COURSE CODE: **VPM 401**

COURSE TITLE: **VETERINARY BACTERIOLOGY**

NUMBER OF UNITS: **3 Units**

COURSE DURATION: **Two hour of lecture and three hours of practical per week**

COURSE DETAILS:

COURSE DETAILS:

**Course Coordinator: Dr. Olufemi Ernest Ojo** *D.V.M****.,*** *M.Sc****.***

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**Office Location: COLVET**

**Other Lecturers: Dr. M. A. Oyekunle, Dr. M. Agbaje**

COURSE CONTENT:

Isolation and identification of pathogenic bacteria of Veterinary importance including *Bacillus,*

*Clostridium, Dermatophilus, Pasteurella, Staphylococcus, Streptococcus, Mycobacterium,*

*Escherichia Salmonella, Listeria, Brucella, Moraxella, Fusobacterium, Corynebacterium,*

*Nocardia, Actinomyces, Actinobacillus, Pseudomonas, Shigella, Vibrio, Bacteroides,*

*Campylobacter, Leptospira, Borrelia, Treponema, Neisseria, Yersinia, Francisella, Bordetella,*

*Acinetobacter, Histophilus, Haemophilus, Erysipelothrix, Burkholderia, Mannheimia and*

*Mycoplasma*

COURSE REQUIREMENTS:

This is a compulsory course for all 400 level students in the College of Veterinary Medicine. In

view of this, students are expected to register for the course and participate in all the course

activities. A minimum of 75% attendance in lecture and practical periods is required to qualify

for continuous assessment tests and the final examination.

READING LIST:

1. Quinn P. J., Markey B. K., Carter M. E., Donnelly W. J. C. and Leonard F. C.: Veterinary Microbiology and Microbial Disease, 4th Edition. Blackwell Science, 2001
2. Gupte S.: Short Textbook of Medical Microbiology, 7th Edition. Jaypee Brothers Medical Publishers(P) Ltd., New Delhi, India, 1999.
3. Koneman E. W., Allen S. D., Janda W. M., Schreckenberger P. C. and Winn Jr W. C.: Color Atlasand Textbook of Diagnostic Microbiology , 5th Edition. Lippincott Williams and Wilkins,1997.
4. Betsy, T and Keogh, J: Microbiology demystified. Published by the McGraw-Hill Companies, NewYork, USA, 2005.
5. Hirch D. C. and Zee Y. C.: Veterinary Microbiology. Blackwell Science Inc., 1999.
6. Quinn P. J., Carter M. E., Markey B. and Carter G. R.: Clinical Veterinary Microbiology.Mosby, Elsevier Ltd. 1999.
7. Gyles C. L., Prescott J. F., Songer J. G., andThoen C. O.: Pathogenesis of Bacterial Infections inAnimals (Third Edition). Blackwell Publishing, 2004.
8. Ojo M. O.: Manual of Pathogenic Bacteria and Fungi. Banola Multi Project Limited. 2009.
9. Hirch D. C. and Zee Y. C.: Veterinary Microbiology. Blackwell Science Inc., 1999.
10. Quinn P. J., Carter M. E., Markey B. and Carter G. R.: Clinical Veterinary Microbiology.Mosby, Elsevier Ltd. 1999

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LECTURE NOTES

**INTRODUCTION**

**(Dr. M. A. Oyekunle)**

**INTRODUCTION**

**What is bacteriology?**

Bacteriology is the study of bacteria

**Why do we study bacteria?**

We study bacteria in Veterinary Medicine or Medicine because bacterial diseases are among the

most important and common problems that animal and fish keepers/managers must deal with.

Therefore, the veterinarian must be equipped to know about these organisms. Because infections

frequently involve more than one system, veterinary microbiologist/bacteriologists have

generally resisted the systemic approach to teaching infection diseases.

However, student may develop tables to assist himself in system orientation to infectious agents.

We study these organisms to know in which diseases they are involved so as to find a treatment.

Therefore, the approach to their study will include knowing fully about their:

- History

- Habitat

- Characteristics – Colony/Culture characteristics

- Cell morphology

- Staining characteristics

- Biochemical characteristics

- Genetic characteristics

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**BACTERIAL PATHOGENICITY**

**(Dr O. E. Ojo)**

§ Majority of bacteria are non-pathogenic saprophytes

§ Bacteria which causes disease in humans and animals are small in number compared to

those that dot not cause disease

§ Bacteria that cause disease are said to be pathogenic

§ The development and severity of bacterial infections are influenced by host-related

determinants such as phy-biological status and immune competence

§ Commensal bacteria can cause opportunistic infection in the host

Steps in bacterial infection

Route of bacterial entrance into the host: skin, mucus, membranes, teat canal and umbilicus

Steps in Bacterial Pathogenesis:

Ø Adhesion to the host cells

Ø Local proliferation or multiplication

Ø Damage to the host tissue

Ø Invasion

Ø Dissemination

Ø Tissue and host specificity

· Virulence of bacteria relates to the ability to invade and produce disease in a normal

animal

· Ability to adhere: virulent pathogens often possess specific surface molecule which

allow adherence to receptors on ost cells

· Adherence factors include: adhesions, fimbriae, intimin, invasion (all in gram-negative

bacteria)

· Adherence factors in gram-positive bacteria: protein F (a fibrionectin-binding protein) is

necessary for adherence of streptococci to respiratory epithelial-the coagulase of

pathogenic staphylococci promotes adherence to fibrinogen-coated surfaces

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· Capsule-like material in *Klebsiella pneumoniae* enhance its interaction with human

intestinal cells

· In general, capsule are thought to hinder bacterial adherence to host cells

· Iron is essential for bacterial respiration

· Most iron in the animal host is bound by iron-binding proteins like lactoferrin and

transferring, and therefore unavailable for the bacteria

· Pathogenic bacteria obtain iron from the host by producing iron-chelating compounds

like siderophores which can remove iron from transferring and lactoferrin

· Other lyse erythrocytes to obtain iron from haemoglobin

· Bacterial multiplication, tissue invasion and avoidance of host defence mechanism

Mechanism employed by bacteria for survival in the host

§ O antigen polysaccharide chain: length of polysaccharide chain hinders binding of the

membrane attack complex of complement to the outer membrane of many gramnegative

bacteria

§ Capsular antigen: incorporation of sialic acid by some gram-negative bacteria has an

inhibitory effect on complement activity

§ Capsule production: antiphagocytic

§ H-protein production: antiphagocytic activity e.g. *S. equi*

§ Production of Fc-binding proteins: *Staphylococci* and *Streptococci* produce protein which

bind to the Fc region of IgG and prevent interaction with the Fc receptor on membranes

of phagocytes

§ Production of leukotoxins: cytolysis of phagocytes by toxins produced by *Manheimia*

*haemolytica, Actinobacillus species* and other pathogenic bacteria

§ Interference with phagosome-lysosome fusion, allows the survival of pathogenic

mycobacterium within phagocytes

§ Escape from phagosomes: survival mechanism used by *Listeria monocytogenes* and

*Rickettsiae*

§ Resistance to oxidative damage: allows the survival of *Salmonella* and *Brucellae* within

phagosytes

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§ Antigenic mimicry of the host antigens: adaptation of surface antigens by *Mycoplasma*

*spp* to avoid recognition by the immune system

§ Antigenic variation of surface antigens: permits survival of *Mycoplasma spp* and

*Borreliae* despite the host’s immune response to these pathogens

§ Coagulase production: conversion of fibrinogen to fibrin by *Staphylococcus aureus* can

isolate site of infection from effective immune response

Dissemination of bacteria in the host

§ Avoidance of host defence mechanism is essential for successful invasion and

dissemination of the pathogen

§ Enzymes such as collagenases, lipases, hyaluronidases and fibrinolysin produced by

bacterial pathogens facilitate breakdown of host tissue

§ Bacteraemia is the transcient presence of the bacteria in the blood stream without

replicating

§ Septicaemia is the persistent presence of bacteria multiplying in the blood stream

Damage to host tissue and associated clinical signs

§ Direct damage is caused by exotoxin and endotoxin production

§ Indirect damage results from the activity of enzymes secreted by the bacteria and host

immune response to infection

**Comparison of exotoxins and endotoxins**

Exotoxin Endotoxin

Produced by live bacteria Released during death and lysis of cells

Secreted actively Component part of cell wall

Produced by both gram-positive and gramnegative

bacteria

Produced by gram-negative bacteria

High molecular weight protein Lipopolysaccharide complex containing

lipid A, the toxic component

Heat-labile Heat-stable

Potent toxins, usually with specific activity Toxin with moderate non-specific

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generalised activity

Not pyrogenic Potent pyrogens

Highly antigenic Weakly antigenic

Readily converted to toxoid Not amenable to toxoid production

Induced neutralizing antibodies Neutralising antibodies not associated with

natural exposure

Synthesis determined extrachromosomally

Encoded by chromosome

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**THE ACTINOMYCETES**

They consist of a group of filamentous microorganisms occupying an intermediate position

between bacteria and fungi.

- Their identity as bacteria was confirmed by:

\* Their prokaryotic cellular organization

\* Their cell wall chemistry

\* Their nitrogen metabolism

\* Their sensitivity to antibiotics and phages.

There are two major groups of actinomycetes namely:

\* Aerobic actinomycetes

\* Anaerobic actinomycetes

- They cause infections in animals and humans.

- There are also a large number of non-pathogenic species

**1. GENUS: *ACTINOMYCES***

- Are pleomorphic Gram positive coccobacilli, rods, filament, branching or non-branching

cells.

- Non-motile, non-spore forming.

- Found on the mucous membranes of the oral and nasal cavities and also on the genital

tract.

- Important species are:

*A. israeli* human actinomycosis obligate anaerobe

*A. bovis* cattle actinomycosis obligate anaerobe

*A. viscosus* Dog peri-odontal disease facultative anaerobe

*A. hordeovulnesis* Human chronic suppurative facultative anaerobe

infection in humans and dogs

*A. naeslundi* Human Periodontal infection, facultative anaerobe

dental caries

*A. pyogenes* Animals Pyogenic infection facultative anaerobic

Disease caused is generally referred to as Actinomycosis

**- Laboratory diagnosis of Actinomycosis**

Direct examination

- Small amount of pus placed in petridish.

- This is washed with water to expose small sulphur granules.

- Transfer granule to a slide, add a drop of 10% NaOH, add cover slip and crush by

gentle pressure.

- Characteristic ray-fungi is seen with club shaped margins under low power if

actinomycosis.

- Then remove cover slip, spread and stained by Gram’sn technique.

- If Actinomycosis, branching Gram positive filaments are observed.

**- Isolation and Cultivation**

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- Can be cultured on blood agar, brain heart infusion agar and thisoglycollate broth.

- An atmosphere containing 5-10% CO2 preferred for incubation.

- Colonies are white, rough, nodular and adhere tenaciously to the medium and

difficult to remove.

- Gram stained smears from growth on media revealed masses of Gram positive rod

and slightly branched filaments.

**- Identification**

Based on characteristic sulphur granules

Demonstration of gram positive filaments

**- Treatment**

- Drainage and antibiotic therapy

**2. GENUS: *NOCARDIA***

- Non-motile, non-spore forming, Gram positive rods which sometimes show

branching.

- Partially acid fast, aerobic.

- Splits sugars by oxidation.

- Are important part of the soil and water flora.

- A number of the members of the genus cause a variety of diseases in both normal

and immunocompromised humans and animals.

- Mechanisms of pathogenesis is complex and not well understood but include the

capacity to evade or neutralize the myriad of antimicrobial activities of the host.

- More than 40 species have been described.

- Important species include

*N. asteroides* - Human and animals

*N. bransiliensis* - Human

*N. cavaiae* - Human, bovine mastitis, guinea pig

*N. farcinica* - Cattle

**Mode of Infection**

- By inhalation, through wounds, hands and feet of laboratory workers.

- Usually exogenous

**Laboratory diagnosis**

**Direct examination**

- Gram strained smears of pus/lesions reveal Gram positive branching filaments

with or without clubs.

- Stains partially with ZN stain.

- Giemsa stain can also be used.

Experimental animal: guinea pig susceptible

**Isolation and Cultivation**

- Organism grows on blood agar or any other enriched media.

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- The colour of the colony varies from chalk white to deep orange.

**Identification**

- Based on demonstration of typical organism, colonial, cultural and morphological

characteristics.

**Treatment**

- Various drugs useful including sulphonamides and antibiotics.

**3. *DERMATOPHILUS CONGOLENSIS***

- Gram positive branching filamentous rods, aerobic and non-spore forming, nonacid

fast.

- Produce motile zoospores.

