# Pathology of imported and infectious diseases

Eric Van Marck, MD PhD

15 th FORPATH
10 & 12 March 2012

Universiteit Antwerpen

# Pathology of imported and infectious diseases

- Pathology of mycotic diseases
- Pathology of parasitic diseases

# Pathology of mycotic diseases

Universiteit Antwerpen

# Pathological specimens

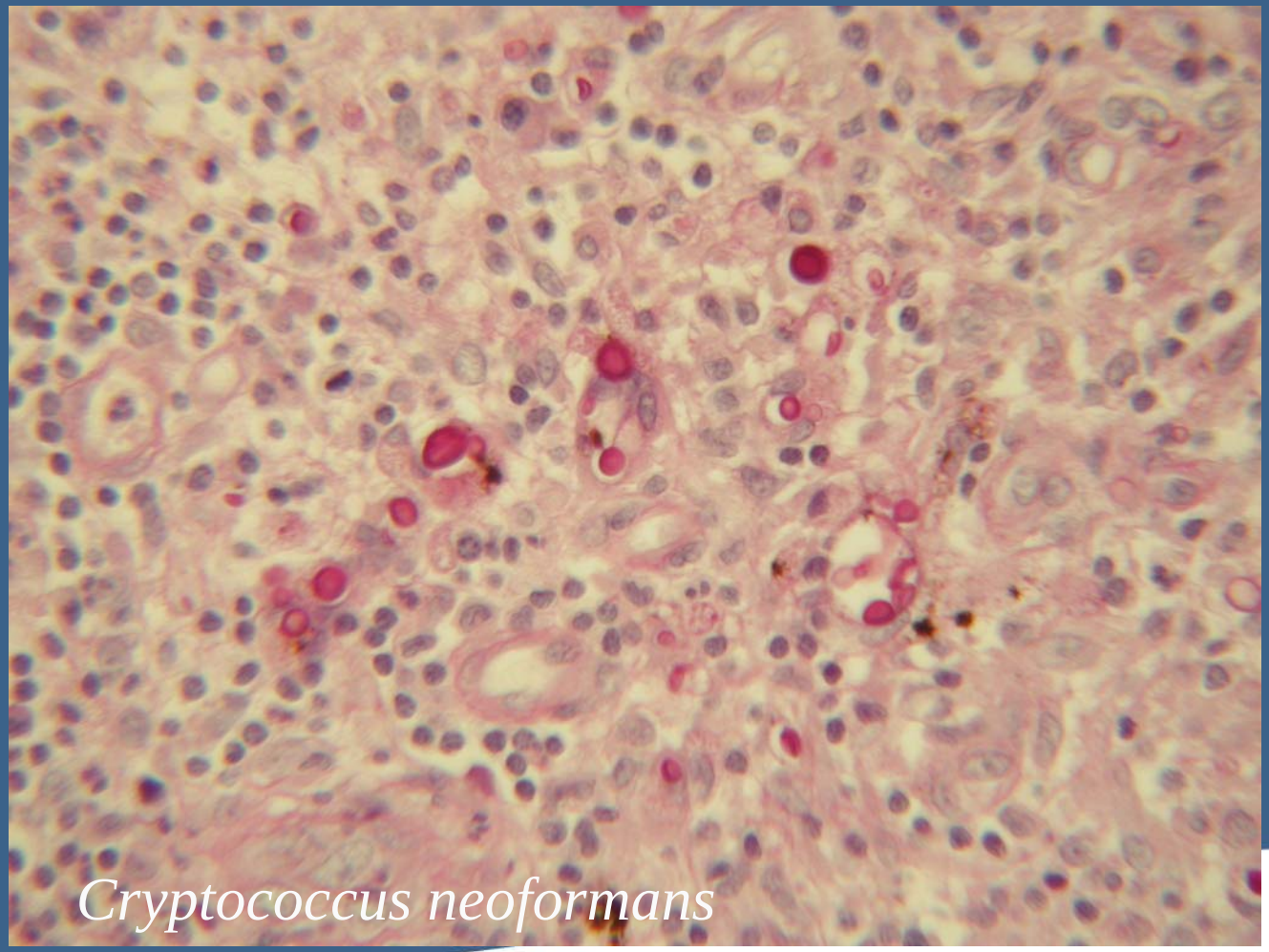
- any tissue specimens: biopsies, surgical specimens, bodies...
- fluids: exudates, urine, aspiration fluid
- cell scrapes from any organ or tissue

# Specimen preparation

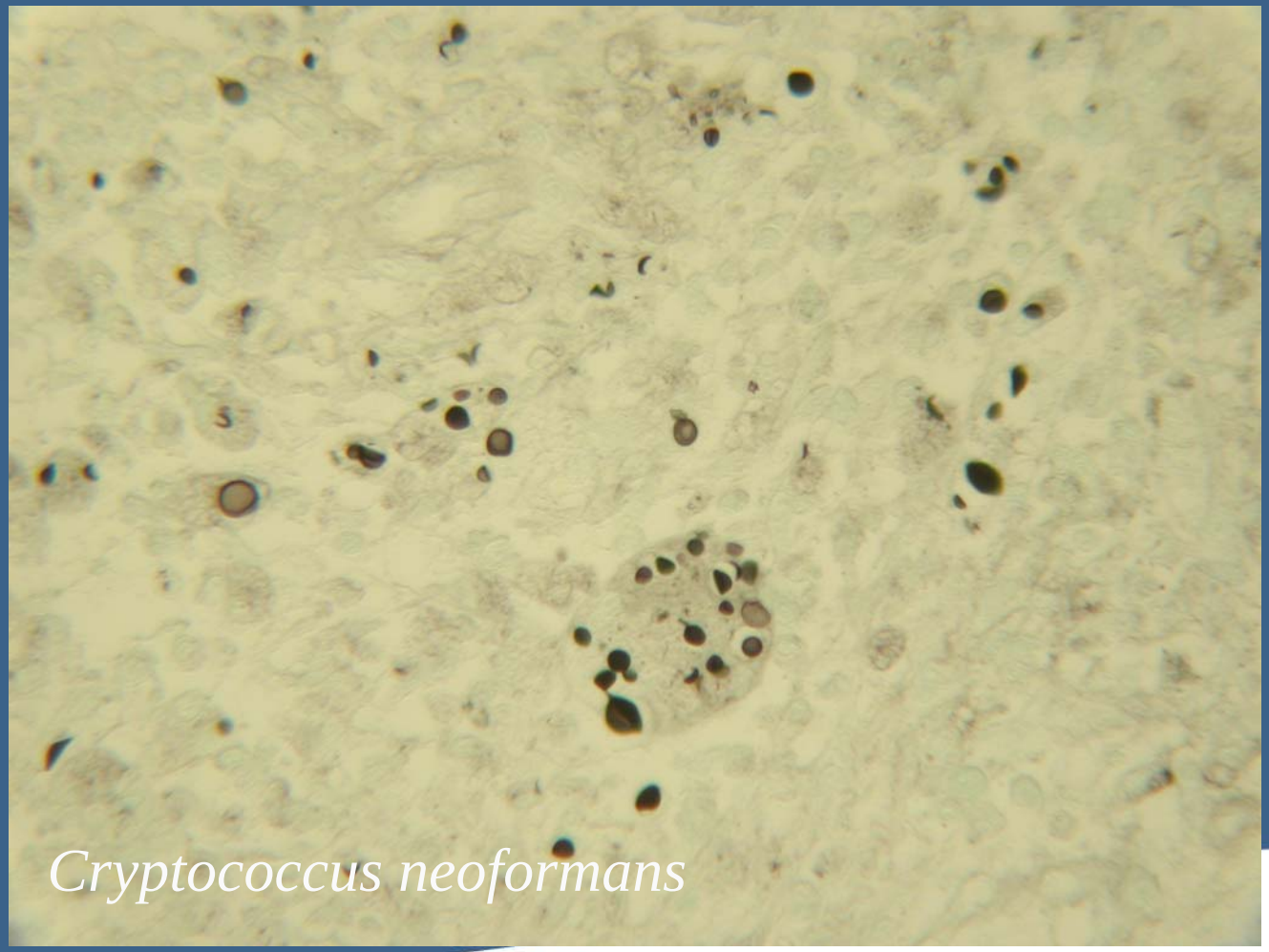
- bodies: cool as soon as possible
- tissue: rapid fixation after having taken on fresh specimen a sample for culture (formalin 10%), or else, keep cool (+4°C)
- fluids: keep cool (+4°C) and transport to laboratory, or add fixative (e.g. Saccomano)

# Tissue specimens

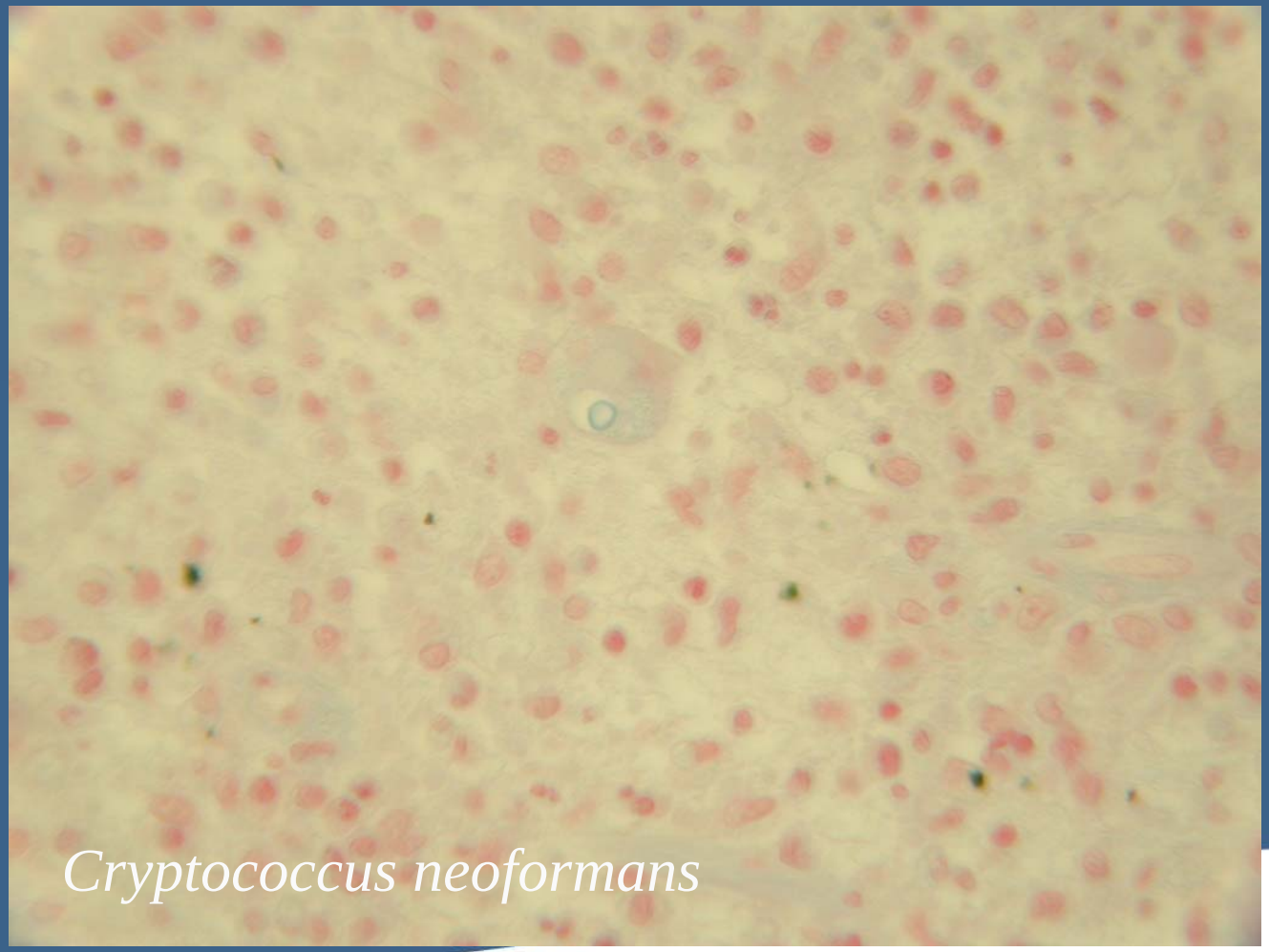
- embed in paraffin after adequate fixation
- prepare paraffin sections
- stain
  - H&E
  - special stains (Grocott, PAS, mucicarmine, alcian blue)



