13/8/19

HISTORY OF MICROBIOLOGY (BACTERIOLOGY)

(1600's)

* Robert Hooke → wrote a book "Micrographia" in which existence of microorganisms are described.

* Antony van Leeuwenhoek → 1st person to see Microorganisms. and also Father of ~~Microbiology~~ Bacteriology.

* Spontaneous Generation Theory (SGT) → Life originates spontaneously from non living matter.

1st scientist who disproved (SGT) → Franseeco Redi (Italian physician)

↓

conducts an expt. i.e, kept meat pieces in 3 vessels kept covered without lid, by paper, by gauge.

(Flies ✓) (Flies ✗) (Flies are over guage)

∴ Maggots are not originated from meat

Lazzaro Spallanzani → conducts expt. i.e, boiled seeds in 2 vessels covered by lid & without lid

↓ (cloudiness ✓ & contaminated).

No contamination

∴ Microorganisms are from air.

Louis Pasteur

Tyndall → Sterilization procedures.

(1700's)

* Lister → conducted surgery with instruments treated with carbolic acid (phenol) so that people are survived. He is regarded as Father of Antiseptic surgery.

(1800's)

* Robert Koch → 1st demonstrated that a particular organism is responsible for particular disease.

eg:- Bacillus anthracis – Anthrax.

Mycobacterium tuberculosis – TB (in Guinea pigs)

Koch's postulates (4)*

1) Causative microorganism of disease must be associated with the diseased host, but absent in healthy host.

2) Microorganism responsible for disease must be isolated from diseased host in pure culture.

3) when pure culture of microorganism is inducted to healthy susceptable host & produce same disease in host.

4) causative microorganism must be again isolated (re isolated) from diseased host.

He was awarded with Nobel prize in 1905 for detecting causative bacteria for TB in Guinea pigs by collecting lung samples.

Exceptions for Koch's postulates

1) Healthy individuals also carry bacteria that do not cause disease.

2) we can't cultivate Treponema pallidum in laboratory. so that we can't prove causative organism.

↓

causes syphilis

Scanned with CamScanner

3) Vibrio cholerae causes enteritis in humans but not in all the experimental animals.

* Louis Pasteur → developed "Pasteurization" technique & vaccines, expts. on silkworms
↓
Milk is subjected to heat so that infections are reduced.

* Initially "Gelatin" is used for bacterial culture. But Gelatin gets liquified.
Fanny Hesse (wife of Hesse) (Assistant of Robert Koch) gave an idea to use Agar for bacterial culture.
Agar is extracted from seaweeds, Red Algae (Gelidia, Gracilaria)

* Richard petri → developed petridish / petriplate.

1/8/19

CLASSIFICATION AND NOMENCLATURE OF BACTERIA

* Taxonomy – Identification, Nomenclature, classification.

* Domains – Arachea, Bacteria, Eukarryota
Kingdom – Prokarryota

Arachea is bridging b/w Bacteria (Prokaryotes) & Eukaryotes.

Characteristic	Prokaryote (Bacteria)	Eukaryote
1) Nucleus	No defined nucleus	well defined nucleus, surrounded by Nuclear membrane.
2) Genetic material	single circular chromosome	Multiple linear chromosomes
3) Extrachromosomal DNA	Plasmids	Plasmids * DNA in mitochondria
4) ER	Absent	Present
5) Golgi bodies	Absent	Present
6) Mitochondria	Absent	Present
7) Ribosomes	70S 30S, 50S Dispersed	80S 40S, 60S Dispersed & present on ER.
8) Size	0.5 – 5 µm (1000 times less than mm)	>5 µm
9) Mode of Reproduction	Binary Fission	Mitosis

Scanned with CamScanner

* Mitochondrial DNA gives clue about maternal ancestors & is very conserved.

* Nomenclature – Carolus Linnaeous.

Genus	Species
Bacillus	anthracis
Mycobacterium	tuberculosis

* Same organism of many variants based on reactive mechanism (Based on reacⁿ with serum) & these variants are called serotypes.

* Charles Chamberland developed filters which have pore size of <0.5 um so that bacteria retain at the top & other organisms comes down into filtrate

* Microscopes Ques * Microorg. are visible only with the help of microscope.

1) Light Microscope → (i) Bright Field Microscope – Bright background, Dark objects
(ii) Dark Field Microscope – Bright objects, Dark background.
(iii) Phase contrast Microscope
(iv) UV Microscope / Flourscent Microscope.
(v) Confocal Microscope

uses light as a source to illuminate object.

* Only refracted & reflected light is allowed to pass through condenser in Darkfield Microscope (we observe dynamic processes of Motility).

* Leptospirum is observed by Darkfield Microscope.

* we can appreciate different organelles within the cell using Phase contrast Microscope.

* The object that is to be observed shall be coated with flourscent dye in UV microscope.

* source of light in UV microscope is UV rays.

* when light falls on flourscent dye flourscence light is emitted.

* source of light in confocal Microscope – laser.

* 3D image in confocal Microscope.

* Motility of objects cannot be seen in Bright field Microscope becoz of Firing & staining. But Motility can be seen in Dark field Microscope.

* we use oil (cedar wood oil) having RI equal to glass in oil immersion lens so that light would not get deflected & passes through same point. In absence of oil there is vaccum b/w object & slide so that light rays gets deflected in different directions.

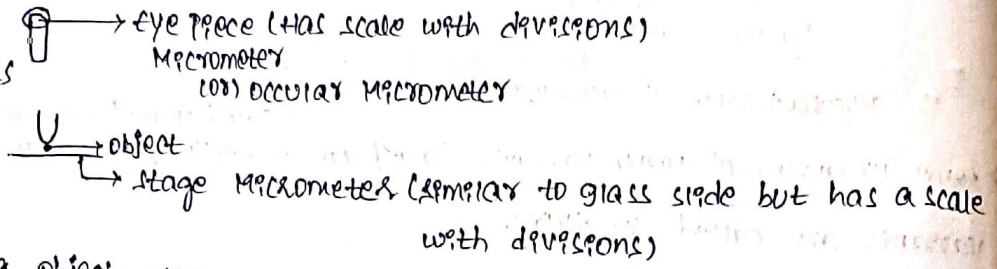
2) Electron Microscope – (i) Transmission EM
(ii) Scanning EM

* Transmission EM – Inner contents of cell.

* Scanning EM – scan surface of objects.

Scanned with CamScanner

* Micrometry – procedure for measuring microorganisms.
↓
Measurement of dimensions of desired microorg. under microscope which use 2 micro scales known as micrometers.

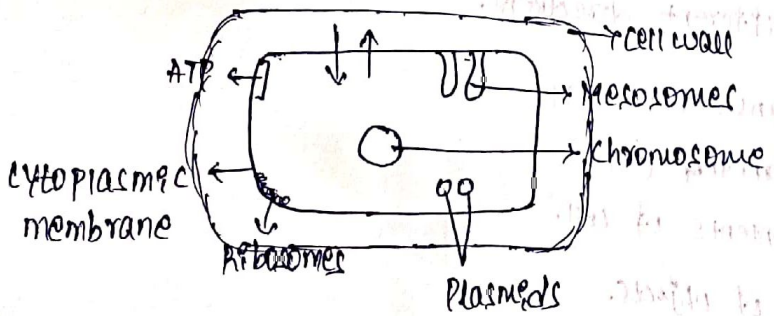


After placing object, align eye piece & stage micrometers, then measure size.

19/8/19 BACTERIAL SHAPES **

* Bacterial structure can be appreciated through microscopic morphology.
* Spherical shaped bacteria – cocci (•)
→ cocci which divide & remain in pairs – Diplococci. (∞)
→ cocci which divide repeatedly in one plane & remain in chains – streptococci (∞∞∞)
→ cocci which divide in random planes & form irregular bunches like grape bunches – staphylococci
→ cocci which divide in 2 planes & remain in square shape of 4 cells – Tetrads (⊞)
→ cocci which multiply in 3 planes & arrange in 8 cells in cuboidal form – Sarcinae
* Rod shaped bacteria – Bacilli (—)
→ Diplobacilli, streptobacilli, staphylobacilli
* Neither fully rod shaped nor fully spherical – coccobacilli (⊂) (short rods)
* Rod shaped bacteria but end is curved – vibrio. (—)
* Spiral shaped bacteria – spirochetes ( ) eg:- leptospira.
* Bacteria like fungal hyphae – Actinomycetes

** BACTERIAL CELL COMPONENTS



Scanned with CamScanner

* Cytoplasmic membrane (CM) → is Diphospholipid layer.

→ contains cellular proteins stuffed over cell membrane.

→ sterols are found in eukaryotic cells but not in prokaryotic cells. so hopanoids are found in prokaryotic cells instead of sterols. → sterol like molecules

(* Bacteria with sterols - Mycoplasma)

→ Allows passage of H_2O, O_2, CO_2, nutrients both in & out.

→ outer & inner surface is Hydrophilic (gets attached to H_2O).

→ passive diffusion.

(If excessive exchange occurs energy is required).

→ Energy is derived by ATP present in innerside of CM cell. so e^- transport in prokaryotes takes place in CM.

→ Innerside of CM contains enzymes, ribosomes

→ Folds of innerside of CM are Mesosomes.

* Cytoplasm → is a liquid phase.

→ contains proteins, enzymes, diff. cell organelles.

→ contains organic & inorganic granules.

Organic granules – β hydroxy butyrate, Glycogen (Iodine stain)
(Sudan black stain)

Inorganic granules – polyphosphates & sulphur granules

Poly phosphates granules / Metachromatin granules / volutin granules

* Ribosome → seat of protein synthesis

→ Found both in cytoplasm & innerside of CM.

→ Ribosomes within cytoplasm are responsible for protein synthesis for proteins responsible for cell metabolism & maintainence.

→ Ribosomes within CM are responsible for protein synthesis that are exported out of the cell.

Eg:- Bacterial toxins.

* Nuclear material → single molecule of circular double stranded chromosome. (DNA)

→ Plasmids (extrachromosomal DNA) are found.

* Plasmid is circular extrachromosomal DNA which replicates autonomously, (irrespective of replication)

→ plasmids are circular in shape.

(linear plasmids are also discovered recently).

→ No distinct nucleus & NO nuclear membrane.

→ plasmid integrated with bacterial chromosome is called episome.

→ sometimes cells loose plasmids during replication & the condition is called curing.

→ Diff. types of plasmids

Scanned with CamScanner

F plasmid (Fertility plasmid) – Bacterial conjugation. (involved in)

R plasmid (Resistant plasmid) – Antibiotic resistance

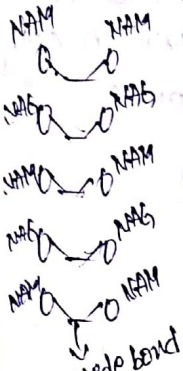
* certain bacteria secretes proteins which kill other bacteria & proteins are referred to as Bacteriocins.

* cell wall → Thick layer ext. to cytoplasmic membrane.

→ Christian Gram used stains to see color of bacteria. certain Bacteria look like purple/violet & certain bacteria look like pink/pinkish red.

→ color difference is due to presence of cell wall.

→ Due to difference in cell wall structure & thickness Bacteria stains differently. so the staining is called Gram's staining.



→ polymer of mesh with diff. sugar units.

(NAG) N-acetylglucosamine
(NAM) N-acetyl muramic acid } 2 sugar units repeatedly present & joined together by peptide bond which contains AA.

→ D-alanine, D-Glutamic acid, Meso diaminopimelic acid – Aminoacids exclusively present in Bacteria.

→ so cell wall structure is referred as Murein / Peptidoglycan.

→ Bacteria which look like violet in color → Gram +ve bacteria.
Bacteria which look like pink/pinkish red → Gram -ve bacteria.

→ Peptidoglycan is very thick & contains teichoic acid in Gram +ve.
Peptidoglycan is very thin & in Gram -ve. bacteria.

→ over peptidoglycan we find another membrane called outer membrane in Gram -ve bacteria.

→ outermembrane contains lipopolysaccharides (LPS).
Lipid present in LPS is Lipid A. which is very toxic.
certain polysaccharides are present in the form of side chains which are called "O" side chains.

→ Becoz of toxic Lipid A LPS is referred to as endotoxins.

→ space b/w cytoplasmic membrane & outer membrane is called Periplasmic space, which is wide in Gram -ve bacteria & contain enzyme.

→ outer membrane consists of proteins called porins which make pores through which transportation occurs.

→ space b/w cytoplasmic membrane & peptidoglycan is called periplasmic space in Gram +ve bacteria which is narrow.

→ Lysozyme breaks peptide bonds b/w NAM & NAG so that peptidoglycan destructs. peptide bonds are generated by Transpeptidases. Penicillin prevents action of Transpeptidases.

20/8/19

Treatment of Gram +ve bacteria with lysozyme & Penicillin makes devoid of cell wall. so Gram +ve bacteria devoid of cell wall is called (Protoplast)
→ Here Peptidoglycan is present.

Gram -ve bacteria devoid of cell wall due to lysozyme & Penicillin treatment retains outer membrane is called as (Spheroplast).

Gram's staining

* He made smear of bacteria
* He poured crystal violet (P stain) stain over smear & waited for 1min & washed smear.
* He poured Grams Iodine (Mordant) ~~stain~~ over smear & waited for 1min & washed smear.
* He treated smear with ethanol (Destaining) for 1/2 min.
* He then poured Eosin/safranin/dil. ~~carbolfuchsin~~ carbolfuchsin. 2° stain/counter stain

→ Grams Iodine facilitates Penetration of P stain. So it is called Mordant

→ Gram +ve bacteria takes violet stain becoz of destaining by ethanol the thick Peptidoglycan shrinks & retains violet, as such & they do not take counter stains. (by bacteria)

→ Gram -ve bacteria takes pink/pinkish red becoz of destaining by ethanol the lipid in outermembrane hydrolyses & lace all violet stain & Peptidoglycan retains counter stains.

* Capsule → layer over peptidoglycan (Gram +ve) / over outer membrane (Gram -ve).

→ comprises polysaccharides (Homo/Hetero) except in Bacillus anthracis where capsule is proteinaceous. So D-glutamic acid is aminoacid rich in proteinaceous capsule.

Geet ji, Geeta ji, Tejal

→ Diffused capsule is called slime layer. (Millions of bacteria with capsule).

Advantages → Bacteria can resist phagocytosis by Host immune system.

→ Bacteria can resist attack by bacteriophages (kill bacteria)

→ Helps bacteria for attachment to host tissues.

→ Helps to prevent dessication.

* Flagellum → originates from CM & passes through cell wall & comes out of bacterial cell.

→ Locomotor Appendage.

→ comprises Filament, Hook, Basal body

→ Basal body contain ring like structures

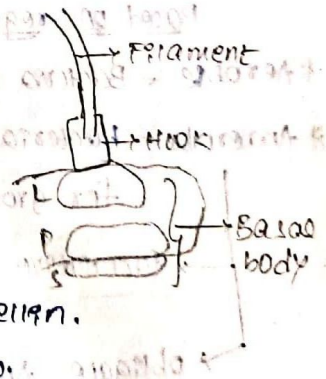
3 rings in Gram -ve

2 rings in Gram +ve.

→ Flagellum grows at tip.

→ Filament of Flagellum contains a protein called Flagellin.

→ Bacterial movement is similar to cork screw.



* Bacteria with single flagellum at one end - Monotrichous
* Bacteria with single flagellum at both ends - Amphitrichous
* Bacteria with group of flagella at one end - Lophotrichous
* Bacteria with flagella around entire cell wall - peritrichous.

→ Leifson method & Gray method are methods of staining for flagellum

(Parasoanilene) (Basic fuchsin)

Scanned with CamScanner

* Fimbriae → short appendages present on bacterial cell.
(100's in no.) → Helpful for attachment of bacteria with host tissues.
→ Pili is long appendages present on bacterial cell
Eg:- F pilus (sex pilus) – exchange of genetic material.

* Endospore → Bacillus sps. (Gram +ve), Clostridium sps. } on adverse conditions the vegetative bacterial cell is transformed into endospore.
→ Tough coat.
→ Resists UV radiation, excess Heat, desiccation, Dry spells.
→ Endospore includes the following layers
Exosporium – spore coat, cortex (outer to Inner)
→ comprises Dipicolinic acid combined with calcium.
↓ ↓
Helpful for protecting bacteria
→ Sometimes Endospores are formed within central, Terminal, subterminal
→ spores germinate into vegetative cells under favourable conditions through Activation, Germination & outgrowth steps.

21/8/19

** BACTERIAL NUTRITION (CLASSIFICATION OF BACTERIA) BITS

* Saprophyte is a bacteria which derives its nutrients from decomposing material.

Based on source of energy
* Phototroph – Bacteria that obtain energy from light sources
* Chemotroph – Bacteria that obtain energy from oxidation of chemical compounds.

Based on source of carbon
* Autotroph – Bacteria that utilize CO_2 as source of carbon.
* Heterotroph – Bacteria that derives carbon source from preformed organic molecules.

Based on requirements of oxygen
* Aerobe – Bacteria which grow in presence of oxygen.
* Anaerobe – Bacteria that grow in absence of oxygen / that doesn't require oxygen for growth.
→ Facultative Anaerobe – Bacteria which don't require presence of oxygen for growth but also can grow in presence of oxygen.
→ Obligate Anaerobe (or) Strict Anaerobe – Bacteria don't require oxygen for growth but presence of oxygen kills bacteria.

* Microaerophilic – Bacteria which require 2-10% of atmospheric oxygen pressure for their growth.

Eg:- Obligate Anaerobe – Clostridium sps.
Microaerophilic – Campylobacter sps.

Scanned with CamScanner

Based on optimum temp. for growth

* Mesophilic – Bacteria which have optimum growth temp. of 20°c – 45°c.
  Eg:- Most of bacterial pathogens which infect Humans & Animals.
* Psychrophils – Bacteria which have optimum growth temp. of 15°c.
  – They also multiply at 0°c.
* Thermophils – Bacteria which have optimum growth temp. of 45°c – 55°c.

→ oxidase test is for (detecting) knowing Aerobe & Facultative Anaerobe.

Nutrition

* Peptone, Beef/Meat extract, Brewer's yeast – 3 components in general bacteriological medium.
* Peptone is partial hydrolysate of proteins like casein, soya, gelatin. It is rich supplement of Nitrogen, carbon.
* Beef/Meat extract provides nucleotides, Nitrogen, carbon, Phosphorus
* Brewer's yeast is rich in B-complex vitamins.

Different forms of Bacteriological medium

* Liquid – Broth
* Solid – Agar 1.7% Agar is used in Bacteriological medium preparation. Agar doesn't provide nutrients, it is only a solidifying agent.
* Semi solid

Based on purpose

* General – Peptone water, Nutrient Broth, Nutrient Agar.
* Enriched – General Bacteriological medium supplemented with serum. (serum)
  (Bacteria which require complex nutrients – Fastidious Bacteria)
* Selective – Mac Conkey Agar
  ↓
  consists of Bile salts & crystal violet dye.
  ↓ ↑ growth of Gram –ve enteric bacteria. ↓ inhibits growth of Gram +ve bacteria.
  E. coli grows on MacConkey Agar & causes diarrhoea.
* Differential – Blood Agar.
  → Autoclave Nutrient Agar & then remove from autoclave.
  → Add 7-10% of Bovine/ovine blood to ~~autocla~~ molten nutrient agar at hand bearing temperature.
  → Place it in laminar air flow cabinet & mix blood with agar.
  → Blood Agar is prepared.

– Certain Bacteria secretes hemolysins so that blood cells in blood agar gets lysed & clear zones appear on Blood Agar.

– No clear zones ⇒ No hemolysins secretion by Bacteria.

Scanned with CamScanner

→ Broth can be in testtubes

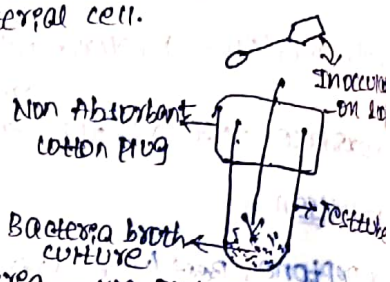
→ Agar can be put in testtubes / petridishes.

If Agar is to put in testtubes, testtube should be kept in slant position so Agar gets solidified in slant position, referred as Bacteriological medium slant

Pure culture

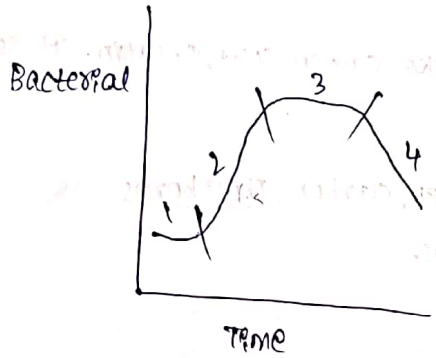
* It is the bacterial culture originated from a single bacterial cell.

Methods of pure culture – Streak plate
– Spread plate
– Pore plate



(Inoculation loop – Made of Nichrome)

Bacterial growth curve / one step growth curve of Bacteria – we plot curve after inoculation of bacteria.



1 – Lag phase
2 – Log / exponential phase
3 – Stationary phase
4 – Decline phase

* Bacteria make preparations for growth – Lag phase.
* Rapid multiplication of Bacteria – Log phase.
* Nutrients start depleting ; Growth rate & death rate became same – Stationary phase
* Bacterial population starts declining – Decline phase.
* After 18–20 hrs of incubation Bacteria will be in Log phase which is the best phase.

26/8/19

PRESERVATION OF BACTERIA

* Chemostat – Instrument for Bacterial preservation.
(Temporary) – Fresh nutrients are infused & metabolites are removed.
* Keep culture media in refrigiration temp. (4 to 8°c)
* Deep freezers (–20° to –80°c)
↓
* Lyophilization / Freeze drying – Process of removing water from bacterial culture in presence of vaccum at freezing temp. so that then bacterial culture will be in powder form & then we store bacteria at –80°c for mts.
* In liquid Nitrogen (–196°c) – Cryopreservation.

Scanned with CamScanner

* TYPES AND SOURCE OF INFECTION, MODES OF TRANSMISSION

* Infection is any organism which causes pathological changes.
* Infection always doesn't lead to disease.
* Disease – Manifestation of severe infection.

Infection by contact

eg:- Abscesses – staphylococcus aureus ✓

Infection by Aerosol

eg:- Mycobacterium bovis → cattle, sometimes Humans also causes TB
Mycobacterium tuberculosis → Humans, sometimes cattle also
} Zoonotic

Bacteria is excreted in sputum & then Bacteria gets mixed with aerosol & then Healthy individuals gets infected by inhalation of contaminated aerosols.

Infection by sexual route & contact

eg:- Brucella sps – infects uterus & causes Abortion.

* If Bull is infected with Brucella → sexual intercourse → cows gets infected → Abortion of cows. (By intercourse)
* If Aborted material is not handled properly. it gets spread to Humans & then Males – infertile ; Females – Aborted. (By contact) (Zoonotic)

Infection by Ingestion (Feco oral route) ✓

eg:- Salmonella – Salmonellosis.

* Bacteria is excreted in faeces & excreted fecal matter contaminates water & food.
* the person excretes bacteria even after 4 wks of curing of salmonellosis.

→ Milk of Animals gets contaminated with bacteria in infection with TB and Brucellosis.

** Leptospira is excreted in urine of animals & contaminates water sources.
↳ penetrates host by causing damage (not intact) & causes leptospirosis. → Damages liver.

Infection by Arthropod vectors

eg:- Plague – Yersinia pestis
↓
transmitted by Fleas.

Borrelia sps. causes disease in poultry & transmitted by ticks.

Scanned with CamScanner

Susceptibility & Resistant Factors

1) Factors associated with Bacteria.

Same bacteria known to be cause disease has many variants & some variant have more virulence & some have less virulence

More virulence → More pathogenicity

Less virulence → Less pathogenicity.

2) Factors associated with Host.

Genetic background of individuals is imp.

(i) sps. of Animal – certain diseases are caused in certain sps.

Eg:- Anthrax is not found in chicken

Polio is not found in Animals (except monkey), humans

(ii) Breed of Animal – certain Breeds are resistant & some are susceptable.

Eg:- Foot & Mouth Disease (FMD). – More in cross breeds & native breeds are relatively resistant.

(iii) Individual difference

(iv) sex difference – Eg:- Abortions only in females.

Terminology

*Bacteremia – presence of viable (live) bacteria in blood of infected Host.

*Septicaemia – Invasion of blood by bacteria leading to their multiplication and Prod^n of toxins.

* Toxemia – presence of bacterial toxins in blood.

Bacterial Toxins

- Endotoxins – Lipopolysaccharide (LPS) of Gram-ve bacteria.
- Exotoxins. (protein)

Character	Endotoxin	Exotoxin
*Biochemical Nature	Lipopolysaccharide	Protein.
*Relationship with bacterial cell.	Present in outer membrane.	Secreted out of bacterial cell.
*Denaturation by boiling.	No.	Yes.

Scanned with CamScanner

* Antigenicity (Ability of body to identify foreign)	yes	yes.
* Toxoid formation. (Any bacterial exotoxin gets converted to toxoid by losing toxicity on chemical treatment) eg:- Tetanus toxoid.	NO	yes.
* Pyrogenicity (Ability to cause fever)	yes	yes.
* Enzymatic activity	NO	yes.
* Potency to cause disease	100 microgms	<1 microgm. <1 nanogm (Clostridium botulinum)

BACTERIAL GENETICS

* Gene is stretch of DNA sequence which codes for protein/character.
* Mutation is stable inheritable Alteration in gene sequence.

→ If purine is replaced with another purine - Transition Mutation.

→ If purine is replaced with pyramidine/ If pyramidine is replaced with purine - Transversion mutation.

→ change in one nucleotide - point Mutation
↓ leads to
silent Mutation.

→ change in codon also codes for same AA's - silent mutation.

→ Mutations are caused by insertions/deletions/inversions/translocations of nucleotides

→ Mutations are caused spontaneously.

→ 1 bacterium in 1 million of bacterial cells develop mutations.

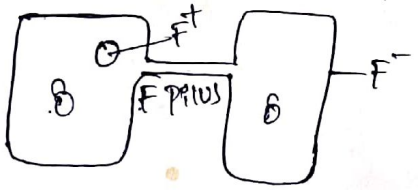
→ Luria & Delbruck by Fluctuation technique developed spontaneous mutations.

→ Auxotrophic mutant - Mutation by which bacterium loses its ability to synthesize a particular nutrient required for its growth & needs supplementation of particular nutrient in medium for its growth

2/8/19

Conjugation R Plasmid - Plasmid mediated Antibiotic Resistance.

* Bacteria which contains F plasmid is called F^+.
Bacteria which doesn't contain F plasmid is called F^-.
* F^+ stimulates bacterium to project outgrowth & it connects F^- forming F pilus.



* F plasmid in F^+ becomes unwound i.e, Double stranded becomes single stranded & one strand is transferred to F^- through F pilus. ~~so that F becomes~~ also F^+
* complementary strands are synthesized in both bacterial cells.
* F^- becomes F^+.

Eg:- E. coli

* HFr^+ strains - Bacterial Plasmid of F^- gets integrated with bacterial chromosome called episome.
- High frequency recombination strains.

Generally seen in Gram -ve bacteria.

In Gram +ve bacteria there is no formation of F pilus & it is by close contact of 2 bacterial cells.

Scanned with CamScanner

Transduction

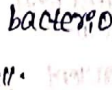
* Transfer of genes b/w bacteria by phages is called Transduction.

(viruses doesn't have cellular machinery so it needs host that may be prokaryotic/eukaryotic. virus which attacks prokaryotes - Bacteriophages.)

