble and when a suspension of the organisms is added to the bile salt, sodium deoxycholeate, the organisms will lyse (turbidity will disappear). Streptococci are not bile soluble and will not lyse in the presence of sodium deoxycholate.
3. Mouse virulence – The encapsulated. Pneumococci are highly virulent for mice whereas alpha hemolytic streptococci are non virulent.
4. Insulin fermentation – penumococci ferment the sugar insulin – most strains of streptococci do not.
5. Capsular swelling test – Quelling reaction: All virulent pneumococci have capsules and can be identified or differentiated on the basis of the reaction of their capsule with specific antibody. When the capsular antigen reacts with its specific antibody the capsule becomes readily visible under the microscope and appears to have enlarged or increased in size.

THE NEISSERIA

They are gram negative diplococci. The pathogenic species, *Neisseria gonorrhoeae* causes gonorrhea, endocarditis, arthritis and conjunctitis in new born. Also *Neisseria meningitisis* cause cerebrospinal meningitis. Other species that are non pathogenic*Neisseria catarrhalis* and *Neisseria sicca*/

Nesseiria organisms produce indophenols oxidase. This enzyme oxidizes dimethyl or tetra-methhyl p-pheonylenediamine hydrochloride to form a colored compound. When a drop of the reagent solution is placed on a colony of gonococcus, meinigococcus or some

other Neisseria, that colony shows a color changes from pink to purple to blue black. *This test, therefore, can be used to detect oxidase positive colonies among the mixed growth*.

Confirmation is obtained by gram stainy smear examination and biochemical tests.

	Gluc	Maltose	Lactose	Sucrose	Oxidase
N. gonorrheae	+	-	-	-	+
N. meningtitis	+	+	-	-	+

Also serologic test with known specific antigen could be used to confirm.

Escherichi coli

Although *Escherichia coli* is part of the normal flora of the intestinal tract, for years it was suspected of causing an occasional moderate to severe diarrhea in humans and animals. It is now established that various *E. coli* strains may cause diarrhea by either of two mechanisms: (1) by production of enterotoxins that indirectly cause fluid loss and (2) by the actual invasion of the epithelial lining of the intestinal wall, causing inflammation and fluid loss.

Enterotoxin-producing *E. coli*, called enteropathogenic*E. coli*, produce one or both of two different toxins. One is a heat-stable toxin called ST and the other is a heat-labile toxin called LT. Both toxins cause diarrhea.\LT, which is destroyed by heating at 65^{o}C for 30 minutes, has been purified, and its mode of action is identical to that of cholera toxin. LT stimulates the activity of a membrane-bound adonylcyclase. This results in the conversion of ATP to cyclic AMP)(cAMP) as shown below:

ATP $\longrightarrow$ cAMP + PP$\rightarrow$ inorganic phosphorus

Extremely minute amounts of cAMP will induce the active excretion of Cl- and inhibit the absorption of Na+, creating an electrolyte imbalance across the intestinal mucosa that causes the loss of copious quantities of fluid from the intestine.

Infection with pathogenic *E. coli* may cause a severe and sometimes fatal infection in newborns. The disease in adults, known by many names such as traveller's diarrhea or Montezuma's revenge may vary from a mild disease with several days of loose stools to a severe and fatal cholera like disease.

Invasion of the blood stream by *E. coli* may result in meningitis in the newborns, the debilitated patient, patients with leukemia or those receiving immunosuppressive drugs.

Klebsiella pneumoniae

It is also called "fried Lander's bacilli". Of interest is the frequency with which it predominates in fecal cultures following oral chemotherapy and participate in urinary tract infection. *Klebsiella pneumoniae* contains a large capsule and the colonies on solid media are quite large and very mucoid due to the presence of the capsule.

LACTOSE-NEGATIVE GROUP ENTERIC BACILLI

The lactose negative group is of major medical importance because it contains many human and animal pathogens as follows:

1. The proteus group – normal members of the intestinal tract which act as opportunists by producing urinary tract infection. Examples

   Proteus mirabilis }
   Proteus vulgaris } *swarmers*
   Proteus morganii

2. *Pseudomonas aeruginosa* – Produces urinary tract and wound infections.