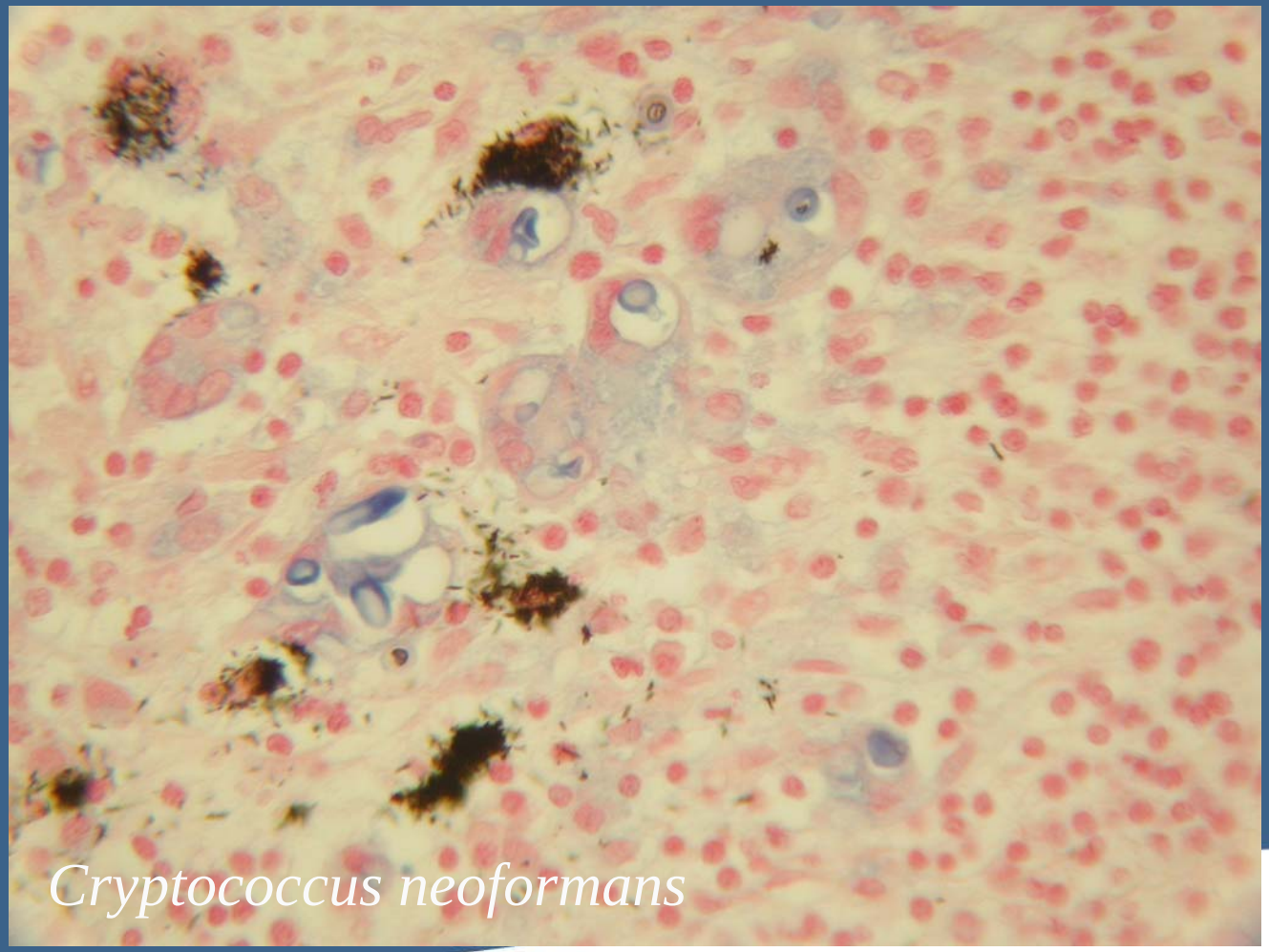
*Cryptococcus neoformans*



*Cryptococcus neoformans*



*Cryptococcus neoformans*


*Cryptococcus neoformans*

# Necessary clinical information

- identification of sample
- age of patient
- nature of the sample
- type of fixation

# Clinical information suggesting possible mycosis

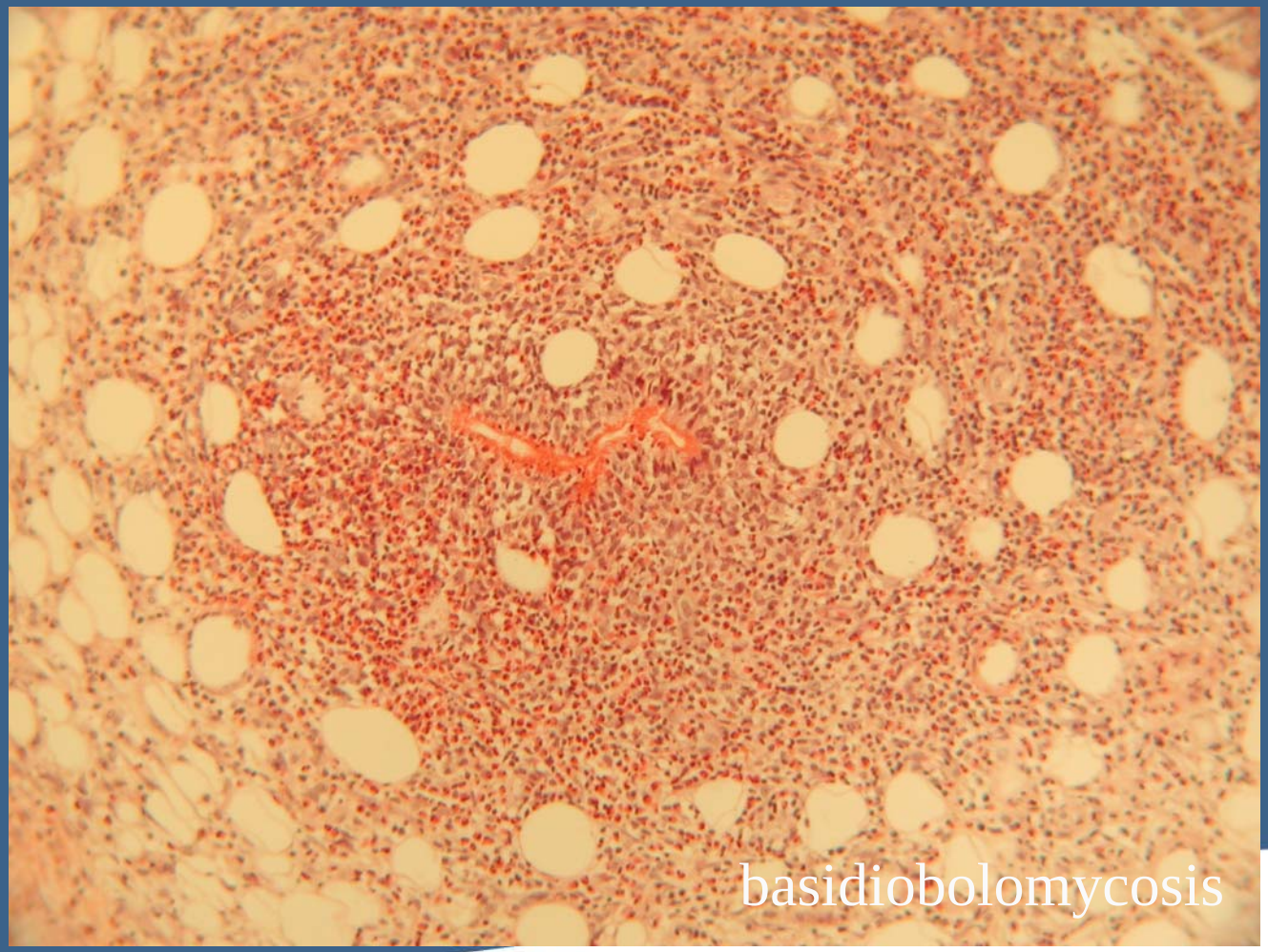
- history of immune depression (immunosuppressive medication in transplant patients or patients with autoimmune diseases, use of cytotoxic drugs, congenital immune deficiency, HIV infection)
- occupational history (work and hobbies)
- travel history