* when viral genome enters host it replicates & attacks protein synthesis machinery (ribosome)
* viral genome produces phage proteins.
* proteins of bacteriophage are packed into new bacteriophages.
* New bacteriophages are released by lysis of bacterial cell.
* such type of bacteriophages are called lytic bacteriophages.

---

* In some bacteriophages, the phage genome which is integrated with Bacterial chromosome instead of replication is called prophage.



* Relation b/w Phage genome & Bacterial DNA is Lysogeny.
* such type of bacteriophages are called Temperate phages
* phage genome gets excised from chromosome of Bacteria on activation by uv rays & in this process phage genome carries a part of bacterial chromosome during its excise.
* 2 types - 1) Generalised Transduction

↓

is the one where phage genome is excised from bacterial chromosome & it takes away random fragments of bacterial chromosome.

- Phage genome with bacterial chromosome replicates & produce progeny containing both phage DNA & bacterial chromosome.
- progeny is released by lysis & they attacks other bacteria. so that bacterial chromosome of one bacteria is then integrated into another bacteria resulting in recombination.

Eg:- P_1, P_{22}.

2) Specialised Transduction

↓

while insertion only, phage genome is integrated into bacterial chromosome at a particular point.

- once phage genome is activated it is excised from bacterial chromosome & it takes away next part to phage DNA of bacterial chromosome. (next to site of insertion)

Same process as in Generalised Transduction.

Scanned with CamScanner

Transformation

* Transfer of naked/cell free bacterial DNA from lysed bacterial cell to the recipient bacterial cell.

* Discovered by Griffith in pneumococci.

Virulent pneumococci (Has capsule) → injected to mice → Died.

↓ Treated with Heat

Killed virulent pneumococci → injected to mice → Alive.

Killed virulent + Non virulent pneumococci → injected to mice → Died.

↓

DNA from lysed pneumococci is transferred to Non virulent & Non virulent becomes virulent & results in death of mice.

* When we perform this in laboratory the recipient cells are called competent cells.

→ Transposans – Mobile Genetic elements.
- Replicate when they enter into bacterium.
- Mediates Antibiotic Resistance.

Classification of Antibiotics

Based on site of Action

* Cell wall synthesis

eg:- Penicillin Antibiotic (Lβ-lactam) – Inhibits cell wall synthesis by preventing action of Transpeptidases

* Cell Membrane

eg:- Polymyxin, Colistin } Damage cell membrane

* Protein synthesis

Inhibit protein synthesis by binding to subunits of Ribosomes (50S, 30S)

eg:- Aminoglycosides – Tetracycline
Macrolides – Erythromycin

Tetracycline is only Antibiotic in Plague discovered by Yellapragada Subbarao.

* Nucleic Acid synthesis

eg:- Flouroquinolones – Ciprofloxacin.

Inhibit bacterial enzymes responsible for bacterial replication.

Scanned with CamScanner

Bacteria require PABA (Para amino Benzoic acid)

← Bacteria die by administration of sulpha drugs becoz sulpha drugs are similar to PABA & bacteria use sulpha drugs instead of PABA.

⇒ Bergey's Manual [redacted]

earlier – Bergey's Manual of systematic Bacteriology.

Presently – Bergey's Manual of Determinative Bacteriology.

Scanned with CamScanner

28/7/19

STAPHYLOCOCCUS

Characteristics (Biochemical):

* Gram +ve Bacteria cocci in irregular clusters resembling bunch of grapes.
* Facultative anaerobes.
* ~~Microscopic Morphology: Bunches of Gram +ve bacteria.~~ (cocci)
* Non motile, non spore formers.
* Staphylococcus aureus subspecies aureus – Most imp. bacterial species of staphylococcus.
* Other sps. include staphylococcus intermedius.

Cause Abscess by contact.

Staphylococcus hyicus.
Staphylococcus chromogenes.
Staphylococcus anaerobius.

* Staphylococcus aureus is prototype i.e, it represents entire genus. Has no. of strains (variants).

Biochemical tests:

* Staphylococcus bacteria secrete enzyme called coagulase.
  - Collect plasma of Rabbit/Human
  - Take a drop of plasma on slide & add bacterial culture to plasma, mix it
  - If bacteria secretes coagulase, it converts fibrinogen in plasma to Fibrin.

$$\text{Fibrinogen} \xrightarrow{\text{coagulase}} \text{Fibrin}$$

  - we observe formation of fibrin clumps.
  - This indicates S. bacteria is +ve for above reac^n. Such staphylococcus bacteria are designated as coagulase +ve.
  - Many staphylococcus aureus are coagulase +ve & some are coagulase -ve so S. aureus are coagulase variable.
  - Coagulase +ve S. aureus are highly pathogenic.
  - Usually coagulase -ve S. aureus are less pathogenic.

Isolate – Bacterial culture isolated from sample is called Isolate.

- Other sps. of staphylococcus are, usually coagulase -ve.
- CoNS → coagulase -ve staphylococci.
- Staphylococcus intermedius – some are coagulase +ve & some are coagulase -ve

Catalase +ve is an enzyme secreted by staphylococcus which acts on H_2O_2 & break it into H_2O & O_2.

$$H_2O_2 \xrightarrow{\text{Catalase}} H_2O + O_2$$

- Take drop of H_2O_2 on glass slide & add bacterial culture from Agar.
- If bacteria secretes catalase, it converts H_2O_2 to H_2O & O_2.

Scanned with CamScanner

- O_2 can be found by bubbles.
- This indicates that staphylococci are catalase +ve.

* Oxidase is an enzyme secreted by aerobic & Facultative anaerobic bacteria
  - Staphylococcus sp. are oxidase +ve.

Cultural characters:

* we have to put sample in bacteriological broth (Nutrient broth) & incubate it for 18-20 hrs at 37°C. - Enrichment of sample.
* we will culture bacterial sample on Agar from enriched sample after incubation by picking inoculum by inoculation loop.
* If you streak culture on Nutrient Agar & incubate for 18-24 hrs we find growth of bacteria.
* If we culture staphylococcus aureus on Agar, we observe Golden yellow colour colonies.
  Colony - Descendence from single bacterial cell.
  only S. aureus of Bovine & Humans.
* S. aureus of other sp. has white colonies.
* Selective medium is best one to demonstrate staphylococcus bacteria.

Eg:- 1) Mannitol salt Agar (MSA)
→ Indicator - phenol red.
  Turns to yellow colour in Acidic conditions.
→ If S. aureus is suspected to be present, it ferments MSA & acid is released so that yellow coloured colonies are observed due to phenol red.

2) Baird Parker Agar
→ Black coloured colonies / Greyish Black - presence of S. aureus.
→ Incorporated with chemical called Potassium tellurite, Egg yolk.
→ S. aureus acts on potassium tellurite & black spots are formed. colour converts into
→ S. aureus on egg yolk hydrolyses & clear colonies are seen.

3) DNA ase Agar
→ S. aureus secrete DNA ase.
→ Included with DNA strands.
→ If we culture S. aureus on DNA ase Agar & incubate then next day we have to add 1N HCl solⁿ.
→ If S. aureus is present DNAase acts on DNA strands of DNA agar & DNA breaks down so that clear zones are observed after pouring HCl.
→ No S. aureus - No DNA breakdown - No clear zones - cloudy appearance.

Scanned with CamScanner

4) VJ Agar (Vogel Johnson Agar)

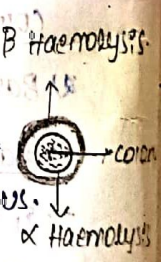
Diseases caused

* S. aureus – Mastitis, Dermatitis } In large animals like cows, buffaloes.
  – Impetigo (Small yellow coloured Abscesses) } In large animals like cows, buffaloes.
  – Mastitis, Tick Pyemia, Abscess } In sheep
  – Botryomycosis (Skin condition), Scirrhous cord (Hardening of Tissues) } In Horses
  – Bumble Foot } Poultry
  – Mastitis } In Goat.
* S. hyicus – Greesy Disease/exudative dermatitis } In Pigs
  – Mastitis } In large Animals.
* S. anaerobius – Abscesses
* Other staphylococcus sps. causes mastitis & skin infections.

3/9/19

Heamolysis

* lysis of Blood cells.
* when we culture staphylococcus aureus on Blood Agar, we see



α Heamolysis – Narrow clear zone of complete Haemolysis around colony.
  – caused by α Haemolysin which is secreted by S. aureus.

β Haemolysis – wider zone of partial/incomplete Haemolysis around colony.
  – caused by β Haemolysin which is secreted by S. aureus.

* so Double Haemolysis is characteristic of S. aureus.
* No Haemolysis – δ Haemolysis.
* Blood Agar plates with β Haemolysis when transferred to refrigerator at 4–8°C for 24 hrs we find very clear zone of Haemolysis. This (incubate at 37°C for 24 hrs) Phenomenon is called Hot-cold lysis.
  ↓
  characteristic of S. aureus.
* Most of S. aureus which are coagulase +ve are also +ve for Haemolyses.
* S. hyicus is non-Haemolytic (–ve for Haemolyses).

Scanned with CamScanner

2 types of coagulase tests

1) Slide coagulase test – To detect bound coagulase of S. aureus. This type of coagulase is also called clumping factor.
(Discussed Earlier)

2) Tube coagulase test.

- collect Rabbit/Human plasma in test-tubes.
- Add bacterial culture.
- Incubate in water bath at 37°c for 24 hrs.
- we find thick clot if coagulase is present, so that it acts on fibrinogen.

This type of coagulase is Free coagulase/staphylocoagulase.

* S. anaerobius is responsible for lymph edemitis.

Laboratory Diagnosis:

* Samples are cultured on selective media (MSA, Baird Parker Agar, DNA ase Agar, VJ Agar, Blood Agar).

But while preparing Blood Agar nalidixic Acid (Antibiotic) should be incorporated to selectively inhibit Gram(–ve) bacteria.

Differential diagnosis with other closely related bacteria* (AM).

Bacteria	coagulase	catalase	oxidase	Bacitracin	oxidation/ Fermentation.
Staphylococci (Irregular bunches)	variable	+ve	–ve (S. aureus → –ve)	(0.04 U) Resistance	Fermentation.
Micrococcus (Tetrads)	–ve	+ve	+ve	susceptable	oxidation.
Streptococci (chains)	–ve	–ve	–ve	Resistant	Fermentation.

Virulent Factors/Toxins.

* Haemolysins
* Coagulase – No Phagocytosis.
* Leucocidin – Kills leucocytes.
* Protein "A" – Binds with Ab's in body & interferes with Phagocytosis
* Enterotoxins – act on intestine & cause Enteritis.

TSST – Toxic shock syndrome toxin.

- Secreted by S. aureus of Human & Bovine.
- Most Potent Enterotoxin.

Scanned with CamScanner

STREPTOCOCCI

(Fastidious organisms require BHI Broth).

* Facultative Anaerobes.
* Usually they colonize respiratory & genital tracts of animals.
* Gram +ve chains.
* Fastidious (Require complex nutrients like serum, Blood)
* Responsible for suppurative infections (Pus forming)
* Certain streptococci are present in Intestine which are called enterococci.
  (Enterococci being Gram +ve will also grow on Mac Conkey Agar) → inhibits Gram +ve.
  → Eg:- Enterococcus faecalis → cause Mastitis. ✓
* Responsible for both 1° & 2° infections.
* Its bacterial cell wall contains "C" Polysaccharide.
* Based on reactivity of "C" polysaccharide with serum streptococci are classified as A to G. This classification is Lancefield grouping/classification.
* Responsible for Meningitis, Metritis (Inflammation of uterus), Mastitis, Arthritis, Respiratory tract infections.
* Toxins secreted include
  Haemolysins (α, β), DNAases, Enterotoxins ✓.
* α Haemolysis, β Haemolysis } exactly opposite to Staphylococcus ✓
* Streptococcus pyogenes – usually infects humans (Scharlet Fever) but causes mastitis in ruminants, Tonsilitis in Dogs, Lymphedemitis in Foals.
  pneumoniae
  suis – Affects pigs but also humans. (Zoonotic)
  canis
  equi
  equisimilis
  agalactiae – Belongs to Lancefield group B. (others belong to group C.)
  uberis – Doesn't belong to any group.
  dysgalactiae
  zooepidemicus
* Streptococcus capsule is virulent.
* S. equi capsule contains "M" protein which inhibits phagocytosis.
* Selective media – Edward's medium
  Blood Agar. ✓

Scanned with CamScanner

14/19

* S. equi is responsible for strangles disease which is more prevelant in young foals.
↓
suppurative infection upper resp. tract.
characterized by Abscessation of lymphnodes. (submandibular).

* pus discharges come from resp. tract of affected foals.
(purulent)

* Abscesses burst & infection from purulent discharges spread to Guttural pouches leading to a condition called "Guttural Empyema". so that animals suffer dyspnea (difficulty in breathing).
↓
Due to lymphedimitis in submandibular lymphnodes.

* S. zooepidemicus & S. equisimilis cause Resp. tract infections & sometimes produce similar symptoms to that of strangles. ✓

Differential diagnosis

Take bacterial culture & culture on broth. (Fastidious – BHI broth)

Then after incubation culture it on selective media.

Sugar Fermentation Test:

	Trehalose	lactose	sorbitol	maltose	(CHO's) ✓
S. equi	–	–	–	Ferments	
S. zooepedimicus	–	Ferments	Ferments	Ferments	
S. equisimilis	Ferments	–	variable	Ferments	

* S. equi – β haemolysis (complete haemolysis). ✓

* S. suis – zoonotic; causes polyarthritis
– Types → I & II ✓

* S. agalactiae, dysgalactiae, uberis – cause Mastitis in Dairy animals (cattle, Buffalo
– so these 3 are called Mastitis streptococci.

S. agalactiae:
↓
Present in mammary ducts of milch animals.
Responsible for acute mastitis in Bovine & Bubaline.

S. dysgalactiae:
↓
Present in buccal cavity & vaginal mucosa in animals
May be acute (or) May not be acute. (Mastitis)

S. uberis:
↓
Present on skin & buccal cavity.
severity varies. (Mastitis)

Staphylococcus & streptococcus Mastitis is contagious Mastitis.

Aesculin Hydrolysis on Edward's medium

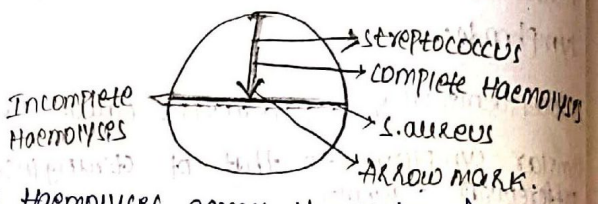
* Aesculin is glycoside. certain bacteria act on Aesculin & break down Aesculin into Aesculetin & glucose when medium is incorporated with 10–40% Bile salts. Aesculetin once produced binds with ferric ions in medium & produces Black spots. ✓

Scanned with CamScanner

Mastitis Differentiation.

Bacteria	Lancefield group	Haemolysin	CAMP test	Growth on Mac Conkey Agar	Aesculin Hydrolysis on Edward's medium
* S. agalactiae	B	β	+ve	−ve	−ve
* S. dysgalactiae	C	α	−ve	−ve	−ve
* S. uberis	Not assigned	α	−ve	−ve	+ve.
* Enterococcus faecalis	D	α	−ve	+ve	+ve.

CAMP test

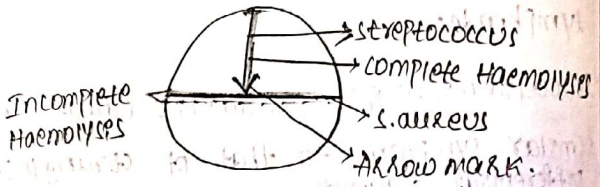


* * Christie Atkins Munch peterson — CAMP
* * Prepare Blood Agar in petriplate.
* * Streak known S. aureus culture producing haemolysis across the centre of petridish.
* Collect sample suspected for streptococcus bacteria & streak this sample ⊥ to central line but not touching central line leaving 2 to 3 cm gap above central line.
* * Incubate for 1 day at 37°C.
* S. aureus haemolysin is secreted & diffuses (α & β) on both sides of streak.
* * If streptococcus agalactiae is present there is β haemolysis, which is responsible for complete haemolysis. β haemolysin acts on incomplete haemolysis of S. aureus making it complete haemolysis forming Arrow mark (V).
* * Presence of Arrow mark → +ve for CAMP test.

Scanned with CamScanner

Bacteria	Lancefield group	Haemolysin	CAMP test	Growth on Mac Conkey Agar	Hydrolysis on Edward's medium
S. agalactiae	B	β	+ve	−ve	−ve
S. dysgalactiae	C	α	−ve	−ve	−ve
S. uberis	Not assigned	α	−ve	−ve	+ve.
Enterococcus faecalis	D	α	−ve	+ve	+ve.

CAMP test



* Christie Atkins Munch peterson – CAMP
* Prepare Blood Agar in petriplate.
* Streak known S. aureus culture producing haemolysis across the centre of petridish.
* Collect sample suspected for streptococcus bacteria & streak this sample ⊥ to central line but not touching central line leaving 2 to 3 cm gap above central line.
* Incubate for 1 day at 37°c.
* S. aureus haemolysin is secreted & diffuses (β) on both sides of streak.
* If streptococcus agalactiae is present there is β haemolysis, which is responsible for complete haemolysis. β haemolysin acts on incomplete haemolysis of S. aureus making it complete haemolysis forming Arrow mark (V).
* Presence of Arrow mark → +ve for CAMP test.

Scanned with CamScanner

11/9/14

CORYNEBACTERIUM

Domain – Bacteria
Phylum – Actinobacteria
Class – A
Order – Corynebacteriale
Family – Corynebacteriaceae
Genus – Corynebacteria
Species – Pseudo tuberculosis, ulcers, Renale

Corynebacterium
* Corynebacterium bovis
* Corynebacterium Diptheriae in humans – Diphtheria.

Morphology
* Gram +ve
* 0.5 μ in width & length varies
* Non Acid Fast
* cell wall consists of lipotechoic Acid, Peptidoglycan, lipids, polysaccharides
* Appears as palisade arrangement/chinese letters – In Gram staining
* Toxins released in addition to cell wall lipids & proteins
  Phospholipase D → (or) sphingomyelinase; Damage to leucocytes, RBC, endothelial cells
* Exhibits Haemolysis Exo
*

Cultural chara
* Facultative Anaerobic
* require enriched media for growth
  on Blood Agar – Half white colonies of 1mm in diameter showing little Haemolysis
* Catalase +ve
* susceptable to Heat, common
* Resistant to Amino glycoside group of Antibiotics.
* 2 types – Equine type → Isolated from equine & Bovine type
  utilize nitrates
  Bovine type → Nitrate –ve.
* Inhibits β toxin of S.aureus & α-toxin of Clostridium perfuringens. } Exhibits CAMP test
  Potentiates

Pathogeneses

* Transmission by trauma
* Intracellular parasite;
* In equines – ulcerative lymphangitis;
* Pectoral muscles, caudal abdomen gets affected.
  Transmitted by flies
  Abscess with
* In sheep – caseous lymphadenitis (lymphnodes appear as onion ring like)
  Due to trauma during time of docking, shearing
  Organism localises in draining lymphnode
  Sometimes progressive leading to of organism causing Thin ewe syndrome.

Diagnosis

* Agar Gel
* Toxin neutralisation test

Corynebacterium renale

* Previously divided into 3 groups
  - C. renale – I
  - C. cystidis – II
  - C. pilosum – III

Morphology

* Non Acid Fast
* Non capsulated
* Harbour glucose in metachromatic/volutin granules.
* Stained by

Cultural char

* Grown on Blood Agar & produce similar colonies of C. pseudotuberculosis.

Biochem

* catalase +ve
* urease +ve
* Produce renalin which is like a CAMP factor
* Virulence Factors – Adhesins by Pili/Fimbriae.
  Urease enzyme
  Cell wall
* susceptable to Heat

Scanned with CamScanner

Pathogenesis

Transmission

- Harbour urogenital tract.
- enters urinary bladder & through ureters reaches kidney - when animal is in stress (during dry period).

~~causing cystitis, ureteritis, Nephritis.~~

Clinical signs

* exert pain, cystitis, urethritis, Pilonephritis
* Blood observed in urine
* red pH of urine towards alkaline.
* History of Animal - stress.

In case of small ruminants c.renale - Pizzle rot
↓
Pizzle rot

* Incidence is more when animal is fed with high legume diet.
* Necro

Diagnosis

* By observation of clinical signs. (Treatment by Pencillins).

C.suis

* Anaerobic
* Associated with urinary tract infections in Boars, Pregnant sows leading to Pilonephritis.

Trueperella pyogenes

* old name - Arcanobacterium pyogenes
↓
old name - corynebacterium pyogenes

Classification

order - Actinomycetale

Family - Actinomycetaceae

Genus - Trueperella

Scanned with CamScanner

Trupello pyogenus

* Gram +ve
* Non spore former, Non capsulated, Non motile
* No volutin granules
* 0.5 x 2 μm size
* cell wall consists of rhamnose, glucose, lysine.
  No mycolic acid
* exotoxins released are Pyolysin o (PLO)
  ↓
  For pore formation on phagocytic cells.
  – several proteins have proteolytic activity.

Cultural characteristics

* Facultative Anaerobic
* Grows at 37°c
* On Blood Agar – Haemolytic colonies

Biochemical tests

* catalase –ve
* Digest proteins like Gelatin, casein,
* Resistant to physical & chemical agents
* susceptable to Drying, Heat, common chemicals used in farm.

Pathogenesis

* Transmission – By flies
* Involves other non spore forming Anaerobics like Peptostreptococcus, Fusobacterium.
* Releases PLO, Damage to area, leading to Abscess formation.
* In Bovines – summer Mastitis (↑ Flies population in dry cows).
  ↓
  Fistulous tracts in udder.
  Pus oozes from Fistulous tracts.
* lesions – Pyogranulomas, Abscess.
* lesions throughout body when progressive summer mastitis is seen in Bovines.

Diagnosis

* cultural characteristics
* staining

Treatment & control

Vaccine

PLO Toxoid.

Scanned with CamScanner

Rhodococcus equi

* Old name – corynebacterium equi
* Classification
  - Kingdom – Bacteria
  - Phylum – Actinobacteria
  - Order – Actinomycetale
  - Suborder – corynebacteriae
  - Family – Nocardicaea
  - Genus – Rhodococcus
  - Species – equi
* Morphology
* Pleomorphic
* 1–5 um in diameter
* cocci shape generally, Not uniform cocci
* Appear as watermelon seeds during
* Non motile, Non spore former, capsulated.
* Gram +ve
* Virulence Factors
  - capsule – no protection from phagocytosis.
  - cell wall – pro inflammatory
  - virulence Associated proteins (7)
* consists of Phospholipase C + Phospholipase D } sphingomyelinases – Enhances Haemolysis.

(C. pseudotuberculosis)

Cultural characteristics

* Aerobic
* Multiplies at wide temp.
* Mucoid colonies (Appear as spit like)
* 70 mm Diameter – colony
* colony acquires pink pigmentation.

Biochemical tests

* Catalase +ve
* urease +ve
* Nitrate +ve
* No results for fermentation test

Scanned with CamScanner

Resistance

* Resists 5% H_2SO_4
* Resistant to β-lactum group of Antibiotics & susceptable to other groups.

Pathogenesis

* i disease of equines – pneumonia.
* Transmits by Inhalation, Ingestion, congenitally via umbilicus / mucous membrane exposure during parturition.
* Inhibits CD_4 / CD_8 cells

↓

↓ γ-interferon prod^n

↓

↓ in Activity of Alveolar macrophages

* very severe in animal below 6 mts.

Clinical Signs

* In equines – pneumonia

Suppurative Broncho pneumonia

large Abscesses

– Abscesses (organism reaches mesenteric lymphnodes through M cells)

* In swine

Diagnosis

* collection of samples
* cultural characteristics.
* PCR

Treatment & control

* Prognosis of disease is guarded if animal is 2 mts old.
* large Abscesses in Thoracic radiography
* Involvement of Hilar lymphnode

Prevention

* colostrum intake.
* Remove animals from contaminated area

Scanned with CamScanner

17/9/19

Dermatophilus

* Belongs to Group 83 & consists of genera with multilocular sporangia.
* Gram +ve, Filamentous, motile, cocoid, consists of zoospores.
* zoospores germinate & produce germtube.
* Because of this germtube appears Filamentous.
* cell wall consists of mesodiamino

Cultural characteristics

* on Blood agar, organism shows hemolytic colonies which appear mucoid to waxy, wrinkled colonies, whitish to greyish yellow

Pathogenesis

* Transmission is by direct contact / indirectly by flies.
* Soaking of trauma with wet → organism reaches deeper cells & germinates resulting in inflammatory lesions.
* Appears in epidermal layers.
* Dermatophilosis / Strepto — Ruminants
* Rain rot — Horses — Greasy Heal / Mud Fever
* Lumpy wool — sheep — Strawberry Footrot

Diagnosis

* By Gram staining
* observation of zoospores, cultural characters

Treatment & control

* self limiting disease if necessary penicillins, Tetracyclins

Streptomyces

* Filamentous, 0.5 μ in width.
* Gram +ve
* saprophytic
* leads to mycetomas (Abscess formation in diff. affected areas)

Antibiotics

S. griseus — streptomycin
S. venezuelae — chloramphenicol.
S. clavuligerus — Clavulanic acid
S. lincolnensis — Lincomycin
S. fradiae — Neomycin

Scanned with CamScanner

S. avermitilis – Ivermectin

S. aureofaciens & S. rimosus – Tetracycline

Antiparasitic drug.

BACILLUS

* Gram +ve, spore formers, Aerobic, Rod shaped
* Domain – Bacteria
  Phylum – Firmicutes
  Class – Bacilli
  Order – Bacillales
  Family – Bacillaceae
  Genus – Bacillus
  Species – anthracis
* Mostly saprophytic
* Post mortem invaders / Anthracoids.
* Bacillus anthracis is the one that shows pathogenesis among Bacillus spp.
* Bacillus ~~cereus~~ cereus shows food poisoning.

Differences

Characteristics	B. anthracis	Anthracoids
Motility	–	+
Hemolysis	Negligible	+++
Reduction of methylene blue	+	+++
Fermentation of salicin	–	+++
Growth at 45°C	–	+
Mucoid colonies on bicarbonate agar	+	–
Susceptable to γ phage	+	+

Anthrax

* Causative agent – Bacillus anthracis
* Gram +ve; 1–3 × 5 μm in size
* Square cut ends
* Appear as short, segmented.

Scanned with CamScanner

* Bamboo stick / Tram-truck Appearance
* Sporulated, but spore donot bulge vegetative cell.
* Virulence factors → capsule (protein – poly D Glutamic Acid)
Prevents phagocytosis ; protection.
→ cell wall – pro inflammatory
→ S layer – in b/w capsule & cell wall
– succumb bacteria to complement component
→ Toxins – encoded by Plasmids i.e PxO_1.
– PxO_1 encodes for lethal factor, edema factor, protective Antigen
– PxO_2 encodes for capsule.
– Role of protective Antigen in pathogenesis is it carries lethal/edema factor into Host cell. In order to carry these factor protective antigen is cleaved by ~~Fixed~~ furins present on cell surface.
– A lethal factor is an metalloprotease & causes death to cell.
– Edema factor is calmodulin dependent adenyl cyclase & is responsible for Edema of affected cells.

* Cultural characteristics
– under aerobic conditions organism forms spores & on nutrient agar organism produces dull, rough spores colonies with varied margin & on close observation appears as Medusae head.
– on Blood Agar, Non Hemolytic colonies are produced which are rough.
– Colonies on serum agar incubated at 5–20% CO_2 containing 0.7% $NaHCO_3$ it produces mucoid colonies. → capsule
– Polymyxin Lysozyme EDTA and Thallous acetate (PLET) medium is used to culture medium from contaminated sample.
– Inverted Fell tree like colonies on lab culture.