   Produces two pigment

   pyocyanin (blue pus) – soluble in chloroform

   fluorescin (yellow) – Not soluble in chloroform but soluble in water

3. *Salmonella typhosa*. Typhoid fever which is an acute infection beginning in lymphoid tissue of the small intestine but usually developing as a generalized infection of the body can also cause perforation of the intestine if not treated.

4. Other salmonella species – Paratyphoid fever and gastroeneteritis (salmonellosis). Sometimes it can cause septisemia.

5. Shigella species – contains species causing shigellosis (Bacillary dysentery). Note that *Entamoebahistolytica* which is intestinal parasite can cause dysentary referred to as amoebic dysentedcy.

   They are classified as

   group A – *Shigelladysenteriae*

   group B – *Shigellaflexneri*

   group C – *Shigellaboydii*

   group D – *Shigellasonaei*

BRUCELLA

The members of the genus Brucella are the etiological agents of brucellosis or undulant fever which occurs principally in animals. Man is infected accidentally ir indirectly through contact with infected animal or their excreta (slaughterhouse workers), the ingestion of unpasteurized milk and other dairy products made from raw milk.

Three species of Brucella are of medical importance. *Brucellaabortus* causes abortion in cattle, *Brucellamellitensis* infects sheep, and *Brucellasuis* infect swine. All three species can infects any of the above animals mentioned.

PATEURELLA, FRANCISSELLA, YERSINIA

Four species in this group of microorganisms are important as etiological agents of infection in man

1. *Yersinia pestis*: the etiological agent of plaque.
2. *Yersinia enterocolitica*: a causative agent – a severe enterocolitis infection with symptoms of dysentedcy.
3. *Francisellatularensis*: The etiological agent of tularemia (Rabbit fever)
4. *Pasteurellamultocida*: This infects man following cat or dog bites and scratches.

rainfluenzae – does not need X factor to grow

THE BACILLUS

The members of the genus Bacillus are widely distributed in nature and are common inhabitants of the soil. They are gram positive, spore-forming aerolysis organism.

The species representing the genus as a type is *Bacillus subtilis*, which ordinarily is nonpathogenic but may involve wounds producing low grade infections and may cause conjunctivitis.

The only member that is regarded as primary pathogen is *Bacillus anthrax*. Two forms of diseases may result in an infection with *Bacillus anthracis*.

(a) Cutaneous anthrax also called “malignant pustule”.

(b) Pulmonary anthrax also called “wool sorters disease”

CLOSTRIDUM

The clostridia are gram positive rods. They are anaerobic.

Clostridial infections manifests themselves in two ways: (1) Toxemia due to the production of an exotoxin (2) Tissue necrosis due to the proliferation of proteolytic enzymes.

Clostridia that cause diseases of man are:

a) *Clostridium tepani* – Tetanus

b) *Clostridium perfringens* – Gas gangrene

c) *Clostridium botulinum* – Botulism

THE PROTEUS GROUP

Members of the genus proteus are non lactose fermenters which are usually highly motile. Because of their extreme motility they tend to form a thin spreading film of growth over the entire surface of solid media making it highly difficult to isolate other bacteria that may be present. This swarming tendency can be prevented by cultivating the organism on a relatively dry surface of 5% agar medium or an ordinary 1-2% agar medium containing chloral hydrate (0.1%). Gram positive organism can be isolated from a swarming proteus by inoculating a blood agar plate containing sodium azide (0.1%) which inhibit the growth of proteus organism but not gram positive organisms. Blood agar containing 0.25% pheny ethyl alcohol (PEA) can also be used for the same purpose. Charcoal agar will decrease swarming also.

Urease production is a distinguishing metabolic characteristic of proteus group and is very helpful in the identification of these organisms.

The species most commonly encountered in the clinical laboratory are *Proteus mirabilis*, and *Proteus vulgaris* which are spreaders or warmers and *Proteus morganii* and *Proteus rettgeri* which usually do not spread or swarm on solid media. These organisms may occasionally cause an acute enteritis particularly in children. They also cause urinary tract infections and may often become the dominant organism in mixed wound infections treated with antibiotics.