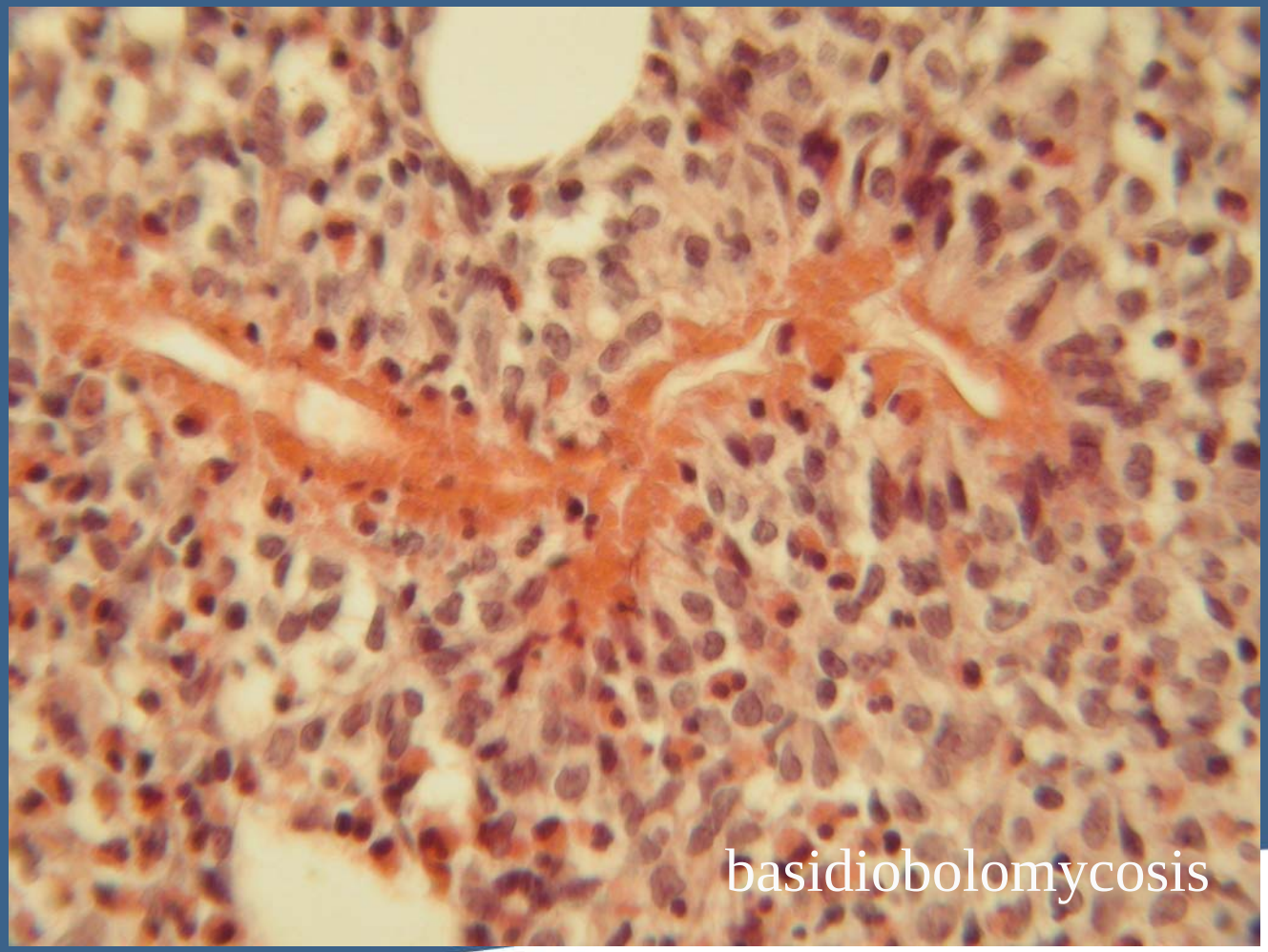
# Pathologist's contribution to the diagnosis of mycoses

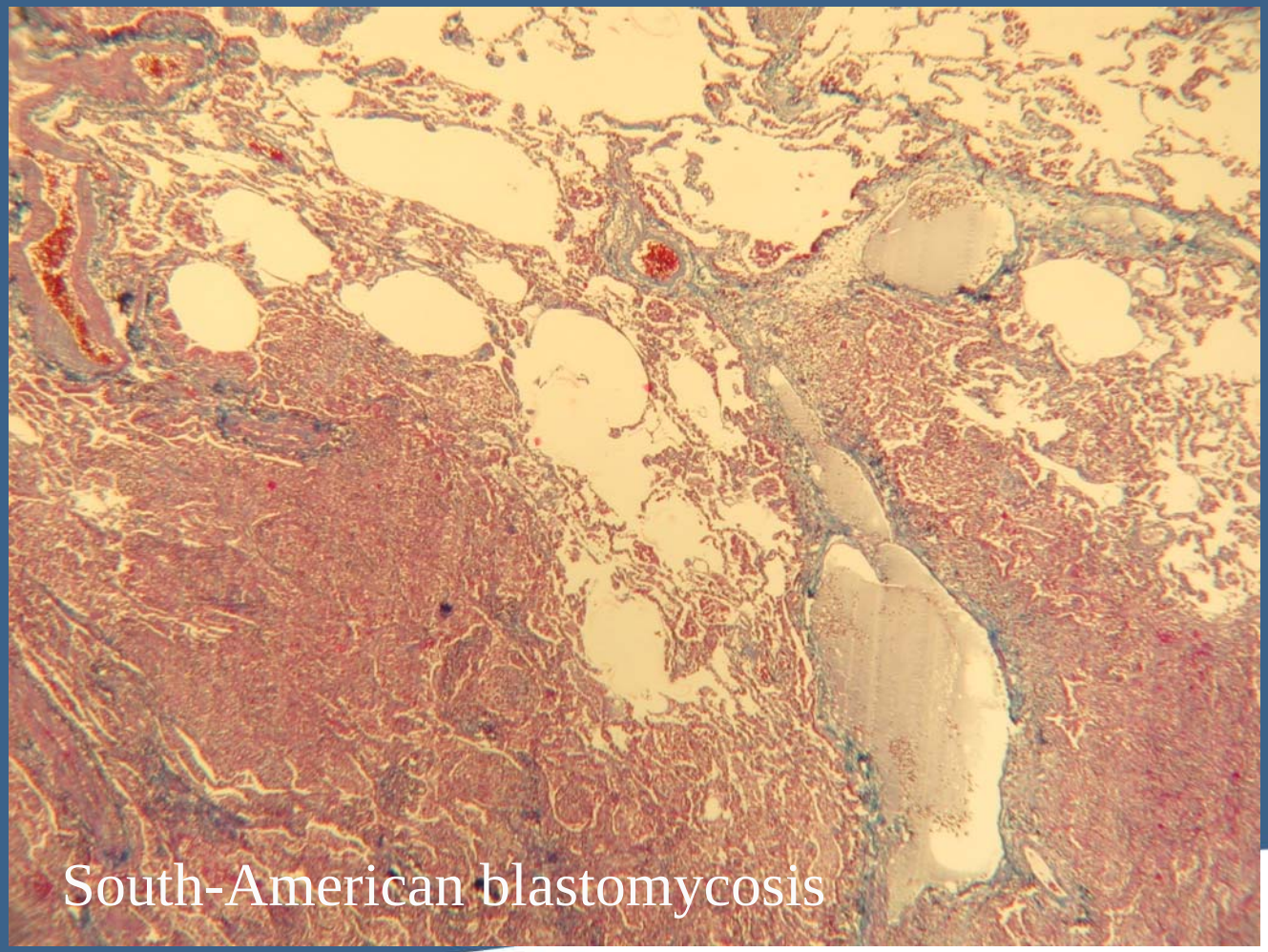
- prove invasive character of a yeast or fungus
- morphologically identify causative organism
- assign a causative organism to a larger class of organisms

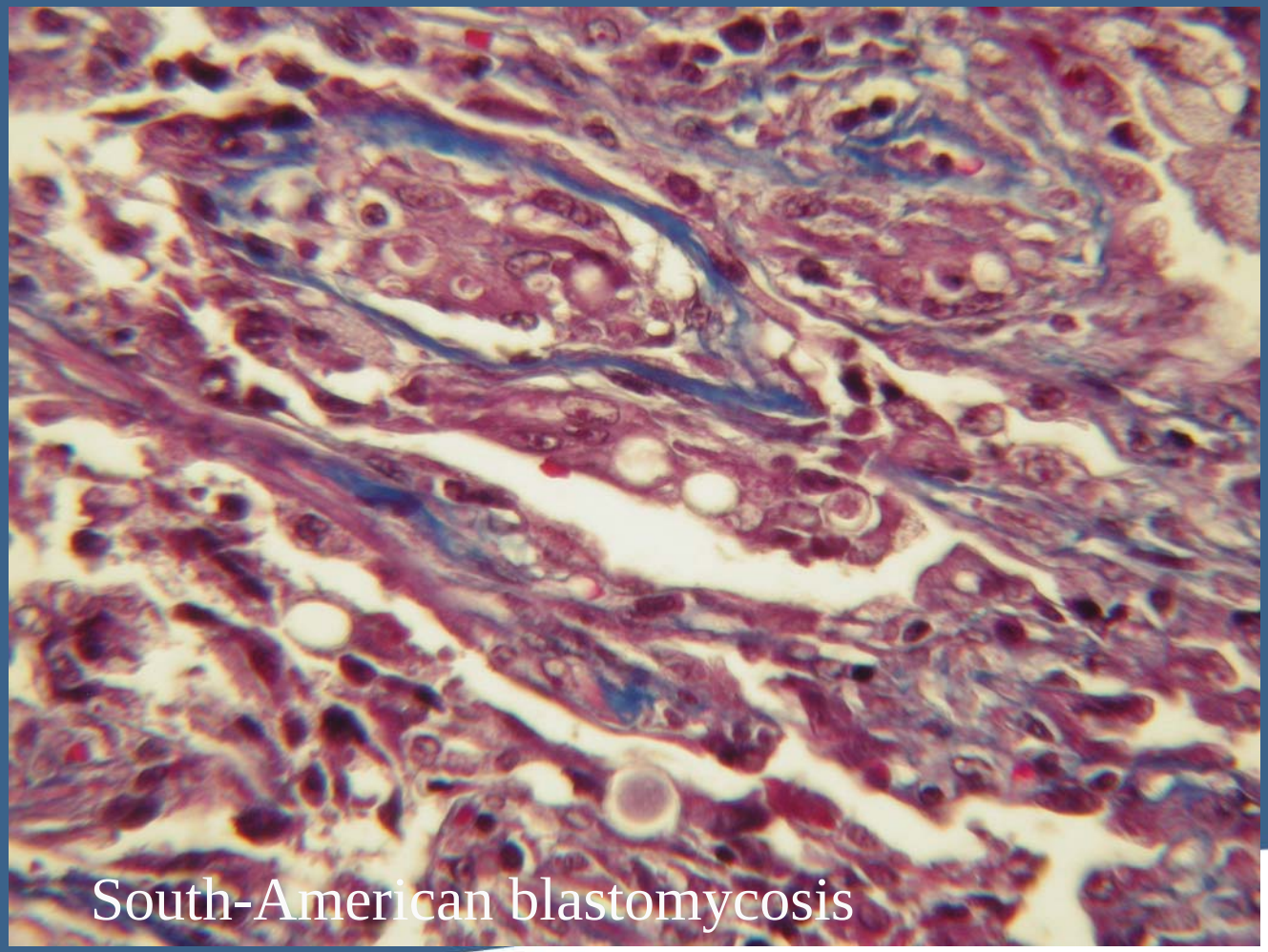
# Host reaction patterns

- Inflammation: no specific pattern
  - "acute" inflammatory reaction (neutrophilic and eosinophilic granulocytes)
  - "chronic" inflammatory reaction (mononuclear cells predominate: histiocytes, lymphocytes, plasma cells, *or* presence of granulomas)
  - fibrosis in long-standing infections
  - a combination of the above