- Unique medically because natural growth cycle is restricted to the living layer of

the epidemics of animal and human skin.

Pathogencity: Causes dermatophilosis in cattle and dermatophilus infection in other animals

which is characterized by scabs formation on the skin.

**Laboratory diagnosis**

Specimen- Infected Scab

**Direct Examination**

- Many procedures employed in making impression smears.

- But better if impression smear is made from the moist concave undersurface of

freshly removed scabs.

- Stains well with dilute carbol fuchsin or methylene blue stain, Gram stain or

preferably 1:10 dilution of Giemsa stain for 30 minutes.

**Isolation and Cultivation**

Organism grows well on media containing blood or blood product.

Colony : - Small, rough, graywhite colonies appear in 24-48 hours of incubation.

- Colonies are yellowish to orange.

- Produces β haemolysis on sheep or horse blood agar. On human blood,

haemolysis is narrow and hazy.

- Motile zoospores are formed as a result of the septation of hyphal element

- Zoospores possess polar flagella.

- Gram positive, branching hyphal elements in various stages of segmentation are

seen.

- Two colony types can be demonstrated.

(i) Rough colony: grows into the agar and difficult to remove and emulsify in water

or saline.

(ii) Smoothcolony: easy to remove from plate and emulsify in water or saline.

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**Antigenic Components**

- Five (5) antigenic types demonstrated using agar gel precipitating test.

**Treatment**

Use of various drugs, chemicals, concoctions/herbal preparations are in practice.

**4. *MYCOBACTERIUM***

- Are Gram positive (not easily stained by Gram method), acid-fast, small rods

- They are non-motile.

- Filamentous and branching forms occur.

- They don’t stain readily, but when they do, may stain with basic dyes.

- They resist decolourization by acid (acid-fast).

- There are more than 50 Mycobacteria species including many that are

saprophytes.

**Classification**

i. **Runyon Classification of Mycobacteria (Runyon’s group)**

**Group Organisms**

Tuberculosis complex *M. tuberculosis*

*M. bovis*

*M. africanium*

Photochromogens *M. asiaticum*

(Produce pigment in light) *M. kansasi*

*M. marium*

*M. simiae*

Scotochromogens *M.flavescens*

(produce pigment in the dark) *M. gordonae*

*M. scrofulaceum*

*M. szulgai*

Non-chromogens *M. avium complex*

(No pigment produced) *M. celatum*

*M. haemophilum*

*M. gastri*

*M. genovense*

*M. malna*

**ii. Rate of growth**

- Rapid growers

- Slow growers

**iii. Anonymous mycobacteria**

Are atypical unclassified mycobacteria that have been recovered from animals and man.

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**iv. Saprophytic or non pathogenic mycobacteria**

Mycobacteria considered to be non-pathogenic or not previously identified are now

becoming epidemiologically important particularly in the AIDS era because of their high

resistance to antibacterial agents.

**v. New species**

**Laboratory diagnosis of tuberculosis**

- Based on (i) Microscopy

(ii) Culture

(iii) Immunological test

(iv) Molecular characterization

(v) Others

Specimen: Different samples may be used depending on the clinical picture of the

disease.

**5. GENUS: *ACTINOBACILLUS***

- Gram negative, small rod, non-motile, non-spore forming, aerobic and fermentative.

- Rarely grows in filaments, and if so, filaments show some branching.

- Has tendency for bipolar staining

Important species include

*A. lignieresii- man*

*A. pleuropneumoniae* - pig

*A. equuli*- Horse (foals) and occasionally pig

- joint illness, navel illness

*A. suis* – pig

*A. seminis* – sheep (ram) – affecting rams’ epididymis

- Natural infections with *A. lignieresii* occur in both cattle and sheep and are characterized

by infections granulomas containing pus affecting the soft tissue in the region of the head

e.g. tongue.

**Laboratory diagnosis**

- Granule/Pus specimen is examined in the same manner as in actinomycosis

- Small Gram negative rods can be demonstrated in the lesion.

**Isolation and Identification**

Specimen - Pus or necrotic material from early lesion seeded on blood or

serum agar.

- Incubated at 37oC under 10% CO2 accelerated growth.

- Subucultured strains grow well in air.

- In media contained fermentable carbohydrate long almost

filamentous form are seen.

Colonies - may be mucoid or stringy when freshly isolated.

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- can be white, grayish-white, yellowish or bluish in colour.

Cultural characteristics: - Are aerobic to facultative anaerobic

- Are micro aerophilic on primary isolation.

Biochemical characteristics: Produce acid but no gas from carbohydrate when fermented.

Pathogenicity: Pathogenic to animals. Some species can affect humans.

Disease produced by *A. lignieresii* can be similar to that produced by *Actinomyces* and

*Mannheimia haemolytica*

**6. GENUS: *MYCOPLASMA***

- They are bacteria

- Members of the genus are characterized by the absence of a cell wall.

- They are pliable and can pass through the pores of filters that retain bacteria.

- Most members have sterol in their membrane which provides added strength and rigidity

protecting the cells from osmotic lysis.

- They are among the smallest form of life.

- Their genomes are thought to be the minimum size for encoding the essential functions

for a free living organism.

- Are facultative anaerobic or obligate anaerobic.

- Are pleomorphic.

- Because they have cell membrane and both RNA and DNA, they differ from

viruses.

- Mycoplasmas can resemble fungi because some produce filaments that are commonly

seen in fungi.

- It is because of these filaments that scientists named them mycoplasma i.e. ‘myco’ means

“fungus”.

- They stain poorly, but giemsa can be used to demonstrate Mycoplasmas in tissues.

- Many are unable to move because they lack flagella but some can glide.

**Cultivation and Cultural features**

- Mycoplasmas have low biosynthetic ability.

- Therefore they need rich medium containing natural animal protein (blood serum) and in

most cases sterol compounds.

- Mycoplasma colonies on solid media produce a characteristic “fried egg” appearance.

**Cell morphology**

- Coccobacilli, coccal forms, ring forms, spiral and filaments seen in smears. Stains poorly,

but giemsa can be used.

- Size 50-60 to 200-250 nm, diameter 0.3-0.8nm.

- Parasitic mycoplasmas contain 10-20% lipid, relatively low content of nucleic acid

compared to other bacteria.

- May grow in chicken embryo.

**Viruses and Plasmids of Mycoplasmas**

- 14 viruses identified to infect mycoplasma

- 6 in *Acholeplasma*

- 4 in *Mycoplasma*

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- 4 in *Spiroplasma*

- There is evidence of integration of viral genomes into mycoplasma chromosomes.

- Release of virus is continuous and not accompanied by cell lysis.

- Plasmids detected in Mycoplasma, Acholeplasma and Spiroplasma.

- Acholeplasmataceae – does not depend on sterol for growth.

- Anaeroplasmataceae – strict anaerobes

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***STAPHYLOCOCCUS SPECIES***

**(Dr. O. E. Ojo)**

§ Gram-positive bacteria

§ Spherical (cocci) in shape

§ About 1 μm in diameter

§ Occur in irregular clusters

§ Staphyle≡bunch of grapes

§ Kokkos≡berry

§ Common commensals on skin and mucous membrane

§ Often cause pyogenic infections

§ Oxidase-negative, catalyse-positive, non-motile, non-sporing facultative anaerobes

§ Important animal pathogens include *S. aureus, S. intermedius*, *S. hyicus*

§ Pathogenic species often produce coagulase

§ *S. aureus and S. intermedius* are coagulase positive while *S. hycus* is coagulase variable

§ Coagulase negative staphylococcus are of low virulence but may occasionally cause

disease in animals and man

Diseases in animals

- Exudative epidermitis in piglets (greasy-pig diseases): *S. hyicus*

- Tick pyaemia of lambs : *S. aureus*

- Bovine staphylococcal mastitis: *S. aureus*

- Botryomycosis (Scirrhous cord) (horse, pigs, cattle): *S. aureus*

- Wound infection (most animals): *S. aureus, S. hyicus, S. intermidus*

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- Mastitis: *S. aureus, S. hyicus, S. intermedius*

- Bumble foot, omphalitis in poultry: *S. aureus*

- Pyoderma, otitis externa, cystitis, endometritis in dogs: *S. intermedius*

Diagnosis

- Sample collection: pus, exudates

- Media: grow on non-enriched media

§ Nutrient agar, blood agar

- Selective medium: mannitol salt agar (staphylococci can tolerate high

concentration of NaCl). Mannitol salt agar contains 7-10% NaCl

- P- agar for cultural differentiation of staphylococci

Colonial characteristics

- Colour: usually white, opaque and up to 4mm in diameter. Colonies of bovine

and human strains of *S. aureus* are golden yelloe. Saprophytic staphylococcus

may be pigmented

- Staphylococcus may produce haemolysis on sheep/ox blood agar. Types of

haemolysis: alpha, beta, gamma, delta haemolysis

- *S. aureus and S. intermedius* produce double zones of narrow complete and wide

incomplete haemolysis on blood agar

- *S. hyicus* is non-haemolytic

Coagulase test: mix a suspension of staphylococcus isolate with rabbit plasma either on

a slide or in a small tube. Coagulase convert fibrinogen to fibrin (strand/lumps)

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- Slide coagulase test detects the presence of bound coagulase or clumping factor

within 1 to 2 minutes

- Tube coagulase test detects free coagulase or staphylocoagulase secreted by

bacteria. It is the definitive test for coagulase. The plasma clots within 24 hours

of incubation at 370C

Differentiation tests

Differentiation of Gram-positive cocci

Organism Appearance of

stained smear

Coagulase Catalase Oxidase O-F test Bacitracin test

*Staphylococcus spp* Irregular cluster ± + - F Resistant

*Micrococcus spp* Packets of four - + + O Susceptible

*Streptococcus and*

*enterococcus spp*

Chain - - - F Resistant

Purple agar: contains indicator-bromocresol purple, sugar (1% maltose)

Species Colony colour Haemolysis

on sheep

BA

Tube

coagulase

Slide

coagulase

Acetone

production

Maltose utilization

*S. aureus* Golden

yellow

+ + + + +

*S. intermedius* White + + V - ±

*S. hyicus* White - V - - -

- Molecular procedure carried out in research and reference laboratories

Treatment: Penicillin and its derivatives

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***STREPTOCOCCUS SPECIES***

**(Dr. O. E. Ojo)**

§ Gram-positive bacteria chain 1.0 μm in diameter

§ Non-motile, non-sporing, oxidase negative, catalase-negative, facultative anaerobes

§ Fastidious organism requiring enriched media for growth

§ Pathogenic species cause suppurative conditions such as mastitis, metritis, polyarthritis

and meningitis in animals

§ *Enterococcus spp* are opportunitistic enteric *streptococci* found in intestinal tracts of

animals and humans

§ Unlike *Streptococcus spp, enterococci* can tolerate bile salt and therefore grow on

MacConkey agar as red pinpoint colonies. Some streptococci are also motile

§ Most *streptococcu spp* live as commensals on the mucosae of the upper respiratory tract

and lower urogenital tract

§ *Streptococci* are fragile and susceptible to desiccation

Diseases:

· Bovine streptococcal mastitis:

*i. S. agalactiae, B,* b *(*a*,*g*)*

*ii. S. dysgalactiae C,*a *(*b*,*g*)*

*iii. S. uberis NA* a *(*g*)*

*iv. Enterococcus faecalis D,*a *(*b*,*g*)*

*v. S. pyogenes A,* b

*vi. S. zooepidemiccos C,* b

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- (i-iii): principal pathogens of mastitis

- (iv-vi) are less associated with mastitis

§ Strangles in horses: *S. equi* C*,* b

· Abscess and other suppurative conditions and septicaemia in many species of animals

*S. pyogenes* (*A,* b*):* humans

*S. canis* (*G,* b*):* dogs

*S. suis* (*D,*a *(*b*)):* pigs

*S. equisimilis* (C*,* b)*:* horses

Diagnosis

· History, clinical signs and pathology may be indicative of streptococcal infection

· Samples are collected and cultured promptly: streptococcal are highly susceptible to

desiccation. Samples include pus and exudates

· Samples can be placed in transport medium

· Stained smear of clinical samples may reveal gram-positive cocci in chains

· Samples should be cultured on blood agar and MacConkey agar

· Incubate agar plates aerobically at 370C for 24-48 hours

· Streptococcal colonies are small, translucent and some may be mucoid

· Differentiation of the streptococci:

i. Type of haemolysis

ii. Lancefield grouping

iii. Biochemical testing

i. Type of haemolysis on blood agar

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- Beta-haemolysis: complete haemolysis of clear zones around colonies

- Alpha-haemolysis: partial, incomplete haemolysis, greenish or hazy zones around

colonies

- Gamma-haemolysis: no observable changes in the blood agar around colonies

ii. Lancefield grouping: a serological method of classification based on the group-specific Csubstance.