* Pathogenesis
– Mode of Transmission by contact, ingestion, inhalation, and directly by insects.
– After entry into host, starts germination within 2–4 hrs, reaches local lymphnodes, then disseminates to other predilection sites, finally results in Acute renal failure, shock, terminal anoxia

* Source of Infection
– contaminated soil, water, carcasses

* Role of enht. in Anthrax endemic areas

- Alkaline soils, soils where tilling is not common practice - supports spore survivability for long duration.
- Heavy rains followed by drought,
  Heavy rains followed by severe climatic conditions (High temp.) make organism available to animals.
- Endemic regions of Anthrax - Anthrax belts/zones.

* Clinical signs

- In cattle organism shows peracute, acute, subacute form of infection.
- In peracute, Animal dies without showing clinical signs.
- Fever, mucosal congestion, terminal convergence, collapse, death - During
- Immediate bloat after death, oozing of tarry coloured blood from natural orifices are observed.
- In acute, fever (1-3 days), depression, Rapid & deep resp., fed Heart rate, bloat, diarrhoea, fed milk prod^n, dysentry.
- In subacute, edema under neck, brisket, Thorax, Jugular pulse.
  * Pregnant animals may abort.
- In Pigs, Resistant to Anthrax compared to cattle
  Acute, subacute to chronic
  Main sign - Edema of Throat, ~~Face~~ Head. Anthrax
  Pulmonary form/Pneumonia - Piglets.
- In Horses, acute, subacute, local forms
  - High rising temp., colic, Blood in faeces, subcutaneous swelling around throat, lower neck, floor of Abdomen.
  - Transmitted by insects
- In Humans, pulmonary, Gastrointestinal (GIT), skin forms.
- Skin forms -;
- Pulmonary form - wool sorters disease
- Highly zoonotic

* Necropsy

- No PM, No rigor mortis, No clotting
- Saw Horse
- Dark blood
- Spleenomegaly (Enlarged spleen) - Black berry jam

Scanned with CamScanner

- Immediate bloat.

*Diagnosis

*Samples to be collected.

* Blood from ear vein

* A piece of muzzle

* If PM changes appeared in carcass, collect soil samples beneath animal at site of anus.

* collect spleen/lymphnodes if PM is done. ✓

- Samples

- Bacteriology: unopened carcass - Blood/Edema fluid
  opened carcass - Direct smears

- Histology

- Staining of smears (capsule - pink
  organism - blue). by polychrome methylene blue

- Cultural characters ✓

- Isolation ✓

*Serology

- Ascoli's test

Precipitation test to identify Thermostable antigen.

- Immunofluorescene test: For capsule

- ELISA: PA antigen

Scanned with CamScanner

18/9/19

Polysaccharide & NA from Zenkowsky strain (or) STI of B.anthracis

Differential

1) Lightening stroke
2) peracute blacking
3) Malignant edema
4) Hypomagnesemic Tetany
5) peracute babesiosis.

Treatment

Pencillin – 20,000 U/kg B.wt.
OTC – 5mg
Streptomycin 8-10g daily in 2 doses
Antiserum – 100-250ml daily for 5 days

Control:

NO PM, Burry 2mt deep with CaO
Quarantine the farm
Destruction of cadavers
Vaccination of
Reduced Human exposure
High acting of pencillin.
Disinfection with 5% lysol, 5-10% NaOH, 3% peracetic acid
Formaldehyde – 10% clothes.

Immunization.

Panteur type vaccine – PxO ; – O e & PxO2 too has 3% mortality
Stern strain – attenuated vaccine strain @ 10×10^6
Culturable spores in cattle & horms 1 % – 5×10^6.
In sheep, goat, pigs in a volume of 1×10^7
* Strain 55 also be used (live spore vaccine)
Ring vaccination
Vaccinated animal prohibit from slaughter after 45 days & discard milk after 72 hrs.

In humans

Bioanthrax vaccine – CC" lysate vaccine
3 injections at 2 weeks interval.

Scanned with CamScanner

... 1 mm in diameter, irregular edges.

Hemolytic

Spore bulges vegetative cells.

Clostridium

Group 18 of Bergey's manual.

Anaerobic spore forming organisms.

K – Bacteria

Phylum – Firmicutes (Gram +ve)

Class – Clostridia

O – Clostridiales

Family – Clostridiaceae

G – Clostridium

General – obligate Anaerobes

All sps. form endospores & have fermentation type of metabolism.

Rod shaped, 2–5 mm, location of spore varies, like terminal, subterminal, central

Motile like by peritrichous Flagella, exception to motility in Clostridium perfringens

Majority are Non capsulated except C. difficile & C. perfringens.

Virulent

cell wall & its components play a little role.

Exotoxins released by bacteria plays imp. role in virulence.

Cultivation

Org. require Anaerobic conditions which in produced in lab by anaerobic jar & chambers.

Blood Agar – commonly used, Enriched Media for growth.

Inorder to test lecithinase activity, one can use egg yolk agar, those org. produce lecithinase enzyme show opalescence on egg yolk, Agar.

Saccharolytic – C. welchii, C. septicum, C. chauvoei

↓

Perfringens – Changed cooked meat particles medium into pink colour.

Proteolytic – C. tetani, C. botulinum, C. sporogenes, C. histolyticum.

– change cooked meat particles medium into black colour.

Diseases

C. Botulinum – Botulism

C. tetani – tetanus Neurotoxic, rod shape (2–4 μm, 0.5 m(w))

C. difficile – Antibiotic / stress associated disease.

C. Hemolyticum – Bacillary Hemoglobinuria

C. novyi – Big Head in sheep, Gas Gangrene

C. perfringens – type A, B, C, D, E

C. pilformae – tyzzer's disease.

C. septicum – Malignant edema in cattle

Pathogenic clostridium sps

- Neurotoxic clostridia: C. tetani, C. Botulinum
- Histotoxic Clostridia: C. chauvoei, C. Septicum, C. Novi type A, Perfringens A, Haemolyticum
- Entero Pathogen: Perfringens (A-E)

Other: C. Nitiformes, Spiroformes, colinum.

Scanned with CamScanner

Tetanus : org. appear drumstick (or) tennis rocket shape.

Gram +ve, Non capsulated

Flagellar antigen, somatic Antigen — Antigens present in Toxins

Toxins responsible for disease is ~~tetanolphasmin~~ 1) Tetanospasmin

C. tetani ⇒ ~~Tetanospasmin~~ → continous stimulation

~~Synapses — Inhibitory transmitter release (excitatory transmission)~~

* Dichain toxin, an endopeptidase encoded by plasmid & highly potent neurotoxin, 0.1 to 3 ~~nanogram~~ ng/kg is enough to kill human.

* ~~sufficient to kill human~~, unstable in liquid & resist autoclave ~~at~~ in solid state. (↓ Temp.)

* Mode of action — ~~Inhibiting stimulus by inhibitors.~~ Inhibits inhibitory NT's like GABA, glycine & causes continous contraction of muscles due to release of Acetylcholine (Ach)

(5) Tetanolysin ~~toxin~~ Pore forming and cell lysin & shows hemolysis on Blood Agar.

* Cleavage of Dichain is required for action of toxin.

Cultural characters strict anaerobe & it requires RCM (or) thioglycolate broth for initialization, RCM broth shows proteolytic activity & turns meat particles into black particles. (Robertson cooked media.)

* Org. shows rhizoid like ~~particle~~ colonies with hemolysis on Blood Agar.

— Sluggishly motile, (catalase -ve, oxidase -ve) — Biochemical characters.

colonies surrounded by zone of hemolysin (tetanolysin).

Pathogenesis — Sporadic disease

* Mode of T — Deep seated wound mainly by rusted nail and contaminated objects.

* Tetanus causes spastic paralysis.

Clinical signs

Ascending tetanus — takes place through deep seated wounds.

Descending tetanus — Through ingestion

Damage to ventral ~~cord of SNS~~ horn of spinal cord.

1. Fever
2. Docking, shearing history
3. Deep seated wound history
4. prolapse of 3rd eyelid (or) nictitating membrane.
5. lockjaw condition
6. Stiffness of body ✓
7. Minor air — causes severe muscle contractions due to mild irritation & severe pain occurs leads to failure of respiration & animal dies.

✓ Diagnosis * Correct samples.
* History of org.

Treatment & conditions : Pencillins, choice of antibiotic along with treatment one has to reduce control: one give muscle spasm giving rest & give antitoxin — 1500 units as preventive dose & therapeutic dose of antitoxin — 10,000 — 15000 units in case of large

Scanned with CamScanner

... 15,000 in case of sheep & Goat.

② Boturinum B'ts/2M Dosage of Antitoxins in sheep & Goat — twice in a week

A, B, F strains — Proteolytic } types (A–G) * under Anaerobic conditions org. produces toxins.

C, D, E strains — Non proteolytic

Saprophytic present anywhere * Generally org. is not endotoxin producing but in putrefied carcass (Hay/silage it produces endotoxin (A to G)

Endogenous (Host multiply in outside & only occur in cells)

Strict Anaerobic

1–μm in width ; 4–5mm in length.

It forms oval spores, usually appears — sub terminal ; Motile.

Virulent Factors

Toxins released by organism named as A to G.

Mode of Action of toxin — Inhibits release of acetylcholine & causes Flaccid Paralysis.

CO — botulinum → synapsis → Toxin block release of Ach form ---

Stimulation : Blocked.

Cultural characters

* Grown at 35°C * Strict Anaerobe * can be grown in RCM

Proteolytic * on Blood Agar shows hemolysis.

* colonies are Flat, irregular.

Disease lame disease in Animals ← "P" deficiency in Animal makes it to lick

Shakers foals syndrome — when animal takes toxin from carcasses & walls or contaminated Hay/silage

Floppy baby syndrome — In humans. → Type B.

Type D — boturinum in "P" deficiency

Type C — In Duck — shows the duck sickness.

	A	B	C	D	E	F	G
RCM B	B	B	P	P	P	B	
Gelatin liquefaction	+	+	—	—	—	—	
Lecithin prod^n	+	+	+	+	+	+	+
Glucose	AG	AG	AA	AG	AG		
Coagulated serum	+	+	+	+	+	+	+
Hemolysis on horns Blood Agar	+	+	+	+	+	+	+
Lactose Fermentation	—	—	—	—	—	—	—
Sucrose "		+	—	—			
Glycerol "		+	+	—			

Treatment
Toxin has to be neutralised by using Antitoxin
Ach synergestic drugs to induce contractions.
Use : To enhance beauty of person
Protect person from muscle twitching.

Diagnosis By observing clinical signs, cultural characters, molecular diagnosis, cultivating org., Boil sample for 60–80°C

Type E — spores require lysozyme for their germination.

Scanned with CamScanner

23/9/19 HISTOTOXIC CLOSTRIDIUM DISEASES

Clostridium Novyi

* Former name – Clostridium oedematiens
* Motile, Gram +ve, obligate anaerobe, rod shaped, Non capsulated
* virulence Factors are exotoxins (A)

α toxin – Necrotising lethal toxin
– Released by clostridium novyi A & B
– causes cell lysis hemolytic

β toxin – Necrotising lethal lecithinase toxin
– Released by clostridium novyi B

δ toxin – Novyilysin
– cytolysin

other toxins are η toxin (myosinase) & ζ toxin (Hemolysin), θ toxin (Lecithinase)

* Type A – Gas Gangrene in humans, Big Head in rams, wound infections in animals
* yellowish edematous fluid is observed; coma, death within 2 days
* Type B – Black disease in sheep, cattle

organism reaches liver through Digestive tract & settle in liver. → liver gets damaged by infection Necroses (or) ↓ O_2 in liver those areas favour sporulation & cause release of toxins.

Anorexia, Depression, Hypothermia

lesions – edema, liver necrosis

* Type C – Osteomyelitis in water buffaloes
* Diagnosis – demonstration of organism in smears

observation of clinical signs, History, PCR.

* control – Giving Antibiotics against Gram +ve organisms.

(*) Bacterin + Toxoid vaccines
↓
Killed bacteria used in preparation of vaccines
↓
Toxin in combination with HCHO; Inactivated form of toxin.

* Clostridium hemolyticum is responsible for Bacillary Haemoglobinuria becoz of toxins released by organism esp. β toxin which is hemolytic.
* Former name – Clostridium novyi Type D

Clostridium Chauvoei

* Gram +ve, sporulated, obligate anaerobe, citron shaped spores in centre/subterminal.
* Black leg
* α toxin – oxygen stable Hemolysin
– similar to pore forming toxins.
* β toxin – DNAase activity
* δ toxin – Hyaluronidase activity (to spread within tissues)

Scanned with CamScanner

*δ-toxin – Chauveolysin, similar to pore forming toxins.
*Neuraminidase (sialidase)

Pathogenesis

* Black leg is seen in highly fed animals
* 6 mts – 2yrs are susceptable to Black leg.
* In cattle pathogenesis is by ingestion & in sheep by trauma.
* organism reaches muscles & settles in muscles in cattle esp. Brachial muscles → Injury
* Further condition is Gangrenous myocytous (led by tension → sporulation → Release of toxins → Gangrenous myoctous)

Clinical signs

* lameness of animal
* Affected area gives crypitating sounds due to gas accumulation
* Skin appears dull at affected portion if disease progresses
* Depression
* Anorexia
* Ruminal stases
* Death occurs after 12–36 hrs esp. in cattle.

Sheep * stiff gait
* lameness
* crypitation of sounds
* Discolouration of skin
* High fever, Depression, Anorexia, Death occurs very – As disease progresses.

Horses * Pectoral edema
* Incoordination
* Stiff gait

Diagnosis

*Observation of impression smears
*conservation of organisms
* Flourescent Antibody technique, PCR

Control

* Bacterin as vaccine

Clostridium perfringens

*Non motile, very short generation time, capsulated, short & stumpy
* virulence factors – Adhesins, capsule (Avoid Phagocytosis)

Type	α	β	ε	ι
A	+	–	–	–
B	+	+	+	–
C	+	+	–	–
D	+	–	+	–

Scanned with CamScanner

E + — — +

* α toxin – Phospholipase C
  – sphingomyelinase
  – causes Hemolysis, Hot cold lysis.
* β – toxin – pore forming toxin
  – Damage to epithelial, endothelial, intestinal, Nerve cells.
  – Requires action of proteolytic enzymes for activation; prototoxin.
* ε toxin – permease
  – Affects cellular cytoskeleton, ↑ed permeability
  – prototoxin
* ι toxin – Ribosilates actin → results in disorganisation of host cell.
* Kappa toxin – collagenase } spread of infection in tissues.
* μ toxin – N acetyl galactosaminidase
* θ toxin – Similar to perfringolysin D (lyses of cells) Pore forming toxin, belongs to Enterotoxigenic group.
* Enterotoxin (sialidase) – released during spore germination in intestine.
  – Affects Apical cells of intestine; Electrolytic Imbalance. ✓

Pathogenesis

* Entry of organism through deep seated wound, multiplication, spread of organism by sialidase, collagenase, hyaluronidase, presence of capsule prevents phagocytosis, Necrotic cellular tissue, emphysema, myocytous infections.

* Enterotoxemia **

Type A

* For Hemorrhagic enterites – cattle, Horses.
  Necrotic enterites – poultry
  Food poisoning – humans ✓
  Diarrhoea – Dogs, cats ✓
* Due to α toxin, perfringolysin D ✓

Type B

* Lamb dysentry – lambs, calves, Foals ✓
* Due to β toxin.
* Gets activated by trypsin.
* Youngones not got colostrum sufficiently may succumb to this infection.
* Anorexia, depression, ~~Abd. pain~~, Incoordination, Diarrhoea – clinical signs.

Type C

* Causes enterotoxemia in neonatal calves. struck in older sheep
* Due to β toxin.

* These toxins ↑ permeability – get absorbed into systemic circulation → Damage to nerve cells.

Scanned with CamScanner

Type D

* In lambs - overeating / Pulpy kidney disease.

* when animal consumes more feed having more CHO's, it leads to bed intestinal motility & then more multiplication of organism, Release of toxins.

* Due to ε-toxin.

↓

causes damage to vasculature, & edema, Reaches CNS & leads to edema by ↑ing intracranial pressure leading to focal symmetrical encephalomalacia.

* ↑ed release of catecholamines

↓

Adenylcyclase activity

↓

Hyperglycemia, Glycosuria

↓

Death of Animal.

Diagnosis

* Clinical signs, History

* All of sudden, animals die.

* convulsions, Diarrhoea.

Type E

* causes hemorrhagic enteritis.
  ulcerative Abomasitis

Cultural characters

* obligate Anaerobe

* on Robertson cooked medium, saccharolytic organism.

* Pink discolouration of meat particles.

* on Litmus milk, Produces stormy clot formation

* on egg yolk Agar, Nagler reac^n (Becoz of presence of lecithinase enzyme organism produce opalescence).

* on Blood Agar, Hot cold lysis

* Determination of toxin - by giving culture supernatant to mice.

Diagnosis

* samples to be collected

* Intestinal loop during PM.

* observation of organism on Gram stained smears.

* observation of cultural characters

* PCR

* Demonstration of toxins

Scanned with CamScanner

Treatment & control

* Antibiotics
* Aquaculture vaccine (Immunisation)
  - Bacterin + Toxoid.

26/9/19

ENTEROBACTERIACEAE

* Belongs to group 5 of Bergey's Manual.
* Gram -ve, Facultative Anaerobic
* Character

Character	Enterobacteriaceae	Vibrionaceae	Pasteurellaceae
Motility	+	+	-
Motile by	Peritrichous Flagella	Polar Flagella	-
Oxidase	-	+	+

* Motile except Shigella, Klebsiella
* Non Fastidious
* Catalase +ve
* Oxidase -ve due to absence of cytochrome c.
* Lactose Fermenters - E.coli, Citrobacter, Klebsiella, Enterobacter.
* Nonlactose Fermenters - Salmonella, Proteus, Yersinia, Shigella

Virulence Factors

* K Antigen - Capsular Antigen
  O Antigen - Somatic Antigen
  H Antigen - Flagellar Antigen
  Fimbriae / pili
  LPS
  Siderophores - Molecules which could able to trap iron from host.

E.coli **

* Gram -ve
* 0.6 μ in width ; 2-3 μ in length.
* Peritrichous Flagella, Motile.
* Capsule may or may not produced by organism.
* Nonspore formers
* Common inhabitat of intestinal tract.
* Most of strains are less virulent.

Scanned with CamScanner

* Indicator for faecal contamination of water.

* $O_{157}:H_7$ is zoonotic pathogen & is responsible for Haemorrhagic colitis syndrome (HCS) & Hemolytic uremic syndrome (HUS).

Cultural Characters

* Non fastidious

* white coloured colonies on nutrient Agar.
  ↓
  Appear mucoid becoz of presence of capsule.

* Non Hemolytic & produce greyish, round shiny colonies on Blood Agar.

* on MacConkey Agar, pink coloured colonies are seen which indicates that it is a lactose fermenter.

* on Eosine Methylene blue (EMB) Agar, metallic sheen colonies

* on Brilliant Green Agar, yellowish green colonies

Indicator is phenol Red.

* on XLD Agar (Xylose lysine desoxycolate), yellow coloured colonies.

* urease -ve

* H_2S -ve

* IMVIC tests ++ --
  ↓ Indole
  Methyl Red

* In nutrient broth, organism produces turbidity.

Resistance

* susceptable to 60°c for 20 min
  50°c for 1 hr

* Able to survive for wks or months in water

* Fecal matter & dust in Animal houses.

Virulence Factors

* capsule - Made up of polysaccharide; Anti phagocytic.

* Endotoxin / LPS - proinflammatory ; pyrogen.

* Fimbriae / Pili - K88 / F_4 pili - receptors are present in pigs

* K99 / F_5 - receptors are present in sheep
  K99, F_{41} - receptors in calves.

* LT (Thermo labile toxin) - Adenylcyclase, causes Hypertension.
  ST (Thermo stable toxin) - Guanylcyclase, ↑ GMP

* verotoxin - similar to shiga toxin of shigella.
  - Causes damage to enterocytes, cell lyses

Scanned with CamScanner

* cytotoxic necrotizing Factors (CNF1, CNF2)
* siderophores
* α Hemolysin.

Enteric diseases

* ETEC – Entero toxogenic E.coli
  - Fimbriae, LT, ST
* EPEC/AEEC – Enteropathogenic E.coli / Attaching effacing E.coli.
  - Release shiga like toxins, damage enterocytes, villi, epithelial cells
  - Malabsorbtion, diarrhoea, dysentry
* VTEC/STEC – Release verotoxins.
  - Edema in pigs, Hemorrhagic colites, Hemolytic uremias
* NTEC – Release CNF1, CNF2
  - cause enteritis, Hemorrhagic colites, diarrhoea, dysentry
* Septicemia – when enter into blood circulation
  - Release endotoxins, cause Arthritis, meningitis, coli septicemia.

Non Enteric diseases

* uropathogenic E.coli – cause cystitis due to release of exotoxins, endotoxins
* Opportunistic E.coli – cause local infections
  Pyometra – pus in uterus
  Metritis
* Non enterotoxogenic diarrhoea – caused by entero haemorrhagic E.coli

Serotyping

* O Antigen – 146
* K Antigen – 91
* H Antigen – 49.

Diseases

* Horses – Joint ill, Navel ill, Sleepy foal disease
  - Salmonella, Actinomyces equuli, streptococcus
* cattle – coli bacillosis, coli septicemia, coli bacillary toxemia (post weaning enteritis).
  - white scours (or) calf scours
  - Diarrhoea (watery, Greyish white with unpleasant smell), Dehydration, weakness.
  - leads to coli septicemia

coli septicemia – organism releases endotoxin into circulation causing hyperthermia, depression, tachycardia, Meningitis, Pneumonia

Scanned with CamScanner

Contagiously toxemia - Toxins released are absorbed causing Toxemia.
- Animal appears weak, dull.

* Poultry - omphalitis (Mooshy chick / Mooshy yolk disease), Arthritis, Hajarres disease/ coli granuloma
↓
chronic inflammatory condition in Adult birds at time of laying.

* Pigs - oedema disease (Toxemia)
- edema of eyelids, face, laryngeal edema, Flacid paralysis, death within 36 hrs are signs. Fecal matter is firm.
- PM lesions contain edema of greater curvature of stomach, mesentry of colon.

* Coliform mastitis - Acute / peracute
watery mammary secretion, sudden swelling of udder.

Diagnosis
* Clinical signs
* Isolation of organism
* observation of cultural characters
* EPEC / AEEC - Urease +ve
* PCR, serotyping

Control
* Avoid stress factors
* Insufficient colostrum
* Build up farm free from E. coli
* Avoid overcrowding, poor hygiene
* vaccines - Killed vaccines but are of less use

Treatment
* Fluid Therapy, Antibiotic Therapy

Scanned with CamScanner

25/9/19 SALMONELLA **

* S. bongori, S. enterica - sps.
  ↓
  S. enterica subsp enterica
  ↓
  S. enterica subsps enterica Typhimurium.

* 1st observed by Eberth
  cultured by Gaffkey

* Bacillus Cholerasuis - Old name of salmonella
  - By salmon & smith.

* ubiquitous

* S. Typhi, S. paratyphi A & C - Man
  S. Abortusequi - Horses
  S. Dublin - Cattle
  S. Abortussuis - Pig

* Gram (-ve, measuring about 0.5 x 2-4 μ in st.

* Motile with peritrichous flagella except S. Gallinarum & S. pullorum

Cultural characters * Facultative Anaerobes

* Tetrathionate broth
  Rappaport medium } selective Broths
  Selenite F broth

Biochemical characters
catalase +ve
oxidase -ve.
urease -ve
H_2S +ve
Nitrate +ve

* Forms turbidity, No pellicle formation - In Broth

* Multiply & produce smooth, round colonies.

* lactose Non fermenters, produce white colonies - on MacConkey Agar.

* Pink coloured colonies - on Brilliant green Agar

* Reddish coloured colonies with black centre - on XLD Agar

* IMVIC results - + - +
  * citrate

* Deoxycolate citrate Agar inhibits multiplication of other Gram (-ve) organisms except salmonella.

* Red coloured slant - on Triple sugar Iron Agar.
  Yellow coloured bed H_2S +ve.

* Nitrate reduction test - (+ve)

* Do not ferment lactose, sucrose, salicin mannose,
  Ferment Glucose, Mannitol, Dextrin, Dulcitol & produces acid

* Urease (-ve).

* Gelatin gets liquified by this organism.

Scanned with CamScanner

Virulence Factors

* LPS
* Flagellar Antigen (H)
* Adhesins (Pef, Agf, Lpf)
* Capsule : Role of capsule is uncertain becoz salmonella is intracellular.
* Enterotoxins - water & electrolyte imbalance
* Iron acquisition
* Salmolysin & virulence plasmids
* Effector & stress proteins
* Cell wall

Pef - Plasmid encoded fimbriae
Agf - Thin Aggregative fimbriae/curli
Lpf - Long polar fimbriae
↓
Helpful for org. to attach to M cells in Intestine.

Typing

* White & Kauffmann scheme based on O, H Antigens.
* O Antigen - divided into diff. groups
* H Antigen - diphasic
* Phase 1 by Alphabets
  Phase 2 by numbers
* S. Typhimurium - 1,4,5,12 : I : 1, 2
* Vi Antigen - Somatic Antigen

Resistance

- To physical & chemical Agents
- 55°C for 1hr
- 66°C for 20min
- Milk heated to 65°C for 1-2 sec
- Meat 65°C for 10-15min
- egg yolk 60°C for 30 sec

O_4 & O_{12} - B group
O_6 & O_7 - C_1
O_6 & O_8 - C_2

Craigie tube method } Phase Identification in salmonella.
Ditch plate Method

Diseases

* Horses - S. Abortusequi (Abortions), S. Typhimurium
* Cattle - S. Dublin, S. Typhimurium
* Pigs - S. Choleraesuis, S. Typhimurium
* Poultry - S. Pullorum, S. Gallinarum.

Mode of Transmission by Ingestion. sometimes by vertical transmission.
↓
S. pullorum.

* Pneumonia, testicular & joint infections, enteritis, pustulous feathers, occasionally by S. Abortusequi.
* If titer is > 1 in 1000 the animal is +ve for infection.
* Widal test - to diagnose S. typhi
* Diagnosis - Isolation
* H Antigen Agglutination test
* resp. for pneumonia, septicaemia.

Scanned with CamScanner

* S. Dublin – Host specific organisms
* Infected adults acts as symptomless carriers. Play main role in spread of infection
* Poor nutrition, intercurrent infections, transport stress predispose occurence of S. dublin
* osteomyelitis, Joint ill, terminal dry gangrene, Abortions, Meningitis, septicemia, Enteritis – symptoms.
* S. typhi (cattle) – Enteritis, septicemia.
* lesions – Necrosis of LI, Blood stained intestinal contents, edematous wall of Gall bladder, Liver Necrosis, pneumonic areas in lungs.
* calves may have haemorrhages on myocardium & peritoneum.

→ Diagnosis: Titer 1 in 60 (or) 1 in 30
↓ O Antigen ↓ H Antigen.