basidiobolomycosis


basidiobolomycosis


South-American blastomycosis



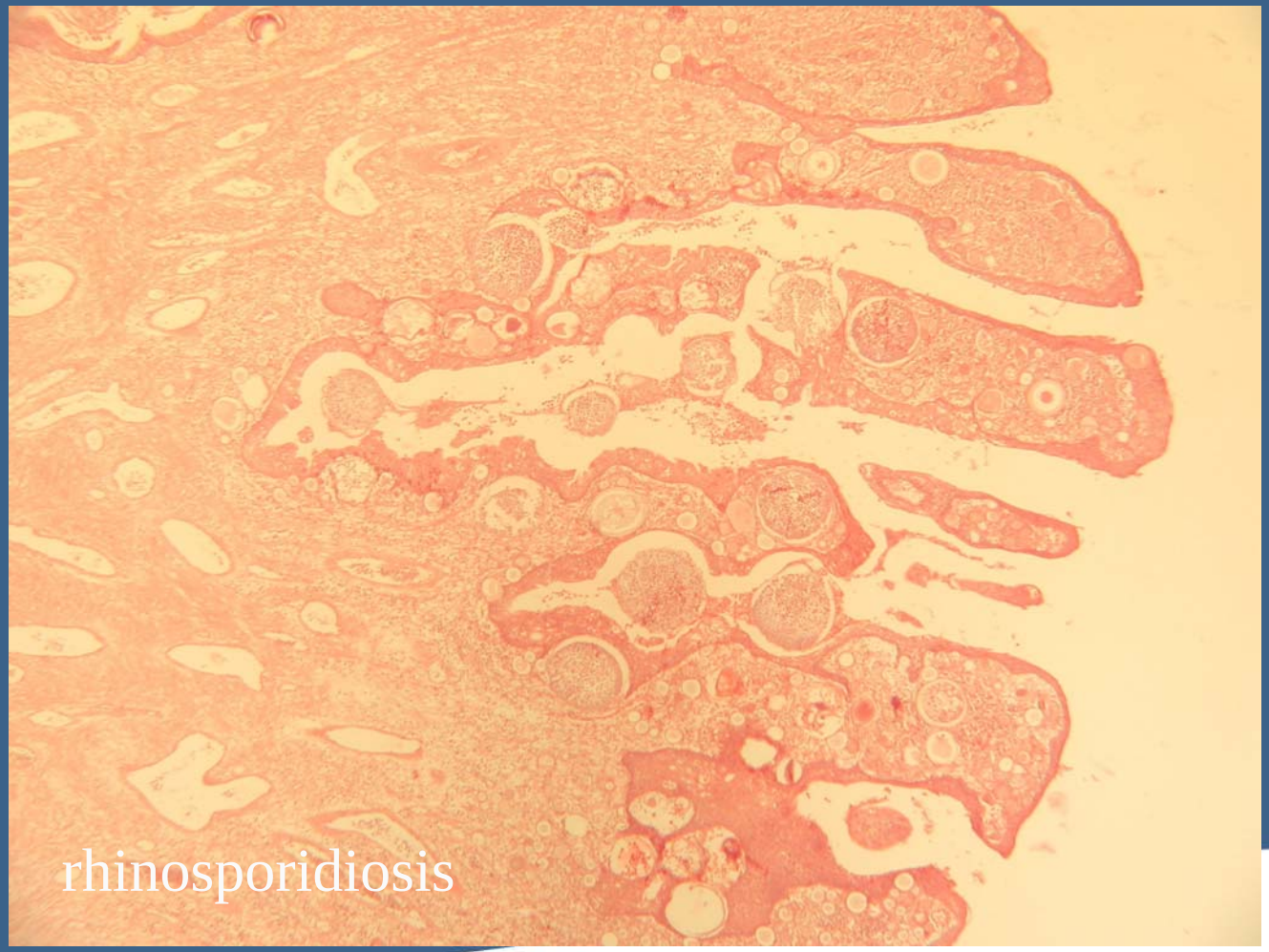
South-American blastomycosis

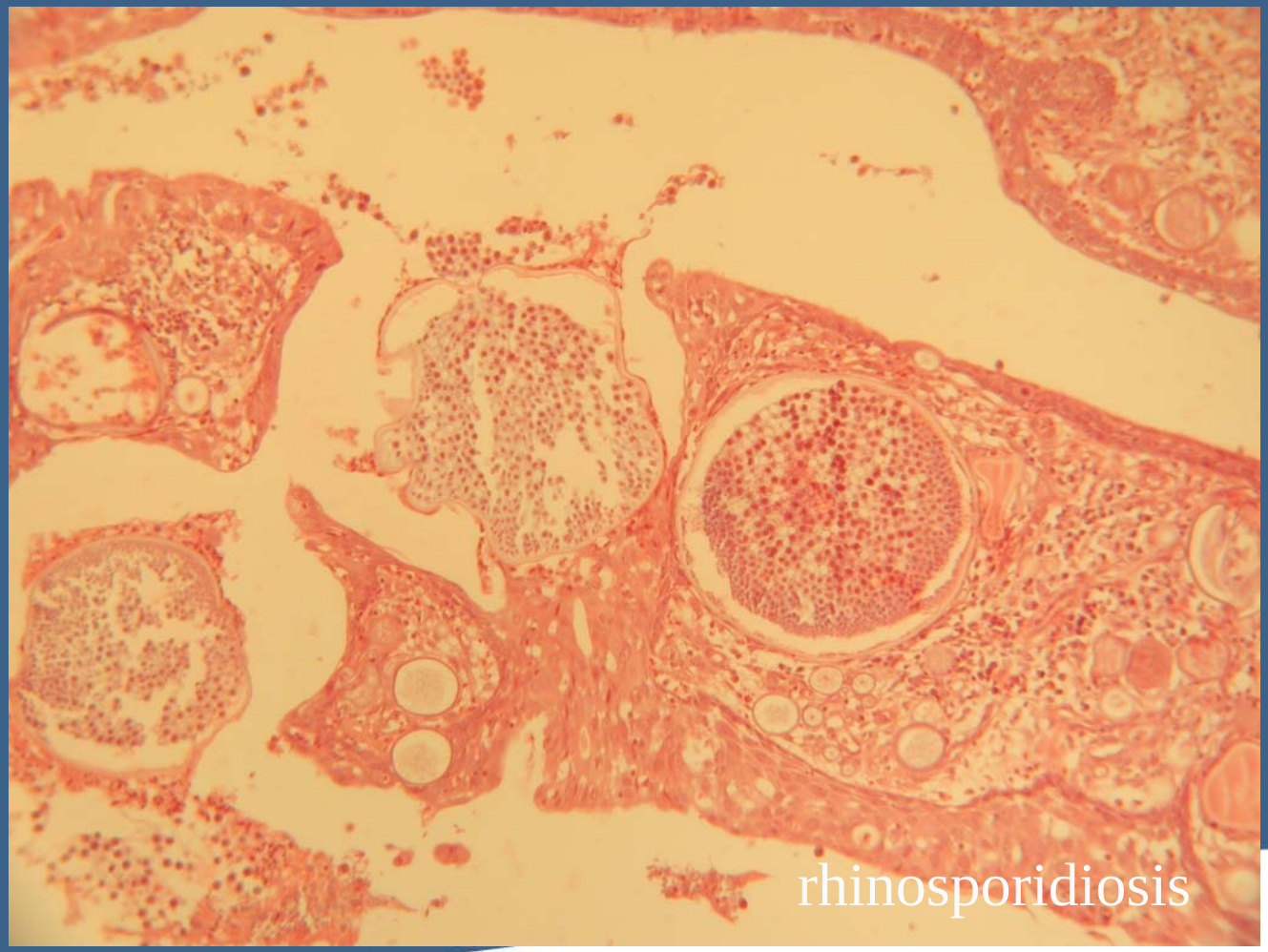
# Granuloma

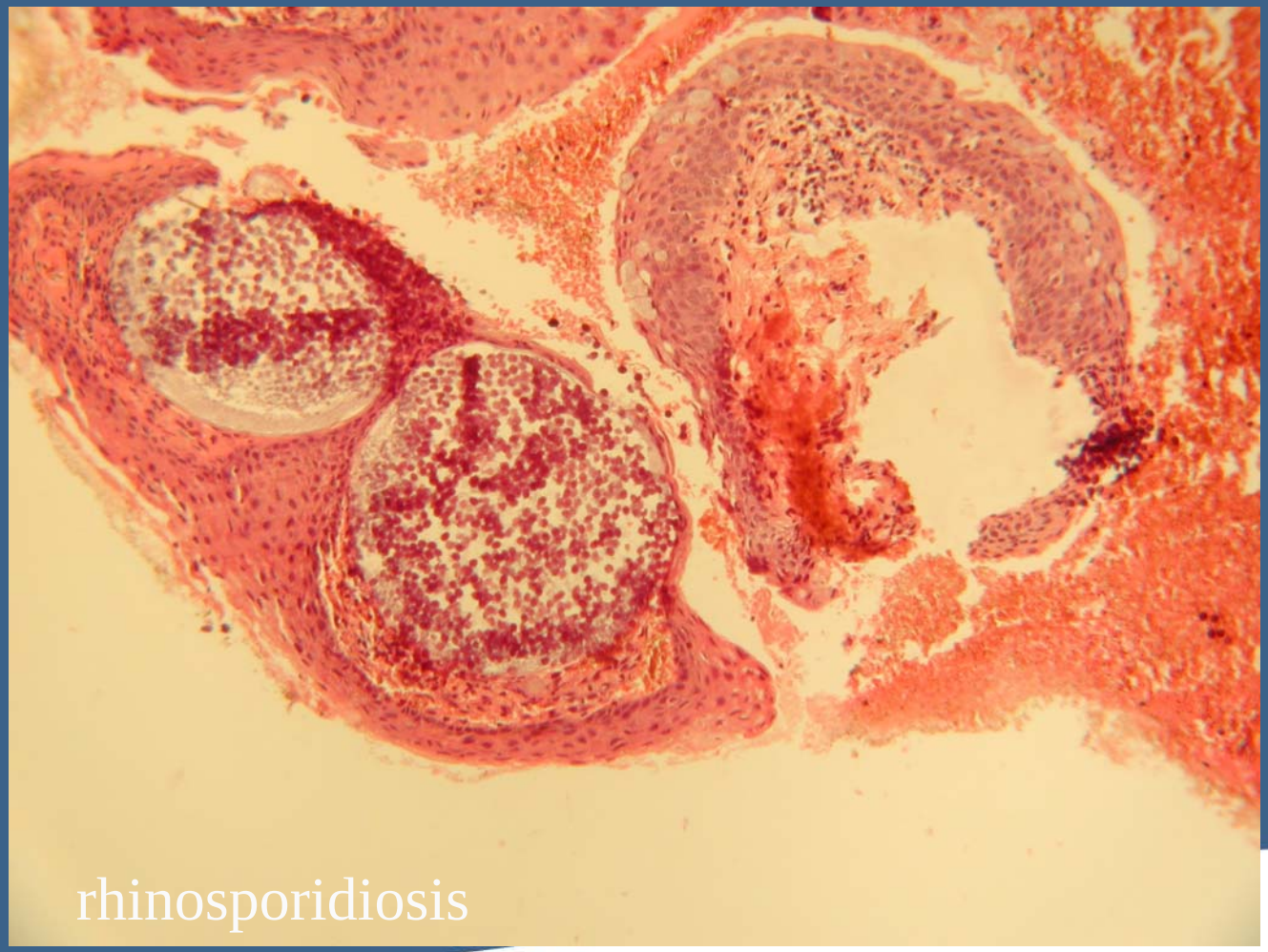
- rounded structure composed of epithelioid cells and a variety of other cells, often also comprising giant cells
- central necrosis can be present
- a causative organism can or can not be present