Lancefield grouping test methods include:

· Ring specification test

- Extract C-substance by acid or heat from the *Streptococcus spp*

- The extract (antigen) is layered over antisera of different specificities in narrow

tubes placed in plasticine on slide

- A positive reaction is indicated by the formation of a white ring of precipitate close

to the interface of the two fluids within 30 minutes

· Latex agglutination test: latex-coated group-specific antibodies are commercially available

for the test. Antigen is extracted enzymatically from the *streptococcus spp* under test

- Mix antiserum and antigen together on a slide

- Positive reaction is indicated by agglutination

iii. Biochemical tests: oxidase, catalase, sugar fermentation tests. Biochemical tests are available

commercially for rapid identification of streptococci

**Biochemical differentiation of equine group C Streptococci**

Species Trehalose Sorbitol Lactose Maltose

*S. equi* - - - +

*S. zooepidemicus* - + + +(-)

*S. equisimilis* + - V +

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**Differentiation of streptococci associated with mastitis:**

*S. pyogenes:* Bacitracin sensitive (all group A)

*S. agalactiae:* CAMP test (Christie, Atkins, Munch and Petersen) positive with *S. aureus* and

*Corynebacterium pseudotuberculosis (*all group B *Streptococci)*

*S. uberis:* Aesculin hydrolysis (black brown zones of discolouration around dark coloured

colonies on Edward’s medium).

*S. pneumoniae:* quellung reaction/capsule swelling test, bile solubility, Optorchin-sensitivity, *S.*

*pneumoniae* appears a lancet-shaped organisms in pairs. It is capsulated

CAMP test: enhanced haemolysis (synergism) of staphylococcal beta-toxin or corynebacterial

phospholipase D

Optorchin: ethylhydrocupreine hydrochloride

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***LISTERIA SPECIES***

**(Dr. O. E. Ojo)**

· Gram positive coccobacillary rods about 2μm in length

· Catalase positive, oxidase negative, facultative anaerobes

· There are six species in the genus, three of which are pathogenic

· *L. monocytogenes* is the most important species. It was first isolated from rabbits

with septicaemia and monocytosis

· Can tolerate wide temperature (40C – 450C) and pH (5.5 – 9.6) ranges

Diseases

*i. Listeria monocytogenes*

Cattle, sheep, goats: encephalitis (neural form), abortion, septicaemia, endophthamitis.

Cattle: mastitis (rare).

Dogs, Cats, horses: abortion, encephalitis (rare)

Pigs: abortion, septicaemia, encephalitis

Birds: septicaemia

ii. *L. ivanovii*

Sheep, cattle: abortion

iii. *L. innocua*

sheep: meningoencephalitis

Diagnosis: (microbiological)

§ Sample collection: cerebrospinal fluid, tissue from brain (medulla and pons), specimen

from abortion cases: cotyledons, foetal abomasal contents, uterine discharges.

Septicaemia: spleen, blood. Collect only fresh samples

§ Smear from cotyledon or liver lesion may reveal several gram-positive coccobacillary

bacteria

§ Immunoflourescence using monoclonal antibodies gives rapid result

§ Isolation

o Inoculate sample onto blood agar, selective blood agar and MacConkey

o Incubate aerobically at 370C for 24 to 48 hours

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o A cold enrichment procedure may be necessary for recovery of Listeria from

clinical specimen

o Inoculate a 10% suspension of sample into nutrient/enrichment broth

o Keep the inoculated broth at 40C in a refrigerator

o Subculture weekly from the broth onto blood agar for up to 12 weeks

§ Two forms are formed on culture media; smooth and rough forms

o Smooth: small, smooth, flat, more common (short filament and coccal forms,

older culture)

o Rough: young culture, entirely of long filament

§ *L. monocytes* colonies are small, smooth and flat

§ *L. monocytes* produces a blue-green colour with oblique illumination

§ Colonies are surrounded by a narrow zone of complete haemolysis

§ It is catalase positive. *Streptococci* and *Arcanobacterium pyogenes* have similar colonies

but are catalase negative

§ It is cAMP test positive with *staphylococcus aureus* but not with *Rhodocossus equi*

§ It hydrolysis aesculin

§ Produces a characteristic tumbling motility after incubation in broth at 250C for 2-4

hours

§ Pathogenicity test in rabbit to confirm virulence: instil a broth culture into rabbit eye.

Virulence strains induce keratoconjuctivitis. This is called Anton test

§ *Listeria spp* are zoonotic

***Laboratory methods for differentiating Listeria species***

*Listeria spp* Haemolysis

on sheep

blood agar

CAMP test Acid production from sugar

*S. aureus R. equi* D-mannitol L-rhamnose D-xylose

*L. monocytogenes* + + - - + -

*L. ivanovii* ++ - + - - +

*L. innocua* - - - - V -

*L. seeligeri* + + - - - +

*L. welshimeri* - - - - V +

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*L. grayi* - - - + V -

+ = positive reaction, - = negative reaction V = variable reaction

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***ERYSIPELOTHRIX SPECIES/ E. RHUSIOPATHIAE***

**(Dr. O. E. Ojo)**

· Gram positive slender rods which may be curved or straight

· Have tendency to form elongated filaments

· May appear in pairs or in groups

· Some thickened filaments are beaded with gram’s staining

· Small rods (small form), filament (rough form)

· Both forms occur on culture media

· Smooth forms are isolated from acute infections

· Isolate from chronically infected animals from rough colonies

· Produce small colonies with incomplete haemolysis in 48 hours

· Grow over wide temperature and pH ranges

· Catalase negative

· Coagulase positive

· Non-motile, oxidase negative, facultative anaerobe

· Form H2S along slab line in Triple Sugar Iron agar

Diseases

§ *Erysipelothrix rhusiopathiae*

o Pigs (swine erysipelas): septicaemia, diamond skin lesions, chronic arthritis,

chronic valvular endocarditis, abortion. Almost 50% of healthy pigs harbour *E.*

*rhusiopathiae* in tonsillar tissues

o Sheep: polyarthritis in lambs, post dipping lameness, pneumonia, valvular

endocarditis

o Turkey (turkey erysipelas): septicaemia, arthritis, valvular endocartitis

Diagnosis

§ Specimen: blood, liver, spleen, heart valves, synovial tissue. Organism rarely recovered

from skin lesions or chronically affected joints

§ Microscopic examination of specimen from acutely affected animals may reveal slender

gram-positive rods

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§ Filamentous elements may be seen in samples of chronic valvular lesion

§ Inoculate specimen into blood and MacConkey agar plates

§ Incubate aerobically at 370C for 24 to 48 hours

§ Selective media containing either sodium azide (0.1%) or crystal violet (0.001%) may be

used for contaminated samples

§ Non-haemolytic, pin-point colonies appear after incubation for 24 hours and after 48

hours, a narrow zone of greenish, incomplete haemolysis develops around the colonies

§ Catalase-negative, coagulase-positive (as in staphylococcus), H2S positive

§ Serotyping for epidemiological studies

o Virulence testing in laboratory animals. Because *E. rhusiopathiae* isolates vary in

virulence, it is necessary to confirm virulence by intraperitoneal inoculation of

mice or pigeons.

o PCR for virulence detection

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***CORYNEBACTERIUM SPECIES***

**(Dr. O. E. Ojo)**

§ Small Gram-positive pleomorphic (coccoid, club and rod forms) bacteria

§ Stained smear reveals cells in palisades of parallel and angular clusters resembling

Chinese letters

§ Non-motile facultative anaerobes

§ Catalase-positive, oxidase-negative

§ Fastidious, require enrichment for growth

§ Cause pyogenic infection

§ Most pathogenic species are host specific

§ Type species: *C. diptheriae,* causes diphtheria in children

Diseases

i. *Corynebacterium bovis*

Host (cattle): subclinical mastitis

ii. *C. kutscheri*

Host (laboratory rodents): superficial absceses, causes purulent foci in liver,

lungs and lymph nodes

iii. *C. pseudotuberculosis* (non-nitrate-reducing biotype)

Host (Sheep and goats): caseous lymphadenitis

iv. *C. pseudotuberculosis* (nitrate-reducing biotype)

Host (horses, cattle): ulcerative lymphagitis, abscesses

v. *C. renale* (type I)

Cattle: cystitis, pyelonephritis

Sheep and goats: ulcerative (enzootic) balanoposthitis

vi. *C. pilosum* (renale type II)

Cattle: cystitis, pyelonephritis

vii. *C. cystitides* (renale type III)

Cattle: severe cystitis, rarely pyelonephritis

*viii. C. ulcerans*

Cattle: mastitis

Diagnosis

§ Specimen: pus, exudates, tissue, sample, mid-stream urine

§ Direct microscopy of Gram-stained smear may reveal coryneform bacteria

§ Inoculate sample onto blood agar, selective media (McLeod’s blood agar, Loeffler’s

medium) containing potassium tellurite, and MacConkey agar

§ Incubate aerobically at 370C for 24 to 48 hours

§ Identification: no growth on MacConkey agar

§ Colonial Characteristics:

o *C. bovis*: a lipophilic bacterium. Small white, dry, non-haemolytic colonies

o *C*. *kutscheri:* whitish colonies, occasionally haemolytic

o *C*. *pseudotuberculosis*: small whitish colonies, surrounded by a narrow zone of

complete haemolysis evident after 72 hours of incubation. Colonies become dry,

crumbly and cream-coloured with age

o Members of *C. renale* group produce small, non-haemolytic colonies after 24

hours incubation. Produce pigment after 48 hours of incubation

§ Microscopy: Gram’s staining and Albert’s staining techniques

Albert’s staining demonstrate metachromatic granule (inclusions)

§ Biochemical tests

o Nitrate reduction: *C. pseudotuberculosis* biotype

o All pathogenic corynebacteria are urease positive except *C. bovis*

***Differentiation of C. renale group***

Feature *C. renale* (type I) *C. pilosum* (type II) *C. cystidis* (type III)

Colour of colony Pale yellow Yellow White

Growth in broth at pH

5.4

+ - -

Nitrate reduction - + -

Acid from xylose - - +

Acid from starch - + +

Casein digestion + - -

Hydrolysis of Tween 80 - - +

§ Enhanced haemolysis by *C. pseudotuberculosis* when inoculated across a streak of

*Rhadococcus equi*

***ACTINOMYCES ARCANOBACTERIUM AND ACTINOBACULUM SPECIES***

**(Dr. O. E. Ojo)**

§ Gram-positive bacteria

§ Require enriched media for growth

§ Non-motile, non-sporing

§ Morphologically heterogenous

§ Anaerobic or facultative anaerobic

§ Modified Z-N staining negative

§ Some members have undergone changes in nomenclature

o *Corynebacterium pyogenes =Actinomyces pyogenes=Arcanobacterium pyogenes*

§ *Actinomyces species* have long filamentous morphology, although short V, Y, and T

configuration also occur

§ *Arcanobacterium* and *Actinobacterium* both have a coryneform morphology

Diseases

§ *Arcanobacterium pyogenes*

Host: cattle, sheep, pigs

Conditions: Abscessation, mastitis, suppurative pneumonia, endometritis, pyometra,

arthritis, umbilical infections

§ *Actinomyces hordeovulneris*

Host: dogs

Conditions: cutaneous and visceral abscessation, pleuritis, peritonitis, arthritis

§ *Actinomyces viscosus*

Host: dogs

Conditions: canine actinomycosis

- cutaneous pyogranulomas

- pyothorax and proliferative pyogranulomatous pleural lesions

- disseminated lesions (rare)

§ *Actinomyces bovis*

Host: cattle

Conditions: bovine actinomycosus (lumpy jaw)

§ *Actinomyces viscosus*

Horses: cutanous pustules

Cattle: abortion

§ *Actinomyces spp* (unclassified)

Pigs: pyogranumatous mastitis

Horses: poll evil, fistulous withers

§ *Actinobaculum suis*

Pigs: cystitis, pyelonephritis

Diagnosis

§ Clinical specimens: exudates, aspirates and tissue samples from post-mortem

§ Direct Gram staining of smear may reveal morphological forms of aetiological agent