* S. Abortusovis } – Abortions
S. typhi } sheep – Enteritis, septicaemia

* S. choleraesuis – Acute Fulminating septicaemia.
  – Symptoms similar to Hog cholera org. so, Also called Hog cholera Bacilli.
  – Disease also called paratyphoid.
  – High Fever, red discolouration of skin, weakness

2M/BIS

* S. pullorum – For pullorum disease, bacillary white diarrhoea in chicks.
* Adult birds carry this org. & laying eggs carry salmonella in their yolk.
* In less acute, chicks appear sleepy, incoordination of limbs, Resp. distress, vents covered with Adherent mass, huddle together, Nervousness, staggering.
* Growing stock shows low mortality rate, slow growth rate, Intermittent diarrhoea.
* lesions – yellow colored liver with streaks of haemorrhages, enlarged spleen, Pale areas on myocardium, caeca may be distended with caseous material.
* Characteristic lesions of ovary (misshapened ovules, observation of ovules in Abd. cavity), Peduncuated ovules), congested liver, spleen is enlarged & congested.
* 95% Mortality.

Diagnosis
* Crystal violet plate Agglutination test
* Tube Agglutination test
* If titer is > 1 in 25, very difficult to isolate
* S. Gallinarum – Fowl typhoid
* Young ones – very difficult to differentiate from S. pullorum
* Green diarrhoea, restlessness – young chicks.
* lesions – congested liver with reddish brown coloured appearance called as Bronze discolouration of liver.

Scanned with CamScanner

Klebsiella Bits

* Friedlander's bacilli - other name
* saprophyte
* Rod shaped, 0.5 x 1-3 μm size
* contains well designated polysaccharide capsule.
* Non spore former, Non motile

Cultural characters

* 37°c for multiplication
* Mucoid colonies becoz of presence of capsule.
* Pink colonies - MacConkey Agar
* Non hemolytic colonies - Blood Agar
* Yellow green - Brilliant green Agar -
* Yellow colonies - XLD Agar -
* urease +ve, H2S -ve
* Yellow, Yellow -ve - TSI Agar -
* IMVIC - - ++
* produce opportunistic infections.
* very high Antibiotic resistance

Diseases

Pneumonia, Metritis, Mastitis, Air sac infection, urinary tract infection - 2° infections

Diagnosis

By isolation:

observation of biochemical properties

PCR

Scanned with CamScanner

30/9/19

PROTEUS BITS

* P. vulgaris, P. mirabilis, P. rettgeri, P. morganii – Diff. sps.

Morphology

* Gram –ve Pleomorphic Rod shaped. Becoz of pleomorphism it appears in coccal forms/ Filaments of 10–12 μ in lt.
* Have peritrichous Flagella
* Non spore forming, Non capsulated, opportunistic pathogen.

Resistance to Habitat

* Present in Intestinal tract of man, Animals, sewage, Animal manure, soil, meat, egg also in wild animals
* Killed at 55°c for 1 hr
  66°c for 30 min
* easily destroyed by Autoclaving.
* susceptable to phenol, Quarternary Ammonium compounds, cresol (Disinfectants)
* O and H Antigens

Cultural characters

* Aerobic
* Grows in range of 20–40°c, optimum 37°c.
* spreads like thin film; Grows like waves (wave like phenomena called swarming) & culture is ring like due to wavy growth – solid media.
* If media contains 6% Agar or sodium Azide swarming is not produced.
* Turns Blood Agar to Brown, very foul smelling; swarming is also seen on Blood Agar.

Biochemical characters

* Non lactose Fermenter
* Pale/white colour on MacConkey Agar.
* variable (vulgaris +ve, mirabilis –ve) – Indole test
* Citrate –ve
* Urease +ve.
* H_2S is produced – Triple sugar iron Agar. (0.1% Glucose, 1% lactose & sucrose).

  Red slant
  Yellow butt – mirabilis.

  Proteus vulgaris – Yellow, Yellow, H_2S +ve.

* Urease test, Lysine decarboxylase broth – To differentiate Proteus from salmonella

  ↓

  Purple – salmonella; Yellow – Proteus (–ve)

* +ve to PPA (Phenylpyruvic Acid) which is produced from Phenyl Alanine.

Scanned with CamScanner

infections
Diarrhoea, Dysentery
Otitis externa in dogs
Urinary tract infections in dogs, Horses

YERSINIA **

* Y. pseudotuberculosis, Y. enterocolitica, Y. pestis, Y. ruckeri – Diff. sps.
* Y. ruckeri is Fish pathogen (affects fish causing Enteric Red mouth / peri oral Haemorrhagic inflammation).
* Mesenteric lymphadenitis – by Y. pseudotuberculosis, Y. enterocolitica
* Y. pseudotuberculoses – sheep, Goat, cattle, Buffalo, Pig, Farmed Deer – enteritis in young Animals
  subclinical Infection in Adults
  cattle, sheep, Goat – sporadic Abortions
  Birds, laboratory rodents – septicemic yersiniosis / pseudotuberculosis
  Humans – enterocolitis, Mesenteric lymphadenitis
* Y. enterocolitica – Pigs, other domestic Animals – enteritis in young Animals
  subclinical infection in Adults.
  Ewes – sporadic (only few cases) Abortions
  Humans – Gastroenterocolitis, Mesenteric lymphadenitis / pseudoappendicitis
* Y. pestis – Bubonic & pneumonic plague – Humans
  Sylvatic plague – Rodents
  Feline plague – cats

Cultural
* Gram –ve, short ovoid rod shaped
* Non motile – Y. pestis
  Y. pseudotuberculosis & Y. enterocolitica exhibit motility at 22°c but not at 37°c.
* Bipolar / safety pin like – From fresh tissues.
* Non spore Forming.
* capsule is seen in smears prepared from tissues.
* Grow at 5–48°c.
  Growth is less rapid when compared to other members of Enterobacteriaceae. (72 hrs)
* Survive cool conditions.
* 21 serotypes with in Y. pseudotuberculosis
  Type 1 – Type 5 cause disease in Animal, Man.
* 5 biotypes, 60 serotypes but isolates with somatic antigen 2, 3, 5, 8, 9 produce clinical infection – Y. enterocolitica.
  serotype O:9 in swine – shares some of Antigens with Brucella.

Scanned with CamScanner

* pseudotuberculosis, Pestis are genetically related.

Pathogenesis

* Intracellular organisms.
* enter mucosa with the help of M cells in peyers patches.
* These bacteria produce Adhesion proteins called invasins which bind to receptors on M cells.
* After invading mucosa, bacteria are engulfed by macrophages
* Finally reach to mesenteric lymphnodes with the help of macrophages producing necrotic lesions.
* Y. Pestis - More virulent, more invasive than enterocolitica, pseudotuberculosis.

Virulence Factors

* plasminogen Activators - Helps in dissemination of bacteria from site of infection.
* Fraction I protein - Antiphagocytic to macrophages, neutrophils
  - Helps in systemic spread.
* Endotoxin.

Clinical infection

* wild & domestic animals serve as reservoirs for enterocolitica
* Pig is natural reservoir of serotype O;3, Biotype 4
* In enteric yersiniosis - stress, poor nutrition, winter season, transportation, cold & wet conditions, weaning.

Symptoms - profuse watery diarrhoea.
Blood tinged Diarrhoea
Fatal if untreated.

PM lesions

* Accumulation of water in lumen of SI, LI
* Mucosa is Hyperemic
* Mucosal ulceration in severe cases.
* Enlarged, edematous mesenteric lymphnodes
* Pale necrotic foci in liver.

* Septicemic yersiniosis

Die suddenly in Acute cases

Symptoms Ruffled Feathers
Restlessness
Death.

PM lesions
Pin pointing Necrosis on liver.

Scanned with CamScanner

Lab Animals

Acquire infection by faecal contamination by wild rodents

Acute – septicemia, Death

Chronic – Diarrhoea, Gradual wt. loss, Emaciation, Death

– Infection gets localised in mesenteric lymphnodes.

PM lesions

* caseous Necrosis, No calcification – Mesenteric lymphnodes.
* white necrotic foci on liver

Feline Plague

* Acquire infection through ingestion of infected rodents
* Bubonic, pneumonic, septicemic
* Most common form, characterised by enlargement of Buboes/lymphnodes.
* Associated with lymphatic drainage from site of infection.
* Fever, Depression, Anorexia, Affected lymphode rupture, Discharges, serosanguinous Fluid/pus.
* Pneumonic – spread infection to Humans through Aerosols.
  – xenopsyllacheopsis/Rat flea – Route from cat to Human.
  – Through cat scratches & Bites.

Diagnosis

* History
* Isolation & Identification.
* Samples from Affected tissues must be placed directly onto solid medium like MacConkey Agar (37°c incubation for 72 hrs)

Y. enterocolitica – selective Agar is CIN

– Faecal samples are directly placed on CIN Agar

– cold Enrichment method

Prepare 5% faeces in PBS held at 4°c for 3wks

weekly subculture onto MacConkey Agar

* Samples like pus, Blood, lymphnode Aspirates – Plague
* Bipolar Appearance of organism.
* PCR
* Serological tests like ELISA to detect Ab's

Passive Haeme Agglutination test for plague.

– Antigen is Fraction I A Antigen, test with paired sera sample (one sample to be collected when animal exhibits symptoms & another sample 3–4 wks apart)

Scanned with CamScanner

9/10/19 Erysipelothrix rhusiopathiae

* Belongs to Group 19 of Bergey's Manual.
* Gram +ve, Non motile, Non Acid Fast, Non spore forming, slender rods.
* under chronic conditions Appears Filamentous.
* virulence Factors – cell wall – Proinflammatory
  Muraminidase – enzyme which forms pores on cell surface
  cleaves sialic acid ; damage to endothelium.
* A B C D E F G & N – As per older classification.
* Basing on Heat stable somatic antigen, organism divides into 23 serotypes – As per new classification.

Cultural Characters

* Microaerophilic ; Growth temp. → 32–37°c ; pH – 7.6
* Growth is enhanced by Presence of Blood/serum in Agar.
* show narrow zone of partial Hemolysis – on Broth Agar
* show bottle brush growth on Gelatin stab culture
* smooth forms – Turbidity } on Broth culture
  Rough forms – Filamentous
* Ferments lactose etc.
* catalase, oxidase – ve
* on TSI agar, Produce acid, turns medium to yellow, H_2S +ve.
* Do not utilise Aesculin, Nitrate, urea, Indole.
* use the media as selective media by using Antibiotics

Resistance

* Resists drying, Alcohol, H_2O_2, cresol, Phenol.
* withstand smoking,
* survive in putrified media for mts. together.
* susceptable to caustic soda, hypochloride sol^n.
* Moist Heat 55°c for 10 min Kill.

Pathogenesis

* Disease of Pigs, Turkey, sheep. (< 3mts – 3yr old pigs)
* source of Infection – either infected Animal secretions – vomit, urine, Faeces.
  Carrier Animal – urine, Faeces
* Mode of Transmission
  Ingestion – Pigs
  Arthropod bite
  wound – sheep.
* contains Antiphagocytic capsule, cell wall, Muramenidase.

Scanned with CamScanner

* Appears in cutaneous, Acute, chronic forms.
* In pigs cutaneous form is through subclinical infections.
* Acute cases – Fever, Anorexia, prostration, conjunctivitis, pink/purplish discolouration of skin esp. ears, Abdomen, thighs, tail, vomition, Death after 3/4 days
* Chronic form – vegetative endocarditis – cauliflower like growth on Bicuspid valve.
  – pathogenesis is embolic Formation.
  – Severe Arthritis
  – Ankylosis of Joints – Due to inflammation of synovial membrane
    Animals exhibit pain during walking.

* Zoonotic pathogen
* In Turkeys synosis – Acute
  Vegetative endocarditis – chronic
* In sheep polyarthritis

Laboratory Diagnosis
* samples to be collected
  Blood
  PM samples like liver, kidney, Heart, synovial tissue.
* examination of impression smears
* culture
* serology

Treatment & control
* penicillins
  Tetracyclins
* Killed vaccines (Formalin killed Al hydroxide Adjuvated vaccine), Attenuated vaccines
* vaccinate breeding stocks regularly twice a year.
* Fattening pigs should be vaccinated after weaning.
* Maintainence of Hygiene.

Listeria monocytogenes
* Group 19.
* Gram +ve, Non capsulated, Motile, Non spore former, Rod shaped.
* Motility – Good at 22°c
  – Not Good at 37°c
* Young cultures appear as palisades, shows similar structures with corynebacterium
  Rough forms shows long rods of 5–7 μ

* Zoonotic pathogen
* Virulence Factors – Act A (protein responsible for cell to cell transmission
  cell tropism
  Actin polymerisation)

Scanned with CamScanner

cell wall – Lipotechoic Acid, Peptedoglycan } pro-inflammatory

Hemolysin / Listeriolysin O – pore forming cholesterol dependent cytolysin
– Most Active in Acidifying envt., in 2 vesicles
– Apoptin for Hepatocytes.

Phospholipase C – cause membrane lyses.

Bile salt Hydrolase – Helps org. to survive in intestinal envt.

Growth & cultural characters

* Facultative Anaerobes
* God Growth in CO_2 tension (capnoic)
* serum, blood, Glucose, liver extract enhances growth.
* Bluish Green, 1mm – smooth colonies; longer – Rough colonies.
* Narrow zone of complete hemolysis
* on semisolid media – umbrella like growth
* shows CAMP test with S. aureus – L. monocytogenes
  Rhodococcus equi – L. ivanovii

catalase +ve
oxidase –ve.

* –ve for Gelatin liquefaction, Nitrate, urea, Indole.
* produce acid during fermentation of lactose, Maltose etc.
* Lithium chloride selective media for isolation
  Moxalactum
  PALCAM Agar (Polymixin Acrifiavin Lithium chloride ceftazidim Aescolin Mannitol Agar)
  ↓ Black colonies
* cold enrichment for isolation
* Basing on O somatic, H Flagellar Antigens there are 16 types

Pathogenesis

* present world wide, show clinical signs in temperate conditions.
* By Ingestion of contaminated feed, soil contamination, by silage.
* If silage pH > 5.5 favours survivability of org. so also called silage disease.

Clinical signs

* In Monogastric Animals – Septicemia, Depression, Fever, Inappetence, Death.
* In Ruminants – encephalitis, Depression, Anorexia, unilateral Facial Paralysis,
  circling disease ← Animal circles in 1 direction, Head Pressing, Ataxia,
  Animal trembles & fall down (Goats)
* Abortions, Macerated Fetus, Conjunctivites ✓
* Humans – Infective endocarditis, Dermatites

Lesions

* CSF Turbidity

Scanned with CamScanner

* * Medulla oblongata shows signs.
* * Miliary disseminated, pyo granulomas - Fetus.
* Diagnosis
* Identification by cultural characters. Kits available for Identification of listeria
* Samples to be collected (CSF, Blood, Brain, Liver, spleen, Ab fluid, mucoserum)
* PCR

Treatment & control

* Penicillins, Erythromycin, Tetracycline, Ampicillins
* Vaccination - Not successful

eatment & control

Fluid Replacement therapy
Broad spectrum Antibiotics } enteritis

vaccine : Y. pseudotuberculosis - Formalin killed - 1, 2, 3 serotypes.

ro, 2 doses 3 wks apart

eventing contamination of food & water from faeces of wild rodents, animals.

opulation, Disinfection, Restocking - In case of lab animals

clusion of wild Rodents

oration of cats in case of plague.

her rodent control measures

Bubonic form - treated with chloramphenicol & tetracycline.

Scanned with CamScanner

Mycobacterium

K – Bacteria
P – Actinobacteria
O – Actinomycetales
SO – Corynebacterineae
F – Mycobacteriaceae
G – Mycobacterium

* Tubercle Bacilli, Lepra bacilli, Mycobacteria causing skin ulcers, Johne's bacillus,
  - Humans – M. tuberculosis
  - Bovine – M. bovis
  - Murine – M. microti
  - Avian – M. avium
  - Human – M. leprae
  - Rat – M. lepraemurium → Mole Bacilli
  - M. ulcerans
  - M. Belnei
  - M. avium ssp. Paratuberculosis (MAP)

M. avium complex (M. avium, M. intracellulare) → Battey bacilli

Saprophytic mycobacteria, Atypical mycobacteria.
- M. butyricum
- M. phlei – used for prod^n of mycobactins
- M. smegmatis

Atypical mycobacteria → Rapid growers, Photochromogens, Scotochromogens, Non photochromogens.

Mycobactins
- Iron chelating Agents
- helpful in inducing t cell activation
  Grabbing in iron from host.

Morphology

* Gram +ve
* Acid Fast in nature
* Require Ziehl-Neelsen stain, Acid Fast staining,
* Non capsulated, Non spore formers, No flagella.
* Stained with flourescent staining using Auramine, Rhodamine (Flourescent dyes)
* Virulence Factors – Lipids present in cell wall – For Pathogenesis, Immunological Purposes.
  - Surface mycocytes like peptidoglycolipids, Glycolipids } survivability of org within macrophage
  - long chain branched mycolic Acid.
  - Keratenoid pigments – in Non tuberculosis bacteria.
  - Alkyl hydroperoxidase reductase – Resistance to superoxides & N_2 intermediates during Phagocytosis
  - Dimycolthrehalose / cord Factor – Immobilises Neutrophils.
    - Evokes Granulomatous inflammation

Scanned with CamScanner

Iron acquisition with exodermins & mycobactins → supply chelated iron to bacteria
→ take iron from Host cells

sulpholipids — Aids in preventing resp. burst
survivability of org. within phagosome.

waxes — Helps org. survivability within cells & prod^n of granulomatous lesions.

Pathogenesis — Acts as Adjuvants

* Mycobacteria taken up by local macrophages through draining lymphnodes, survive within macrophages, binds to TLR's, induce proinflammatory cytokines & induce release of GMCSF, MIF, IL-12, IL-8, Monocyte chemoattractant protein, Monocyte inhibitory protein & also activate T-cells. Induce nodule formation with central caseous material surrounded by epithelioid cells, Macrophages, Langerhans Giant cells etc.

Cultural characteristics (M. bovis)

* Aerobic.
* 37-38°c for growth.
* 18 hrs — Generation time
* Take 2-3 mts for prod^n of single colony.
* Slow Growth rate is observed.
* Require serum, potato, egg for their growth.
* Diff. media used are
  - Dorset egg medium
  - Lowenstein Jensen medium
    - contains Malachite Green which is inhibitory agent for contaminants & also contains potato,
    - Eugonic colonies — M. tuberculosis (luxuriant growth)
    - Dysgonic colonies — M. bovis. (No luxuriant growth) Very long duration for their growth.
  - Stone brink medium
    consists of Malachite Green, Sodium pyruvate, egg.
    → Bovine strains multiply on this medium.
  - Middle brook's Agar
    Oleic Acid & Albumin medium.

In order to grow mycobacteria in broth culture it requires Tween 80 which reduces surface tension

Resistance

* Survive in droplets for 8-10 days.
* Survive in 5% Phenol
  15% H_2SO_4
  3% HNO_3, 5% oxalic acid.
* Killed at 60°c in 15-20 min.

Scanned with CamScanner

* survive for 10-20 min in sputum.
* sensitive to HCHO, Glutaraldehyde.
* Ethanol is suitable application to superficial surfaces & skin gloves.
* susceptable to pasturelization pasteurization.

Bovine Tuberculosis

* 1° host – cattle
* Maintainence Hosts – Brush tailed opposums (New zealand)
  Badgers (UK, ireland)
  bison & elk (canada)
  kudu, African buffalo (southern Africa)

Diseases

* cattle – Tuberculosis
  – lungs are highly affected.

Clinical signs

Chronic wt. loss / wasting
Animal feeds normally but shows wt. loss, decreased prod^n & productivity.
Animal shows contenous temp.'s. due to toxemia.
unthriftness
Animal goes down in condition.

lesions

Nodules in lung, liver, spleen etc.

Prevalance of infection

Bovines – 8%
Camels – 11%

* sheep & Goat – less susceptable to infection.
* Horses – Resistant
  – show lesions with M. avium.
  – 1° lesions in Alimentary tract, Pharynx.
  – 2° lesions in lung, liver, spleen
* Swine – M. bovis produce progressive disease

Diagnosis

* Microscopy
  conducting Acid Fast staining
  Cultural characteristics.
* cell mediated immunity test.

Scanned with CamScanner

Purified protein Derivative / Tuberculin
- whole cell sonicates
- Lipoarabinomannon

Single intradermal test - 0.1 ml of PPD intradermally at neck / caudal region
- Diff. in skin thickness after incubation of 72 hrs.
  - >3mm - Doubt case
  - >4 mm / above - +ve for Disease

Double intradermal test / Stormont test - Both mammalian & avian tuberculin
- comparative test.
- Diff. in skin thickness
  - >4mm - +ve for disease.

Anergy

Short thermal test - 4 ml of PPD subcutaneously & rise in body temp. from 39 - 42°c results +ve.
↓
Also as Mantoux reac^n in Human beings.

* γ - Interferon Assay
* Lymphocyte proliferation Assay
* ELISA
* Complement fixation test.
* Flourescent Ab technique
* Bacterial Agglutination test
* Wattle Tuberculin test - In poultry
* PCR.

Control

* No vaccines in
* Through milk org. spreads
  ↓
* org. localises in supramammary lymph glands.
* Possibility for DCG vaccine but requires large doses so it is useless.

(Anergy - Desensitization of animals after intradermal tests
- Period is 60 days in cattle - single intradermal test
  6 mts in cattle - Stormont test)

MAP (Mycobacterium avium subsp. Paratuberculosis) - Johne's Bacillus.

* Acid Fast
* 1 - 2 μ - length
  0.5 μ - width
* Exhibit iceberg phenomena →

(1 - clinically infected.
1 - 8 - clinical
4 - 8 - subclinical
10 - silent.)

Scanned with CamScanner

Cultural characteristics

* 38-39°c for multiplication
* Media for multiplication used are
  - Herrold's egg yolk medium (HEY)
    contain Mycobactin J, pyruvate.
    → From M. Paratuberculosis.
* Takes 2-3 mts for its multiplication.

Disease

* Fecooral route
  Milk contain organism.
  Infected animal as source of infection
  org. secretes in semen.
  Intrauterine transmission
  Stressed Animals - susceptable to infection.

} Mode of Transmission.

Pathogenesis

* Through ingestion org. enters intestine.
* Through M-cells in ileocecal junction org. is taken up & settles in adjacent lymph nodes, enters mesenteric lymphnodes, survives in phagocytic cells, starts granulomatous inflammation esp. in lamina propria & submucosa.
* Takes very long duration from point of infection to appearance of clinical signs

Clinical signs

* Feed intake - Normal
* Chronic wt. loss
* Show Diarrhoea (shooting diarrhoea)

sheep & goat
Diarrhoea - Not a sign
Unthriftness
Goes down in condition.

lesions

* Intestine shows granulomatous inflammation & appear permanent transverse corrugation esp. at ileocecal junction.

Diagnosis

* Rectal pinch & go for Acid Fast staining.
* Cell mediated immune responses using Johnin (PPD)
  Frothingham, Johne - observed Johne's disease
  ↓
  Intradermal tests - 0.1 ml of Johnin
  Intravenous tests - 2-4 ml of Johnin.
  rise in 1.5° F temp. gives +ve result.

* Isolation

Scanned with CamScanner

* Lymphocyte proliferation Assay.
* Agar Gel immunodiffusion test
* ELISA
* PCR

Control

* Live avirulent culture of MAP in oil adjuvent can be used as vaccine.

15/10/19 ** BRUCELLA (textbook** causes Abortions in late pregnancy.

* Disease caused by Brucella is Brucellosis (Zoonotic Disease).
* Brucellosis cause Abortions in Females
  Orchitis in Males
* B. abortus – cattle / Bangs disease (BY Bang in 1897)
  B. melitensis – Goat & sheep (Humans – Malta Fever)
  B. suis – pigs (Humans – undulant Fever (wave like)) (BY Traum in 1914)
  B. canis – Dogs (BY Carmichael & Bruner in 1968)
  B. ovis – sheep (BY Buddie & boyed)
  B. neotame – Desert wood rats
  (species.)
* 1st org. that was identified was B. melitensis in the area malta in 1887 by Bruce.
* Main symptom of Brucellosis in man – Hygroma (Inflammation of knee Joint).
* these org. reside in Reproductive tract of male & female.

Morphology

* Gram –ve, coccobacillary, Non motile, Non spore former, Intracellular pathogen
* org. often occur in groups, singles, in laboratory cultures as short chains.
* This org. is resistant to 0.5% Acetic Acid. So org. appears pink with modified ZN staining.
* org. appear pink against blue background – Koster's method of staining.
* Koster's stain is used esp. to demonstrate tissue smears.

Cultivation

* Serum Dextrose Agar – SDA
* Albini
* Columbia Agar with serum and Antibiotics.

Cultural characteristics

* Glistening smooth colonies – B. melitensis, B. suis, B. abortus.
  Smooth are more virulent.
  Colour of colony is bluish.

Scanned with CamScanner

* Rough colonies – Not virulent
  – Appear pale, yellowish.

* Aerobic

* capnophilic – B. abortus, B. ovis
  ↓
  Require 5% CO_2
  for their growth.

~~Biochemical char~~

Differentiating features of diff sps. of Brucella based on Biochemical tests (Textbook)

Except B. ovis & B. neotome all others are oxidase +ve.

B. ovis – American → H_2S +ve
  – Danish → H_2S –ve.

Thionin inhibit growth of B. abortus
Basic fuchsin inhibits growth of B. suis.

Pathogenesis

* Mode of transmission – contact, ingestion, inhalation, skin penetration.
* once org. enters, it is engulfed by macrophages, macrophages reaches RES & enters blood stream. once it enters blood stream it settles in knee causing Joint pains / Hygroma.

Clinical signs

* Cattle – sporadic Abortions
  Orchites
* Rams – Epydedimitis
* Humans – Abortion is Rare.

Diagnosis

* Staining of impression smears from placenta, cervuterine discharge (or) aborted fetus.
* Isolation of organisms.
* Serological tests – Rose Bengal Plate Agglutination test
  (3.6 – pH of Rose Bengal Antigen).
  – Serum Agglutination Test (+ve titre – 1:n 40; Doubtful – 1:n 20).
  (can find only IgM Antibodies which is limitation)
  ↓
  Present in early Infections.
  – complement Fixation test
  confirmatory Test.

Scanned with CamScanner

- way Agglutination test.
- Comb's Test
  (used to detect non Agglutinating Antibodies esp. IgG)
- ELISA.
- Competitive ELISA
  (used to detect all types of Antibodies due to infection (or) vaccination).
- Abortus Ban Ring Test (ABR test) / Milk Ring Test (MRT)
  Herd test
- Brucella skin Test
  Allergic Test & Brucellin is Allergin.
- PCR.

Control

* Made virulent into non virulent/Attenuated by S-19 (smooth virulent made as avirulent)
  ↓
  calfwood vaccination (S-19)
  - Attenuated strain, live Avirulent.
  - Protects Female calves not in males
  - Becoz S19 cause orchitis.

* 45/20 is bacterin
  used as Booster vaccine
  killed vaccine.

* Test & slaughter method.

* RB51 — stable rough Attenuated mutant.
  - Gives Good protection against Abortions.
  - Not interfere in serological test.

16/9/19

Campylobacter

* C. fetus subsp. fetus — Present in intestinal tract of sheep, Goat, cattle.
  C. fetus subsp. venerealis — present in Genital tract
  C. jejuni subsp. jejuni — zoonotic, present in intestinal tract of mammals, birds.
* Reside in reproductive Tract & as commensales in intestinal tract.

Morphology

* Gram -ve, slender rods & during multiplication if 2 organisms join they appear like seagull wing shaped.
* Microaerophilic
* Non spore formers
* Motile with Polar flagella.

Pathogenesis

* venerealis cause infertility (temporary), Early embryonic deaths in cattle
* Fetus cause still births, weak, kids & lambs, Abortions — sheep & Goat
  sporadic Abortions — cattle.

Scanned with CamScanner

* Jejuni causes enterocolitis – Humans
  Avian hepatitis – Birds.
  Enteritis – Dogs
  Abortions – sheep.

Cultural characteristics * stained in Dil. carbolfuchsin for 4 min & org. appear red.