The antigenic structure of *Proteus vulgaris* is of particular medical interest because strains possessing certain O antigens (O x K, O x 2, o x 19) are agglutinated by the sera from patients with vickettsial diseases. This is known as the Weit-Felix Test.

SALMONELLA

Typhoid fever is an acute infection beginning in the lymphoid tissue of the small intestine but usually developing as a generalised infection of the body, particularly the lymphatic system. The symptoms of the disease are produced by endotoxins liberated by the disintegration of the bacilli.

The genus salmonella, of which there are more than 700 species or types, produce three distinct kind of infections: (a) Enteric or paratyphoid fever, a disease clinically indisgushable from typhoid fever (b) A gastroenteritis commonly referred to as food poisoing and (c) septicemia.

ISOLATION AND IDENTIFICATION OF SALMONELLA

ISOLATION: Differential media such as Eosin methyl Blue (EMB) agar or MacConkey agar serve to distinguish colonies of lactose fermenting organisms (coliforms) from those negative for lactose. But EMB agar or MacConkey agar does not favour or selectively inhibit the growth of one enteric bacillus over another. And so, the isolation from fecal specimens of the occasional salmonella or shigella organism is rendered difficult by the overgrowth of the more numerous coliforms. This problem was solved by the introduction of selective media of lipids (SS (Salmonella-Shigella) aga is an example. The principal ingredient of SS agar are lactose bile salts, brillian green dye and a pH indicator neutral red. If not only differentiates between lactose-fermenting and lactose-negative colonies, but it also inhibit most of the coliform organisms. The production of colourless coloures on EMB, MacConkey or SS agar is a property which the Salmonella share with several other general. Therefore, identification

of a salmonella organism requires the use of other methods such as biochemical and serologic tests.

Xylose-lysine deoxycholate agar (XLD and Hektoen Enteric agar (HE) are two other selective media used for the isolation of salmonella and shigella from stool specimens. The percentage of isolations of salmonella and shigella obtained using either of these two media is much higher when compared with SS agar and for this reason XLD and HE agars have replaced SS agar in most well developed laboratories.

IDENTIFICATION: Certain of the biochemical tests are used as a screening method is that they serve to indicate the probable identify of an enteric bacillus. These are lactose fermentation, glucose fermentation, sucrose fermentation, production of hydrogen sulfide, breakdown of urea and citrate utilization. The first four characteristics are combined in one medium known as Triple Sugar Iron Agar (TSI). A gram negative bacillus that is negative for lactose, sucrose and urease but is positive for glucose and H_2S is a salmonella suspect. The final identification is established by serologic method in this instance by agglutination tests which is identification of an unknown organism of a salmonella by means of known antisera. As well a type of test also commonly used as the clinical laboratory in which the reagent are reserved is available. In this case, it is the agglutination of suspension (antigen) of known organism by an unknown (patient) serum demonstrates the presence of antibody specific for the antigen. Agglutination tests of this type constitute an important laboratory step in the diagnosis of salmonella infections (WIDAL TEST).

SHIGELLA

The genus shigella are the etiology agents of shigellosis or dysentery and in contrast to typhoid fever caused by *Salmonella typhi* or Salmonellosis caused by paratyphoid bacilli

or other salmonella species which may cause generalized infection. Shigellosis is primarily an infection involving the large intestine with the production of conditions that range from a mild diarrhea to a severe and toxic dysentery. To differentiate this disease from the dysentery caused by *Entamoebahistolytia*, shigella enteritis is known as bacillary dysentery or shigellosis and the other one is known as amoebic dysentery.

The shigella are morphologically instinguishable from the salmonella. The growth requirements of the salmonella and shigella are so similar that isolation methods are of the same general nature. The dysentery bacilli however are more delicate. For example SS agar, which is somewhat valuable for the primary isolation of salmonella, is toxic for many shigella strains. Therefore, the less inhibitive EMB or XLD and HE agars are the media of dose for shigella.