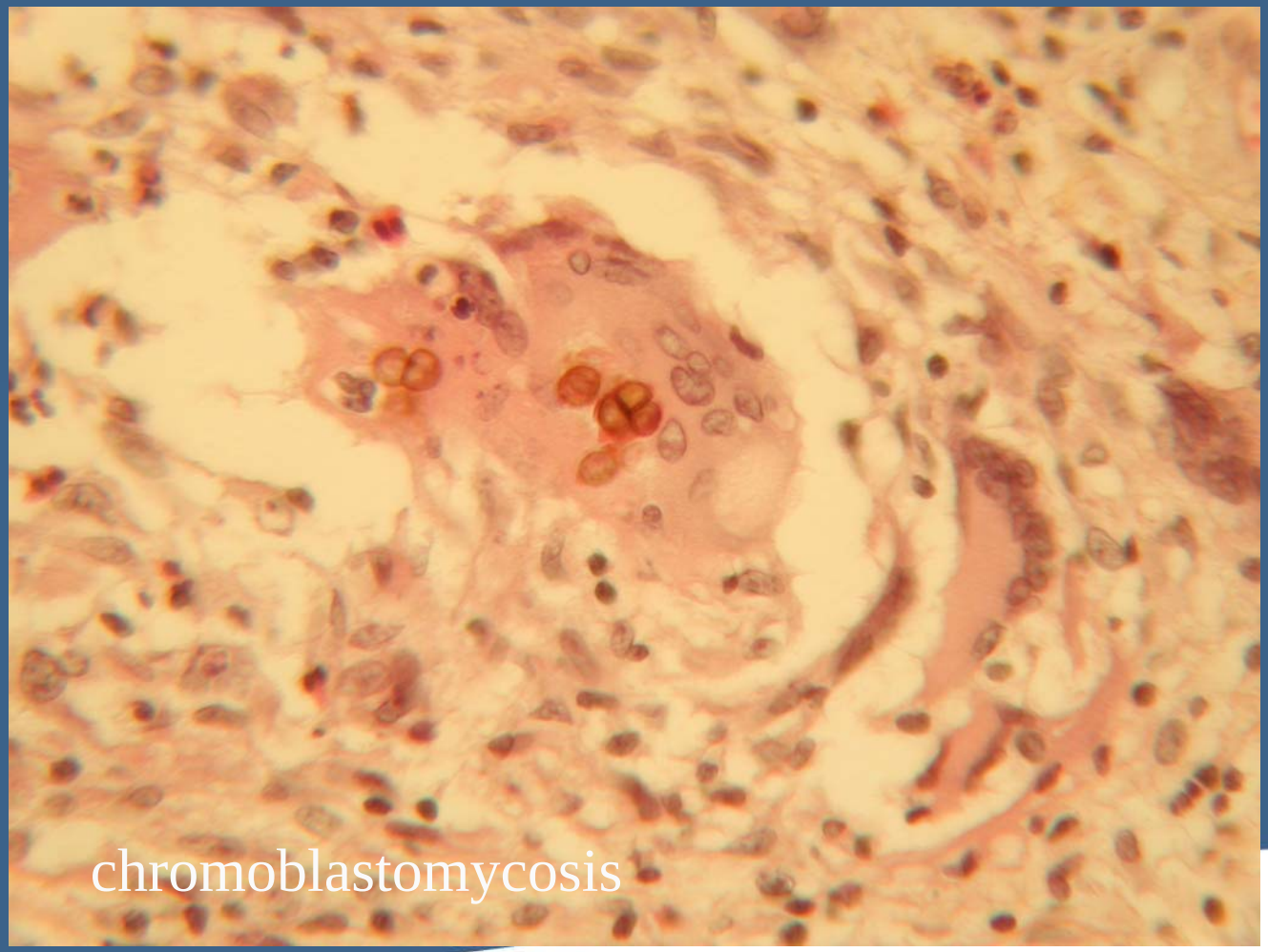
# Mycotic organisms

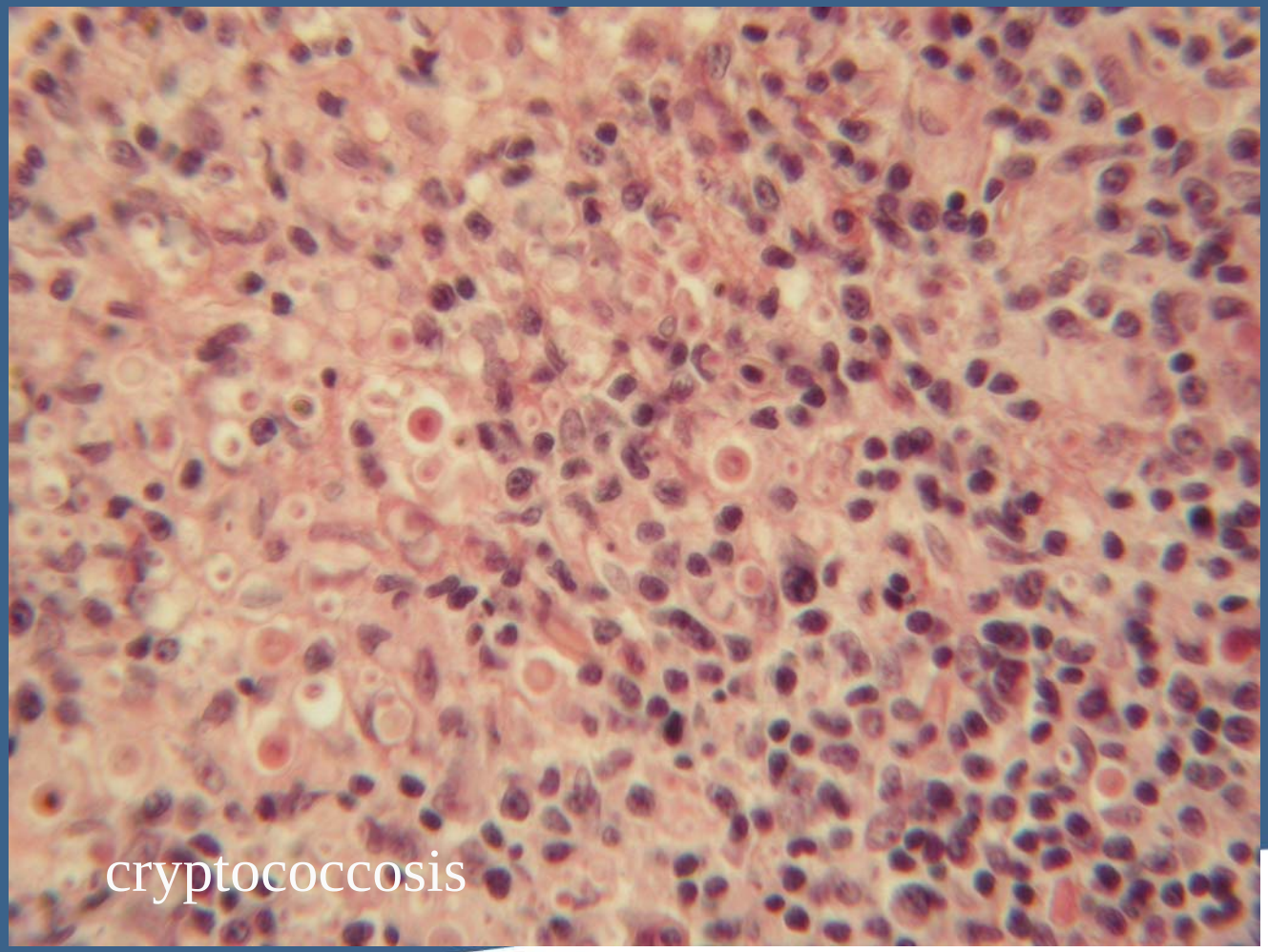
- some are readily visible in routine H&E stains as a result of their size or because they are pigmented
- others require special stains to be revealed