* Fetus, venerealis produce small, round, translucent colonies with dewdrop appearance
* Jejuni produce small, flat, grey colonies with spreading watery like colonies.

Differentiating characteristics of campylobacter sps.

C.sps.	catalase	Growth at 25°C	42°C	Growth in 1% glycine	Growth in 3.5% NaCl	H2S Prodn
venerealis	+	+	–	–	–	–
Fetus	+	+	–	+	–	+
Jejuni	+	–	+	+	–	+

Clinical Infections

* Bovine genital campylobacteriosis
  - cause: C. fetus subsp. venerealis.
  - Mode of transmission is through coitus.
  - Present in glandular crypts of prepuce, bulls remain infected & act as carriers.
  - Persists in vagina of carrier cows & condition comes normal in 3–5 mts due to natural immunity by IgA, IgG Antibodies → present in secretion.
  - Early embryonic deaths, sporadic Abortions.

*
  - causes: Fetus, Jejuni.
  - Abortions, org. excreted through faeces
  - Transmission is by Fecooral route.
  - Org. localises in uterus in pregnancy causing Abortions due to Necrosis.
  - Still born/weak lambs, Aborted ewes are major source of infection.
  - Necrotic lesions on liver surface in Aborted fetus.

* Intestinal campylobacteriosis in dogs.
  - Diarrhoea in dogs attributed to infection with campylobacter esp. Jejuni.
  - Dil. carbolfuchsin stained fecal/rectal smears.

* Avian vibrionic Hepatitis.
  - Cause: Jejuni.
  - Chick acquire infection from feed, water.
  - Infection is Asymptomatic.

Scanned with CamScanner

- Multifocal Necroses in liver.
- Drop in egg prod^n.
- Impression smear taken from bile should reveal curved rods with darting motility.

* Intestinal campylobacteriosis in Humans
- causes Food poisoning.
- causes: C. Jejuni, C. coli in pigs.
- poultry meat is major source for infection.
- Fever, Abdominal pain, Diarrhoea, dysentry - symptoms.

Diagnosis

* staining of smears with dil. carbolfuchsin for 4 min.
* Flourescent Antibody technique
  cervico mucous from bulls.
* Isolation & determination of C. fetos subsp. venerialis.
  venerialis from prepucial/vaginal mucous is confirmatory
→ skarrow
* vaginal mucous Agglutination test.
* ELISA - used to demonstrate IgA Antibodies in vagina.
* PCR.

* zoonotic - Brucella, campylobacter jejuni subsp. jejuni, leptospira

control

* Injections of Dihydrostreptomyces.

* Abortions - Brucella, campylobacter, leptospira

* Brucella - coccobacillary
  Leptospira - spiral shaped, Hooked ends.

21/10/19 Leptospira *

* L. interrogans - Pathogenic
  L. biflexa - saprophytic.

interrogans
biflexa
bratislava
tarassovii

* zoonotic.

* Leptospirosis - weil's disease (well described leptospirosis in man).
  ↓ occupational disease.
  Stuttgart disease.
  Rice field Fever, sugarcane Fever, Autumnal fever.
* org. is responsible for stormy Abortions, still births.

* 25 serogroups, 250 serovars } L. interrogans.
  ↓
  L. interrogans serovar canicola — Dogs
  hardjo — cattle
  Icterohaemorrhagiae — Dogs
  Bratislava

Icterohaemorrhagiae serovar Grippotyphosa – cattle.
tarassovi – pigs
pomona – pigs

* present in mud, wet envt.
* present in PCT of kidneys, Genital tract } Maintainence Host → carrier.
spreads infection into
Incidental Host

* leptospirosis is also called Rice field fever becoz Rats are important maintainers hosts for leptospira.
* Mode of Transmission – Inhalation, ingestion, penetrates skin.
vertical Transmission esp. in pigs.

Morphology

* Gram –ve
* Family: spirochetaceae (spiral shaped with Hooked ends)
* Motile but doesn't contain true Flagella; it contains endoflagella which is present in b/w periplasmic space.
* Movement is serpentine like.
* org. can be examined by Dark field Microscopy.
* silver impregnation method of staining – Fontana.

Cultural characteristics

* Fastidious, Microaerophilic.
* common commercial liquid medium – Ellinghausen McCollough Johnson Harris (EMJH)
  - contains 1% bovine Albumin Fraction B
    Rabbit sera – supplies B_{12}
  - For isolation of leptospira.
  - Incubation at 30°C & should wait upto 8 wks.
  - org. grow just below the surface.
  - Addition of 5 fluorouracil in medium is inhibitory for contaminants except leptospira.

Ellinghausen, McCollough, Johnson, Harris

Stuart's, Fletcher's

* Stuart's liquid medium, Fletcher's semisolid medium } other media.

* colourless colonies
Not visible until held against opaque light.

Resistance

* killed at 50°C for 10 min
  80°C for 10 sec

Scanned with CamScanner

* Destroyed by Gastric juice in 30 min

Antigens & Toxins

Based on surface Antigens, protein polysaccharide complexes L. interrogans is classified into 25 serogroups
250 serovars.

Pathogenesis → Textbook.

Symptoms

* Acute disease (Haemorrhagic septicemia – Haemorrhages, Hepatitis, Nephritis, Meningitis.
* Sub Acute
* Moderately severe disease – Agalactia, Hepatitis, Meningitis.
* Chronic – Infertility, still births, Abortions
* Subclinical – Mild disease, development of Ab's.

Acute
Chronic

Cattle

* Acute – Drop in milk prod^n, Pyrexia
* Chronic – Abortions, still births

Pigs

* Haemoglobinuria, Jaundice, Still births, Abortion in last 3rd of pregnancy.

Dogs

* Acute – Stuttgart disease
  – Fever, Haemorrhage, Anemia, Jaundice.

Specimens → Textbook.

* Kidney / liver sections.
* Collection of samples – Blood (upto 1-7 days), Urine, Vaginal discharges.
* FAT ELISA – To check Antibody Titre.
  PCR
  Control
* Liver Impregnation (Immunoblotting) Technique.
* MAT – Microscopic Agglutination Test. For diagnosis of leptospira.
  – Should have serve leptospira serovars
  Titre 1:400 & Above is +ve.

* Using bacterins

Treatment

* Streptopenicillins
  – Amoxycillin
  – Dihydrostreptomycin

Pathogenesis

* Mode of transmission
* Presence of limited virulence Factor
* Leptospira invade host tissues through moist/softened skin or through mucous membranes.
* Tissue invasion is possible by their motility.
* Spread throughout body via blood; as a result of infection Ab's are appeared within 10 days resulting in clearance of infection from circulation.
* Some org. may evade immune response & persists in body esp. in renal tubules, also in uterus, eye, meninges.
* Susceptible animals – Hemolytic Anemia, Jaundice, Haemoglobinuria, Haemorrhage associated with Acute leptospirosis.

Scanned with CamScanner

Section

Pasturella

* Present as commensale in upper resp. tract
* Gram -ve, coccobacillary
* Show Bipolar staining. (o) safety pin like
* Non motile, Non spore formers, capsulated.

Cultural characteristics

* Fastidious
* BHI Agar to which 5-10% of Blood
* on serum Agar - smooth, Rough, Mucoid colonies
  - smooth → virulent to Rabbits
  - Rough → Non virulent to Rabbits
  - Mucoid → due to capsule which contains Hyaluronic Acid.
* Granular sediment

P. hemolytica — later named as Mannhemia hemolytica

Biochemical characterisation

Infective to cattle, sheep causing Shipping Fever

	P. multoseda	M. hemolytica
Growth on MacConkey Agar	-ve	+ve
Catalase	+ve	+ve
Oxidase	+ve	+ve
odour	sweetish odour	odouriess
Urease	-ve	-ve
Haemolysis	-ve	+ve (Dewdrop Appearance of colonies)

Clinical signs

* Necrotic Foci of lever - characteristic for Fowl cholera - Birds.
* Snuffles - Rabbits by Pasturella.

Control

* Pas Bacterin

* Type E - HS in cattle, Buffalo - Africa (E:2)
  Type F - Turkeys.
* Also based on LPS - somatic Antigen. (no.)
* sps. - P. multoseda ↓ Type A, B, C, D, E, F By carter based on diff. Infective to (capsular Antigen) letter polysaccharides in capsule.
  - Type A - cattle, sheep, Goat, Birds, Pigs
    HS, Bovine pneumonic Pasturellosis
    Fowl cholera - Birds.
  - Type B - Buffalo, cattle
    HS - Asia (ATR) → (B:2)
  - Type D - Pigs - Atrophic Rhinitis, Pneumonia

23/10/19

Haemophilus

* sps. – H. Paragallinarum, H. Somnus, H. influenza, H. Parasuis.
* commensales of upper resp. tract of mucous membrane of Humans & Animals.
  Genital tract.

* H. Paragallinarum – upper resp. tract
  – In chicks
  – Infectious coryza.

* H. somnus – Genital tracts of Male & Female.
  – In cattle
  – Thrombotic Meningoencephalitis – young cattle (pneumonia, Arthritis,
  – Epididymitis – Rams
  Mastitis, vulvitis – ewes
  septicemia, Arthritis, pneumonia, Meningitis – lambs

* H. Parasuis – In Pigs
  – Glasser's disease (polyserositis & Meningitis in young piglets).

* H. influenza – In Humans.
  – Resp. infections to Meningitis.

Morphology

* Gram –ve
* coccobacillary; occassionally form filaments.
* Motile
* Facultative Anaerobes
* Non spore Forming.
* capsules can be produced.
* They require X, V Growth Factors for their growth except H. somnus.

cultural characteristics

* Fastidious; Aerobic, Facultative Anaerobes; 37°c.
* will not grow on Nutrient Agar, MacConkey Agar.
* X Factor is Heat stable, porphyrin, Iron, Hematins; present in RBC.
  required for synthesis of catalase
* V Factor is Heat labile Factor.
  synthesized by staphylococcus aureus.
  is a coenzyme NAD.
  present in RBC.
* Growth can be enhanced by 10% CO_2.
* Chocolate Agar – Heat the blood; expose agar at 48°c but for Hemophilus at 80°c
  for 10 min

NADases will get destroyed. (enzymes)

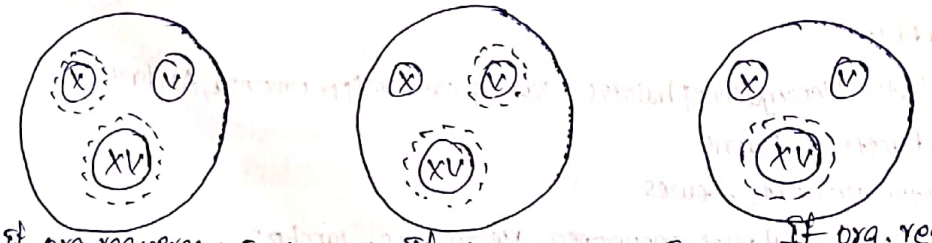
so that V factor is protected/Not destroyed

Recog NADases which destroy NAD are destroyed.

Scanned with CamScanner

* Pin point / dew drop like colonies.
* Hemophilus exhibits a phenomena called satellitism (Hemophilus grows close to staphylococcus aureus).
* Levinthal's medium
Porphyrin test
* For detection of X factor.

Disc method



If org. requires X factor — If org. requires V factor — If org. requires both X & V factors

Actinobacillus

* commensales on mucous membrane of upper resp. tract & oral cavity.

Morphology

* Non motile
* Gram -ve.
* coccobacillary
* Non spore Former
* Non Acid Fast.
* Small Focal elements lying at pole of bacilli giving porescode appearance.
* Require enriched media.
* Fastidious
* sps. — A. lignereseii, A. pleuropneumonia, A. suis.
* A. lignereseii — In cattle
  * — wooden tongue / timber tongue.
  ↓
  — Granulomatous lesions around head, neck & ...

  * club shaped structures around Actinobacillus which are called chemically calcium phosphate crystals — white granules which reveals pink coloured club shaped structures & blue coloured bacilli.
* A. equiales — In horses
  — sleepy Foal disease / Joint ill.
* A. suis — In pigs under 3 mts
  — Acute

Cultural characteristics

* Non Hemolytic colonies on Blood Agar
* Grows well on MacConkey Agar & produces pink coloured colonies — Late lactose fermenter.
* +ve for CAMP Test (β hemolysis of staphylococcus aureus is enhanced by A. pleuropneumonia).

Biochemical Tests

* IMVIC tests : - - - -
* catalase +ve
* oxidase +ve.

Ehrlichia — Rickettsiae

* Placed in b/w bacteria & viruses.

Classification

O — Rickettsiales

F — Rickettsiaceae, Anaplasmataceae

Tribe	Genera	Sps.
Rickettsiae	Rickettsia	R. rickettsi, R. prowazekii
	orientia	O. tsutsugu
	Coxiella	C. burnetii
Ehrlichiae	Ehrlichia	E. canis, E. bovis
	Neorickettsia	N. helminthica
	Cowdria	

Morphology

* Related to Gram -ve bacteria.
* Presence of Metabolic enzymes
  O2 requirement (Aerobic)
  Nucleic Acid consisting of both DNA, RNA
  Replicate by Binary fission
  sensitive to Antibiotics
  viewed under light microscope..

  } Similar to bacteria.
  Require spl. staining methods like
  Machiavello stain — Bright red
  castaneda stain — Blue
  Zeinsa stain — Bluish purple.

* Don't grow on Artificial media
  Exhibit Intracellular replication.

  } Similar to viruses.

* Small, Non-motile, Pleomorphic, Gram -ve bacteria.
* Replicate in Host cells.
* Bright red in colour against blue background — Machiavello stain
  Blue colour against red background — castaneda stain
  Bluish purple colour — Zeinsa stain.

Resistance

* Very Fragile except C. burnetii
  ↓
  Forms endospore like structure & it is viable for upto 50 days

Scanned with CamScanner

* Coxiella - Doesn't get killed with pasteurization.

Replication (Pathogenesis).

* Exhibit Intracellular replication.

* liberation of phospholipases whenever org. is in phagosome.

↓

break membrane of phagosome

↓

org. come into cytoplasm of cell.

↓

Divide by Binary Fission.

↓

Generation time (Doubling) - 8 hrs.

Transmission

* Require Arthropod Vectors for transmission from one host to another host

Mostly ticks like Dermacentor sps.
Amblyomma sps.
Rhiphicephalus
sanguineus.

* R. rickettsi - By both Transovarian & Transstadial Transmission.

↓ Transmission by ticks except C. burneti.

↓ Generation to Generation

↓ within generation (stage to stage).

* E. canis - By Transstadial Transmission.

* whenever there is tick bite org. enters intestinal tract epithelial cells

↓

Org. Multiply

Excreted in Faces.

reaches upward to salivary gland.

Pathogenesis

* Most of org. in rickettsiae are zoonotic.

* Humans, cattle, sheep, - susceptable.

* Predilection for endothelial cells of small blood vessels.

↓

settles & org. replicates

↓

thrombus formation due to replication of org.

↓

Rupture of endothelial cells
loss of blood vol.
shock & Death.

Scanned with CamScanner

* clinical manifestations are due to endotoxin prodn.

C. burnetii

* shows predilection to Female reproductive tract & mammary glands of ruminants.
* Affects macrophages, epithelial cells.

Neorickettsia

* localises in lymphnodes
* produce generalised lymphadenopathy

Ehrlichia

* predilection for leucocytes & platelets

Disease

Rocky mountain spotted Fever

* caused by R. rickettsii.
* Host - Human, Dog.
* Both Transovarian, Transstadial Transmission.
* By ticks like Dermacentor sps., Amblyomma sps., Rhipicephalus, sanguineus.

Clinical signs

* course → < 2 wks
* Incubation period → 8-10 days
* Fever, Depression, Retinal Haemorrhages, conjunctivitis, Muscles & Joint Pains, dyspnea, coughing, edema of extremities, Neurological disturbances in 80% of affected Dogs.
  ↓
  Ataxia, stupor, Neck rigidity, seizures, coma.
* Renal damage, cardiovascular damage - complications.

PM Findings

* Haemorrhages
* Spleenomegaly
* lymphadenopathy

Diagnosis

* prevalent in American countries.
* Dogs exposed to ticks must be suspected for disease.
* Immunofluorescence Antibody test - To know rising Ab titres.
* ELISA - To kno
* Thrombocytopenia, leucopenia in Acute cases in Blood Picture.
* Must be differentiated from canine ehrlichiosis.

Scanned with CamScanner

Treatment & control

* Tetracycline Therapy
  → Broad spectrum Antibiotic.
* Frequent removal of ticks.
* As it is zoonotic disease, one should wear Gloves.

Canine Monocytic Ehrlichiosis

* caused by E.canis.
* confined to tropical, subtropical countries.
* Tick – Rhiphicephalus, sanguineus.
* Transstadial Transmission
* Dogs remain as carriers for > 2yrs after recovering from acute disease.

Clinical signs

* Incubation period → upto 3 wks
* Forms – Acute, subclinical, chronic.
* Acute – Fever, Thrombocytopenia, leucopenia, Anemia
  – Most of dogs recover from Acute phase,
* Tropical canine pancytopenia – when dogs are exposed to stress, condition becomes chronic & all values of blood cells becomes down.
  ↓
  Persistent bone marrow depression
  Haemorrhages
  Neurological disturbances
  Peripheral edema
  Emaciation
  Shock & death

Factors that contribute to development of chronic form

1) Immunosuppressiveness
2) Virulence of E.canis
3) Breed susceptability

Diagnosis

* Based on clinical signs & Hematological blood picture from those dogs in history of exposing to ticks in endemic areas.
* Morulae of E.canis is detected in mononuclear cells if smear is prepared from buffy coat.
* Seroconversion (paired sera samples)
* This org. can be cultured in canine Macrophage cell lines.

Treatment & control

* Fluid Replacement Therapy
  Blood Transfusion.
  Tetracyclins, chloramphenicols.

Scanned with CamScanner

* Tetracycline can be given as prophylactic Agent.

Salmon poisoning

* caused by N. helminthica

* Due to Flukes – Nanophyetus salmincola
  Not due to ticks. Maintains its lifecycle from snail, fish, dog.

* Raw fish containing fluke metacercaria gets ingested to dog.
* From metacercaria org. enters blood circulation of dog.
* Most common in North America.

Clinical signs

* Develop after 7 days of ingestion of raw fish
* Fever, Anorexia, weakness, Depression, Persistent vomitions, Bloody diarrhoea.
* Death occurs within 7–10 days in 90% of untreated cases.
* Those that survive they resist reinfection.

Diagnosis

* History of access to raw fish in endemic areas.
* Observation of fluke egg samples in Dog fecal samples. ✓
* Must be differentiated from canine distemper.

Treatment & control

* Tetracyclins, chloramphenicols
* Supportive therapy
* Should not offer raw fish to dogs in endemic areas. ✓

Bovine Anaplasmosis / Gall sickness – By A. marginale.

* Affects cattle in tropical & subtropical regions.
* Young calves – Mild infection – Development of carrier state.
* Adult cattle – 50% Mortality.
* Transmission is by tick Boophilus.
  also by biting diptera.
  Through contaminated instruments.

Clinical signs

* Incubation period → 2–12 wks.
* Inappetence, depression, red milk yield, Marked Anemia, Jaundice, wt. loss
  ↓ because of
  Predisposition for erythrocytes
* Recovered animals resist reinfection (do not develop disease again).

Scanned with CamScanner

Diagnosis

* Based on clinical signs.
* Hematological findings in stressed cattle as well as naïf cattle.
* Giensa stained blood smears contain density stained bodies located to periphery of erythrocytes
* After 10 days of onset of fever org. are numerous in blood picture.
* Immunofluorescence
* PCR
* To detect latent Infections – CFT
* ELISA

Treatment & control

* long acting oxytetracyclins
* Imidocarbdipropionate
* Supportive Therapy.
* Minimise stress to cattle.

Q Fever

* caused by C. burnetii.
* Influenza like occupational disease of farmers, veterinarians, other people which are in contact with farm animals & their products, Abatoir & workers.
* Transmit through Aerosols originating from parturient sheep/cattle.
* It localises in reproductive tract of animals & mammary glands.
* Laboratory infection is common.
* Through tick bite Q fever is very rare to occur.
* Domestic animals exhibit subclinical phase.
* Sporadic Abortions, Infertility, Birth of weak offspring, Placentitis, endometritis, Hepatitis, Myocarditis, Interstitial pneumonia

Diagnosis

* Proper Handling of material.
* Diagnostic procedure in biohazardous envt.
* Smears from placental tissues, uterine discharges by MZN reveals clumps of cocobacillary bodies.
* Org. is demonstrable by immunofluorescence in placental smears.
* PCR.
* cultivated in chicken embryos through yolk sac route.

Scanned with CamScanner

* CFT
* ELISA
* Indirect Immunofluorescence.

Control

* segregate animals when abortions are noticed.
* care for disposal of placenta & Aborted fetuses
* Inactivated egg yolk vaccines

5/11/19

Chlamydia

* Gram -ve
* obligate Intracellular parasite
* Placed b/w bacteria & viruses.
* properties similar to bacteria in rickettsia — same in chlamydia.
* sensitive to Interferons along with Antibiotics. ✓
* exhibit Growth/development cycle during its lifecycle } Not seen in Rickettsia

O – chlamydiales
F – chlamydiaceae
G – chlamydia – c. trachomatis, c. suis, c. muridarum.
chlamydophila – c. psittaci, c. pneumoniae, c. abortus, c. pecorum, c. caviae, c. felis.

SPS.

* Classification based on host preference

Iodine staining for presence of Glycogen. ✓
sulfonamide susceptability
c. psittaci – resistant to sulfonamides
Inclusion body morphology. ✓

Disease

* c. trachomatis – Trachoma in humans
  ↓ A–K serotypes.
  ↓ Inner surface of eyelid roughens
  – Inclusion conjunctivitis of infants.

Agents of c. trachomatis which cause Trachoma & Inclusion conjunctivitis are called TRIC Agents

– Arthritis
– Non specific urethritis
– Resp. disease of Infants.
– Genital tract infections.

Scanned with CamScanner

* C. suis – Resp. infections in pigs
  - Intestinal infections.
* C. muridarum – In Guinea pigs – Resp. infections.
* C. psittaci – In Birds, pneumonia & Air sacculitis
  - Intestinal infection
  - Diarrhoea
  - conjunctivitis
  - Pericarditis
  - Encephalitis
  - In Humans disease condition is psittacosis / ornithosis. (Abortions, conjunctivitis).
* C. Abortus – In sheep
  - EAE – Enzootic Abortion of ewes.
  - In Goat, cattle, pigs – Chlamydial Abortions.
* C. felis – In cats – conjunctivitis
  - Feline pneumonitis
* C. cavriae – In Guinea Pigs – Guinea pig Inclusion conjunctivitis
* C. pecorum – In sheep – Intestinal infection
  conjunctivitis
  polyarthritis
  - In calves – sporadic Bovine encephalomyelitis
  polyarthritis.
  - In older Animals – Metritis
* C. pneumoniae – In Humans, Horses – Resp. infections.

Developmental cycle * D

* 2 stages – Elementary body – Infectious form
  Reticulate body – Reproductive form.

* Elementary body – Infectious extracellular forms.
  - small, circular 200–300 nm with dense centre.
  - NO peptidoglycan layer in periplasmic space. (Diff. b/w rickettsia & chlamydia)
  - outer envelope is similar to outer membrane LPS.
  - Metabolically inert & osmotically stable structures.
  - osmotic stability is due to disulphide cross linked envelope proteins.
  - enters Host cell through receptor mediated endocytosis
  - Due to structural reorganisation gets converted to reticulate bodies.

Reticulate body – large, 1000 nm

Scanned with CamScanner

- Metabolically active & osmotically Fragile.
- Replication of reticulate bodies is by Binary fission (or) Budding.
- start reprod^n in 10-15 hrs of infection.
- After 20-30 hrs of infection these newly formed reticulate bodies gets reverte-d to elementary bodies.
- This happens for upto 72 hrs of infection.
- After 72 hrs all these come out by lysing host cell.

* Intracytoplasmic Inclusion bodies - All daughter cells looks like Inclusion bodies.
* Replication is delayed with addition of γ-Interferons, penicillins (or) whenever availability of AA's like TRP, cys are limited resulting in persistent infection.

Habitat

* GIT - Mostly chlamydophila sps.
  comes out through Fecal shedding. ✓
* latency & persistency - Imp. factors for chlamydial infections.

Mode of Infection

* Inhalation of infectious dust & droplets.
* In ewes - Through Ingestion (reaches epithelial cells of placenta). ✓

Pathogenesis :-

* Group specific & species specific Antigens
  - Complement Fixation Antigen
    - Lipopolysaccharide
    - Heat stable.
    - Associated with cellwall
    - Common Antigen.
  - Heat labile
    - Present in inner part of cell.
* Elementary body produce Haemagglutinins but reticulate body doesn't produce.
  (By Haemagglutination Inhibition test - To detect chlamydia).
* Lipid A - Endotoxic similar to Gram -ve bacteria.
  - Release of IL-1 which inturn causes inflammation & then leads to Tissue injury. ✓

Delayed Type Hypersensitivity Reaction - Since chlamydial infections are persistent, org. present constantly inside, so constant stimulation of immune system, more destruction of tissues.

* Ewes - Through ingestion
  ↓
  org. localises in placenta
  ↓
  Affects Fetus during Gestation.

Scanned with CamScanner

Psittacosis/ornithosis/Avianchlamydiosis

* Caused by C. psittaci
* worldwide in distribution.
* All birds (psittacine & non-psittacine), Humans – zoonotic.
* Symptoms are exhibited by birds when they are exposed to stress conditions such as during egg laying, overcrowding, Transportation, captivity etc.
* Mostly it is subacute/chronic form
* Resp. & digestive tracts are affected.
* Signs – ocular & Nasal discharges
  sinusitis
  conjunctivitis
  weakness
  loss of condition.
  Green pasty diarrhoea which is evidenced by pasting of feathers around vent.
  Spleen, liver also affected.
* During PM – enlargement of spleen, liver characterised by multifocal necrosis of spleen liver.

Diagnosis – Intracytoplasmic basophilic inclusions

* ELISA kits – For detection of Antigen in faeces
* CFT
* Latex Agglutination test.
* Humans – By Ingestion/Inhalation of dried feathered parts (mostly)
  – Influenza like symptoms Fever, Headache, pulmonary involvement
  Meningitis, Meningoencephalitis – severe cases
* Mice inoculation intraperitoneally
  Fatal infection – Parrot strain (psittacosis).
  No Fatal infection – Pigeon strain (ornithosis)

Treatment

* Tetracyclins
  Chloramphenicol } prolonged periods (5–6 weeks).

Enzootic Abortions of sheep & goat

* caused by C. abortus
  characterised by placentitis.
  leads to premature lambing Abortions in late gestations, retention of placenta
  Acquire infection through ingestion.

Scanned with CamScanner

* If ewes acquire infection in late pregnancy they donot abort in that pregnancy & Abort in next pregnancy.
* If ewes acquire infection in early pregnancy they abort in same pregnancy.
* some of ewes may die due to 2° bacterial infections.
* Neonatal lambs of acquired infection show signs in its 1st pregnancy.
* Necrosis of cotyledons
  Dirty pink uterine exudate } Imp. Findings.
  Necrotic placenta

Diagnosis

* Fresh placenta & fetus are required for preparing smears. (By MZN & Giemsa)
* Isolation & cultivation of org. by using embryonated eggs through yolksac route.
* By using cell culture.
* Paired sera sample - To detect elevated Antibody levels.
  - Four fold ↑ in titre is significant.