A gram-negative bacillus originating from a colorless colony on EMB or SS agar is supported of being a shigella if it is H_2S-negative, urease-negative. Final identification of the shigella is carried out by specific agglutination test using "O" antisera. The shigella are classified as:

Group A (*Shigelladysenteriae*)

Group B (*Shigellaflexoner*)

Group C (*Shigellaboydii*)

Group D (*Shigellasonnei*)

MYCOBACTERIA

Infection with mycobacteria usually occurs in the lungs, however the organisms can invade almost any tissue of the body. These organisms are gram positive, however the gram stain is never used in their identification. They are called "Acid fast Bacilli (DFB) because they resist decolorization with acid-alcohol after staining. The procedure most frequently

used is the Ziehl-Nelsen (ZN) method which utilizes carbolfuschin, acid alcohol and the methylene blue or malachite green. With the exception of the genus Moriadia, only the Mycobacteria are acid fast. The most important pathogenic species for man are:

1) *Mycobacterium tuberculosis* – the etiological agent of tuberculosis and (2) *Mycobacterium leprae* – the etiological agent of leprosy (Hansen bacilli) *M. leprae* has not been cultured on laboratory media.

Neither the tuberculin test nor any serologic test gives evidence of active disease due to tubercle bacilli. Only isolation of tubercle bacilli given such proof. Acid fast bacilli stain by Ziehl-Nelsen technic or fluorescence microscopic can give presumptive evidence of tuberculosis. Sputum is first treated with 2% NaOH are liquefied sputum is then neutralized and centrifuged. The sediment could be acid fast bacilli stained and also inoculated on Lo Westein-Jensen medium for isolation of *Mycobacterium tuberculosis* after incubation for 2-8 weeks.

The final diagnosis could be done by serologic test (complement-fixation) test, agglutination test with known antibody or fluorescent antibody technique.

The most widely use antituberculosis drugs at present are isoniazid (Isonicolusic and hydrazide, INH), ethambutol, rifampin and streptomycin.

VIBRIO

Vibrio cholera is a small, slightly curve gram-negative organism possessing a single polar flagellum. The organisms have many similarities to the members of the Enterobacteriaceae but can be readily differentiated by their positive oxidase reaction and their ability to grow at pH between 9.0 and 9.5. Growth is rapid on peptone agar, blood agar with pH near 9.0 or thioglycolate-citrate bile sucrose agar. A number of serologic types have been reported, based on antigenic differences in their O antigen. Of these, three strains have

been given specific names: Inaba Ogawa and Hikojima. Those strains which produce a soluble hemolysin have been designated as EI Tor strain of *Vibrio cholera*.

Cholera is spread as faecal-oral disease and is acquired by ingestion of faecally contaminated water and food. The organisms do not spread beyond the gastrointestinal tract, where they multiply to very high concentrations in the small and large intestine. Unlike the shigella, they do not penetrate the epithelial layer but remains tightly adhere to intestinal mucosa.

The foremost symptom of cholera is a severe diarrhea in which a patient may lose as much as 10 to 15 liters of liquid per day. The feces, containing mucus, epithelial cells and large number of vibrios, have been referred to as “rice water stools”. Death, which may occur in as many as 60% of untreated cases, results from severe dehydration and loss of electrolytes.

Diarrhea from *Vibrio cholera* is the result of the secretion of an enterotoxin, called choleragen, that stimulates the activity of the enzyme adenylcyclase, which converts ATP to cyclic AMP (cAMP). This activity is identical to that described for one enterotoxin produced by the enteropathogenic*Escherichia coli*, however, the two toxins are not identical and antitoxins to one does not neutralize the other. The cAMP stimulates the secretion of CL^- and inhibits the absorption of Na^+ resulting in a cupious fluid loss and an electrolyte imbalance.

The mortality of cholera can be reduced to less than 1% by the replacement of fluids and electrolytes. The observation that the inclusion of glucose in a salt solution permits oral replacement of electrolytes has made treatment of this disease (particularly in rural areas) much more effective.