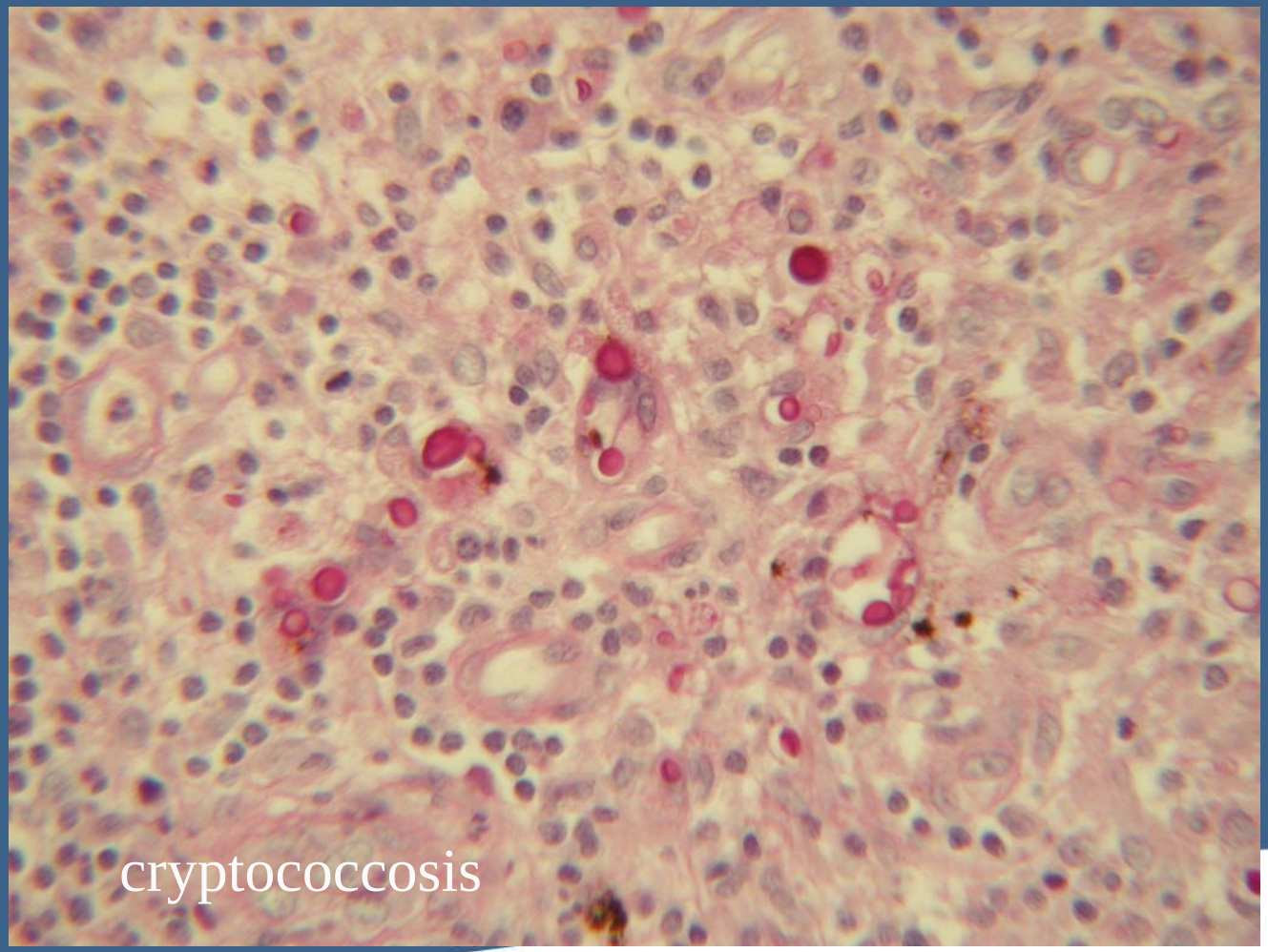

rhinosporidiosis

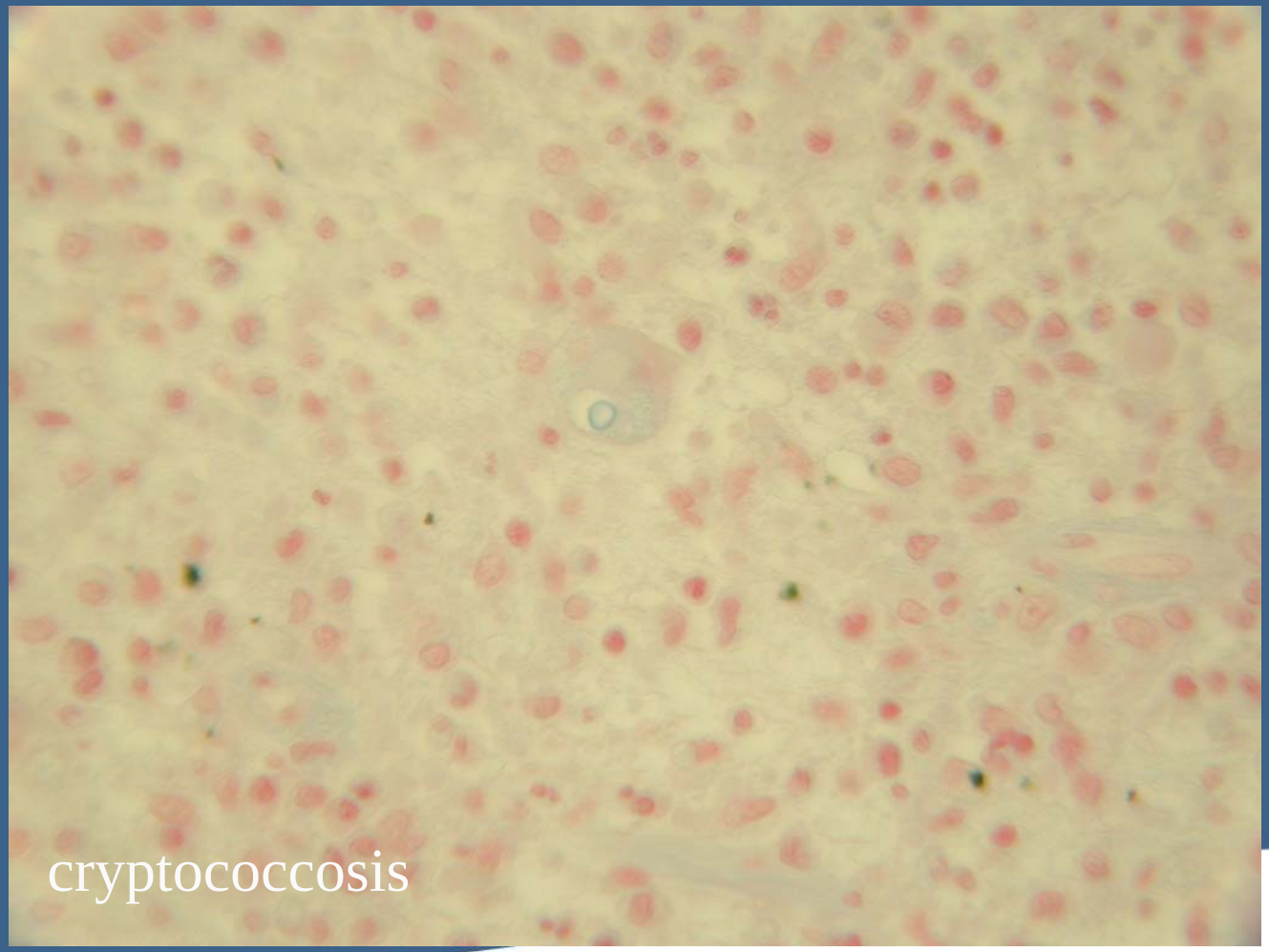

rhinosporidiosis


rhinosporidiosis


chromoblastomycosis

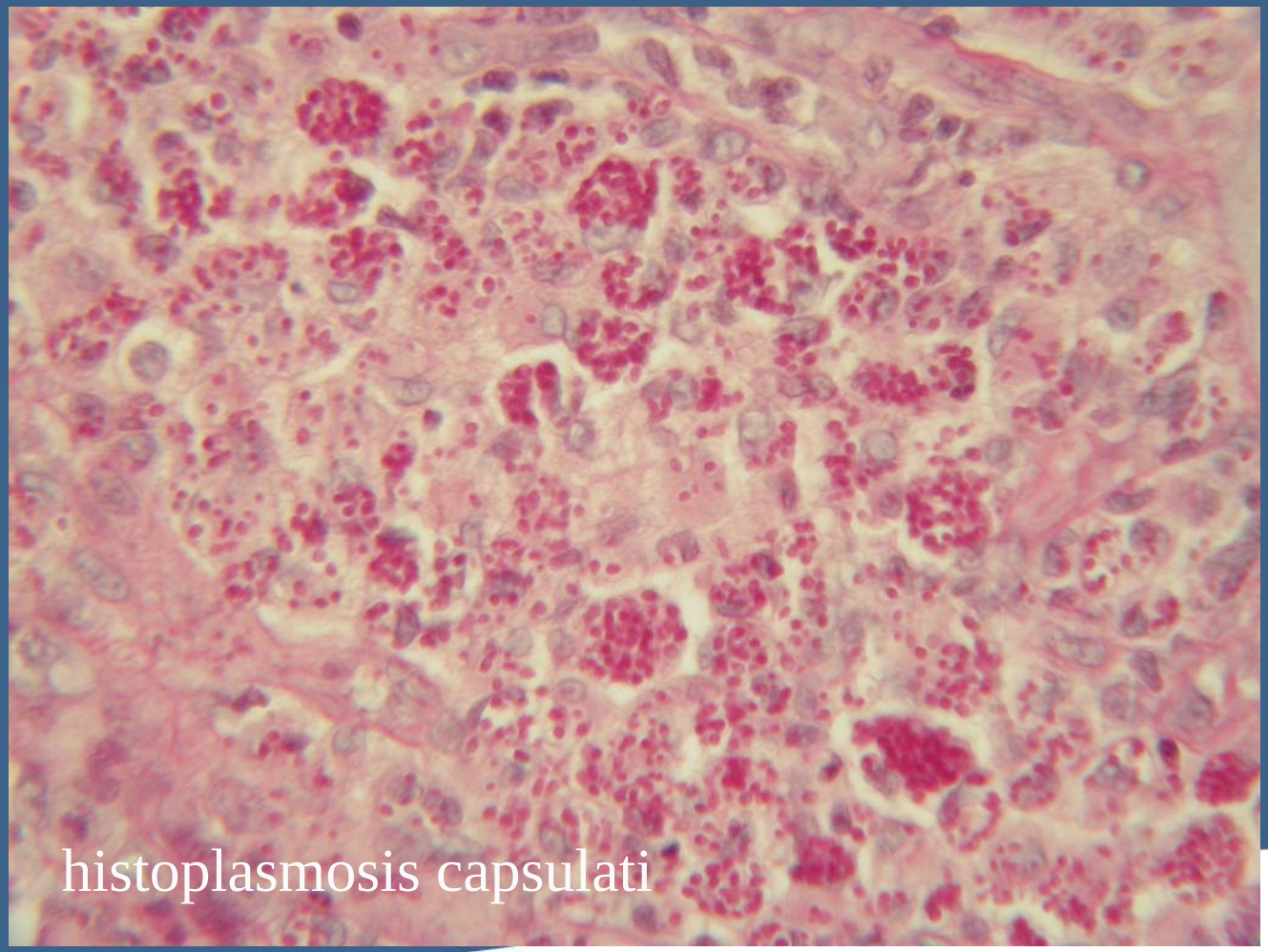

cryptococcosis


cryptococcosis

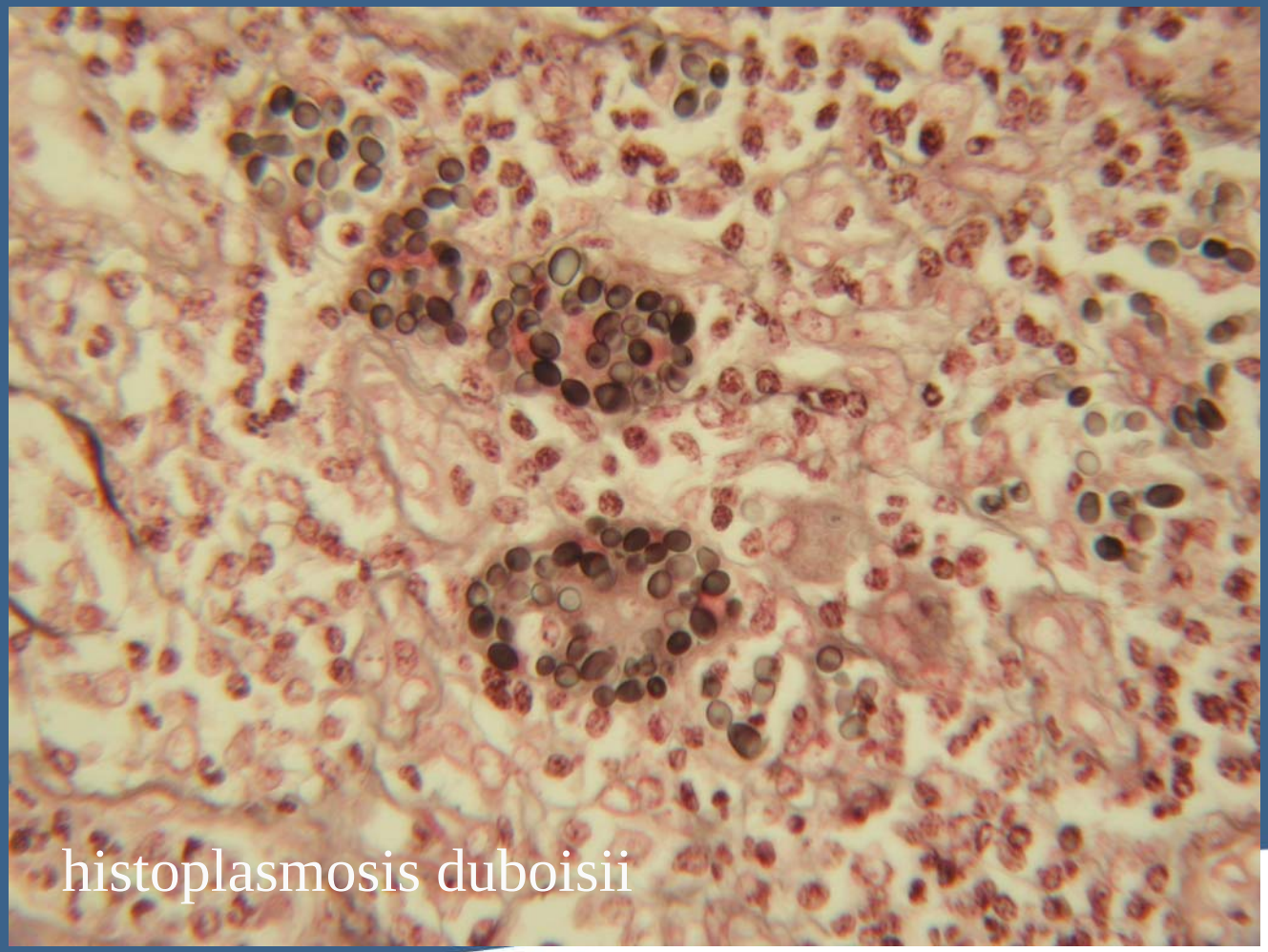

cryptococcosis

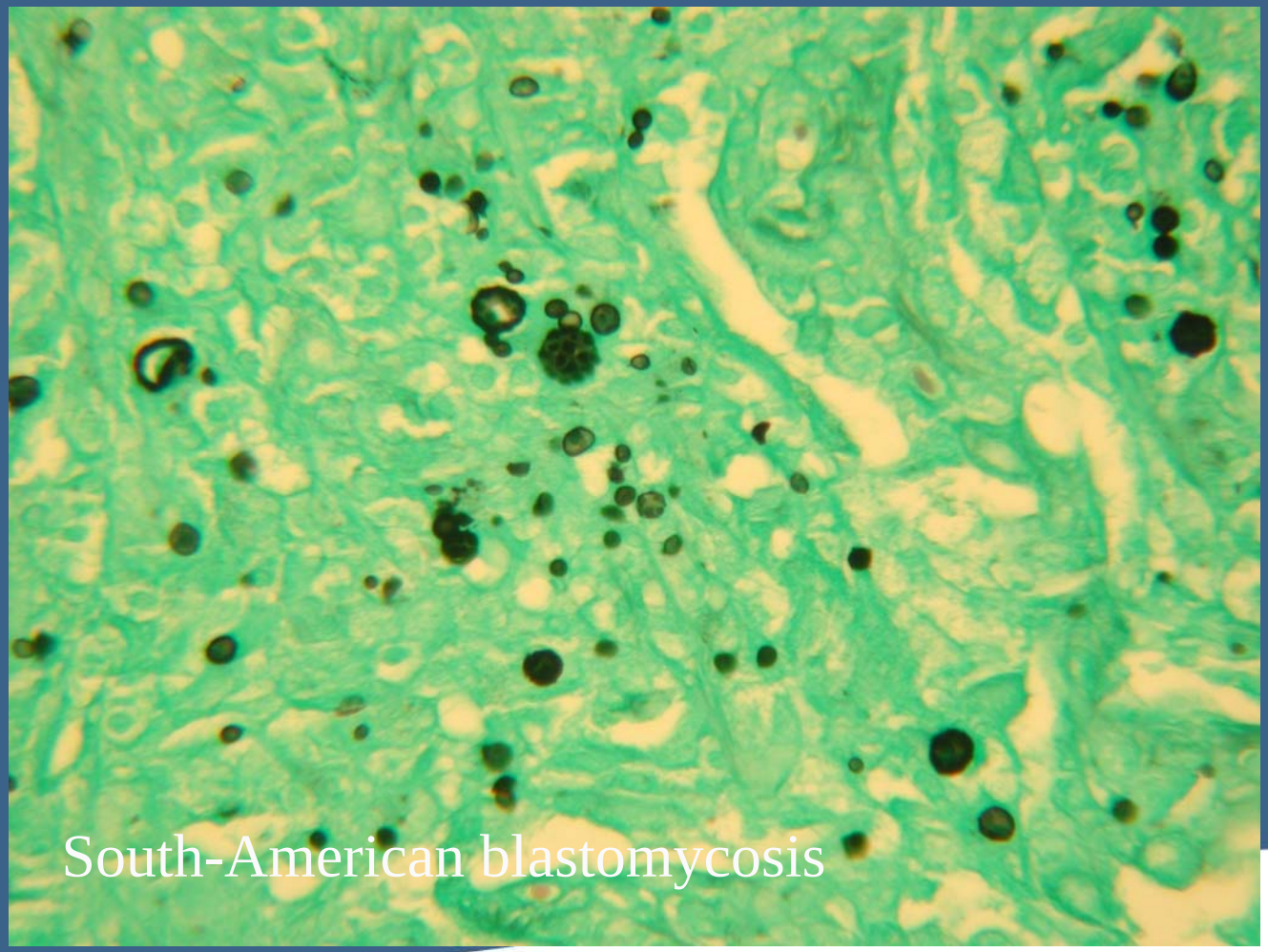
# Accuracy of histopathologic diagnosis

- some organisms have distinctive morphological characteristics:
  - histoplasmosis capsulati (*Histoplasma capsulatum*)
  - histoplasmosis duboisii (*Histoplasma duboisii*)
  - paracoccidioidomycosis (*Paracoccidioides brasiliensis*)
  - blastomycosis (*Blastomyces dermatitidis*)
  - lobomycosis(*Loboa loboi*)
  - coccidioidomycosis (*Coccidioides imitis*)
  - cryptococcosis (*Cryptococcus neoformans*)
  - rhinosporidiosis (*Rhinosporidium seeberi)*
  - adiasporomycosis (*Chrysosporium parvum*)