§ Inoculate blood and MacConkey agars and incubate at 370C for up to 5 days. Different

species have peculiar atmospheric requirement for culture

§ Identification criteria

o *Arcanobacterium pyogenes* produce a characteristics hazy haemolysis along

streak lines after 24 hours of aerobic incubation. Pin point colonies are seen

after 48 hours. Proteolytic, hydrolyses gelatine

o *Actinomyces bovis:* adhere to agar media and produces no haemolysis

o *Actinomyces hordeovulneris:* same as *A. bovis*

o *Actinomyces viscosus:* produce two colony types

§ Large and smooth: V,Y, and T cell configurations

§ Small and rough: short branching filament

o *Actinobaculum suis*: poor haemolysis on ruminant blood agar. Colonies have a

shiny raised centre and a dull edge. It is urease positive

***SPECIES DIFFERENTIATIONS***

Characteristics *Actinomyces*

*bovis*

*Actinomyces*

*viscosus*

*Actinomyces*

*hordeovulneris*

*Arcanobacterium*

*pyogenes*

*Actinobaculum suis*

Morphology Filamentous

branching,

some short

forms

Filamentous

branching,

short forms

Filamentous

branching,

short forms

Coryneforms Coryneform

Atmospheric

requirement

Anaerobic

+ CO2

10% CO2 10% CO2 Aerobic Anaerobic

Haemolysis on

sheep blood

agar

± - ± + ±

Catalase

production

- + + - -

Pitting on

Loeffler’s serum

slope

- - - + -

Granules in the

pus

Sulphur

granules

White granules No granules No granules No granule

§ Granules in lesion is caused by *A. bovis* contains characteristic clubs. Club colonies are

also produced by *Actinobacillus ligniersii* and *Staphylococcus aureus* botryomycosis

***RHODOCOCCUS EQUI***

**(Dr. O. E. Ojo)**

§ Gram-positive aerobic bacteria

§ Non-motile catalase-positive, oxidase-negative

§ Weakly acid fart

§ Grows on non-enriched media

§ Rod or coccibacillus in shape

§ Produces pigments, colonies are pink

§ It forms capsule. Produces large, moist, viscid/mucoid colonies

Diseases

Foals of 1 to 4 months of age: suppurative bronchopneumonia and pulmonary abscessation

Horse: superficial abscessation

Pigs, Cattle: mild cervical lymphadenopathy

Cats: subcutaneous abscesses, mediastinal granulomas

Diagnosis

§ Specimens: tracheal aspirates, pus from lesion

§ Inoculate blood and MacConkey agar

§ Incubate aerobically at 37 oC for 24 to 48 hours

§ No growth on MacConkey

§ Does not ferment carbohydrate

§ Does not haemolyse on blood agar. It is cAMP test positive. (enhanced haemolysis) with

*S. aureus*

§ Most strains are urease and H2S positive

**Tutorial Questions**

1 Describe the type of colouration produced when *Listeria monocytogen* colonies are

viewed under oblique illumination

2 What is the significance of Anton’s test in the diagnosis of *Listeria monocytogenes*

3 Describe the cold enrichment procedure for the diagnosis of *Listeria monocytogenes*

4 What is the aetiological agent of diamond skin disease of pigs

5 List two selective media for the isolation of *Corynebacterium spp*

6 What staining technique is employed for the demonstration of *Corynebacterial*

*metachromatic* ranules

***PSEUDOMONADACEAE***

**(Dr. O. E. Ojo)**

§ Pathogenic members that infect animals include:

*Pseudomonas aeruginosa*

*Burkholderia mallei*

*Burkholderia pseudomallei*

§ Gram negative rods of medium size

§ Obligate aerobes

§ Oxidase-positive and catalase-positive

§ *Pseudomonas species* and *Burkholderia pseudomallei* are motile by polar flagella

§ *Burkholderia mallei* is non-motile and require 1% glycerol for enhanced growth

§ *P. aeruginosa* produces pigments which diffuse into culture media

§ Pigments of *P. aeroginosa* include:

o Pyocyanin: blue-green

o Pyoverdin: greenish-yellow

o Pyorubin: red

o Pyomelanin: brownish-black

Diseases

§ *P. aeruginosa:* causes opportunistic infection in many species of animals

Cattle: mastitis, metritis, pneumonia, calve enteritis, dermatitis

Pigs: Ear infection, respiratory tract infection

Horses: genital tract infection, pneumonia, eye infection

Sheep: mastitis, pneumonia, otitis media, fleece rot/ suppurative dermatitis

(predisposing factor: heavy rainfall)

Dogs and Cats: pneumonia, ulcerative keratitis, cystitis, otitis externa

Minks: haemolytic pneumonia, septicaemia, farmed minks very susceptible

Rabbits: pneumonia, septicaemia

Reptiles: necrotic stomatitis, especially in captive reptile (found in oral cavity of snakes)

§ *Burkholderia mallei:* glanders (a contagious disease of equidae characterized by the

formation of nodules and ulcers in the respiratory tracts or on the skin

§ *Burkholderia pseudomallei*: causes melioidosis-chronic debilitating disease with

disseminated abscesses in many organs of the body

§ *Pseudomonas flourescene and P*. *putida:* pathogens of freshwater fish

Diagnosis

§ Sample collection: based on observed clinical signs and lesions. Samples may include

pus, respiratory aspirates, ear swab, mastitic milk, discharges, blood (for serology) etc.

§ Inoculate blood agar and MacConkey agar plates

§ Incubate aerobically for 24 to 48 hours at 370C

§ *B. mallei* grows on media containing 1% glycerol and also on MacConkey agar

§ Identification criteria:

o Colonial morphology

o Microscopy

o Biochemical reactions

§ Serology

o Compliment fixation test and agglutination technique for *B. mallei* detection

o Slide agglutination, ELISA, CFT, indirect haemagglutination test used for

detection of *B. pseudomallei* serum antibodies

§ The mullein test: an efficient field test for screening and confirmation of glanders in

animals. Mallein is a glycoprotein extract of *B. mallei*

o It is injected intradermally just below the lower eyelid

o A local swelling with mucopurulent ocular discharge is evident after 24 hours in

positive cases

§ *P. aeruginosa*: produces pigments detectable in media that contains no dye e.g. nutrient

agar. It also has a characteristic fruity, grape-like odour

***Comparative features of the Pseudomonadaceae***

Feature *P. aeruginosa B. mallei B. pseudomallei*

Colonial morphology Large and flat with

serrated edges

White and smooth

becoming granular and

brown with age

Range from smooth and

mucoid to rough and

dull becoming yellowish

brown with age

Haemolysis on blood

agar

+ - +

Diffusible pigment

production

+ - -

Colony odour Grape-like None Musty

Growth on MacConkey

agar

+ + +

Growth at 420C + - +

Motility + - +

Oxidase production + ± +

Oxidation of

carbohydrate:

Glucose

Lactose

Sucrose

+ + +

- - +

- - +

**ENTEROBACTERIACEAE**

**(Dr. O. E. Ojo)**

§ Members are Gram-negative rods about 3 μm in length

§ Oxidase-negative, catalase-positive

§ Ferment glucose and a variety of other sugars

§ Non-sporing facultative anaerobes

§ Reduce nitrates to nitrites

§ Mostly enteric organisms

§ Motile members possesses peritrichous flagella

§ Grow well on MacConkey agar because they tolerate bile salts

§ Categorised into two broad groups based on lactose fermentation

o Lactose fermenters e.g. *E. coli, Klebsiella spp*

o Non-lactose fermenters e.g. *Salmonella spp, Proteus spp*

§ Major animal pathogens (cause both enteric and systemic diseases)

§ Examples:

o *E. coli*

o *Salmonella* serotype

o *Yesinia spp*

- *Y. pestis*

- *Y. enterocolitica*

- *Y. pseudotuberculosis*

- *Y. intermedia*

- *Y. kristensenii*

- *Y. frederiksenii*

- *Y. ruckerii:* pathogen of fish

§ Opportunistic pathogens cause disease outside the GIT

§ Major pathogens, cause disease in both enteric and non-enteric locations

*Yersinia species*:

- Yesiniae stain bipolar on primary isolation

- Yersiniae are intracellular organisms localizing in macrophages

o *Y. pestis*:

- It is pleomorphic

- It produces little or no turbidity and small deposit in broth culture

- Haemin required for aerobic growth on nutrient agar

- Two forms of colony: smooth and rough

- Causes plaque: bubonic plaque, (septicemic, pneumonic sylvalstic forms).

Characterized by lymohadenitis

- Virulence factor F1 or fraction I (capsular/envelope heat-labile protein), V

(protein), W (lipoprotein), F (factor antigens)

- Probably produces toxin

- Virulence strains kill mice or guinea pigs following intraperitoneal or

subcutaneous injection with as low as 10 viable organisms

- Transmission: Wild rat (through flea) to town rat (through flea) to

humans

Diagnosis

- blood sample, materials from lymph nodes

- grow on blood agar and selective media

- Fluorescent antibody test on cerebrospinal fluid and in aspirates

Note:

- Colonies of non-lactose fermenting bacteria are alkaline due to utilization of

peptone in medium. They are pale

- Colonies of lactose fermenters are pintk due to acid production from lactose

- Somatic (O), flagellar (H), and capsular (K) antigens are used for serological

identification and classification of the enterobacteriaceae

**Differentiation and Identification of the Enterobacteriaceae**

*E. coli Salmonella*

serotype

*Yersinia*

*species*

*Proteus*

*species*

*Enterobacter*

serotype

*Klebsiella*

*pneumonia*

Clinical importance Major

pathogens

Major

pathogen

Major

pathogen

Opportunistic

pathogen

Opportunistic

pathogen

Opportunistic

pathogen

Cultural characteristics Some

strains

haemolytic

- - Swarming

growth

Mucoid Mucoid

Motility at 300C Motile Motile Motile Motile Motile Non-motile

Lactose fermentation + - - - + +

IMViC test

Indole production + - V ± - -

Methyl red test + + + + - -

Voges ProsKauer test - - - V + +

Citrate utilization test - + - V + +

H2S production in TSI

agar

- + - + - -

Lysine decarboxylate + + - - + +

Urease production - - + + - +

*Yersinia pseudotuberculosis*

§ Causes infection in many animals including guinea pigs, mice, rats, rabbits, chicken,

turkey, pigeons, and canaries

§ Sporadic cases reported in horses, cattle, sheep, goats, pigs and cats

§ Produced in necrotic nodules in ileum and caecum as well caseous necrosis of

mesenteric lymph nodes and omentum

§ Grows on blood agar, MacConkey and Salmonella-shigella agar at 370C and at room

temperature (220C – 280C)

§ Samples of isolation of organism: liver, spleen, heart blood

*Yersinia enterocolitica*

§ Grows on blood agar, Salmonella-shigella agar, desoxycholate citrate agar (DCA) and

MacConkey agar

§ May require enrichment in phosphate buffered solution (pH 7.6) or peptone broth at

40C for 3weeks

§ Must be differentiated from *Pasteurella*

*Note the following characteristic of Pasteurella: MR negative, Oxidase positive, no growth on*

*MacConkey except Manhemia haemolytica*

§ *Yersinia enterocolitica* grows at 40C unlike other enteric bacteria

§ Pig is a major reservoir

§ Isolation requires enrichment them subculture on agar then do identification tests.

*Proteus*

§ *P. vulgaris*

§ *P. mirabilis*

§ Pathogenic role doubtful

§ May cause diarrhoea in young animals

§ Otitis media in dogs

§ Often causes infection only when found outside the intestinal tract

§ Associated with chronic urinary tract infections

Diagnosis

· Produces characteristic smell and swarms on solid media

*Klebsiella*

*K. pneumoniae*

§ Pneumoniae in humans

§ Klebsiella and Enterobacter cause neonatal meningitis in children

§ Opportunistic infections in animals

§ Pneumonia in fowls, metritis in mare and sow

§ Mastitis (chronic) in cow

§ Complicate air-sac infection and pullorum disease in poultry

§ Other species: *K. ozaenae, K. rhinoscleromatis*

*Providencia*

*P. stuartii, P. rettgeri, P. alcalifaciens*

§ Involved in urinary tract infection, sepsis, pneumonia and wound infections

§ Hospital infection

*Morganella*

*M. morganii*

§ Hospital infection

§ Implicated in summer diarrhoea in children

***Biochemical differentiation of Proteus species***

*Proteus*

*vulgaris*

*Proteus*

*mirabilis*

*Providencia*

*rettgeri*

*Morganells*

*morgani*

Maltose

fermentation

+ - - -

Mannitol

fermentation

- - Delayed -

Indole

production

+ - + +

Gelatin

liquefaction

+ + - -

H2S production + + - +

Citrate

utilizatio

- - + -

Urease

production

+ + - +

*Salmonella*

Selective media:

§ Desoxycholate citrate agar: slightly opaque often with central black spot

§ Brilliant-green agar: *S. typhi, S. gallirum, S. pullorum, S. cholerae-suis and S. typhi-suis* do

not grow on the agar. Colonies are pale-pink usually surrounded by a pink zone.