The organisms can be viewed in stools, particularly with a dark-field microscopy. Fluorescently labeled antiserum can be used to confirm the identification of the observed organisms.

Control of cholera requires proper sewage disposal and adequate water sanitation. Immunization with killed *vibrio cholera* or formation treated enterotoxin appears to give some protection and recovery from the disease imparts of an unknown degree or duration. Current research efforts are directed toward the preparation of a vaccine consisting of degraded enterotoxin which will stimulate IgA antibody production to the toxin.

HAEMOPHILUS

The Haemophilus is as grown negative baccilus which require blood for growth as their genus name implies and blood agar is used routinely for their isolation in the laboratory. It has been known that in the blood, there are true factors, designated as the X and the V factors which are required for the growth of Haemophilus. These factors are hemin and nicotinamide adenine dimucleotide (NAD). The hemin (X factor) is required for the organisms to synthesize their enzymes containing heme such as cytochromes and catalase. X factor or hemin, a hermostable substance present in erythrocytes is required by all haemophilus Sp. for growth. The thermolabile V factor or NAD is not required by all haemophilus Sp. For growth.

The genus haemophilus contains known pathogens for man as well as nonpathogenic species that are part of the normal flora of the mucous membranes. The following is a list of the important pathogenic species of Haemophilus and the disease they produce.

H. influenza serotype b: Serotypes other than b seldom cause disease. It cause meningitis in children, pharyngitis, tracheobronchitis.

H. aegytius: conjuncturtis (pink eye)

H. chicrey: venereal disease – chancroid

H. vaginitis: vaginitis, cystitis, cervicitis

H. parainfluenzae: rare case of endocarditis – pneumonitis

Haemophilus influenza will grow on blood agar and chocolate agar. Growth is not luxuriant and particularly on blood agar, the fine delicate colonies may be overlooked.

SATELLISM: When *H. influenza* is cultured on blood agar in conjunction with *Staphylococcus aureus* as a "spot" inoculation on the surface of the plate, colonies of *H. influenza* will become distinctly larger in the area adjacent to the *Staph*. Colony. This area of heavier growth is called the satellite phenomenon. The staphylococci elaborate the V factor which enhances the growth of *H. influenza*.

H. parainfluenzae can be easily differentiated from *H. influenza* by testing for satellitism on plain nutrient agar with a *Staphylococcus aureus*. *H. parainfluenzae* will give a satellite phenomenon on plain nutrient agar since it does not require X factor. *H. influenza* will not grow on plain nutrient sign since it needs the X factor present in blood agar.

BRUCELLAE

The members of the genus Brucella are the etiological agents of brucellosis or undulant fever which occurs principally in animals. Man is infected accidentally or indirectly through contact with infected animal or their excreta (slaughter house workers), the ingestion of unpasteurized milk and other dairy products made from raw milk.

Three species of Brucella are of medical importance. *Brucella abortus* causes abortion in cattle, *Brucella mellitensis* infects sheep and *Brucella suis* infects swine. All three species can infect any of the above animals mentioned.

Bordetella pertussis

Bordetella pertussis is the causative agent of pertussis or whooping cough. This organism was first isolated and described by Bordet and Gengou and is sometimes called Bordet-Gengou bacillus. It is a small grown negative coccobacillus which is non motile and

Bordetellapertissis required Bordet-Gengou (BG) medium for its isolation (special medium which contains penicillin to which *Bordetella pertussis* is resistant).

The disease whooping cough is an acute infection of the respiratory to involving both the trachea and the brochii. It begins with a catarrhal stage characterized by sneezing and a mild but irritating cough. The paroxysms of coughing may be so severe that cyanosis, vomiting and convulsions follow and completely exhaust the patient. Endotoxins may be released from disintegrating organisms, causing local inflammation as well as fever. The organisms also excrete a hemagglutinis, and disrupted cells release a heat-labile toxin (called pertussin) which is responsible for some of the symptoms of whooping cough. The organisms do not invade the bloodstream but remain localized in the respiratory tract. The incubation period is usually seven to ten days.