Treatment & Prevention

* Tetracyclins for infected newborn lambs, Aborted ewes
* Destruction of placenta carefully
* Disinfection of premises.
* Administer long acting tetracyclins to prevent Abortions during pregnancy
* Give attenuated vaccines to ewes prior to breeding.
* Inactivated/Killed vaccines to pregnant ewes.
* Avoid contact with affected ewes during lambing season.

Sporadic Bovine encephalomyelitis - calves.

* caused by C. pecorum
* Neurological disease.
* Age group is under 3yrs
* Nervous signs - High fever, Opisthotonous condition, Excessive salivation, Depression, Incoordination, Diarrhoea.
* 50% mortality
* Isolation of org. from brain tissue.
* Histopathological changes in Brain tissues.

Scanned with CamScanner

6/1/19

FUNGI

* Mycology – Study of Fungi.
* Mycoses – Diseases due to Fungi
* Mycotoxicoses – Toxins released by Fungi

Importance of Fungi

* Fungi are either saprophytic (or) parasitic.

General Features of Fungi

* Fungi is either unicellular (or) multicellular.
* Unicellular Fungus – Yeast.
* Multicellular Fungus – Mould.
* Hyphae < Septate < Coenocytic, Monocytic; Aseptate – Coenocytic
* Eukaryotic
* Aerobic (obligate Aerobes) but some are anaerobes found in rumen of cattle.
* Non motile
* Chemoorganotrophic org.
* Resists Acidic pH
* 3.8–5.6 – preferrable pH for their growth.
* Spore Former
* Absorb nutrients
* Non photosynthetic
* Vegetative structure of Fungus is called Thallus.
* 10–40°C (22–30°C) – optimum Temp. (saprophytic)
  32–37°C – optimum Temp. (parasitic)
* Reproduce both sexually & Asexually.
* At 37°C in Host – yeast form
  At 25°C in lab – Mould form
  Fungi that exists in both yeast & Mould forms is Dimorphic Fungi (YM shift)

Morphology

* Unicellular = Yeast.
* Yeast like Fungi – Has pseudohyphae – candida
* Mould – Distinct Filamentous structures (multicellular) – Dermatophytes
* Dimorphic Fungi – Histoplasma capsulata, Blastomyces.
* Mould – Thallus has 2 parts < Mycelium – Produce Asexual spores at tip of hyphae; spores.
* Hyphae – sided by cell wall like bacteria.
  – cell wall contains chitin, hemicellulose.

Scanned with CamScanner

- Hyphae divides into cells by crosswalls due to inward growth of cell wall.

Hyphae
- Septate with
  - Uninucleate
  - Multinucleate
- Aseptate with many nuclei

} Based on morphology

* Hyphae
- Vegetative
- Aerial (or) Reproductive mycelium – Resp. for spore prod^n.
  - Extend from medium into air

} Based on Function.

* Hyphae are sometimes surrounded by Fimbriae.
  Enriched with crystals of Ca oxalate that strengthen hyphal wall.

* Stalk that contains sac in which spores are present & sac is called sporangium.
  & such stalk is called sporangiophore.

* Some stalks contain Macro conidia / Microconidia & such stalks are called conidiophores.

Nutrition

Grow best in Dark, moist envt., presence of organic material.

- Glycogen is 1° storage polysaccharide in Fungi.

Classification Based on tissue invasion

1) Superficial mycoses – confined to outermost layer of skin, hair.
2) Cutaneous mycoses – Invade & destroy keratin, hair, skin, nails
3) Subcutaneous mycosis – Invade muscle tissue.
4) Systemic mycoses – Invade deep tissue.

Based on type of ~~Fungi~~ Hyphae

1) Septate Hyphae 2) Aseptate Hyphae 3) Pseudohyphae.

Based on type of spores

1) Asexual spores – Chlamydospores – Thick walled resistant spores
   - Arthrospores
   - Blastospores
   - ~~Conid~~ Sporangiospores.
   - Conidia
     - Macro conidia
     - Micro conidia.

2) Sexual spores – Ascospores, Basidiospores, Zygospores.

* Imperfect Fungi – No sexual activity in its lifecycle.
  - Placed under Deuteromycetes.

Based on macroscopic morphology

1) Yeast form – Unicellular, round structures reproduced by budding.
2) Mould form – Multicellular, Filamentous structures called Hyphae.
3) Dimorphic form – occurs in 2 forms (Yeast, Mould) eg:- Histoplasma, Blastomyces.
4) Yeast like Fungi – They grow partly as yeast & partly as elongated cells called Hyphae & we call these Hyphae as pseudohyphae.
   eg:- candida

Scanned with CamScanner

Classification

K : Fungi

P/D : mycota

SD : mycotina

C : mycetes

SC : mycetidae

O : mycetales

F : mycetaceae

*6 Fungal groups

Chytridiomycota

Zygomycota

Glomeromycota

Ascomycota

Basidiomycota

Microsporidia – develops spores outside Host & Mycelium inside body.

11/11/19

Growth & Reproduction of Fungi

* Fungi grow in feeds containing high conc. sugars.

	Fungi	Bacteria
Optimum pH	3.5 – 5.6	6.5 – 7.5
Optimum Temp.	22–30 (saprophytic) 30–37 (pathogenic)	20–37 (Mesophils)
oxygen requirement	strictly Aerobic for moulds & most of the yeasts but some yeasts are Facultative.	Aerobic to Anaerobic.
light requirement	Not required	Required for photosynthetic groups
Sugar conc. in media	4–5 %	0.1 – 1 %
Carbon requirement	organic like Glucose	organic & Inorganic like CO_2.
cell wall structural component	chiten, cellulose (or) Hemicellulose.	Peptidoglycan.
Antibiotics susceptability	Griseofulvin Resists penicillins, Tetracyclins, Chloramphenicol etc.	Resists Griseofulvin susceptable to other Antibiotics.

Scanned with CamScanner

cultivation — Sabraud's Dextrose Agar. (Maltose & peptone are principle components)

cottony Airy mycelium like growth in moulds & these mycelia are transferred by Teasing needles.

Reproduction → sexual & Asexual Reprod^n.

somatic (or) vegetative.

↓

Fission of somatic cells yielding a similar daughter cells

Budding of somatic cells (or) spores.

Fragmentation (or) Disjoining of Hyphal cells, so that each fragment becomes new cell.

Spore Formation

Asexual spores

Sporangiospores

* Produced in sac like structure called sporangium.
* Motile sporangiospores are known as zoospores.
  Nonmotile sporangiospores are known as Aplanospores.

Conidiospores (or) conidia

* Depending on size, divided into
  Microconidia — small, unicellular structures
  Macroconidia — large, multicellular structures.
* Produced on tip (or) sides of Hyphae.

Oidia (oidium)/Arthrospores

* Formed by disjoining of Hyphal cells.

Chlamydospores

* Thick walled, single celled, Highly resistant to Adverse conditions.
* Formed from cells of vegetative Hyphae.

Blastospores

* Formed by budding.

Scanned with CamScanner

Sexual Reprod^n

* due to fusion of 2 nuclei of 2 parental cells. this happens in stages as
Plasmogamy – Fusion of protoplasm.
Karyogamy – Fusion of Nuclei
Meiosis

* sex organelles of Fungi are known as Gametangia which are made up of cells known as Gametes made up of Gametic Nuclei.
* Male Gametangium – Antheridium.
* Female Gametangium – oogonium

Methods

Gametangial copulation
Gamete Gametangial copulation
Gametic copulation
Somatic copulation
Spermatisation.

} Prod^n of sexual spores

* Ascospores – unicellular, 8 in no., produced in sac like structure called Ascus (Asci)
* Basidiospores – single celled, 4 in no. produced on club shaped structures
* Zygospores – large, thick walled spores formed by fusion of Gametangia.
* oospores – Formed within oogonium.

* Both Asexual & sexual spores are protected by Fruiting body.
* Fruiting bodies of Asexual spores – acervulus, pycnidium.
sexual spores – Apothecium, perithecium

* Mycoses
  - superficial
    - cutaneous → Dermatophytes, Malassezia
    - subcutaneous → Sporotrichosis, Rhinosporidiosis, Mycetoma.
  - Deep
    - systemic → Dimorphic Fungi
    - opportunistic systemic → Candidiasis, Aspergillosis, Mucormycosis

Scanned with CamScanner

Malassezia * superficial cutaneous infection
Pachydermates

* commensale on skin of Animals & Humans.
* Aerobic, Non Fermentative.
* Urease +ve.
* Grow at 35-37°c.
* Bottle shaped which measures about 7.5 µm in diameter.
* Thick walled yeast.
* Reproduce by Budding (Multiple budding occurs from same site.)
* Habitat - skin of mammals & Birds.
  - In areas rich in sebaceous Glands like ext. earcanal, lips, Anal region, interdigital skin of Dogs.
* Pathogenesis
- Produces 2 clinical conditions in Dogs
  1) Otitis Externa
  2) Seborrhic Dermatitis
- Breed susceptability is a major predisposing Factor.
  Immunosuppression
* Symptoms - Frequent Head shaking
  Oozing of dark purgent discharges from ear
  Rubbing of ears
  Pruritis
  } Otitis externa.
  - pruritis
  - Erythema
  - Foul smelling with greasy exudate
  - Matting of Hairs.
  } Seborrhic Dermatitis
* Diagnosis - collect exudate from ear canal.
  - Biopsy of skin → If it is dermatitis
  - 3 to 4 days time to grow on SDA.

Scanned with CamScanner

12/11/19 Candida – candidiases
→ 800 sps.

* C. albicans, C. krusei, C. tropicalis, C. parapsilosis.
↳ Pathogenic.

* Exists in oral cavity, Digestive tract, Genital tract.

* yeast measuring about 5x8 µm; Reproduce by budding.

* It appears in 3 forms

Yeast form
Pseudohyphae — Repeated budding
True Hyphae — Elongated budding cells.
} Animal tissues & laboratory media.

* can grow in all media at 30-37°c & produce smooth, creamy bacteria like colonies.

* Elongated buds (pseudohyphae) penetrate deep into ↓ media.

* Growth is both superficial & submerged.

* submerged growth is due to pseudohyphae.

* Cornmeal Agar – Differentiate candida from ~~other sps forms~~ yeast like org. like cryptococcus.
↳ Nutritionally poor media – produces chlamydospores.

* In bovines it causes mastitis – parapsilosis, krusei, albicans.
Abortions by tropicalis, parapsilosis.

* In poultry – sporadic disease of Alimentary canal with high mortality.

* Candidiasis in poultry & human infants is known as Thrush / Aftan, Moniliasis

* candidiasis is endogenous infection.

* Isolate org. from faeces, GIT of ~~mammals~~ Normal, birds, man, lower Animals.

* Poor managemental practices & housing
Nutritional deficiencies / disorders
Avitaminosis
Bacterial & viral infections
} predisposing factors

* Prolonged Antibiotic therapy enhancing candidiasis.
↳ Direct stimulus to fungi; eliminate org. which compete for nutrients.

* Removal of org. ~~with~~ which secrete Antifungal substances like probiotics.

* If Antibiotic is tetracycin this flourishes more becoz it utilises tetracycin for its N2 source.

* In swine – Prolonged Antibiotic therapy
– Fed on high glucose diet

* In Bovines – Intramammary infusions of Antibiotics which are of poor quality

Pathogenesis

Org has ability to produce various virulence factors like extracellular

Scanned with CamScanner

cytotoxic - Phospholipases, Proteases.

* phospholipases helps yeast to invade the tissues & helps the yeast to adhere to host cell.
* cell wall Glycoproteins have endotoxin like action.
* Fungus produces an enzyme leucine amino peptidase which destroys local tissue & helps in establishment of infection.
* Through Hematogenous spread, they reach to other int. organs producing systemic lesions.
* Mostly youngones are highly susceptable.
* puppies, kitten, calves, Foals - candida produces mycotic stomatis, enteritis.
* In swine lower oesophagus gets affected.

  Diarrhoea

  cutaneous candidiasis.
* In chicken, Turkeys & other birds Mouth, oesophagus, crop are affected.
* If it affects crop the condition is Avian Monoliasis / thrush. (Infection of upper Alimentary canal) symptoms are

  stunted Appearance, unsatisfactory growth, listlessness, ruffled Feathers
* In cows - Mastitis, Abortions.
* In mares - Metritis, vaginitis.
* In stallions & bulls - Genital candidiasis.
* In Humans - Mucous membrane of mouth, tongue & genital tract.

  - Fecal material contamination of yeast & slaughter houses.

  - No man to man transmission.

Diagnosis

* Direct microscopic examination of clinical material

  clinical material is mucosal scrapings on slide stained by Gram's staining

  - wet mount by using lactophenol method.

  Rounded/oval budding cells, Fragments of mycelium, Attached budding cells may be demonstrated.
* Tissue sections stained by PAS (or) methanamine silver method for observing hyphal & pseudohyphal structures.

  Periodic Acid schiff

Cultural tests

* Isolation of culture from SDA with cycloheximide & chloramphenicol

  cycloheximide → Prevents growth of saprophytic fungi

  chloramphenicol → Antibacterial.
* Incubate at 30°c for 3 - 7 days.
* colonies are cream, smooth (young colonies).
* submerged mycelial growth - Old colonies.

  with feathery appearance at their margins - pseudohyphae.

Scanned with CamScanner

* Prodn of chlamydospores on cornmeal Agar.

* Germ tube test/serum tube test - ~~Reynolds Broad~~ Reynauld's Braude phenomenon

Germ tube growth / Pseudohyphae - are formed in the serum tube with culture incubated at 37°C.

* Histopathological Findings

* Animal Inoculation

Mice & Rabbits - I/V inoculation

- Die within 1/2 wks.

Not useful to differentiate spl.

Preventive measures

* Eliminate all predisposing factors.

Cryptococcosis

* yeast, round to oval, 3.5 x 8 µm

* capsulated yeast.

* Chemical nature is polysaccharide with Mannose units.

* Based on capsular Antigen, 4 serotypes

C. neoformans var neoformans - A & D

C. neoformans var gattii - B & C.

* Exogenous Infection.

* Exists in soil, skin of healthy humans as well as animals.

* can be isolated from insect bodies, milk, butter.

* Also found in Aged Pigeon droppings. bcoz droppings contain creatinine which is utilised as substrate for its growth.

* No Animal to Animal transfer (Non contagious)

* Non contagious chronic systemic infection which involves lungs, skin & other parts but it has marked predilection for brain, meninges.

* World wide in distribution.

Pathogenesis

* capsule acts as Antiphagocytic & Immunosuppresant.

* secretions of capsule are Antigen, binds to Antibody so that there is no Ab to lyse yeast cells.

* Granulomatous reacn develops due to infiltration of macrophages, Neutrophils.

* Phenol oxidase releases melanin, melanin gets accumulated in cellwalls of yeast & this protects yeast from toxic effects of free radicals.

* Cryptococcosis occurs through inhalation of capsulated yeast.

Scanned with CamScanner

* cattle – cryptococcal mastitis
* Dogs, cats – Infection of pharynx, paranasal sinuses.
* Horses – Paranasal sinuses, Brain, Abortions.
* Humans – Infection involving lungs & CNS.
* Horses – Resp. symptoms like nasal discharges due to involvement of paranasal sinuses.
* cattle – swelling & firmness of udder, reduced milk yield, milk is visibly Abnormal, mucoid, viscous, Greyish white.
* Dogs – CNS involvement resulting in incoordination, hyperesthesia, Nasal discharges, oral & cutaneous lesions like s/c granulomas around ears, face, feet.
* cats – chronic nasal & occular discharges, lesions in nose, lungs, CNS.

Diagnosis

* Direct examination

  yeast cells are seen in wet mounts of CSF, pus.
* Tissue sections for demonstration of capsule – Mayer Mucicarmine stain.
* If we perform Gram staining – Gram +ve.
* If we see capsulated org. in spinal fluid – cryptococcal Meningitis.

Cultural tests

* Smooth, mucoid colonies on various agars like SDA, blood Agar etc. at 20–37°c for 3–5 days.
* At 7 days characteristic budding cells with thick capsule may be seen.
* Cornmeal Agar – Differentiate it from candida.
  – Non mycelial yeast. Mycelial yeast.
* Birdseed Agar – seeds of Guizotia abyssinica
  – phenol oxidase utilises caffeic acid & produce melanin.
  – Tan to reddish brown coloured colonies becoz of melanin.
* Urease +ve
* Non Fermentative yeast.
* Produces Acid from sugars not gas.
* Melanin is demonstrable in tissue sections by Fontana masson stain

Pathogenicity test

* Animal inoculation

  Intracerebral inoculation into mice about 0.02–0.03 ml of saline suspended org.

  Animal dies in 4d – 2 wks.

Scanned with CamScanner

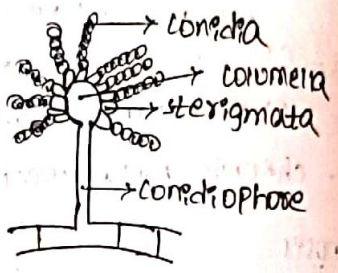
Treatment

Ampho-tericin – B

Prevention

Avoid contact with pigeon droppings.

13/11/19 Aspergillosis



* A. fumigatus, A. niger, A. flavus – species.
* ubiquitous, saprophytic moulds.
* In soil, dust, rotting plants & Animal matter
* Mostly associated with Hay, straw, grains.
* Frequent & common contaminant in laboratory media
* A. fumigatus – Bovine & Avian Aspergillosis
* A. flavus, A. parasiticus – Aflatoxicoses
* A. fumigatus – Mycoses of resp. tract
  - Humans → pulmonary Aspergillosis
  - Disease of Agricultural workers & others who are exposed to high conc. of spores.

Brooder pneumonia / Aspergillosis

* In Birds
* Acquire through inhalation of spores which are present in envt., litter, & on floor.
* Poor sanitation
  Overcrowding
  Poor ventilation
  Prolonged storage of feed
  Nutritional deficiency like vit A deficiency
  Prolonged Antibiotic therapy
  Corticosteroid therapy
  } Pre disposing factors.
* Disease occurs in 2 forms
  - Acute – Brooder pneumonia
    - common in youngones
  - Chronic – common in old birds

Acute form causes high mortality & morbidity than chronic form.

Pathogenesis

* produces enzymes like proteases (predominant is elastase which causes lung damage, & Invasion.
* Produces Gliotoxin
  ↓
  Inhibits activity of cilia as well as phagocytic activity of macrophages
  ↓
  spores develop into hyphal forms.

Scanned with CamScanner

↓
Hyphae invade to blood vessels
↓
Thrombus formation, vasculitis
↓
Mycotic granulomas in lung & other int. organs.

Symptoms

Acute Aspergillosis - High morbidity & mortality
- Rise in Temp.
- loss of Appetite
- fed Respirations
- listlessness
- Foul smelling Diarrhoea
- Rapid loss of condition
- convulsions
- Die within 1/2 days after onset of symptoms.

* chronic Aspergillosis - All symptoms in Acute form will be in chronic form but at milder degree
- Anemia, yellowish faeces, Resp. rattles, Rapid loss of body wt.

↓

* Principle lesion is yellowish nodule in lungs & int. organs.
* These nodules contain tightly packed masses of hyphae & spores.

* Cattle - Abortions bcoz infection involves uterus, fetal membranes, fetal skin.
- ocassionally Mastitis.
- calves → occular infection, diarrhoea

* Horses - Abortions, keratomycosis, ocassionally pulmonary Aspergillosis, Mycosis of Guttural pouch.
↓
- Auditory
- sporadic life threatening disease characterised by necrotised infection with formation of diphtheritic membranes in Guttural pouch.
- From Guttural pouch infection invades int. carotid Artery, vein, Glossopharyngeal Nerve.
- Due to this epistaxis, dysphagia, Parotid pain, Abnormal Head position, unilateral nasal discharges (mucopurulent) are exhibited by Horses.
- seen in summer esp. in horses in stables.

* Dogs - Nasal Aspergillosis, unilateral/bilateral nasal discharges (mucopurulent/mixed with blood), sneezing, open mouth breathing.
- The disease is not systemic though it is classified under systemic.

* Cats - Nasal & Intestinal Aspergillosis

* Humans - lungs, skin, Nasal sinuses, Ext. ear canal, Bronchi, Bones, Meninges are affected.
- Seen in immunocompromised patients.

Scanned with CamScanner

Diagnosis

* Based on clinical signs.
* Birds – Impression smears from lungs & Air sac
  – Gram's staining
  – By observing certate mycelium under microscope, +ve for Aspergillosis.
* wet moulds can be prepared by lactophenol cotton blue & observe under low & high power microscopes for typical structures.
* Grow on SDA in 4–5 days
* Colony colour initially – white, fluffy
  later – dark bluish green, powdery → due to spores.

  A. fumigatus – Bluish Green, Flask shaped vesicle.
  A. niger – Granular, Black
  A. flavus – Yellowish Green
* Depending on colony colour & shape of vesicle sp. can be identified.

Treatment

* Maintain sanitation in hatcheries, incubators, incubation room.
* Remove mouldy hay & litter
* Spray with bleach / cusol around feeders & waterers to eliminate Fungus.
* ↓ the time of confinement
* Improve ventilation.
* Avoid offering poor quality hay & silage.

Mycetoma

* Refers to Tumefaction of extremities
* Caused by Fungi belonging to classes of Ascomycetes, Deuteromycetes.
* Mycetoma ← Eumycetoma – Mycetoma caused by Fungi.
  Actinomycetoma – Mycetoma caused by Bacteria, Actinomyces, Nocardia etc
* These tumors are chronic, affect skin, s/c tissue, fascia & bones.
* It is tumorous swelling → Abscess with multiple draining sinuses.
* Exudate contain granules → Masses of Hyphae with/without spores.
* Humans – Mycetoma is recognised in Madhura. so the disease is also referred as Madhuramycosis / Madhura Foot.

Scanned with CamScanner

* curvularia geniculata
* Helminthosporium specifferum } Produces dark colored granules in pus. In Animals mostly causes mycetoma. saprophytes.

* Allescheria boydii – In humans mostly causes mycetoma
  ↓
  produces light colored granules in pus

Pathogeneses

* Through local trauma
* Neutrophilic response is initiated.
* development of granuloma.
* Reaches to bones also.

Allescheria boydii

* Rapidly growing, initially produces white, cottony, Aerial mycelium.
* After 2 wks it become brownish grey.
* Broad, septate mycelium → Microscope.
  unicellular oval to pear shaped conidia attached to conidiophores by broad, flat bases
* Some of isolates produce sexual fruiting bodies called perithecia/cleistothecia
* Dark then walled structures with delicate Asci & Ascospores – cleistothecia.
* Yellowish, oval, slightly smaller than conidia – Ascospores.

curvularia geniculata

* Grows on SDA
* Initially white → Greyish brown → Black
* Then, lightly pigmented at periphery of colony
* Fusiform, dark brown conidiophores which have 3–4 septa
* conidiophore has tendency to slightly curve on its long axis & hence named as curvularia geniculata.

Helminthosporium specifferum

* white → Grey → Black depressed area with raised Greyish periphery.
* conidiophores develop as branch from mycelium & are swollen at terminal, Appear as twisted/knotted.
* conidia may be single/multiple
* conidia formed on lat. sides of swollen ends of conidiophores.
* conidia resemble Helminth eggs & hence named as Helminthosporium specifferum.

Treatment

* Surgical removal of tumours

Scanned with CamScanner

Rhinosporidiosis

* Chronic granulomatous condition by R. seeberi
* zoonotic infection.
* Resides in ponds, lake water, stagnant water
* Mostly affects Nose, Nasopharynx.
* Affected sps. - cattle, Horses, mules, Dogs, Aquatic birds.
* characterised by formation of polyps on nasal & occular membranes
* other sites - lips, palate, conjunctiva, epiglottis, larynx, trachea, Bronchi, skin, vulva, vagina.
* Lifecycle includes 3 stages

Trophic stage

→ Trophocyte (with clear cytoplasm)

6-8 µm

↓ consists of outer chitinous layer

↓

Development of sporangium

Matures & formation of immature endospores

↓

200-300 µm

→ Inner cellulose layer

→ Immature endospores (12000-16000)

→ outer chitinous layer

Liberation of endospores

After acquiring outer chitinous & Inner mucinous layers the spores mature & released from sporangium with the formation of Germinal pore.

Treatment

* whitish dots are seen on polyps which are sporangia.
* Surgical excision
* chance of reoccurence.

Fungi

Coccidiomycoses

* coccidiodes immitis - soil
* South USA & south America
* subclinical inf - Animals & Man
* Two Asexual reproductive structures

Arthroconidia - Grows in environ. & infectious to humans & Animals.

spherules/sporangium - in body

Transmission

Inhalation of Arthrospores

Inoculation (penetrating objects)

Scanned with CamScanner

dust covered meles.

* direct transmission b/w people is rare.

clinical cases common - Dogs, llamas, Non human primates

Disease less common - cattle, Horses

Disease rare - cattle, sheep, Pigs

Incubation in Animals

* 1° Pulmonary Infection
  symptomatic within 1-4 wks
* Disseminated disease
  Mts. to yrs after initial exposure
* Illness similar to human disease.
  i.e resp. disease
  Dissemination to any tissue/organ occurs
* Nodules (or) granulomas localised.
* cattle - lesions resemble TB - Bronchial & mediastinal lymphnodes.
* less frequent in lungs.
* Dogs - lesions in lungs, brain, liver, spleen, bones, kidney.

Diagnosis

* cough, fever, anappetence recover spontaneously
* Extensive pulmonary lesions
* Persistent coughing
* weakness, depression, fluctuating fever, loss of wt.
* Horses - Non specific, fever, Abd. pain, loss of wt.
* Pulmonary - coughing
  Sk. muscle - osteomyelitis

Diagnosis

* Tissues, exudates, transtracheal (or) bronchoalveolar lavage fluids, lymphnodes, Pleural Fluids - visualisation of parasite
* Double walled
  Most 80-80 µm
  contain endospores } spherules.
* Multiple stains effective

Scanned with CamScanner

Culture

* Lab Diagnosis
* SDA & BA – 25°C & 35°C in 1-2 wks since it is highly infectious.
* Colony morphology
* Flat moist & membranous at both temp. later turns to coarse cottony aerial mycelium white to brown
* Arthroconidia – Barrel shaped, white, multinucleated.
* Tissue phase is not seen in art. medium – SPI spherule medium at 40
* Inoculating into mice – yeast form & formation of spherules in vivo.
* Lactophenol cotton blue: branching septate mycelium that forms chains of thickwalled barrel shaped arthrospores or arthroconidia 2-4 um seperated by clear spaces, remnants of empty cells.
* CFT
* ID – Immunodiffusion Test
* Tube precipitation
* Latex Agglutination Test
* Skin test – Coccidioidin filtrate of mycelial culture.
* Amphotericin – B
* Imidazole – Ketaconazole, Fluconazole.

Prevention

* Limit animal's exposure to large conc. of Arthroconidia.
  - Desert soils
  - Areas of soil Disturbance
  - Dust storms following rain
  - Other dusty conditions.
* No vaccine available.

Histoplasmosis

* H. capsulatum var capsulatum – systemic histoplasmosis in dogs, cats.
* H. c. var farciminosum (H. farcy) equine epizootic lymphangitis
* H. c. duboisii – Human
* Dimorphic fungus
* Soil & decaying vegetation – mycelial form
* Yeast in Animal tissues.

Scanned with CamScanner

Life cycle

Mycelial form in soil → Inoculation of conidia by Human →

* Faeces of bats, birds, pigeons – high N_2 content fav. for multiplication.
* Inhalation, Ingestion.
* pathogenicity
* RES
* Resp. tract
* Dogs, cats, cattle, Non human primates, sheep, swine, human, wild Animals
* Dogs, cats – ulceration of intestinal tract
* Tubercle like lesions.
* Acute & chronic forms.
* Chronic cough, persistent diarrhoea, emaciation.