Colonies have a translucent dew-drop appearance

§ Wilso and Blair agar: colonies are black

§ Salmonella-shigella agar: colonies are pale or colourless

§ Hektoen enteric agar: blue-green with black centre

§ Motile except *S.galinarium* and *S. pullorum*

§ Enrichment media:

o Selenite F. broth

o Tetrathionate broth

o Rappaport broth

**Reactions of Members of Enterobacteriaceae in Triple Sugar Iron (TSI) agar**

pH change H2S production

Species Slant Butt

*Salmonella* serotype Red (alkaline) Yellow (acid) +

*Proteus mirabilis* Red Yellow +

*P. vulgaris* Yellow Yellow +

*E. coli* Yellow Yellow -

*Yersinia enterocolitica* Yellow Yellow -

*Y. pseudotuberculosis* Red Yellow -

*Y. pestis* Red Yellow -

*Enterobacter aerogenes* Yellow Yellow -

*Klebsiella pneumonia* Yellow Yellow -

*Shigella species* Yellow Red -

*Shigella*

§ Non-motile

§ Non-sporing

§ Non-capsulated

§ Oxidase-negative, catalase-positive

§ *Shigella dysenteriae* type I is catalase negative

o Species

o *Sh. dysenteriae* (Tropics):dysentery in human and monkey (shigellosis, colitis)

o *Sh. flevneri* (Tropics): dysentery in human and monkey (shigellosis, colitis)

o *Sh. boydii* (Tropics): dysentery in human and monkey (shigellosis, colitis)

o *Sh. sonnei (*temperate): dysentery in human and monkey (shigellosis, colitis)

Diagnosis

§ Sample: fresh stool

§ Small colonies on DCA and MacConkey agar

§ *Shgella dysenteriae* type I does not grow on DCA

§ No growth on Wilson and Blair medium

§ Grow on S-S agar and Hektoen enteric agar producing pale and green colonies

respectively

§ May be inhibited to a certain extent by selenite F broth

Biochemical reactions:

Glucose fermentation Positive (acid only)

Lactose fermentation Negative

Sucrose fermentation Negative

Mannitol fermentation Variable

Indole production Variable

MR reaction Positive

Voges-Prostkauer Negative

Citrate utilization Negative

H2S production Negative

Urease production Negative

Motility Negative

**Biochemical differentiation of *Shigellae***

Test *Sh. dysenteriae Sh. Flexneri Sh. boydii Sh. Sonnei*

Glucose Acid (A) A/A and G (gas) Acid Acid

lactose - - - Late fermenter

Mannose - Acid Acid Acid

Sucrose - - - -

Dulcitol - -/A - -

Xylose - - - -

ONPG test -/+ - - +

Indole Variable Strain variation Variable -

ONPG: Orthonitrphenol (-b-D-galactopyranoside)

*Escherichia coli*

*E. coli* diseases (enteric and exraintestinal)

§ Enteric colibacillosis

§ Colisepticaemia

§ Oedema disease in pigs

§ Post-weaning diarrheoa in pigs

§ Coliform mastitis

§ Urinogenital tract infection

Other diagnostic procedure

§ Serology/serotyping

§ PCR

§ Toxin detection

o Cytotoxicity

o Loop ligation test

o Sereny test (invasiveness)

§ Animal inoculation

*Salmonella* diseases

§ Septicaemic salmonellosis

§ Enteric salmonellosis

§ Fowl typhoids

§ Pullorum (bacillary white diarrhoea)

§ Ipuman infection

§ Abortion in cattle

Diagnosis

Sample from Suspected Animals

§ Tissue

§ Faeces:

o Inoculation into enrichment broth e.g. selenite F, rappapoort, Tetrathionate

(370C for 48 hours aerobically)

o Subculture at 24 and 48 hours onto MacConkey agar, brilliant green and xyloselysine-

deoxycholate

o Direct inoculation: MacConkey agar, brilliant green and xylose-lysinedeoxycholate

(370C for 24 hours aerobically)

- Suspicious colonies

- Inoculation of TSI agar and lysine decarboxylase broth

- Typical salmonella reactions

- Serological confirmation with polyvalent antisera

- Definitive serotyping into specific ‘O’ and ‘H’ antisera

- Biotyping or Phagetyping

*E. coli*

Commensal

Opportunistic

Enteric

Extraintestinal:

Urogenital (uropathogenic), cystitis

Avian

Mastitis

Pyometria (dogs and cats)

Septiceamic (endotoxin): cystitis mainly in bitches

Virulence factors of *E. coli*

CNF: Cytotoxic Necrotizing Factor

ETEC: Adhensins, K88 pigs, and K99 calves and lambs for colonization, heat stable enterotoxins

(ST), and heat labile enterotoxins (LT)

Diarrhoea in neonatal piglets, calves, lambs, post-weaning diarrhoea in pigs

EPEC: A/E factor, intimin, haemolysin, destruction of microvilli, shedding of enterocytes,

stunting of villi malabsorption, diarrhoea in piglets, lambs, pigs

VTEC: VT1, VT2, VT2e, damage to vasculature in intestine and other locations, oedema disease

in pigs, haemolytic colitis in calves, post-weaning diarrhoea in pigs,haemolytic uraemic

syndrome (HUS) and haemorrhagic colitis (HC) in humans

Necrotoxigenic: CNF1 amd CNF2 (Cytotoxic Necrotizing Factor). Damage to entrocytes and

blood vessels, HC in cattle, enteritis in piglets and calves, diarrhoea in rabbits, dysentery

in horses

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***BACILLUS SPECIES***

**(Dr. O. E. Ojo)**

· *Bacillus species* are large Gram-positive rods about 10.0 μm in length

· They produce endospores

· They appear singly, in pairs or in long chains

· They are aerobic or facultative anaerobes

· *Bacillus species* are catalase-positive and oxidase negative

· They are motile except *B. anthracis* and *B. mycoides*

· Most species are saprophytes but often contaminate clinical specimen and laboratory

media

· *B. species* can tolerate extreme adverse conditions such as high temperature and

desiccation because of their endospores

· *B. anthracis* produces capsule

Diseases

*B. anthracis*

Cattle and sheep: total peracute or acute septicaemic anthrax

Pigs: subacute anthrax with oedematous swelling in pharyngeal region, intestinal form

with higher mortality is less common

Horses: subacute anthrax with localised oedema, septicaemia with enteritis and colic

Human: skin, pulmonary and intestinal forms of anthrax

*B. cereus*

Cattle: mastitis

Human: food poisoning, eye infection

*B. licheniformis*

Cattle, sheep: sporadic abortion

Diagnosis:

§ Ability to produce catalyse and grow aerobically distinguish *B. species* from *Clostridium*

*spp*

§ *Bacillus species* are differentiated based on colonial characteristics, biochemical test and

genetic composition

§ Colonial characteristics:

- *B. anthrax* colonies are up to 5mm in diameter, flat, dry, greyish and with a

‘ground-glass’ appearance after 48 hours incubation. At low magnification,

curled outgrowth from the edge of the colony impart a characteristic ‘medusa

head’ appearance. Isolates are rarely haemolytic. When present, haemolysis is

weak

- *B. cereus:* colonies are similar to those of *B. anthracis* but slightly larger with a

greenish tinge. The majority of strains produce a wide zone of complete

haemolysis around the colonies

- *B. licheniformis*: colonies are dull, rough, wrinkled and strongly adherent to the

agar. Characteristic hair-like outgrowth are produced from streaks of the

organisms on agar media

**Distinguishing features of *B. anthracis* and *B. cereus***

Feature *B. anthracis B. cereus*

Motility Non-motile Motile

Appearance on sheep blood

agar

Non-haemolytic Haemolytic

Susceptibility to penicillin Susceptible Resistant

Lecithinase activity on egg

yolk agar

Weak and slow Strong and rapid

Effect of gamma phage Lysis Lysis rare

Pathogenicity for animals Death in 24 -48 hours No effect

Diagnosis of Anthrax:

- History of sudden death

- Pathology: carcass is bloated, putrefies rapidly and no rigor mortis

- Collect peripheral blood and make smear

- Stains smear with polychrome methylene blue

- *B. anthracis* appears as blue-staining rods with square-end surrounded by pink capsules

- Culture on blood and MacConkey agar

- Incubate aerobically at 370C for 24 to 48 hours

- Study colony morphology

- No growth on MacConkey agar

- Study microscopic appearance

- Do biochemical test

- Conduct pathogenicity test

- Do Ascoli test:

o A thermoprecipitation test

o Detects *B. anthracis* antigen

o A ring precipitation or gel diffusion test with *B. anthrcis* antiserum

- Other tests: agar gel immunodiffusion, CFT, ELISA, IFT and PCR

***CLOSTRIDIUM SPECIES***

**(Dr. O. E. Ojo)**

§ Large gram-positive rods

§ Produces endospores. *C. perfringens* rarely produce spores

§ Anaerobic

§ Catalase and oxidase negative

§ Motile except *C. perfringens*

§ Require enriched media for growth

§ Size, shape and location of endospores used for species differentiation

§ They are toxigenic. They are non-capsulated except *C. perfrigens*

*C. perfringens:* large wide rods. Rarely form endospores in-vitro

*C. tetani:* thin rods. Characteristically produce terminal endospores (drumstick appearance)

*C. chauvoei:* medium-size rods. Produce lemon shaped endospores

Diseases:

Categorised into three major groups based on toxin activity

- Neurotoxic clostridium: *C. tetani, C. botulinum*

- Histotoxic clostridia: localized lesion in liver and muscle*: C. chauvoei, C. septicum, C.*

*novyi type A, C. perfrigens type A, C. sordelli, C. haemolyticum, C. novyi type B*

- Enterotoxigenic clostridia: *C. perfrigens type A-E*

- Less important groups

o *C. piliforme:* spore-forming, filamentous gram-negative intracellular pathogens

(atypical member of the clostridia). Has not been cultured artificially on media

- Grows only in tissue culture and fertile egg

- Causes Tyzzer’s disease (a severe disease causing hepatic necrosis) in

laboratory animals foals rarely in calves, dogs and cats

o *C. difficile:* chronic diarrhoea in dogs and haemorrhagic anterocolitis in newborn

foals

o *C. spiroforme*: enteritis in rabbits

o *C*. *colinum:* enteritis in birds

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· Neurotoxic clostridia:

i. *C. tetani:* locked jaw/tetanus

- Infect wounds

- Terminal endospores

- Toxin produced in wounds

- Toxin production in regulated by genes encoded

in plasmids

- One antigenic type of toxin (tetanus plamin)

- Toxin causes synaptic spasms

- Prevented by toxoid

- Treated by antitoxin

ii. *C. botulinum:* botulism

- subterminal endospores

- preformed toxin in canned foods, carcasses, decaying vegetation etc.

- toxin production regulated by genomes

- eight antigenically distinct toxins (A-G)

- toxins inhibit neuromuscular transmission

- produces flaccid paralysis

- most potent biological toxin known

- prevented by toxoids, treated by antitoxin

· histotoxic clostridia: they produce toxins (a,b,g,d toxins)

o *C. chauvoei* (a,b,g,d)*:* blackleg in cattle and sheep.

o *C. septicum* (a,b,g,d): malignant oedema in cattle, pig and sheep. Braxy

(abomastitis) in sheep and occasionally calves

o *C*. *noryi type A (*a): big head in young rams, wound infection

o *C*. *sordell* (a,b)*i*: myositis is cattle, sheep, horses, abomastitis in lambs

o *C*. novyi type B (a,b), infectious necrotic hepatitis (black disease) in sheep and

occasionally in cattle

o *C*. *haemolyticum* (b): bacillary haemoglobinuria in cattle and occasionally in

sheep

o *C*. *perfigens type A* (a): necrotic enteritis in chicken, necrotizing enterocolitis in

pigs, gas gangrene.

· Enterotoxaemia clostridia: toxins (a,b,e,i) *C. perfrigens* type A – E

o Type A (a toxin): necrotic enteritis in chicken, necrotizing enterocolitis in pigs,

canine haemorrhagic gastroenteritis

o Type B (a,b (major),e,): lamb dysentery; haemorrhagic enteritis in calves and

foals

o Type C (a, b (major)): struck in adult sheep, necrotic enteritis in chickens,

haemorrhagic enteritis in neonatal piglets, sudden death in goats and feedlot

cattle

o Type D (a, e (major)): pulpy kidney in sheep, enterotoxaemia in calves, adult

goats and kids

o Type E (a and i(major)): haemorrhagic infection in calves, enteritis in rabbits

Diagnosis

· Clostridia are fastidious and anaerobic

· Samples are collected from live or recently dead animals

· Tissues or exudates for culture should be placed in anaerobic transport media

· Samples should be cultured promptly

· Ideal medium is blood agar enriched with yeast extract, vitamin K and haemin

· Robertson cooked medium is used for anaeronic enrichment

· Media should be freshly prepared or pre-reduced to ensure absence of oxygen

· Test for toxin production in laboratory animals. Toxin neutralisation by antitoxin.