Treatment is not entirely satisfactory, but several antibiotics are of value. Chloramphenicol, erythromycin or tetracycline may be used. Antibiotic therapy causes a reduction in the number of secondary infections, such as bronchitis and pneumonia, caused by other organisms.

The introduction of an effective vaccine has markedly reduced the incidence of whooping cough. Formerly almost every child had this disease during his early years of life. Today, cases are rare. The vaccine consists of killed encapsulated phase I organism which are usually incorporated with diphtheria and tetanus toxoids. Because of the very high mortality of the disease in infants under one year of age, the vaccine should be administered by the second or third month of life.

NOTE: The encapsulated strain of *Bordetella pertussis* is of a single antigenic type and of maximum virulence. This state has been designated as Phase I. After this strain is cultivated in the laboratory on artificial media, the capsule is host as a result of mutation. These stepwise ranges have been designated as Phases II, III and IV; Phase IV is entirely avirulent.

BACTEROIDES

The genus *Bacteroides* includes a number of species but the one that is of clinical significance is *Bacteroides fragilis*. *Bacteroides* are gram-negative, nonsporulating, anaerobic bacilli, which constitute a major portion of the normal flora of the gastrointestinal tract and can be found on the mucus surfaces of the nose and throat. Occasionally they are isolated in blood culture or from the wounds and joint fluids. Bacteroides may be difficult to culture, requiring special media and prolonged incubation. Most strains grow well in fluid thioglycollate medium but may require much as 5 days to grow.

EFFECTS OF PARASTES UPON THE HOST

A parasite, by definition, is an organism which to some degree injures its host. In many instances it cannot be said with certainty whether or not an organism injures the host. Even if we can be fairly sure that some injury is produced, we may not be able to detect it. Thus a distinction is made between hookworm disease and hookworm infection, on the basis of the presence or absence of clinical symptoms. Overt symptoms of infection with parasite may depend upon the number of worms present, the nutritional status of host or both.

Injury to the host may be brought about in a wide variety of ways. The most widespread type of injury is that brought about by interference with the vital processes of the host, through the action of secretions, excretions or other products of the parasite. Parasites producing such effects may be the tissues or organs of the host, in the blood stream, within the gastrointestinal tract, or may even be ectoparasitic. When the giant intestinal fluke, *Fasiciolopsis buski*, is present in large numbers, absorption from the intestinal tract of its secretions and excretions may lead to severe toxicity. *Entamoeba histolytica* erodes the intestinal wall, destroying the tissue locally by means of a proteolytic enzyme. Malaria parasites invade and multiply in red cells, which are destroyed in the process. Ascaris may perforate the bowel wall, cause intestinal obstruction if present in large number and invade the appendix, bile duct or other organs. Hookworms suck blood and by so doing may deprive the host of more iron than is replaced by his diet and so bring about an anemia. The broadfish tapeworm, *Diphllobothrium latus* selectively remove vitamin B12 from the alimentary tract, producing a megaloblastic anemia in some infected persons.

PARASITES OF VETERINARY IMPORTANCE

The parasites of major importance to livestock include trypanosomiasis, gastrointestinal (especially hookworm) infection, fascioliasis, taeniasis (cysticercosis), hydatidosis and tick diseases. Apart from the direct economic losses resulting from the effects of these diseases, they are of importance to man because many of them are zoonotic disease (diseases capable of transmission between man and animals). They also undermine the nutritional status of the community by depleting the amount of protein available to the people, resulting in protein-calorie malnutrition.

Parasitism has been incriminated as the major cause of the low productivity of livestock poultry in the tropics. For example, calf mortality as a result chiefly of parasitism is estimated at 30% of the world's meat. Thus while U.S.A. produces 93kg per head of cattle, Africa produces only 15kg per head. These are the visible, measurable facts, but it is not so easy to estimate the loss due to reduction in quality and quantity of meat and milk and the reduced length of productive life etc resulting from infection.