Diagnosis

* Samples – scrapings of ulcers, lymphnode cut surface, buffy coat.
* Giemsa/wright – intracellular small round (or) oval yeast like single (or) budding cells.
* when it grown on SDA at 25°c – white cottony mycelium after 2-3 wks.
* The mold phase of H. capsulatum is characterised by thin, branching septate hyphae that produce microconidia.
* & a very distinct spore called Tuberculate macroconidium.
* Also known as tuberculate chlamydospore.
* Round thick walled covered with knob like projections sunflower like.
* Grown at 37°c the budding yeast form appears
* white to tan colony.
* Yeast cell is 1-6 μ in diameter & slightly oval in shape & found exclusively within macrophages.
* Mouse inoculation of contaminated specimens yield pure culture.
* CFT
* ID
* CLEP
* Skin test using Histoplasmin +ve test indicates exposure to fungus
* Azole group

Scanned with CamScanner

* Amphotericin - B

Epizootic lymphangitis

* H. farcininosum in equidae
* wounds, abrasions, blood sucking Arthropods, contaminated grooming equipment.
* chronic disease - lymphnodes, superficial l. vessels, skin of limbs, back, neck.
* suppurative nodular ulcerating lesions along lymphatics of legs
* pulmonary & disseminated forms also recorded.
* 15% Mortality
* cutaneous form, after which the disease was named is most common
* Initial lesion is open granulomatous wound along the course of lymphatic vessels which has tendency to ulcerate.
* ophthalmic form - less frequent.
* Resp. form - lesion to upper resp. tract

on nasal mucosa - lesions as yellowish papules (or) nodules & form granulating ulcers that bleed easily

* lesions are usually found near ext. nares
* Asymptomatic carriers.

Direct smear examination & culture

* Diagnosis - based on demonstration of typical yeast like, double contoured cells in pus collected aseptically
* serum agglutination test

+ve titre is 1:80

Treatment

* Iodine treatment
* killed vaccine is available
* culling infected horses.
* test & slaughter policy

~~Bia~~

Scanned with CamScanner

Blastomycoses

* Blastomyces dermatitidis
* wooded areas, moist soils
* Acidic soils rich in organic matter
* Resp. tract
* Lungs, Hematogenous spread to skin & generalised infection
* Granulomatous Nodules
* 2 Phases — Asexual - B. Dermatitidis
  sexual phase - Ajellomyces dermatitidis
* Clinical presentation - Depends on site of infection
  Lung disease - exercise intolerance, coughing
  loss of Appetite, fever, wt. loss
  skin - ulcerated lesions, Draining Abscesses
  eyes - Hyperemic & painful with discharges
  Bone - lameness.

Diagnosis

* wet mounts - large spherical thick walled cells.
* Grow slowly in SDA & BA.
* white cottony mycelium, later tan dark brown to black.
* conidia on septate hyphae close to septa common conidiophore.
* yeast colonies cream to tan wrinkles & waxy.
* cytological (or) Histopathological preparations - M.B/Giemsa.
* ELISA, CLEP

Sporotrichoses - Textbook.

* Non contagious chronic infectious disease of equine, humans, other domestic animals
* caused by Sporothrix shenkii
* Characterised by cutaneous nodules & ulcers on limb with (or) without lymphangitis & lymphadenitis.
* Etiology - Grows on SDA
* Predisposing Factors
  - cutaneous wounds
  - Presence of Animals in muddy, unhygenic stables
  - Entry: on thorny plants & traumatic introduction (Rose Gardeners disease)

Scanned with CamScanner

Epidemiology - Textbook

* Horses - Ascending lymphocutaneous infection of leg
* Infected Animal - Main source for spread of disease

Pathogenesis

* Affects s/c tissues causing nodular ulcerative lesions & spread through lymphatics.
* org. gain access through wounds, Abrasions in skin.
* Clinical signs

3 forms - cutaneous

lymphocutaneous - Development of small s/c nodules at site of inoculation usually at fetlock.
- Serohaemorrhagic exudate
- Fever, listlessness

Disseminated

Cats - Nodules on extremities, Head, Tail
large no. of yeast cells in discharges

Field Diagnosis - Depends on History, epidemiology, clinical signs of disease.

Diagnosis

* Pus, blood & serum sample.
* Direct microscopic examination of stained smears reveal cigar shaped bodies in neutrophils.
* Methanamine staining of nodules reveal characteristic Asteroid bodies
  ↓
  cluster of yeast cells with peripheral eosinophilic rays.
* Isolation - Animal Inoculation
* Latex Agglutination Test

→ Hyphae with microconidia - characteristic flowerett like arrangement
→ Cauliflower like clusters of small conidia born on delicate sterigmata on septate Hyphae.

Differential Diagnosis

* Glanders, ulcerative lymphangitis etc.

Treatment

Scanned with CamScanner

Control
Adequate hygiene to prevent spread of infection.

Dermatophytosis

Dermatophytoses (or) cutaneous mycoses are diseases of skin, hair, nail.
Generally called ringworm infections (or) tinea versicolor.
Caused by homogenous group of closely related fungi known as Dermatophytes.
Dermatophytes – septate fungi that require & use keratin for growth
Affect only stratum corneum of skin.
Dermatophytes are placed in Deuteromycota (or) Fungi Imperfecti in three anamorphic (asexual (or) Imperfect state) genera

Trichophyton – infections on skin, hair, nails
Microsporum – infections on skin, hair
Epidermophyton – infections on skin, nails.
Sexual forms of perfect states of about half the microsporum & trichophyton sps. have been identified.
They belong to two genera of Ascomycetes.
Dermatophytes on basis of their natural habitat & host preferences
Anthropophilic sps. – Human (E. floccosum)
Zoophilic sps. – animals (M. canis, T.m, T.g) → Trichophyton gallinae.
Geophilic sps. – soil fungi. (Microsporum gypseum & M. nanum)
Dermatophytes usually grow only on keratinised skin & its appendages.
Do not penetrate the living tissue beyond stratum corneum bcoz of serum & body fluids.
Do not tolerate >35°C.
Aerobic, Non fastidious
Moist environment.
Highly host adapted parasites
M. canis – Dogs, cats, Humans
M. gypseum – Dogs, Horse, cats.
M. nanum – swine.
T. mentagrophytes – all domestic animals
T. verrucosum – cattle, sheep
T. gallinae – Fowl

* T. equinum – Horse.

Asexual spores

* Microconidia – unicellular, small (2–5 µm), round (or) pear shaped, borne laterally (or) terminally singly (or) in clusters.
* Macroconidia – Multicellular, septate.
* Their shape, size, surface are used to differentiate the genera.

Spindle shaped Macroconidia of microsporum | Cigar shaped Macroconidia of trichophyton | Macroconidia of epidermophyton.

Boat shaped macroconidia in M. canis
M. nanum – unique in being round & 2 celled.

* Ectothrix
- Fungal branches (hyphae) & spores (arthroconidia) cover outside of the hair.
- Eg:- Microsporum sps., T. mentagrophytes – small spore Ectothrix.
- T. verrucosum, T. equinum – large spore Ectothrix.

* Endothrix
- Hair shaft is filled with fungal branches (Hyphae) & spores (arthroconidia)
- Eg:- T. violaceum.

* Infective arthrospores germinate within 6 hrs of adhering to keratinised structure
* Minor trauma of skin & dampness may facilitate infection.
* Ability of dermatophytes to hydrolyse keratin causes damage to epidermis, hair shafts, hair follicles & Feathers.
* Nature of lesions is affected by
  virulence of fungus
  Immunological response of host
* Very young & very old animals
* Debilitated (or) Immunosuppressed. } Highly susceptible

Pathogenesis

* Host mounts an inflammatory response to the fungal metabolic product i.e, harmful to fungus.
* So dermatophyte moves away peripherally towards normal skin.

Scanned with CamScanner

* The result is the commonly seen circular lesions (ringworm) of alopecia with healing at the centre & inflammation at edge.
* In hair infecting sp. Hyphae grow down the hair follicle enter the shaft & affect keratinised cells just above root bulb.
* Septation of hyphae result in formation of arthrospores - only spores formed during asexual phase.
* CMI - DTH reac^n try to eliminate infection.
* Lifelong immunity if recovered.

Subclinical cor/inapparent infections
* Classical round ringworm lesions.
* Serious generalised lesions that may be complicated by mange mites lor/by 2° bacterial infection.
* Nodular (or) tumourous lesions called (Kerions), seen most commonly in dogs.

Clinical signs
Dogs & Cats

* M. canes
* Thick circular scaly patches upto 3cm on any part of body.
* Broken stubs of hair cor/complete hair loss.
* Vesicles & pustules at periphery.
* Large no. of multiseptate boat shaped macroconidia.

Cattle
* All ages are susceptible
* Calves & young ones - vulnerable
* Winter months in housed
* Large discrete confluent raised scaly lesion on head (ear, eyes, muzzle).
* Neck, less frequent on back, flank, limbs.
* 2-4 mts later thick yellow brown asbestos like scabs.
* T. verrucosum - large spores
* T. mentagrophytes - small spores.
* Microconidia absent in verrucosum

Horses
* T. equinum
* Young horses - highly susceptible
* Withers, saddle, girth - Affected parts.
* Small no. of smooth cigar shaped macroconidia.

Scanned with CamScanner

Poultry

* Ringworm / Favus

* White patchy crusts on comb, wattles.

Diagnosis

* If possible examine patient (dogs & cats) in dark with wood's lamp.
* Filtered u.v. light 3650A°.
* Flourescent Hairs from edge of lesion.
* Apple green Flourescence – M. canis
* Nails, Hair, skin scrapings – 10-20% KOH – arthrospores conthyphae.
* warm & wait for 30 min to digest prot. debris
* Lactophenol cotton blue.
* SDA – 1 month incubation.
* For T. verrucosum at 37°C.
* For Prod^n of macroconidia in verrucosum – Inositol & thiamine.
* T. equinum – Nicotinic Acid.
* 2-4% yeast extract provide these supplements.
* Examine colony Morphology.
* Texture & pigment.

Identification

* For sps. identification
* size & shape of macroconidia & thickness of cellwall.
* urease prod^n.
* Geographic occurence.
* Req. of vitamins & a.a
* Temp. stimulation of growth – T. men & T. verr grow well at 37°c.
* Hair perforation Test

M. canis, T. mentagrophytes – perforate Hair shaft forming wedge shaped dark blue structures stain darkly with lactophenol cotton blue.

* Trichophyton agars 1-7 contain various growth factors for speciation.
* Dermatophytes Test medium – modified SDA containing Phenol red they change yellow medium to red within 2 wks.

Scanned with CamScanner

Treatment

* Topical & systemic
* 5% lime sulphur, Na hypochlorite, Chlorhexidine, Povidone Iodine.
* Systemic in dogs & cats.
* Griseofulvin - 2-40 mg/kg - 10 days for hair infections (orally)
* whitfield's ointment - due to human strains - feet - T. mentagrophytes.
* Ketoconazole, Itraconazole.

Humans acquire infection from

T. verrucosum - cattle

M. canis - Dogs

T. mentagrophytes - Horses

Control

* Isolation of suspicious Animals
* Early lab confirmation
* In contact animals should examine under woods lamp.
* Vaccum cleaning of contaminated areas to remove infected debris & Hair.
* Disinfect sheds, stables, cages etc.
* Blow lamping of walls

20/11/19 Mycotoxicosis

* Disease syndrome results due to ingestion of mycotoxins present in feed.
* Mycotoxins - 2° metabolites produced from certain 2° toxins under defined conditions on crops, stored feeds & Grains.

It requires moist, humid climatic conditions, optimum temp., O_2 requirement.

* low molecular wt. compounds
* Very stable compared to actual fungus.
* Heat resistant.
* Major problem in cattle & poultry becoz of recycling of food.
* ↓ed Growth rates in Broilers

↓ed milk yield in cattles.

Etiology

* 800 kinds of Mycotoxins are present.

Field Fungi - They attack crops before harvesting.

- Helminthosporium, Chladosporium

Storage Fungi - They attack crops after harvesting during storage

Scanned with CamScanner

— Aspergillus, Penicillium.

A. flavus, A. parasiticus — Aflatoxins

A. ocheraceus, Penicillium viridicatum — ochratoxins

DHS, Fusarium rosium, F. trisinctum — Trichothecins (or) T_2 toxin.

F. graminearum — Zearalenones

Ergot peptide Alkaloid — sclerosia of claviceps purpurea.

* Through Ingestion, Inhalation, direct skin contact — Animals acquire mycotoxins
* In low conc., Mycotoxins cause carcinogenesis, Mutagenesis, Keratogenesis.

Toxigenic Fungus
↓
Growth on plants — Determined by regional, seasonal climatic conditions

Growth in stored feed — Determined by quality of Feed

Growth on crops — Determined by Age, species of plant

Prod of Mycotoxins (require presence of Fungus, substrate (plant), Envt.al conditions).

* After ingestion Mycotoxins are metabolised by liver into toxic & Non toxic compounds. Toxins binds to macromolecules like Nucleic Acids which results in ↓ed protein synthesis, carcinogenesis, Keratogenesis, Aplasia of thymic cortex, ↓ed CM Immunity.

Symptoms

* 1° & 2° Mycotoxicoses
* 1° — Acute / Chronic

Acute 1° Mycotoxicosis

* when high to moderate toxins are consumed host exhibit marked signs (or) even death. Diff. systems gets affected based on type of mycotoxin.

* Vascular system if affected — Aflatoxins
  - ↑ed vascular fragility
  - Haemorrhages on body tissues

* Digestive system if affected — Trichothecins
  - Diarrhoea
  - Intestinal Haemorrhages
  - Hepatotoxic effects (liver necrosis & fibrosis)
  - Mucous membranes are affected by Trichothecins

Scanned with CamScanner

* urinary system if affected – ochratoxins
  Nephritis
  Uremia
* Reproductive system if affected – zearalenones
  Infertility
  Prolonged oestrus
* Cutaneous if affected – Fusarium toxins.
  Photosensitisation
  Necrosis & sloughening of extremities.

Chronic 1° Mycotoxicosis

* when moderate to low levels are consumed it results in slow growth rate, led reproductive efficiency, reduced mortality.

2° Mycotoxicosis

* when very low quantities are consumed.
* No open signs but there is predilection to 2° bac. infections becoz of impaired immunogenesis, suppression of CM immunoresponses.
* candidiasis, salmonellosis, coccidiosis – poultry

Diagnosis

* Accurate detection of toxin in suspected samples is imp.
* PM Findings
* History collection (sudden drop in egg/milk prod^n)
* Biological testing of toxin by feeding toxins to susceptable species

Prevention

* By adding certain Fungistats like Acetic Acid – Propionic Acid in the ratio of 60:40, 2%. Gemsian violet, 1-2% of NH_3 treatment.
* Toxin binders like Hydrated Na Ca Al silicate.
* Prevention of mould growth during harvesting, transportation, storage is possible by controlling moisture at all these levels.
* Strict enforcement of rules & regulations on feed manufacturers to control mycotoxicosis.
* Supplementations like Vit-K to check development of haemorrhages.
* Broad spectrum Antibiotics for treatment of 2° infections.
* B-complex injections.

Scanned with CamScanner

Mycotic Mastitis

* Mastitis – characterised by change in milk secretion of affected quarters, presence of clots, discolouration, large no. of leucocytes.
* Around 26 sps. of fungi are associated with mastitis.
* Trichosporon sps, Saccharomyces sps., A. fumigatus, A. niger, Alternaria sps., Rhizopus sps., Mucour, Geotrichum candidum, Prototheca sps., C. neoformans, Cryptococcus, C. albidus, All 3 sps. of candida (C. albicans, C. krusei, C. parapsilosis). ↓ Cause severe mastitis
* Ability of fungus to set up inf. in mammary gland ↓ depend on

1) Fungal characteristics.
   - Ability to occupy area of Mammary tissues.
   - To survive in the envt. of Mammary Gland.
   - To resist influence of cleaning, disinfection etc.
   - To resist Antifungal Antibiotics

2) Transmission Mechanism ↓ depend on
   (i) Animal Factors → - Susceptibility (old Animals)
   - Stage of lactation (1st stage)
   - Inherited resistance ↓ Due to shape of teat, Anatomical structure of Teat canal.
   Funnel shaped Teats – low incidence
   cylindrical Teats – More incidence.
   - Immune status.

   (ii) Source of Infection
   - Brewer's yeast.
   - Pigeon droppings in soil & dust of dairy sheds.
   - contamination of utensils with yeast.
   - Spores in air of cowsheds
   - Contaminated intramammary infusions.

Yeast better adapt in mammary duct

Aspergillus penetrate walls of teat cistern & teat canal

Candida – Chronic Fibrotic thickening in septum of mammary gland.

Cryptococcus – Granulomatous lesions

Prototheca – Induration in udder, clots formation in milk making milk more watery.

Symptoms (similar to Mastitis caused by bacteria)

* mucilaginous milk
* Multiple Abscess
* Yellowish clots in milk.

Scanned with CamScanner

Diagnosis

* Microscopic examination of fungi from milk sample.
* smear – lactophenol cotton blue – 0.5% Gemsian violet : Modified silver methanamin -e.
* Isolation
  streak this deposit on SDA, incubate at 37°C, observe for 1 week.
  If growth occurs examine under microscope using lactophenol cotton blue.
* yeast – prefer blood Agar.
* presence of Arthrospores & Blastospores – Trichosporum sps.
* presence of Arthrospores – Geotrichum candidum
* sporangiophores branched – Mucour
* corn meal Agar – wide filaments – prototheca
* sporangiophores with Rhizoides – Rhizopus sps.

Treatment & control

* Avoid offering feeding with fungal growth.
* Iodine Treatment – Candida
* cleanliness.

Mycotic Abortions

More
* Frequent in Dairy Animals.
* less frequent in sheep, Horses, Pigs
* A. fumigatus – responsible for Abortions at 6–7 mts of Gestation period in cattle.
* Animals acquire inf. through inhalation of fungus/spores.
* Through Hematogenous spread reach placenta.

Symptoms

* Inf. of Genital organs, Abortions
* Aborted fetus exhibit discrete raised ringworm like lesion on skin of head & back.
* Placenta is thickened, Haemorrhagic, Edematous, Necrotic.
* Infertility
* In males – orchitis, Epididymites, Inflammation of vesicular gland.
* Max. no. of cases recorded during monsoon.

Diagnosis

* Placental, Foetal stomach contents
* Direct & cultural detection of Hyphae – structures observed in direct & cultural examination should be same.
  on Malt Agar plates
  look to observe Fungal Hyphal growth.
  Lactophenol cotton blue.

Scanned with CamScanner

* Various precipitation tests are available to detect antibodies of A. fumigatus.

Prevention

* Keep animals in well ventilated sheds.
* Avoid offering mouldy hay/straw to pregnant animals.
* Treat hay with fungistats.

Zygomycetes * Hyphae are coenocytic

* Mucor, Absidia, Rhizopus, Rhizomucor – Thermotolerant saprophytes in soil & decaying vegetation.
* Transmit through inhalation/ingestion
* Systemic opportunistic fungi
* 2° to infections like metabolic Acidosis, immunosuppression.
* Affect lymphnodes of Resp. & Alimentary tract
* lesions are granulomatous, occassionally lesions – ulcerative caseo calcareous
* In swine, cause gastric ulcers, embolic tumours in liver & lungs
* In cattle – Abortions, lymphnode infection.
* Affect all the animals.

Diagnosis

* Direct examination of smears prepared from lesions or tissues.
* Rapidly growing on SDA at room temp.
* Repeated observation of same structure under microscope.

Mucor

* Thick, colourless mycelium without rhizoids
* simple/branched sporangiophores with globose sporangia containing small spores.

Rhizopus

* Dense, cottony, aerial mycelium
* Initially white in colour, turns to grey
* stolons contact medium through rhizoids

Absidia

* Resembles Rhizopus grossly but sporangiophores do not arise from stolon.

Rhizomucor

* Thick, grey mycelium.
* Short sporangiophores with black, spherical sporangia.

Scanned with CamScanner

Penicillium

* common Air borne contaminant
* Grow on various substrates like ~~blood~~, bread, Jam, Fruit, cheese
* Bluish Green colonies with white border & powdery surface.
* septate Hyphae with branched conidiophores.
* conidiophore with 2 rows of sterigmata (or) with chain of spores giving brush/broom like Appearance.
* opportunistic infections known as penicillosis.
* Rarely Pathogenic affecting pulmonary & Genital Areas.

Emerging, Re-emerging & Transboundary Bacterial diseases

* Emerging diseases – Appeared for 1st time in population (or)
  - have existed previously but are rapidly ↑ing incidence (or) Geographical range. (or)
  - A new infection/infestation resulting from evolution/change of existing pathogenic agent (or) previously unrecognised pathogenic agent/disease diagnosed for 1st time & which has significant impact on Animal/public Health. (OIE)
* Re-emerging diseases – A known infection/infestation spreading to a new geographic area/population (or)
  - A known endemic disease considered to be re-emerging if it shifts its geographical setting (or) expands its host range (or) significantly ↑ its prevalance.
* Transboundary diseases – Diseases with an essential impact on economy, trade (or) food security of group of countries which can be easily spread to other countries reaching epidemic proportions & that require control & eradication. (FAO)
* Factors – Host, Agent, Envt·al Factors.

Agent

1) Evolution of Agent – Change in genome.
2) Development of Resistance to Antibiotics – Due to improper use of Antibiotics.
3) Resistance of vectors to pesticides – eg:- Mosquitoes, Ticks, mites

Host

* Human demographic changes leads to development of intensive farming system.
* Animal susceptability becoz of immunosuppression.

Scanned with CamScanner

Envt.al Factors

* Climate
* Change in ecosystem
* urbanisation — leads to development of diseases due to deforestation.
* Global warming — due to ↑ in temp., many effects on health of Animal.
* ↑ in Transboundary diseases due to globalisation.
  ↓
  Trading has bee ted b/w countries.
* About 2/3rds of emerging infections are zoonotic like Brucellosis, TB, Paratuberculosis, leptospirosis, Multidrug resistant E.coli, Multicellular resistance staphylococcus.

Scanned with CamScanner

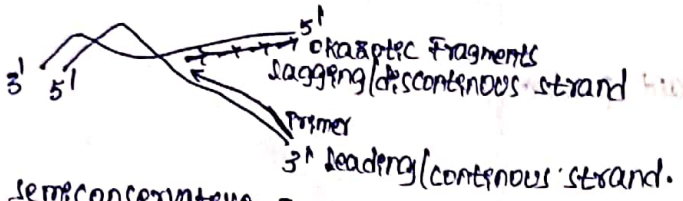
Recombinant DNA Technology

→ Herbert Boyer & Stanley Cohen (Father)

* Recombinant Insulin (or) Humulin.

* DNA replication

- unwinding of double stranded DNA helix – by enzyme Helicase.



semiconservative Replication.

* prokaryotes – Transcription & Translation occur together.

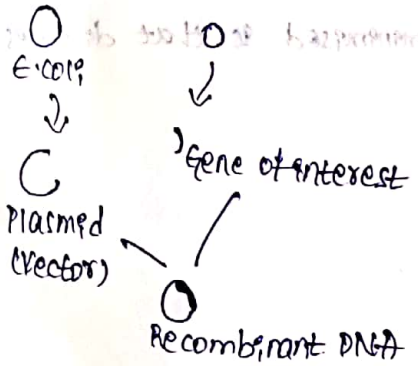
* eukaryotes

RNA processing – splicing
↓
Introns/non coding Agents are removed & exons/coding Agents are joined.

DNA
↓ Transcription
m RNA
↓ Translation
Protein

5' – cap
3' – poly-A tail, } after post transcription.

*



* Covalent insertion of DNA fragment from one cell (or) org. into replicating DNA of another – DNA recombination (or) molecular cloning.

* GM Plants

- Bacillus thuringeneris cotton
(BT cotton)

→ Genome of cotton
↓
Bacillus thuringeneris
↓
Able to produce 200 toxins
Among 200 toxins, $\boxed{1 \text{ toxin}}$ is resp. to kill Boll worm which infects cotton.

Scanned with CamScanner

* 1st Transgenic Animal – Dolly (sheep).

*GM Animals

GM pigs, GM cows, GM salmon, GM birds.

Applications

* Prod^n of Hormones
  - Insulin, GH
  - Humulin (1st recombinant product)

* vaccines
  - sub unit vaccines
  - Marker vaccines (DIVA)

*Antibiotic producing org.
  - Streptomyces sps.
  - Penicillium sps.

* Mab's – Monoclonal Antibodies – Ab's produced against a single epitope
  - Hybridoma technology
  - Diagnostics.

  A smaller part of Antigen

* Prod^n of chemicals, enzymes – Industrial Applications.

* Gene therapy – Introduction of extra gene for correction of diseases.
  - Delivery of sp genes for correction of diseases.

* Gene silencing – Expression of extra gene is minimised so that diseases can be minimised.

* sequencing of DNA/Genes.

* study of mutations

* Molecular Analysis of various diseases.

* Application in Forensic sciences

* Biotec Prod^n, Envt. Protection.

* Genetically engineered microbes.

Human Genome project

* used to study polygenic disorders, cancers, Diabetes etc.

Scanned with CamScanner

Cloning – Require vector, Gene of Interest, Enzymes (RE, ligases), competent cells.

Fragment DNA from any org. + Broken Plasmid vector

RE digests will cut DNA into 1000's of fragments ↓

Recombinant DNA molecule.

Host chromosome

competent cell Bacterium

Transformation of bacterium & selection of a cell containing Plasmid

Bacteria containing Plasmid.

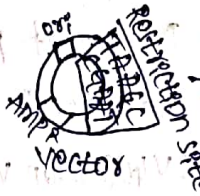
Growth & cell division

uses bacteria to purify & make many exact replicas (clones) of DNA fragments.

Clone of Plasmid containing bacteria (amplify)

Vector

* It should be self replicating.
* It should have unique restriction sites.
* It should have High copy no.
* It should have selectable markers
  ↓
  Antibiotic resistant Genes
  β galactosidase markers



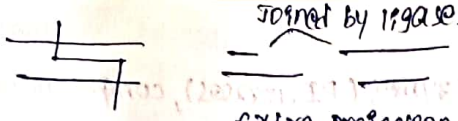
→ recognised by particular restriction enzyme & gets cleaved by RE

* It should be extrachromosomal.
* vectors – 3 types → pBR322, pUC 16, 17, 18, 19 (Most commonly used.)
  1) Cloning vector – ori, restriction site, selectable Markers, Gene of interest.
  2) Expression vector – A Promotor infront of Gene of interest is present along with ori, restriction site, selectable markers.
     – Promotor is also called TATA box.
     ~~Induced vector~~ – Inducer is present which binds with promotor & helps in expression of gene by promotor.
  3) Shuttle vector – can replicate in more than one type of Host cell.

Restriction Enzymes

* Enzymes that cleave Genome/DNA at specific site.
* Type I, Type II, Type III
* Type II Endonucleases – commonly used RE.

Scanned with CamScanner

* 

extra projection part – overhang

sticky ends.

Eg:- EcoR1, Hind III, KpnI – Restriction sites.

↓ Hemophilus influenza ↓ Klebsiella pneumoniae

* RE recognise palindromic sequences as restriction sites.

Competent cells *Easy to handle & Easy to propagate

* living cells either bacteria which can hold a vector including gene of interest.

* It should allow vector to replicate

* commonly used host cells – E.coli, yeast cells

* 2 selection processes

– During insertion of Gene of interest into vector.

2nd – During transformation of vector with Gene of interest into Host/competent cell.