· Cultured plates are incubated in anaerobic jar containing hydrogen supplemented with

5 to 10% carbon dioxide

· Identification and differentiation among *C. species* are based on colonial morphology,

biochemical tests, toxin neutralization methods and gas-liquid chromatography for

profiling organic acids

· Fluorescent antibody techniques, immunoassay such as ELISA and molecular technique

like PCR are of diagnostic importance

Special Features

*C. tetani* produces filmy growth on blood agar with narrow zone of haemolysis. Prevent

swarming by using 4% agar (stiff) or sodium azide

*C. perfrigens*: produces double zone of haemolysis on blood agar (narrow zone of

incomplete haemolysis and wide zone of partial haemolysis). Produces marked opalescence

on egg yolk medium because of the lecithinase action of alpha toxin (Nagler reaction).

CAMP test positive with *Streptococcus agalactie*

*C. novyi type A*: give a characteristic ‘pearly layer’ on egg yolk medium due to the lipase it

produces. It is also Nagler’s reation

Principle of Nagler’s reaction: lecithinase action on lecithin in egg yolk leading to the opacity

due to insoluble fatty acid accumulation

**NEISSERIAE**

**(Dr. M. Agbaje)**

· Gram-negative cocci, kidney shaped and usually occurring in pairs (diplococcus).

· Normal inhabitants of the human and animal respiratory tracts, and are extracellular.

**Media and Growth**

· The organisms prefer well enriched media while the pathogenic ones are normally

cultured on selective media. They prefer solid medium to liquid medium.

· *N. gonorrhoeae* and *N. meningitides* grow best on media containing complex organic

substances such as blood or animal proteins, atmosphere of 10 percent CO2..

· Although they grow well on chocolate agar, the popular selective medium used in the

laboratories is the Thayer-Martins medium.

· Thayer-Martins medium contains sodium colistimethate and vancomycin to inhibit

bacterial contaminants while Nystatin is also added to prevent the growth of fungal

contaminants.

**Species**

· Aside the pathogenic species earlier mentioned, other non-pathogenic species include *N.*

*flavescens, N. flava, N. sicca, N. pharyngis, N canis, N. ovis,* and *N. lactamica.*

· The nonpathogenic species can grow at the low temperature of 22oC while the

pathogenic species have optimum temperature of 35oC-36oC, minimum and maximum

temperatures of 30oC and 38oC respectively.

**Biochemical reactions**

· Pathogenic species are scarcely saccharolytic, fermenting very limited carbohydrates with

acid production but no gas.

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· Biochemical tests on serum slope sugars, with 5 percent (human, rabbit or guinea pig

serum) in addition to 1 percent carbohydrate, are preferred. Horse serum is usually not

added because of it tendency to contain maltase, which splits maltose, resulting in false

positive result.

· Non-pathogenic species are biochemically more active. Such *Neisseria* species are

oxidase and catalase positive, indole and methyl red (MR) negative.

**CAMPYLOBACTER AND HELICOBACTER**

**(Dr. M. Agbaje)**

**Campylobacter:**

· First isolated by Mcfadyean and Stockman in 1913 but were classified as vibrios because

of their curved shape and rapid motility.

· Because of their association with infectious infertility and abortion in cattle and sheep,

they were named *Vibrio fetus.*

· In 1963, Sebald and Veron proved that they were a different genus and hence, the name

Campylobacter (Greek; meaning curved rod).

· Nonsaccharolytic and microaerophilic and exhibit unique cork screw motility. In direct

smears from clinical materials, they appear S-shaped or may have a “seagull” appearance.

· In cultures, they are longer and more variable. They may form spherical or coccoid

bodies.

· There is variability in colony forms. On blood agar they are shiny, pale, grey, semitranslucent,

flattened and nonhaemolytic.

· Biochemically they are relatively inactive. They do not ferment any carbohydrates but

they are oxidase positive while some produce catalase.

**Media:**

· Media for isolation include blood agar, brucella medium and brain heart infusion broth

and agar, the later is supplemented with 5 per cent blood.

· Basic selective media which suppress contaminants include Skirrow’s or Butzler’s

medium. They are usually supplemented with antibacterial agents, such as, vancomycin

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(10μg/ml), polymycin B (2.5 i.u/ml), trimethoprim (5 μg/ml), novobiocin (5μg/ml) and

cephalothin (15 μg/ml). These media are commercially available.

· Because of their microaerophilic nature, they require atmospheric condition of 10 per

cent CO2, 5 percent O2 and 85 per cent nitrogen. Incubation is at 37o – 42oC and

incubated cultures are examined daily up to 7 days.

· Thermophilic campylobacter sp. such as *C*. *jejuni* and C. *coli* grow at 37oC and 42oC and

not at 25oC, whereas the non-thermophilic grow at 37oC and 25oC but fail to grow at

42oC.

**Resistance:**

· The organism is sensitive to acid pH. It is rapidly killed by HCl at pH 2.3, hence the

gastric acid is an effective barrier against infection.

· It can survive for 2-5 weeks in bovine milk or water kept at 4oC.

**Virulence Factors:** Factors associated with virulence and infections include:

(a) **Motility and chemotaxis:** associated with the flagella. Campylobacter move in response

to chemotactic stimuli which direct motility and enhances the effectiveness of mucosal

colonization.

(b) **Adhesion:** Fimbriae have not been demonstrated in *C. jejuni* and *C. coli.*

(c) **Enterotoxin:** heat labile enterotoxin has been demonstrated in *C. jejuni*. There is also

some evidence that C. *jejuni* and *C. coli* secrete a cytotoxin which is toxic for mammalian

cells, for example, bovine kidney and HeLa cells.

(d) **Invasiveness:** The organism penetrates intestinal mucosa and proliferates in the lamina

propria and mesenteric lymph nodes. This results in low grade damage of the affected

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tissues. The virulence factors may not be manifested by all strains of *C. jejuni* and this

may explain variation in symptoms of Campylobacter enteritis.

**Laboratory diagnosis:**

· Isolation and identification of *C. jejuni* and *C. coli* seem more common. *C. jejuni* is the

more important pathogen and frequently associated with *Campylobacter* enteritis.

· Direct field microscopy of stool specimens may be carried out for presumptive

identification. Care must be taken not to misdiagnose the organism for *V.cholerae.*

*Campylobacter spp*. exhibits corkscrew motility while *V.cholrtae* exhibits darting

motility. The later is coma-shaped while the former is spiral or s-shaped.

· Immunofluorescent technique can be used to detect *C. jejuni* in various specimens. Other

tests which are promising are ELISA and bacteriophage typing using c-phages specific

for *C. jejuni.*

***Campylobacter foetus***

**Virulence factors:**

· They are associated with the cell wall LPS.

Infection is acquired during coitus or by artificial insemination procedures. Bull to bull

transmission may take place during mounting when many bulls are enclosed together. Bovine

venereal campylobacteriosis is a chronic infection of the female genital tract, characterized by

mild endometritis and transient infertility. The infection is confined primarily to the surfaces of

the mucous membrane. Soon after infection the organism can be found in the vagina, the cervix,

uterus and oviduct of susceptible cows. The infection is transient in the uterus but becomes

established in the cervix and vagina. Abortion may be due to bacterial inflammatory placentitis

or allergic response to endotoxin of the organism. The endotoxin has been shown to be

abortifacient in pregnant cows.

The earliest antibodies to appear are IgM followed by IgG and IgA. IgG predominates in the

uterine secretion of convalescing animals, and IgA is found in the cervico-vaginal secretions.

IgA helps to immobilize the organism thereby limiting its entry to the uterus while IgG plays a

role in opsonization during phacogytosis.

The organism persists in the vagina for up to 2 years. The persistence may be associated with

antigenic variation resulting from phase conversion. Asymptomatic vaginal carrier may arise in

animals which regained their fertility but continue to harbour the organism in the vagina or in

convalescent animals which have become susceptible to reinfection due to decline or loss of

immunity.

**Laboratory diagnosis**

(a) Bacteriological diagnosis in the bull is carried out by culture of the preputial materials.

From the cow, materials are obtained from the vagina and cervix by aspiration. In the

case of abortion, specimens are obtained from the placenta, cotyledon and the aborted

foetus including the stomach content. Cultures are made on selective media. Suspected

colonies may be identified by the fluorescent antibody technique or biochemically.

(b) Serological test

(i) Vaginal mucus agglutination (VMA) is useful as a herd test but of little value in

identifying individual infected animals.

(ii) Indirect haemaglutination (IHA) using tanned sheep red blood cells, sensitized with

phenol-extracted antigen. False positive results may occur in about 1 percent of

mucus samples from non-infected cattle.

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(iii) Immunoflourescent technique. It is a useful rapid screening method as an adjunct to

cultural examination. It cannot distinguish the two subspecies though it is specific for

*C. fetus.*

*Campylobacter fetus ss fetus.* The organism has been isolated from gall bladder, intestinal tract

and occasionally from the genital tract of healthy animals. The important infections in animals

are abortion and enteritis.

**VIBRIO**

**(Dr. M. Agbaje)**

**Vibrio:**

· Organisms in this genus are Gram-negative, non-sporing, curved rods, with single polar

flagella.

· They are oxidase and catalase positive. Generally they ferment glucose with acid

production only.

· They are aerobic and facultatively anaerobic,

· some are proteolytic and liquefy gelatine.

The type species of this genus is *V. cholerae* and was first isolated by Robert Koch in Egypt in

1883.

***Vibrio cholerae*:**

· Gram-negative curved rod with single polar flagella.

· It is aerobic and facultative anaerobic.

· It ferments glucose with acid production only

· Grows well in alkaline pH with little or no growth in acidic pH. The optimum

temperature of growth is 37oC with a range of 15oC to 42oC.

**Growth Media:**

*V. cholerae* can be cultured on ordinary media such as nutrient agar, blood agar or nutrient broth.

Selective and enrichment media may also be used when contamination is suspected. Alkaline

peptone water (pH 8.4) is a good enrichment medium for the growth of this organism. Examples

of selective media include, thiosulphate-citrate-bile slat sucrose (TCBS) agar and taurocholate

tellurite gelatine agar (TTGA). The two media are popularly used in cholera laboratories.

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**Biotypes:**

Two biotypes have been identified. They are EI Tor and classic biotypes.

**Antigenic groups:**

With reference to the use of the O antigen, three serotypes are known.

(a) Inaba with antigenic components A and C,

(b) Ogawa with components A and B and

(C) Hikojima with components A, B and C.

The first two are more common. Serotype Ogawa can change to serotype Inaba but the reverse

does not occur.

**Other Vibrios**

*V.parahaemolyticus.*

· Halophilic marine *vibrio*, which was first recognize as a cause of food poisoning in Japan

in the early 1950s.

· Shares some characteristics with *V.cholerae* but tolerates a high concentration of salt in

contrast to *V. cholerae.*

· Causes two types of enteritis in humans

(1) Watery diarrhea with abdominal cramp, nausea, vomiting and fever, and

(2) Dysentary-like infection with a shorter incubation period, (2.5 hours or more) than

the former (15 hours). In both cases the illness is usually self-limiting.

· Enteritis caused by this organism is mainly transmitted by food, particularly seafood.

Infection is most common during the warmer months. This may reflect both enhanced

opportunity for *V.parahaemolyticus* to multiply in unrefrigerated foods and increased

prevalence of the organisms in the environment during the warmer months.

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· Generation time of this organism is 9 minutes under normal conditions in food and can

quickly reach the rather large infectious dose (ID50) of 105 – 107. Growth is inhibited at

temperatures below 15oC and above 65oC.

Other vibrios which have been associated with human infections include: *V. vulnificus, V.*

*fluvialis* and *V .mimicus, V.anguillarum* and *V. ordalii.*

***V.metchnikovii:*** It was oringinally isolated from the blood and gut contents of chickens dying of

fowl cholera-like disease. It grows rapidly in peptone water and grows well on DCA. It is aerobic

and facultative anaerobic. Temperature range of growth is 30o-40oC.

***BRUCELLA***

**(Dr. M. Agbaje)**

· Organisms in this genus are Gram-negative cocco-bacilli, which are non-sporing and

non-motile.

· They are strict aerobes but some strains require 5-10 percent CO2 for growth.

· Utilize various carbohydrates with negligible acid production.