These factors combine to produce a deficiency in the amount of animal protein available to the people of the tropics. Thus while Americans consume 69g of animal protein per day, Africans consume only 10.8g. It has been proposed that since animal protein is not essential to man's diet, it would make more sense to derive protein requirements directly from eating cereals and legumes rather than going through the wasteful and expensive process of first converting them into animal protein. This is all very well in the strict context of human nutrition. However, in the socio-economical context, this undermines a man's estimation of the quantity and enjoyment of life when he is constrained to depend on vegetarian diet after he has developed the taste for meat.

THE VARIOUS CONTROL METHODS DIRECTED AGAINST MALARIA AND THEIR LIMITATION

Control methods directed against malaria is aimed at achieving:

1. Reduction in longetivity of the parasite
2. Reduction in longetivity of the mosquito
3. Reduction in man-mosquito contact.

The reduction in longetivity of parasite can be achieved by the use of drugs, the reduction in longetivity of mosquito by the use of insecticides and the reduction in man-mosquito contact by the use of repellants, mosquito net and the removal of mosquito breeding sites in the vicinity of human habitation.

The first antimalaria drug to be discovered was quinine and its use has persisted from seventeenth century to the present day. There are now four drugs listed by WHO as being acceptable for human use, quinine, chloroquine, primaquine and pyrimethamine. Quinine and chloroquine only act against the blood stage whereas pyrimethamine and primaqine are effective against the liver stage also but are not as effective against the blood stages. The most useful drug is chloroquine, particularly in bringing a raging infection and fever under control. Unfortunately resistance to chloroquine is now becoming widespread. Fortunately, resitant to the other antimalarials is less frequently encountered and particularly in chloroqine resistant cases acombination of pyrimethamine and sulphonamide is effective and useful. There is however no cheap long lasting antimalarial drug available for mass prophylaxis.

The reduction in longetivity of mosquito the use of insecticides, and in particular the use of residual insecticides against resting adults has long been the main weapons against malaria. From 1945 onwards, intensive spraying campaigns eradicated malaria from many temperate areas, for example Italy, Greece and parts of South America and severely reduced the prevalence of the disease in Asia. Unfortunately, a series of factors has combined to lessen the impact 0f such campaigns. The first problem is that of insecticide resistance which has now spread to over 40 species of *Anopheles* in 60 countries.

The third method of control is the reduction of man-mosquito contact. For the individual, the use of mosquito nets or insect repellants can be adequate but for a population at large it is necessary to remove sources of mosquitoes from around human habitations. This source reduction as it is called involves for example the removal of temporary water and removing or covering other possible breeding sites. At one extreme this could simply involve not discarding empty cans and the other, the use of controlled irrigation systems in rice fields.

Various kinds of biological control have been suggested but it is now felt that the biological control of mosquitoes by such diverse method as the use of predators and pathogens or the release of sterile male mosquitoes is probably only of academic interest at the present time despite its obvious attractions. Similarly the possibility of immunization is remote and the present hope must lie in the development of better and long-lasting drugs and insecticides together with an increased realization of the need for source reduction.

EPIDEMIOLOGY OF ENTAMOEBA HISTOLYTICA

Facors of epidemiological importance include the following:

1. Infection arises from ingesting cysts in contaminated food and drinking water.
2. The cysts survive the action of the acid in the stomach. The ideal conditions for encysment include the presence of the right bacterial flora, glucose and vitamin B , anaerobic condition etc.
3. Cysts are resistant and can withstand extremes of environmental conditions for several; weeks. They are however, susceptible to decication, and temperatures as low as -5oC or as high as 40Oc. Of particular significance in epidemiology is the fact that they are not harmed by the standard treatment of water with chlorine nor by washing salads etc. with potassium permanganate.
4. They survive in the intestine of houseflies which spread the cysts either mechanically with their legs or by vomiting or defecating while feeding on foodstuffs.
5. Food vendors with poor personal hygiene who are symptomless carriers help to spread the cysts through careless food handling.
6. Use of untreated human feces as fertilizers spread the disease.
7. Generally, transmission is associated with a low level of sanitation in which food and water are very easily contaminated in human feces and where poverty and ignorance combine to inhibit adequate treatment and purification of food and drink.