

CRYPTOCOCCOSIS

Learning objectives

To know in detail about,

- Diseases caused by *Cryptococcus neoformans*
- Cryptococcosis or Torulosis
- Morphology and cultural characters of *C.neoformans*
- Media for cryptococcosis
- General approaches used to isolate and identify *C.neoformans*

INTRODUCTION

- Cryptococcosis also known as European blastomycosis or Torulosis is a subacute or chronic mycotic infection of man and various species of animals involving the CNS, the respiratory system and eye.
- It is caused by encapsulated yeast – *Cryptococcus neoformans*. Of the 19 species of *Cryptococcus*, only *C.neoformans* is pathogenic for animals and humans.
- Diseases and main hosts of *Cryptococcus neoformans*

Host (s)	Disease (s)
Dogs and cats	Subcutaneous and nasal granulomas, Meningitis, Blindness
Horses	Nasal granuloma
Cattle	Cryptococcal mastitis
Human	Cryptococcal meningitis

MORPHOLOGY

- There are two varieties *Cryptococcus neoformans var neoformans* (serotypes A and D) and *Cryptococcus neoformans var gattii* (Serotypes B and C).
- *C. neoformans* is a member of the fungi imperfecti. But *Filobasidiella neoformans* is the teleomorphic (sexual stage) state of serotypes A and D, *Filobasidiella bacillispora* is the teleomorphic state of serotypes B and C.
- *C. neoformans* is a spherical to oval, thin walled, budding yeast that varies greatly in d.m.
- The cells are surrounded by a mucoid polysaccharide capsule that varies in thickness, but in animal tissues it is usually very large, the width of the capsule exceeding the d.m. of the parent cell.
- Daughter cells are usually single and bud from the parent cell by a thin neck.
- Yeast cells are Gram positive. Lactophenol cotton blue or nigrosin stains are commonly used to demonstrate the spherical budding yeast surrounded by a capsule.

HABITAT

- *C. neoformans* is present in dust and has been isolated from the skin, mucous membrane and intestinal tract of normal animals and birds.
- The reservoir of types A and D is the faeces of birds, particularly pigeons and soil contaminated by avian excreta.
- The pigeon is not infected, the organism colonise the faeces after they have been passed.
- The organisms are concentrated in pigeon faeces due to their high content of creatinine.
- The creatinine inhibits many other micro organism but can be utilized by *C. neoformans*.

- It can survive in pigeon droppings for more than a year. *C. neoformans* has a worldwide distribution.

CULTURAL AND BIOCHEMICAL CHARACTERS

Cultural characters

- *C. neoformans* will grow well on blood agar or on SDA (without cycloheximide).
- The plates are streaked out as for bacteria and incubated aerobically at 37°C for upto two weeks.
- Capsular growth can be enhanced by culture on chocolate agar under 5% Co₂ at 37°C.
- The majority of the *Cryptococcus* species are unable to grow at 37°C, where as *C. neoformans* can grow at temperature upto 40°C.
- Colonial growth is often not apparent until after nearly two weeks incubation.
- The colonies are smooth, moist, shiny, white and become wrinkled, cream to brownish granular colonies on further incubation.
- Bird seed agar (which contain *Guizotia abyssinica* seeds) is the selective medium for *C. neoformans*.
- It contains di and polyphenolic compounds. *Cryptococcus species* use creatinine and produce melanin-pigmented (brown) colonies.
- The dark brown pigment colonies occur after the plates are incubated at 37°C for atleast a week.

Biochemical characters

- *C. neoformans* is highly urease positive.
- It will produce urease on heavily inoculated Christensen's urea agar slope.

PATHOGENESIS

- Infections are exogenous and are usually acquired by inhalation. Animal to animal transmission is not known to occur.
- The route of infection is usually respiratory, often with localization in the nasal cavity or paranasal sinuses and later extension to the brain and meninges.
- The virulence of *C. neoformans* is largely associated with the antiphagocytic and immunosuppressive capsule and a unique enzyme diphenol oxidase.
- The cryptococcal lesions, on gross examination, resemble myxomatous neoplasm; infection of the meninges can resemble tubercular meningitis.
- Infections extend to the optic nerve resulting in blindness. Subcutaneous granuloma is often occurring in cervical or pedal regions.

PATHOGENECITY

- In cryptococcal mastitis cows with mild infections often show no clinical signs except swelling of the affected glands.
- In severe infections, animal exhibits typical clinical signs of bacterial mastitis (fever, swelling and firmness of the udder).
- Milk secretion gradually diminishes. Milk will appear grey, white, highly viscid and mucoid.
- In dogs, it affects the CNS, causing incoordination, hyperaesthesia and nasal discharge.
- Subcutaneous granuloma around the ear, face and feet. In cats, chronic nasal and ocular discharge, granulomas and blindness are common.

DIAGNOSIS

Diagnosis

- Specimens: CSF, lesions or exudates, mastitic milk, biopsies and tissues should be collected.
- Direct microscopy
 - Demonstration of budding yeast with a large capsule by India ink, Nigrosin and LPCB staining methods.
- Histological sections on biopsies of tissue from lesions can be stained by the PAS-haemotoxylin stain. This will stain or outline, the yeast cell but not the capsule, which appear as a clear area surrounding the cell.
- Based on isolation and identification: production of brown pigment on birdseed agar.
- Based on urease production
- By Animal inoculation
 - Mice are susceptible to pathogenic strains.
 - When intra cerebral or intra peritoneal inoculation of suspected material, the mice will die within 4 days to two weeks.
 - PM reveals gelatinous lesions in the abdominal cavity and lungs.
 - The budding encapsulated yeast can be demonstrated from the lesions.
- By serology
 - Slide LAT, tube agglutination, CFT, ELISA, IFAT are useful to detect antibodies.

Treatment

- Amphotericin B is the drug of choice. Imidazole derivatives such as ketoconazole and fluconazole are also useful.