· *Brucella* species are obligate intracellular organisms.

**Media**

· Include liver infusion agar, blood agar, chocolate agar, glycerol glucose agar and

serum dextrose agar. The latter which is more popular in the isolation of this

organism consists of 5 percent serum and 1 percent dextrose.

· Antibacterial and antifungal agents such as bacitracin, actidione, fungizone,

polymyxin B, cycloheximide and vancomycin may be added to a medium to

suppress contaminant for primary culture.

· CO2 requirement.-

*B. abortus* and *B. ovis* require optimum 10 percent CO2 for growth.

*B. melitensis* and B. *suis* would rather prefer addition of CO2 for growth.

· Maximum temperature of growth is 37oC and growth occurs in 2-4 days with

small and transparent to translucent colonies.

· They are catalase positive and some species reduce nitrates to nitrites.

· Both *B. abortus* and *B. suis* will agglutinate monospecific antiserum to *B. abortus*

while B. *melitensis* agglutinates only its monospecific homologous antiserum.

**Antigens:**

· Two antigens designated A and M are possed by smooth strains of *B. abortus, B.*

*suis* and *B. melitensis*.

· Cross-reactions occur with other Gram-negative bacteria such as *E. coli O116* and O157,

*F. tularensis, Salmonella sp., Y. enterocolita* O9 and *V. cholerae* O1*.*

**Bacteriophage typing:**

· The bacteriophage, Tbilisi (Tb) is specific for smooth strains of *B. abortus* in routine test

dilution (RTD). At 10,000 RTD, the phage will lyse *B. suis* but not *B. melitensis .* The

phage is stable at 40C for a long period.

**Virulence factors:**

· Virulence of *Brucella* species is derived from their intracellular niche within the

reticuloendothelial systems.

· Cell Wall - the cell wall lipopolysaccharide (LPS) of brucellae aid in its survival within

macrophage.

· *Erythrito*l- a four-carbon alcohol, is one of several “allantoic fluid factors” found in the

gravid uterus, and appears responsible for the preferential localization of brucellae to the

reproductive tract of the pregnant animals. “Allantoic fluid factors” stimulate the growth

of brucellae.

· Outer Membrane Proteins- Porin proteins in the outer membrane are thought to stimulate

delayed-type hypersensitivity and account for the varying susceptibility to dyes observed

for the different species.

· Miscellaneous Productsi.

Production of adenine and guanine monophosphate by Brucella inhibit

phagolysome fusion and activation of the myeloperoxidase-halide system. *Brucella*

are able to inhibit apoptosis in infected macrophages, thereby preventing host cell

elimination.

ii. Soluble protein products inhibit tumour necrosis factor alpha (TNF-α) production. iii.

The Vir (for virulence) operon encodes a Type IV secretion system, which appear

to be involved with intramacrophage survival.

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**Laboratory diagnosis:**

Diagnosis in animals is based on microscopy, culture and serology.

(a) **Microscopy:** The foetal stomach content of infected animals is a good source of *Brucella*

and can be examined for *B. abortus* by staining preferably by the modified Ziehl-Neelsen

method. The same method is applicable to *B. melitensis* or any other *Brucella* species.

*Brucella* organisms stain pink and are coccobacillary. They are usually present in large

numbers.

In the absence of the foetus, smears are made from other foetal-maternal tissues interface

such as cotyledon or placental materials. However, care must be taken to exclude other

that may be bacteria present. *Brucella* organisms normally stain readily by the modified

acid-fast staining technique.

(b) **Culture:** Samples like content of the foetal stomach, the placental materials or ground

cotyledon is streaked on serum dextrose agar plates or on any other suitable media, with

or without antibacterial and antifungal agents. For primary cultures, antimicrobials like

antibacterial and antifungal agents need to be added to suppress contaminants. The plates

are incubated in 5-10 percent CO2-enriched atmosphere at 37oC for 4 days. If growth is

absent after 4 days, the plates are incubated further for another 4 days before plates are

discarded. If there is growth, slide agglutination with monospecific serum is then carried

out for the identification of the *Brucella* species.

**(c) Serology**

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(1) Rose Bengal Plate Test (RBPT) is very useful screening tool in the field. *Brucella*

organisms are stained with rose Bengal stain at pH 3.5. The stained antigen preparations

may be obtained commercially or from Reference Laboratories.

A drop of the serum from sampled blood is mixed with a drop of the stained antigen

preparation. The suspension is rocked for 2-3 minutes to produce homogenous mixture.

Occurrence of agglutination within 2-4 minutes certifies the sample positive. On the other

hand, serum sample may be diluted up to 1:8 with phenol saline and spot agglutination

carried out with each dilution. This test is specific, sensitive and useful in screening and

survey work.

(2) Serum agglutination test (SAT): This test reliable and specific in bovine, caprine and

ovine brucellosis. Its reliability in swine brucellosis diagnosis is in doubt. The RBPT is

more reliable for swine brucellosis test. The SAT is sufficiently standardized regardless

of the modification in the test in some countries, that the results can be reported in

international units (i.u.). Standard serum can be obtained from the International

Reference Centre for Brucellosis, Weybridge, England.

(2) Milk ring test (MRT). It is generally carried out on individual animals or on bulk milk

sample. For MRT, 1ml of the milk in a 1ml – tube is mixed with a drop of stained

suspension of the organism and shaken. The mixture is incubated in a water bath at 37oC

for 30 minutes or 1 hour. A positive reaction occurs when a blue ring is formed at the top

due to antigen – antibody reaction. The cream is white or colourless when the test is

negative. False positive results may occur in mastitis cases, particularly in goats. If the

milk contains little cream, sterile cream is added to the milk to aid diagnosis.

**(3)** Other tests include Whey agglutination test, Enzyme linked inmmuosorbent assay

(ELISA), etc.

**MORAXELLA AND ACINETOBACTER**

**(Dr. M. Agbaje)**

***Moraxella:*** (formerly referred to as Diplobacillus Morax-Axenfeld)

· Organisms of the genus are bacilli or coccobacilli, usually in pairs.

· They can be pleomorphic and grow on simple media containing blood.

· They do not ferment carbohydrates.

· They are oxidase and catalase positive. Different species may liquefy gelatin and

coagulate serum.

Important species are:

**Species Host Disease**

*M. lacunata* Human Conjunctivitis

*M. liquefaciens* Human Corneal ulceration

*M. bovis* Cattle, goat Kerato conjunctivitis

(“pink eye”)

*M. anatipestifer*

*(*formerly *Pasteurella)*

Duck Septicaemia and serositis

*M. catarrhalis* Human,

Cattle, sheep & dog

Bronchitis,

Pneumonia

Commensal

*M. osloensis* Human Commensal of genital tract

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***M. bovis:***

· It is the most important animal species affecting cattle and to a less extent goats. It is

opportunistic and was first isolated by Jones and Little in 1923 from cattle suffering

from keratitis and conjunctivitis.

· It is Gram-negative plump coccobacillus often in pairs (diplobacillus) and exhibits

Pleomorphism cultures.

· Fresh isolates from lesions are capsulated.

· It is an obligate parasite of the eye of cattle transmitted directly or via flies from carriers

to other animals.

· It is the cause of infectious bovine kerato conjunctivitis(IBK), “pink eye”, a highly

contagious and an important disease of beef cattle.

**Virulence factors:**

· Virulence is associated with the following; fimbriae or pili, haemolysin, fibrinolysin an

dermonecrotic factors.

**Laboratory diagnosis:**

· Eye swab is taken and cultured on blood agar aerobically at 37oC.

· Virulent *M. bovis* usually show β-haemolysis and may pit the agar.

***Acinetobacter:***

**(Dr. M. Agbaje)**

· They are Gram-negative diplococcobacilli

· Unlike *Moraxella* species, members of this genus are oxidase negative and grow well at

22oC.

· They grow on MacConkey agar and are resistant to penicillin.

· Like *Moraxella* species, they are not fermentative.

· They are found in soil, water and sewage and as part of the normal flora of animals and

humans.

**Important species**

· *A. calcoeceticus* and *A. iwoffii.*

Both may be opportunistic pathogens of humans and animals. Their pathogenic roles are not well

known.

***HAEMOPHILUS***

**(Dr. M. Agbaje)**

· Members of this genus require for propagation one or both of two growth factors:

porphyrins (heme) or nicotinamide adenine dinucleotide (NAD, NADP) originally

called X (heat-stable), and factor V (heat-labile), respectively.

· *Haemophilus paragallinarum* (the cause of infectious coryza in chickens) is one of the

most important type species of veterinary importance.

· *H. parasuis*, (the cause of a septicemic disease called Glasser’s disease or

polyserositis, and seconday respiratory disease of swine), and

· *Histophilus somni* (the cause of septicemic, respiratory, and genital tract disease in

cattle and sheep). *Histophilus somni* is the name now given to those micro-organisms

previously knwon as “*Haemophilus somnus*” “*Haemophilus agni*”, “*Histophilus ovis*.”

**Characteristics of the genus;**

· Gram-negative coccobacilli and facultative anaerobes.

· Typically oxidase-positive (differentiating them from members of the family

*Enterobacteriaceae*).

· Most are commensal parasites of animals.

**Descriptive Feature**

***Morphology and Staining***

· Though members of the genera *Haemopilus* and *Histophilus* are gram-negative

rods/coccobacilli, they can sometimes form long filaments.

· Some species (*H. paragallinarum* and *H. influenza* are encapsulated.

· The genus name *Haemophilus* is inferred from the fact that these organisms require

factors X and V in blood for growth. Species designated with the prefix ‘para’ require

only V factor. On blood agar, *Haemophilus* colonies cluster around a *Staphylococcus*

streak line in a phenomenon called *satellitism*.

**Virulence factors**

· Adhesins. allow the organisms to adhere to cells lining a particular niche, as well as to the

surface of so-called “target” cells prior to the initiation of disease (in some cases, niche

and target cells may be the same). This is common among members of this genus.

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· Capsules. This component found on the cell wall of some bacteria is used to interfer with

phagocytosis (antiphagocytic) by preventing deposition of membrane attack complexes

generated by the activation of the complement system. *Haemophilus influenzae* and *H.*

*paragallinarum* produce capsules.

· Call wall. Lipopolysaccharde (LPS) component of gram negative bacteria elicits

inflammatory response when they bind to lipopolysaccharide binding protein (LBP) (a

serum protein). The complex formed by LPS-LBP is transferred to the blood and

combines with the blood phase of CD14. The CD14-LPS-LBP complex binds to Toll-like

receptor proteins located on the surface of macrophage cells, thereby triggering the

release of pro-inflammatory cytokines.

· The cell well lipopolysacharide of *Histophilus somni* is termed lipooligosaccharide

(LOS). LOS under the control of the gene *lob* (for LOS biosynethesis), undergoes phase

variation resulting in the production of various epitope expression with resultant changes

in the carbohydrate portions of the *LOS*.

· Iron Acquisition iron is an absolute growth requirement in bacteria existence, hence,

bacteria must acquire this substance. *Haemophili* and *Histophilus* bind transferrin-iron

complexes through the use of iron-regulated outer membrane proteins expressed in these

bacteria when there is iron depletion in their environment (so-called transferring binding

proteins, or Tbps). Iron is then acquired by these bacteria from the transferring-iron

complexes bound to their surface.

**Growth Characteristics**

· Members of the genera *Haemophilus* and *Histophilus* are facultative anaerobes.

· Typically oxidase-positive, and attack carbohydrates fermentatively.

· Carbon dioxide enhances growth of some strains.

· Growth factors may be supplied as hemin (X factor) and NAD (V factor). A medium

naturally containing them is chocolate agar, a blood agar prepared by addition of blood

when the making regular blood agar). This procedure liberates NAD from cells and

inactivates enzymes destructive to NAD.

**Biochemical Activities**

· *Haemophilus* and *Histopilus* of animals are oxidase and nitrates-positive and ferment

carbohydrates.

· In non specialist laboratories, a presumptive identification of the fastidious *Haemophilus*

species is based on host species, clinical signs and lesions, colonial and microscopic

characteristics, X and V factor requirements, oxidase and catalase reactions and whether

or not CO2 enhances growth.

· *H. somnus* is variable in biochemical activities and the most reliable reactions are

oxidase-positive, catalase-negative with CO2 giving a considerable enhancement of

growth. Indole positive reaction is usually diagnostica.

**Variability**

· There are three serotypes; A-C in the so-called Page scheme, or I-III in the Kume

scheme) of *H. paragallinarum*

· There are at least sevenserotypes of *H. parasuis*.

**Transmission**

· Transmission of haemophili and *Histophilus* is by airborne or through close contact.