Phage vectors

~~Viral~~ vectors – Bacteriophages (λ Phage, M13 Phage)

Plasmids – pBR322, pUC 19

cosmids – carry portions of plasmids & phages.

– Phages have "cos".

Viral vectors – SV40 (Simian virus 40)

Retroviruses

Baculoviruses

Competent cell

* Introducing foreign DNA into Prokaryotic Host cell (E.coli) – Transformation

* Introducing foreign DNA into Eukaryotic Host cell (Saccharomyces) – Transfection

Methods of Transformation

→ Calcium chloride method

* cells are exposed to very cold $CaCl_2$ soln, then subjected to 42°c for 2 min so that

→ Electroporation

* cells are subjected to High voltage electric field which results in formation of pores

→ Rubidium chloride method

conjugation

when Host cells are unable to take foreign DNA, donor cell is used & there will be conjugation b/w Host & Donor cells resulting in conjugation tube formation so

Scanned with CamScanner

that foreign DNA enters host cell from donor cell.

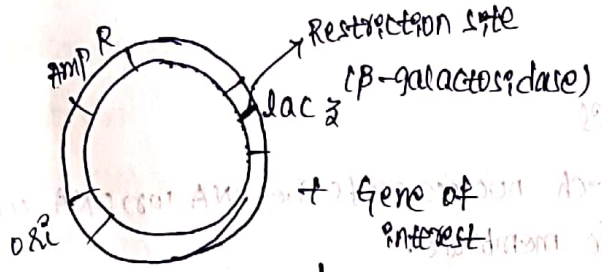
Methods of Transfection

Chemical Methods – calcium chloride method
– DEAE – dextran method
– Liposome
Lipofection is process where liposomes are used as vacant to introduce DNA into host cell

Non chemical Methods – Electroporation.
– sonoporation
Host cells are subjected to high intensity ultrasound waves
– optical transfection – use laser beam.
– cell squeezing – Destabilization of cell membrane introducing recombinant DNA

* Host cell without plasmid
* Host cell with religated plasmid – only vector
* Host cell with religated ~~vect~~ gene – only Gene of interest.
* Host cell with recombinant DNA

pUC19



X – Gal media
1) Ampicillin
2) Lactose

+ E. coli – inoculate in media & stored at –80°C
↓
(37°C) Room Temp
↓
IPTG – Inducer

Insertional Inactivation – Enzyme, β-galactosidase is not expressed due to insertion of our Gene of interest in b/w lac Z.

Lactose $\xrightarrow{\text{β-galactosidase}}$ Galactose + Blue colour dye

Since β-galactosidase is not expressed, No splitting of lactose, NO blue colour formation & we see Appearance of white colour.

Blue-white selection.

Scanned with CamScanner

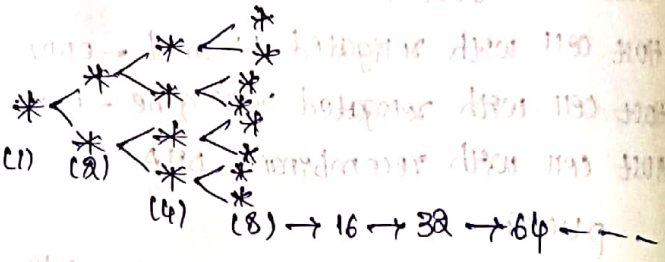
PCR

* Pioneer of PCR – Kary Mullis – 1983.
* Rapid method of diagnosis.

eg:- HS caused by pasturella multocida.

whole Blood
↓
Isolate DNA
↓
50–60 copies of DNA
↓ By PCR.
Multiply to million copies of DNA
(visualised by Agarose Gel electrophoresis)

* Denaturation
* Annealing
  - Primers
    - Forward primer
    - Reverse primer
* Extension.

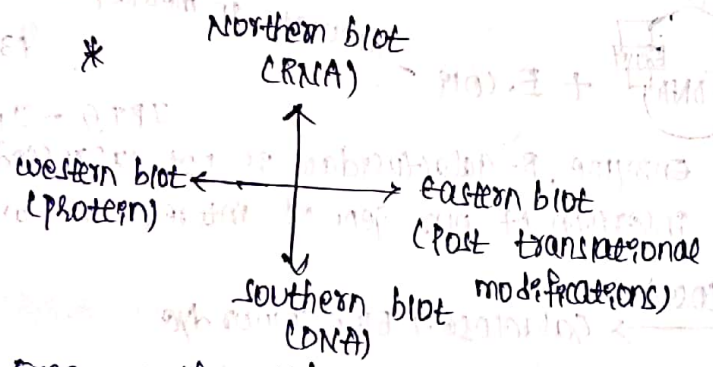


3/12/19

Blotting Techniques

* Blotting is a technique in which nucleic acids i.e, RNA (or) DNA (or) proteins are transferred onto a specific membrane.
* This membrane may be nitrocellulose / nylon membrane / PVDF (poly vinylydene Flouride)

*



* Probes – Fragment of DNA/RNA of variable length
  - 100–1000 bp
  - Detects complementary sequences in target nucleic acid / protein samples
  - SS DNA probes – preferable & convenient.

Types – Genomic DNA probes
- c-DNA Probes
- synthetic oligonucleotides as probes
- RNA probes / riboprobes

(complement-ary)

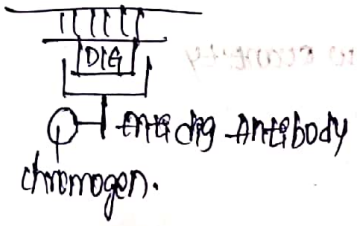
Scanned with CamScanner

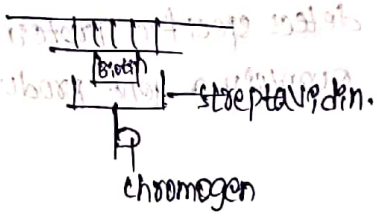
RNA → DNA
BY Reverse Transcriptase
called as cDNA.

Labelling of Probes
Radioactive labelling
Non radioactive labelling

Radioactive labelling
* ^{32}P, ^{3}H, ^{35}S, ^{125}I → Radioisotope labels
* 3 methods for labelling ← Nick translation
Oligonucleotide labelling
Riboprobe preparation

Non radioactive labelling
* Biotin, Digoxigenin





Southern Hybridisation
* Sir Edwin Southern

1) * Digest DNA with an appropriate RE
2) * Run the digest on an Agarose Gel ← Gel electrophoresis.
3) * Denature DNA
4) * Transfer Denatured DNA onto the membrane → lots of dry paper / Membrane / Gel
5) * Probe membrane with labelled ssDNA (hybridisation)
* Visualise labelled target sequence
6) Prehybridisation
7) Hybridisation.

Applications
* DNA fingerprinting
- RFLP of VNTR's
* Dot (or) slot blot
* Colony (or) Plaque lifts
* Microarray technology

Scanned with CamScanner

Northern Blotting

* Alwine

* Disease Diagnosis
  Gene expression } Applications

* used to detect & analyse RNA in given sample.

Western blotting

* W. Neal Burnette

* used for detection & Analyses of protein in given sample

extraction of protein → SDS-PAGE

[Sodium dodecyl sulphate poly acrylamide Gel electrophoresis]

* we used 1° Antibody

2° Antibody in place of Antidig Antibody

* Applications – used in clinical purposes
  – used to detect specific protein in low quantity
  – used to quantify a gene product

Eastern Blotting

* Bogdanov

* used to identify CHO epitopes including glycoconjugates & lipids

* Extension of western blotting.

* Mostly blotted protein after transferring onto the membrane are analysed for PTM's by using a probe & hence identify CHO's & lipids

* Applications – used to purify various phospholipids
  – used for binding studies by using various ligands
  – Detection of protein modification.

4/12/19 Bioinformatics

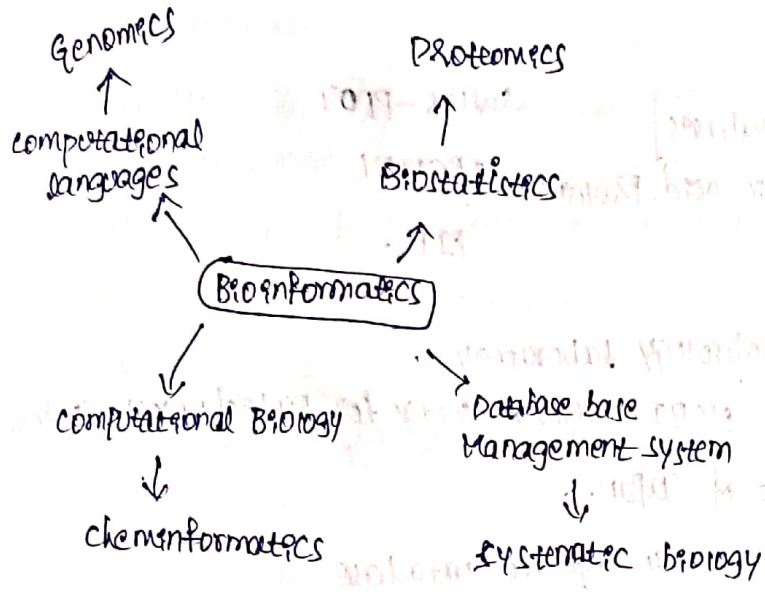
* requires Knowledge from different subjects.

* computational Approach for solving biological problems.

* Emerging & Advanced branch of biological sciences.

* It requires knowledge of biology, statistics, mathematics, computer science.

Scanned with CamScanner



Applications

computational Biology – comparative Homology modeling
- Phylogenetic Analyses (Based on ·% of Divergence).
- Protein structure prediction
- Protein Folding problem.
- Microarray analyses
- Docking Approaches & New drug Discovery

Internet & Bioinformatics

* Mathematical portion helps to understand algorithms used in Bioinformatics software & tools

Biological databases

Type of database	Information contain
Bibiographic databases	Literature
Taxonomic databases	Classification
Nucleic Acid databases	DNA information
Genomic databases	Gene level information
Protein databases	protein information
Enzymes, Metabolic pathways	Metabolic pathways.

* Databases – 3 types

1° Databases – contain raw information
2° Databases
composite Databases

Scanned with CamScanner

EMBL

Genbank

DDBJ

1° databases
(Nucleic acid, Protein)

SWISS-PROT

TREMBL

PIR.

EMBL – European Microbiology laboratory

Genbank – Maintained by NCBI (National centre for Biotechnology Information)

DDBJ – DNA Data Bank of Japan.

SWISS-PROT – Annotated protein sequence data base

TREMBL – Protein sequence database.

PIR – Protein Information Resource

2° databases

PROSITE

Pfam

BLOCKS

PRINTS

Composite databases

NRDB

(Analyse 1° & 2° databases & form composite database)

OWL

Collaboration of International sequence Database

DDBJ

EMBL

Genbank

2D gel Database

SWISS-2DPAGE

REPRODUCTION-2D PAGE

3D structure database

Analyse protein structure, protein Folding.

Scanned with CamScanner

Gene Banks

* Guardians of Diversity.
* Type of biorepository which preserve genetic material.
* Facilities where genetic material can be conserved & made available for users such as breeders, researchers & even farmers.
* CGIAR, Gene bank platform, the network conserves & makes available more than 773,112 accessions of crop collections that contributes
* $-80°c / -196°C$ ← common Freezing temp.

Seed Bank

* preserved dried seed by storing them at very low temp.

Cryobank

* utilised for cryo conservation of Animal Genetic resources.

Q12/19

PCR – Polymerase chain Reaction.

* Polymerase is enzyme which catalyses synthesis of new DNA.
* Template DNA
  Primer
  DNA polymerase
  dNTP's – A, T, G, C
  → Deoxy nucleotide trephosphates

  } Components/ Things required to PCR.

* 95°C → Two strands get seperated
  40–65°C
  72°C → Extension of new DNA.

* "Thermal cycler" → Machine used for PCR.

Use of PCR

* Diagnosis

* Template DNA
- DNA may be genomic/complementary/plasmid DNA.

* Primers
- Short stretches of nucleotide sequences.
- Single stranded.
- 18 to 20 bp in length.

Scanned with CamScanner

- For each reac^n we take both forward primer & reverse primer.
- usually bind at 3' end of strand.
- primers are selected in such a way to amplify specific target gene.

* DNA polymerase

→ Klenow fragment - 1st used DNA polymerase, derived from E.coli
- limitation is (it is heat sensitive)

→ Taq polymerase - scientists identified Thermus aquaticus (thermophilic) in Hot springs in Yellow stone National park, USA.
- cheap & commonly used
- Amplify upto 10-15 kbp.

→ Vent DNA polymerase - derived from thermococcus litoralis
- Amplify 50 kbp.
- More stable than Taq

→ T^th polymerase - extracted from Thermus thermophilus
- Highly Heat stable

* dNTP's

dATP's, dGTP's, dTTP's, dCTP's - present in equal conc.'s

* other components

↳ Mg, Mn, KCl, Ammonium ions, Glycerol, DMSO → Dimethyl sulphoxide

All these are present in a sol^n called Buffer & helps in conducting reac^n efficiently

*All these are used in preparation of Master mix (or) Reaction mix.

DNA - 2 µl
Forward primer - 1 µl
Reverse primer - 1 µl
Master mix - 11 µl
DNA polymerase, dNTP's, other components - 5 µl
FW
20 µl

Mix well & place in thermal cycler.

~~Cycling conditions~~ Steps in PCR

* Denaturation → 94-98°C — strands
* Annealing → 40-65°C — primer
* Extension → 72°C — DNA polymerase

Exponential Amplification

Scanned with CamScanner

we carry out this process usually for 30–40 cycles.

cycling conditions

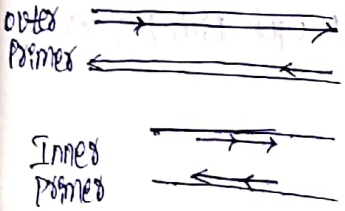
* Initial denaturation – 94°c/10 min – occurs only once.
* Denaturation – 94°c/30 sec.
* Annealing – 40–65°c/1 min.
* Extension – 72°c/1 min

These 3 steps continue for 30–40 cycles.

* Final extension – 72°c/10 min – occurs only once.

whole process takes on 2–3 hrs.

* Uniplex PCR – Targetting a single gene (or) a part of gene.
* Multiplex PCR – Targetting several genes. (we should have primers for all the genes)
* Nested PCR – use 2 sets of primers
  - outer < Forward primer, Reverse primer
  - Inner < Forward primer, Reverse primer.



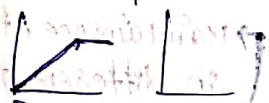
Product of 1 PCR act as template for other → Inner primers are used.

* Reverse Transcription PCR – when genome is RNA this type of PCR is used.

RNA → (Reverse Transcriptase) → DNA → called as complementary DNA.

* Agarose Gel Electrophoresis (AGE) – To detect whether product is amplified (or) not.
  - Takes 1/2 hr – 2 hrs.

* To minimise time consumption in AGE, Real time PCR is used.
  → Advanced technique.



Peak (Amplification) | No peak (No Amplification).

SYBR green reaction
Taq Probe chemistry } Real time PCR.

SYBR green reac^n – Flourescent dye gives Flouroscence by binding to loop of double stranded DNA.

– Flouroscence ↑es with ↑ in no. of double stranded DNA indicating whether the product is amplified (or) not.

Taq Probe chemistry – Taq man probes → used along with primers.
– Primers.

* Hemenested PCR
* Colony PCR
* In situ PCR – performed directly on a slide containing tissue sections.

10/10/19

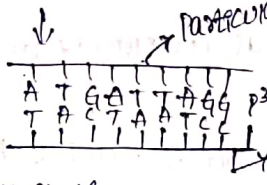
* Real time PCR – To upregulate/downregulate gene expression.
* Master mix should also include Intercalating dyes (or) Flourescent tags.
  - Intercalating dyes → Goes & sits in the grooves of ds DNA.
  - Flourescent tags → primers themselves tags with Flourescent probes

Intercalating dyes – SYBR Green
Eva Green

Flourescent probes
- Primer probes – scorpion primer probe
- Hydrolysis probes – Taq man probe
  Flourescence emitted by Hydrolysis depends upon DNA polymerase
- Hybridisation probes – Molecular beacon

DNA Hybridisation

Principle: sample → Extract DNA/RNA.



* To identify similarity (or) divergence b/w sps.

* Hybridisation may be DNA – DNA ; RNA – RNA ; DNA – RNA.

DNA libraries * 2 types
1) Genome libraries → Maintainence of whole genome in different pools not in the single pool.
2) cDNA libraries → when Genome is RNA.

* org.
↓
Genome DNA/RNA
↓
Denaturation
↓
Restriction Digestion
↓
Seperation of fragments
↓
Cloning of each fragment into seperate plasmid. → Transformation of recombinant plasmid into Host cell.

when Genome is DNA.

RNA $\xrightarrow[\text{enzyme (from retrovirus)}]{RT}$ DNA → cDNA
↓
incorporated into host cell.

RT enzymes → AMV – RT (Avian myeloblastosis virus).
MMLV – RT. (Moloney Murine Leukemia Virus)

* Main aim is to preserve whole genome.

Scanned with CamScanner

DNA sequencing

To determine sequence of particular DNA to know mutations (or) to compare sequences.

i) Maxam – Gilbert Method.

- Developed by Allan Maxam & Walter Gilbert.
- Also be called as chemical sequencing.

sample
↓
extract dsDNA.
↓
Denaturation
↓
ssDNA + should be radiolabelled at 5' end (P^{32})
↓
whole ssDNA is divided into 4 test tubes

1% DMSO → Cleave ssDNA wherever Adenine is there (A)

60% Formic Acid → Cleave wherever Guanine ~~Thymidine~~ is there & at two places of Adenine. (A+G)

Hydrazine → Cleave wherever cytosine & thymidine is there. (C+T)

4M Hydroxylamine → Cleave wherever thymidine is there. (T)

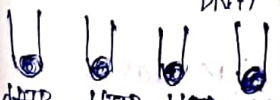
↓
Agarose Gel electrophoresis
↓
AutoRadiograph.

ii) Sanger sequencing – Most commonly used till now.

- Developed by Frederick Sanger in 1977.
- Also called as chain termination method becoz terminates the extending chain.

eg:- ddATP – Terminates at Adenine, & doesn't extend after A.

sample – sequence 800 bp.
↓
Extract dsDNA
↓
Denaturation
↓
ssDNA. (using ssDNA as template we have to synthesize a new complementary DNA which require primers, dNTP's, enzymes, dd NTP's)
↓



ddATP ddTTP ddCTP ddGTP.
Radiolabelled.

(synthesized by removal of Hydroxyl group) dideoxy ribo triphosphates

All the 4 tubes contain primers, enzyme, dNTP's, dd NTP's.

↓
Agarose Gel electrophoresis → Autoradiograph.

Scanned with CamScanner

(iii) Shot gun sequencing
sequence more than 1000 bp

(iv) High throughput sequencing / Next Generation sequencing (NGS)
Very Advanced sequencing.

(v) pyro sequencing.
Sequencing based on light emitted when each base is incorporated.

11/12/19

DNA Fingerprinting / STR Analyses / DNA profiling.

* 99.9% Genome is same but 0.1% varies which contributes to 3 million bases.

* Short Tandem repeats (STR's)
variable number Tandem repeats (VNTR's) } Minisatellites.

* DNA
↓
PCR — STR's / VNTR's using primers which are Flouroscent labelled.

* 11 STR's are sufficient to detect person involved in crime & 1 STR will be on sex chromosome which is to differentiate whether the person is male or female

* To identify Parentage
To detect criminals (Forensic) } Applications.
To identify Biological parents

Intellectual Property Rights (IPR)

* These are rights given to persons over the creation of their minds.
* This gives the creator an exclusive right over the use of his/her own creation for certain period of time.
* These are applied to wide variety of categories

eg:- 1) Copyright © — regarding novels, Textbooks, Movies.

2) TM — Trademark
R — Registered Trade Mark. — facebook, whatsapp, nike etc.

3) GI — Mysore sandal from Karnataka. 30 GI of Karnataka.
↳ Geographical Indicators.
GI of Haleem — Hyderabad.
GI of Teapowder — Assam.

Scanned with CamScanner

4) Patents – Mostly related to science & Technology.

* Patent means granting of special exclusive rights to a person for developing a new ~~person~~ process (or) product (or) technique.
* patent includes inventions, trade secrets, industrial designs.
* Patents are necessary for promoting scientific progress.
* 1st patent filed was by Louis pasteur (usage of yeast for Fermentation)
* some other patents by Alexander Fleming (extracted penicillin from penicillium notatum)
  (west Bengal) ← Dr. chakravarthy (Identified that pseudomonas take all the oil in pacific ocean so that fishes may survive).

Ethics (or) Bioethics

Bioterrorism – Anthrax in worldwar, 9/11 attacks in 2001 (by Binladen).
→ Using Biological Agents to destroy org.

Ethical Issues

* There are several legal issues surrounding genetic & biological researches like Ethical considerations, genetic diversity.
* Biological integrity will be destroyed.

Regulatory Authorities which control Biotechnological Advances.

National Institute of Health, NIH, US.

Environmental protection Agency, EPA

United states Drug Authority, USDA

Food & Drug Authority, FDA

Occupational safety And Health Administration, OSHA.

Agriculture Biotechnology Recombinant DNA Advisory committee, ABRAC.

Scanned with CamScanner

23/12/19

Immunology

* Immunis (→ Latin word) = to exempt from

* Chinese people in 5th century observed people with small pox disease (vesicles formation). Certain people died & certain people recovered. They (Chinese people) collected vesicular fluid from recovered people & applied to children by making scar. Amazingly children survived.

* 17th century in Turkey
  Lady Mary Montague, wife of British Ambassdor observed the same practice in Turkey as in China & did to her children & spreads the practice to Britain also.

* Eduard Jenner, 1798 observed vesicles formation in cows - cowpox disease
  He thought "infectious agent responsible for causing small pox might be related with infectious agent responsible for cowpox".
  He collected nodules/vesicles from cowpox & applied to children by making scar. Amazingly children survived.

* Vecca = cow (∵ since 1st material used is cow) "vaccine"

* Rinderpest → Affected Animals - Nasal discharges
  collected strings of Nasal discharges from Affected animals & applied to dewlap of Healthy Animals. The Healthy Animals survived.

* Cattle pleuropneumonia
  collected lung pieces from affected animals & they inserted at tail region of Healthy animals by incision. Tail is degenerated & dropped but animals survived.
  But since Tail is dropped, the infectious Agent (Mycoplasma bovis) spreads in envt. & other Animals gets infected.

* Louis Pasteur - started working on Fowl cholera (Pasturella multocida).
  - Collected material from infected birds, cultured on bacteriological medium, inoculated to healthy birds & healthy birds die.
  - Aged bacterial culture (Not preserved properly), inoculated to healthy birds, but healthy birds survived.
  collected material directly from infected birds, inoculated to birds with Aged bacterial culture, birds survived.
  - observed that "Any infectious agent which is not (or) grown unconventionally preserved properly lose its Pathogenesis but retained protective properties".

Scanned with CamScanner

worked with Bacillus anthracis (Anthrax) & it is grown at high temp. & it loses its pathogenesis but it started retaining protective properties. He introduces this org. into 1st flock & no introduction of this org. into other (2nd) flock. 1st flock protected & 2nd flock gets infected after introduction of live infection.

- Then he coined the term "vaccine" in remembrance of Edward Jenner.
- He closely observed the Rabies dogs. Then he found excessive salivation, dogs behave madly. He observed that CNS is responsible. He collected saliva from mad dogs & inserted to healthy dogs & healthy dogs die. It was confirmed that infectious agent in saliva is responsible. He also injected saliva to rabbits & they die. He collected spinal cord from infected dried rabbits & spinal cord is dried in sun, made into powder, inserted to healthy rabbits & challenged with Rabie dog saliva, surprisingly Rabbits survived. He did the same in dogs & the dogs survived.
- Joseph meisher, - 1st vaccine against rabies virus - on July 6th 1885 → zoonosis day.

* Small pox, Rinderpest (cattle) } Eradicated. Last small pox disease is in 1977.

24/10/19

Types of Immunity

Immunity
- Innate
- Acquired/specific
  - Humoral
  - Cell mediated

Innate

* Rapid response against foreign substance.
* Pre existing Immunity.
* Ancient in evolutionary origin.
* Recognises a set of molecular patterns present on microorganisms.
* PAMP's - pathogen associated molecular patterns are the molecular patterns that are present on microorganisms.
eg:- LPS of Gram -ve bacteria
Potechoic Acid in Gram +ve bacteria
Mannans in Fungal cell wall
Bacterial DNA - unmethylated CpG → poly cysteine & Guanine.

Acquired/specific

* 3 important Hall marks
1) specificity 2) Memory 3) self vs Non self.
* Basically mediated by lymphocytes
* 2 parts - Humoral Immunity
cell mediated Immunity.
* Antibody mediated Immune response is called as Humoral Immunity. Antibodies are secreted by lymphocytes.
* Lymphocytes (cells) directly participate in fighting with foreign such type of immunity is cell mediated Immunity.
* chief cells in Humoral - B lymphocytes
cell mediated - T lymphocytes.

Scanned with CamScanner

* PAMP's are recognised by receptors present on host like PRR's.
* Pattern Recognition Receptors – PRR's on ~~Neutrophils & Macrophages~~ Host.

Eg:- Toll like receptors – TLR's on Neutrophils & Macrophages. (present: surface, cytoplasm)

1st discovered in Drosophila.

* lacks Memory, short lived Immunity, Doesn't differentiate individual/molecular Patterns specifically – Disadvantages.

* Humoral & cell mediated Immunity are interdependent.

Maturation takes place upto puberty

Thymus – Thymostimulin, Thymulin (Harmones required for maturation in Thymus)

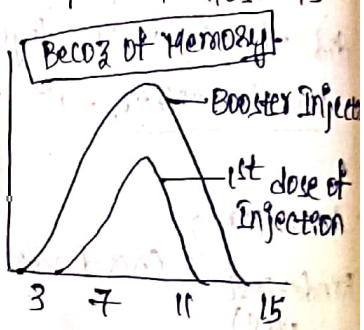
Bursa – Bursin.

Bonemarrow – Acts as both 1° & 2° lymphoid organs.

*Antigen – Any foreign molecule/substance that elicits immune response in Host is called Antigen.

*Antigenicity – property of Antigen to elicit immune response.

*Toxoid lacks Toxicity, retains Antigenicity.



* 2nd dose of Injection – Booster Injection
* 1st Immune response – 1° Immune response
2nd Immune response – 2° Immune response / Anamnestic Immune response.

* organs which produce lymphocytes are categorised into

1° lymphoid organs – organs where lymphocytes are matured.
– Maturity is required to identify foreign antigens & to ignore self Antigens.
– Eg:- Thymus – Present below Thoracic cavity infront of Heart.
(2n) → required for thymus growth – lymphocytes that mature in medulla of thymus → T-lymphocytes.
– lobular structure & contains cortex & medulla.

Bursa of Fabricious – B lymphocytes were 1st discovered in birds & their maturation was in Bursa of birds.
– Above Cloaca.
– comparative organ in humans → Bone Marrow.

Peyer's patches of Ileum – diffused lymphoid patches present in Intestine.
– B lymphocytes mature in peyer's patches in large & small ruminants (sheep & Goat).

MALT (Mucosa Associated lymphoid tissue) → Resp. system.
– GALT (Gut Associated lymphoid tissue) – Intestine.

2° lymphoid organs – lymphocytes reach 2° lymphoid organs after maturation.
– lymphnodes, spleen, Bonemarrow, Tonsils.
– interact with

Hippocampus, Pig → Medulla outside, cortex inside.

– lymphnode – Bean shaped ← cortex, Medulla, paracortex.
– Spleen – Red & white pulp

Scanned with CamScanner