**Laboratory diagnosis**

Specimen

Specimen collection should be based on clinical disease and must be protected from

desiccation as these organisms are fragile.

Refrigeration at 4oC and use of transport media are of little relevance in the preservation

of these genera. Hence, deep freezing at -60oC is the most reliable.

Direct microscopy

Demonstration of Gram negative rods in smear is difficult. Fluorescent antibody

technique is advisable

Isolation

To culture successfully, X and V factors must be supplied for members of genus

*Haemophilus* except *H. somnus*.

X factor is present in 5% blood agar while V factor is available in red cells but

susceptible to NADases enzymes present in blood.

During the preparation of chocolate agar, V factor is released from the red cells, the

NADases are destroyed by heat, and the heat stable X factor remains unaffected. Also, a

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streak of *Staphylococcus aureus* made across blood agar plate will provide V factor. Vfactor

requiring haemophili grow as satellite colonies around the streak.

Successful culture of many *Haemophilus* species is enhanced in an atmosphere of 10 per

cent Co2. Hence, inoculated chocolate agar plates should be incubated under 10 per cent

Co2 at 35-37oC for 3-4 days, although some may grow in 24 hours.

**Identification**

Colonial morphology

Colonies may appear small and dewdrop-like after 24-48 hours of incubation and are not

consistently haemolytic. A few strains of *H. somnus* may show clearing around the

colonies especially on Columbia-base sheep blood agar. *H. somnus* colonies may also

appear yellowish in a loopful of growth in a confluent lawn.

**Microscopic appearance**

· Haemophili are small gram-negative rods that can be coccobacillary in form. More rarely

short filaments occur.

**Tests for X and V factor requirements**

· V factor:

The need for the V factor can be demonstrated by satellitism around V factorproducing

bacterium such as *Staphylococcus aureus*. The test is carried out on

tryptose agar which does not contain either the X or V factor.

· Disc Method for X and V factors:

Three different commercial discs impregnated with V factor, X factor and XV factors,

respectively, are placed on a streaked lawn of suspected bacterium inoculated on a

trptose agar plate. Colonies will cluster around the disc(s) supplying the required growth

factor(s). However, the results of this test can be invalidated:

a) If there is a carry-over of, particularly, the X factor from a previous richer medium.

b) If a contaminating colony is present on the plate, this may act as a feeder-organism.

c) If the test medium contains traces of X or V factors.

· Porphyrin test:

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This test is used for the determination of the requirement for the X factor. A loopful of

growth from a 24 hour culture is suspended in 0.5ml of a 2mM solution of delta-aminolevulinic

acid (ALA) hydrochloride and 0.8mM MgSO4 in 0.1 M phosphate buffer at pH 6.9. It is

incubated for at least 3-4 hours at 370C and exposed to a wood’s UV lamp in a dark room. A

red fluorescence glow indicates the presence of porphyrin and suggests that X factor is not

required. The test principle is based on the ability of X factor-independent strains to convert

ALA, a porphyrin precursor, to porphyrin (an intermediate in the haemin biosynthetic

pathway). Haemin- dependent strains lack the appropriate enzymes and cannot convert ALA to

porphyrin.

**Serology**

· Serological techniques such as slide and tube agglutination tests, agar gel precipitation,

latex agglutination and haemagglutination and haemagglutination-inhibition tests are

capable of detecting antibodies to *H. paragallinarum* in poultry after 1-2 weeks of

infection and in career birds.

· Although evidence abound of the presence of antibodies to *H. somnus* in cattle

populations, diagnostic test for clinical cases are scarce.

**BORDETELLA**

**(Dr. M. Agbaje)**

· The bordetellae are small, Gram-negative rods that tend to be coccobacilliary

· They are strict aerobes and do not attack carbohydrates.

· *B. avium* and *B. bronchiseptica* are motile by peritrichous flagella but *B. pertusis* and *B.*

*parapertusis* are non-motile. All are catalase-positive and oxidase-positive.

*B.bronchiseptica* and *B. avium* will grow on MacConkey agar.

**Natural Habitat**

· The bordetellae are primarily inhabitants of the upper respiratory tract of healthy and

diseased humans, animals and birds.

· *B. pertussis* and *B. parapertussis* are human pathogens causing whooping cough and mild

form of whooping cough, respectively.

· *B. bronchiseptica* can be present in the upper respiratory tract of infected pigs, dogs, cats,

rabbits, guines-pigs, rats, horses and possibly other animals. *B.bronchiseptica* and

toxigenic *Pasteurella multocida* type D are the primary agents of swine atrophic rhinitis.

*B. bronchiseptica* is also associated with kennel cough of dogs.

· *B. avium* inhabits the respiratory tract of infected poultry, majorly turkeys. The organism

was formerly named *Alcaligenes faecalis.* It causes turkey coryza, a severe rhinotracheitis

of young poults.

· Mode of transmission of infection is majorly by aerosols but in turkeys indirect spread

can occur via water and litter.

**Laboratory Diagnosis**

Specimens

· Include nasal swabs, tracheal washings and pneumonic lungs.

· In young animals and other animals with narrow nasal orifices, such as dogs and

laboratory animals, the narrow gauge, flexible swabs designed for human infants (such

as Mini-Tip Culturette swabs) can be adapted for use.

**Direct microscopy**

· bordetellae are small Gram-negative coccobacilli.

· Rather than direct smears from specimen, fluorescent antibody technique is preferrable.

**Culture**

· *B. avium* and *B. bronchiseptica* grow well on both sheep blood and MacConkey agars

media. The plates are incubated aerobically at 37 oC for 24 – 48 hours.

· Selective media include;

a. MacConkey agar with 1 per cent glucose and 20 μg/ml furaltadone or blood agar

with 2 μg/ml clindamycin and 4 μg/ml neomycin.

b. Smith- Baskerville (SB) medium is an indicator medium. This medium is very

specific for the isolation of *B. bronchiseptica* from pigs. However, it can also be

used is used for the isolation of strains from dogs or rabbits

c. B.avium will grow well on SB medium, with or without the antibiotic supplement.

The inoculated SB medium is incubated aerobically at 37 oC for 48 hours.

**Identification**

**Colonial morphology**

· On sheep or horse blood agar;

a*. B. bronchiseptica* produces very small, convex, smooth colonies with an entire

edge. Some strains may be haemolytic.

b. *B. avium* are similar but are non-haemolytic.

Phase modulation exists in both species and attributed to loss of a capsule-like structure during

subculture. The phases are;

i. Phase I- This encapsulated virulent phase appear convex and shiny,

ii. Phase II appear larger, circular and convex with a smooth surface and

iii. Phase III is avirulent and colonies are large, flat and granular with an irregular edge.

· On MacConkey agar, colonies are small, pale with a pinkish hue and amber

discolouration of the underlying medium. *B. avium* and *B. bronchiseptica* colonies appear

very similar on MacConkey agar.

· Smith-Baskerville (SB) medium which is an indicator medium contains the pH indicator

bromothymol blue and the agar is green at PH 6.8.

i. After 24 hours’ incubation, *B. avium* and *B. bronchiseptica* colonies appear small (0.5

mm diameter or less), blueish with a lighter blue (alkaline) reaction in the medium

around them.

ii. After 48 hours’ incubation, the colonies increase to about 1.0-2.0 mm diameter, blue or

blue with a green centre and the surrounding medium is blue. Fermentative bacterial

contaminants produce acid reaction change colonies and their surrounding medium to

yellow.

**Biochemical reactions**

· *B. bronchiseptica* is oxidase, catalase, citrate, urease and nitrate positive. It is motile and

does not ferment carbohydrates

· *B. avium* and *Alcaligenes faecalis* show similar reactions to those of *B. bronchiseptica*

but are negative to urea and nitrate tests.

· *A. faecalis* is a ubiquitous organism found in the soil, water and faeces. This contaminant

has many similar properties with *B.avium* and can be mistaken for it.

**Haemagglutination test**

· *B. bronchiseptica* possesses a haemagglutinin and will haemagglutinate washed sheep

erythrocytes. 24-hour culture colonies are more reliable for detecting haemaglutinin as

older colonies tend to lose their haemagglutinating ability with age.

· Two colonies of a suspected *B. bronchiseptica* culture should be suspended in a drop of

physiological saline on a slide. An equal volume of a 3 per cent suspension of a washed

sheep red cells is added and mixture gently rocked. To rule out autoagglutination,

controls should be set-up to include a suspension of colonies without erythrocytes and a

suspension of erythrocytes alone. *B. bronchiseptica* haemagglutinate the red cells within

1-2 minutes.

**Serology**

· Microagglutination, Tube agglutination and ELISA procedures have been developed for

*B. avium* and *B. bronchiseptica.*

**Animal inoculation**

· *B. avium* and *B. bronchiseptica* produce dermonecrotising toxins which are intracellular,

heat-labile and form part of their virulence factors. No evidence of cross-reactivity has

been demonstrated between these two toxins.

· The dermonecrotising toxin of *B. bronchiseptica* has been shown to be lethal when

inoculated intraperitoneally into mice and causes skin necrosis when injected

intradermally into guinea-pigs. Fatal infections can also be produced in guinea-pigs by

injection of young, intact cells given intraperitoneally.

***Taylorella equigenitalis***

**(Dr. M. Agbaje)**

· Gram-negative, facultatively anaerobic rods.

· The genus contains two species;

*T. equigenitalis*, the cause of contagious equine metritis (CEM), and

*T. asinigenitalis* an inhabitant of the genital tract of clinically normal male donkeys.

Because of its clinicial and economic importance, *T. equigenitalis* will be discussed in

detail. Because of its phenotypic similarity to *T. equigenitalis, T. asinigenitalis* will only

be mentioned.

*Taylorella equigenitalis* (formerly *Haemophilus equigenitalis*)

· Gram-negative rod, facultative anaerobe and non-motile.

· Oxidase-positive, catalase-positive, phosphatase-positive and produces no acid from

carbohydrates.

· *T. equigenitalis* is a fastidious and slow-growing. Optimal growth is obtained on

chocolate agar with a rich base (Eugon or Columbia agar) at 37 oC under 5-10 per cent

CO2.

· It does not grow on MacConkey agar.

**Natural Habitat**

· *T. equigenitalis* is the causal agent of contagious equine metritis (CEM).

· It resides exclusively in the equine genital tract. Stallions develop no signs of this highly

contagious disease

**Transmission**

· Transmission is essentially veneral, but can also be by attendants and via instruments

especially in mares.

· The organisms can be isolated from neonatal and virgin animals.

· *T. equigenitalis* can be found on the surface of the penis, in preputial smegma and in the

urethral fossa. The infection in mares causes a temporary infertility and occasionally

abortion within 60 days of pregnancy.

**Laboratory diagnosis**

**Specimens**

· Mares: cervix, uterus, clitoral fossa and clitoral sinuses.

· Stallions: urethra, urethral fossa and diverticulum, prepuce and pre-ejaculatory fluid.

· Samples can be obtained from stallion after servicing two maiden mares. The mares are

then sampled instead of the stallion. The specimens are carefully collected using sterile

swabs which are placed into Amies transport medium with charcoal for transportation to

the laboratory not later than 48 hours.

**Direct microscopy**

· Gram-negative rods, coccobacilli or short-filaments

· Gram-stained smears are only useful on uterine exudates from mare suspected of clinical

*T. equigenitalis.*

**Isolation**

· Chocolate agar with a highly nutritive base such as Eugon or Columbia agar and

preferably equine blood. The inoculated plates should be incubated at 37 oC under 10 per

cent CO2. Although growth may be seen at 48 hours, negative plates should only be

discarded after 7 days no growth.

· Selective media are required to suppress contaminating bacteria. If streptomycin is used

as one of the selective agents, two plates should be inoculated in parallel, with and

without streptomycin, as some strains of *T. equigenitalis* are susceptible to this antibiotic.

**Identification**

**Colonial morphology**

· Colonies are less than 1mm in diameter and appear, shiny, smooth and grayish-white.

**Microscopic appearance**

· Gram-negative pleomorphic coccobacilli are seen in smears.

**Biochemical reactions**

Catalase and Oxidase positive colonies with macroscopic and microscopic appearances

consistent with the organism are subcultured onto Eugon chocolate agar without antibiotics and

subjected to further tests:

· Inability to grow in air.

· Agglutination with *T. equigenitalis* specific antiserum in a slide test. Weak spontaneous

agglutination may sometimes occur in the saline control.

· Phosphatase activity: 0.5ml of p-nitrophenyl phosphate solution (1mg/ml) is added to a

suspension of the suspect colonies in 0.5 ml of Tris buffer (PH 8.0). The mixture is

incubated at 37 oC for up to 2 hours. A yellow colour indicates a positive result.