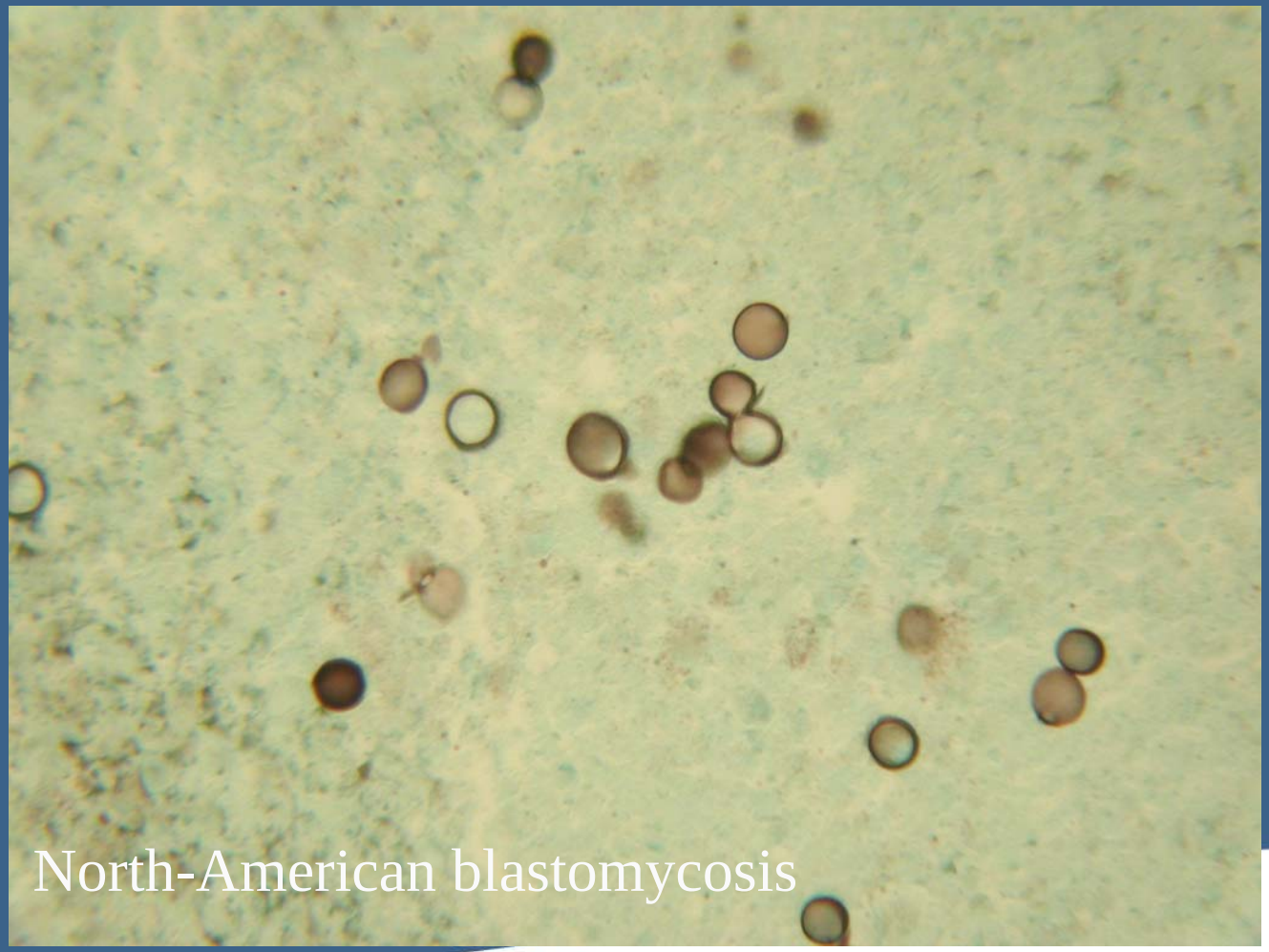


histoplasmosis capsulati

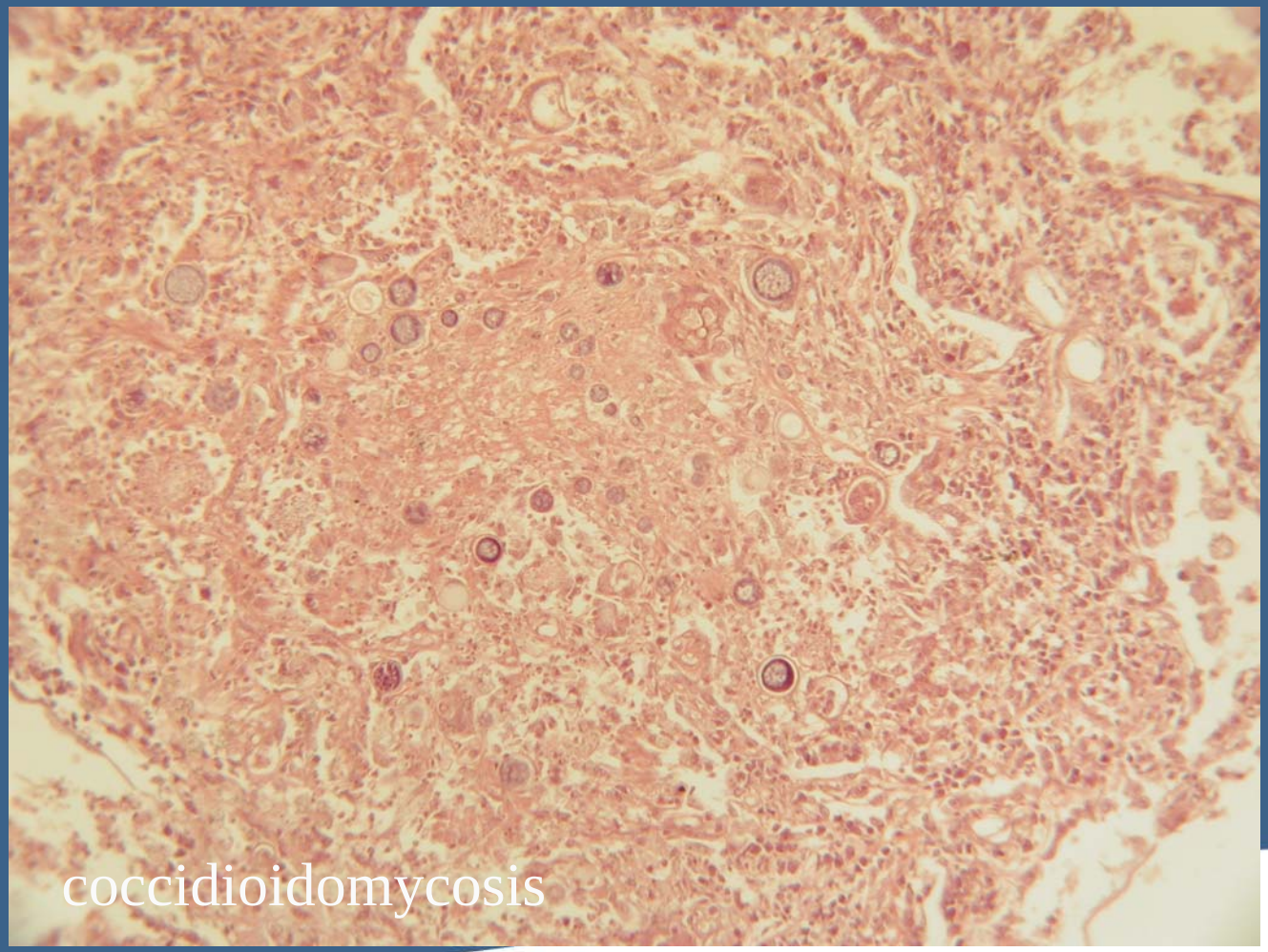

histoplasmosis duboisii

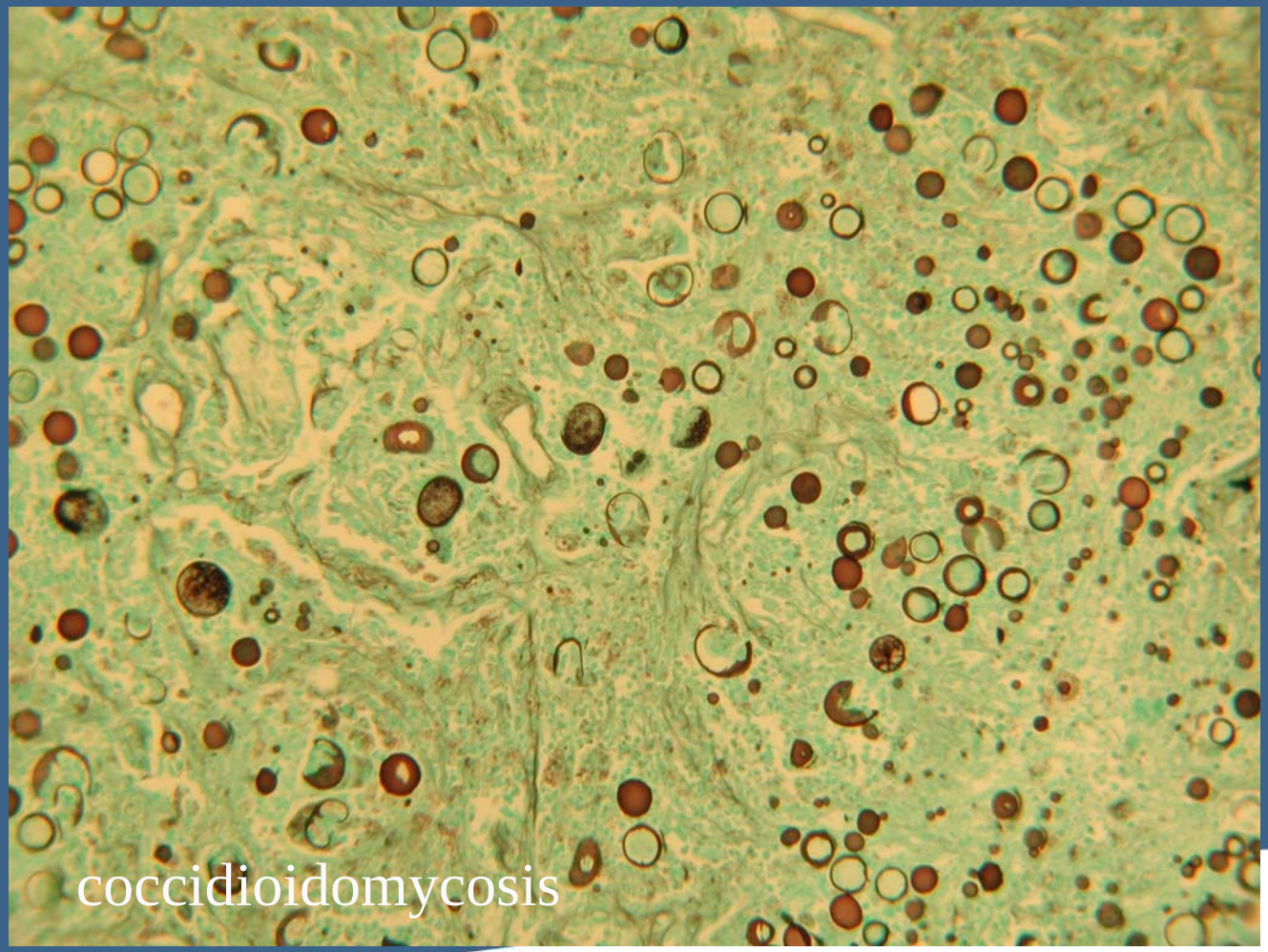


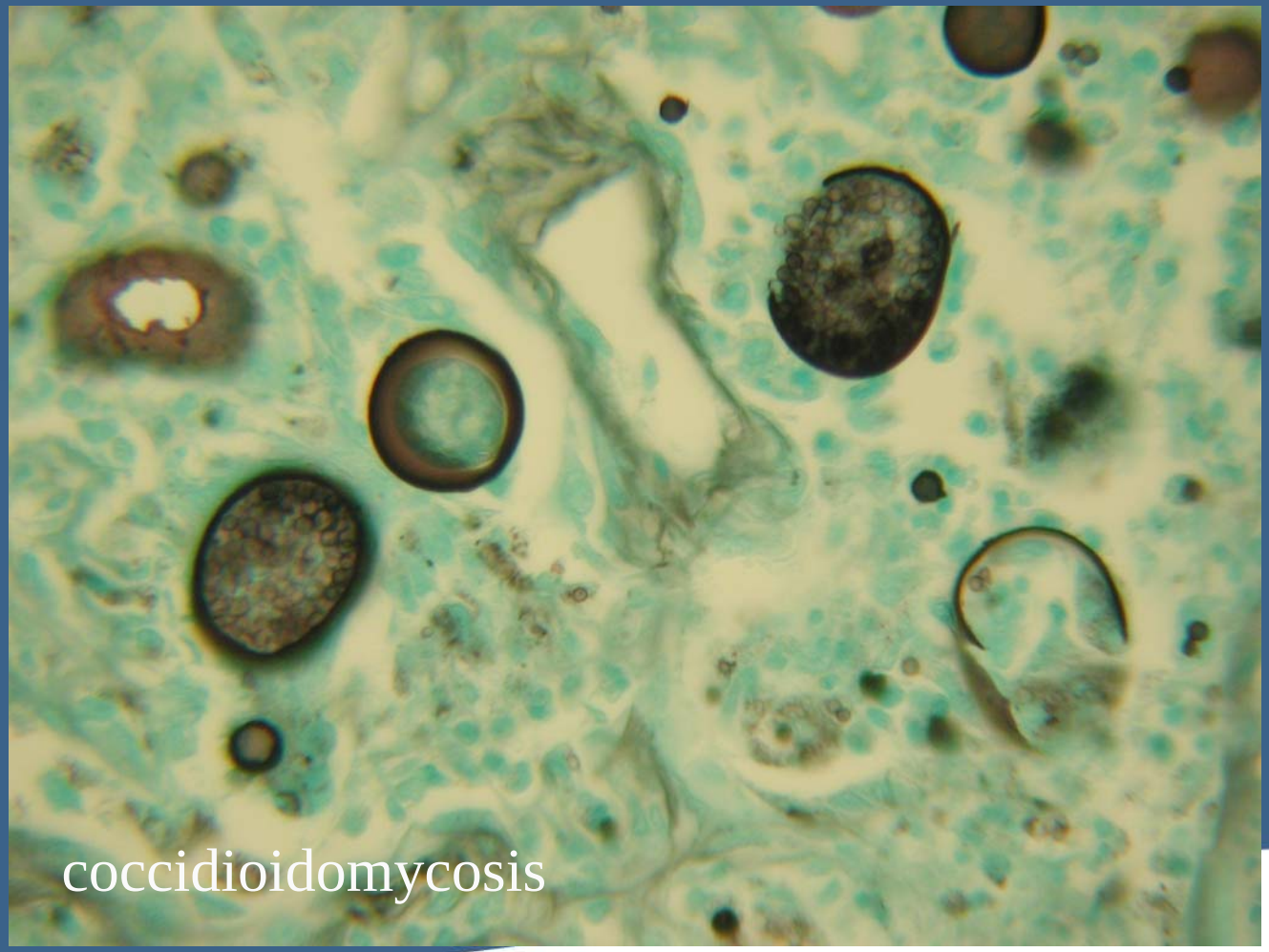
South-American blastomycosis



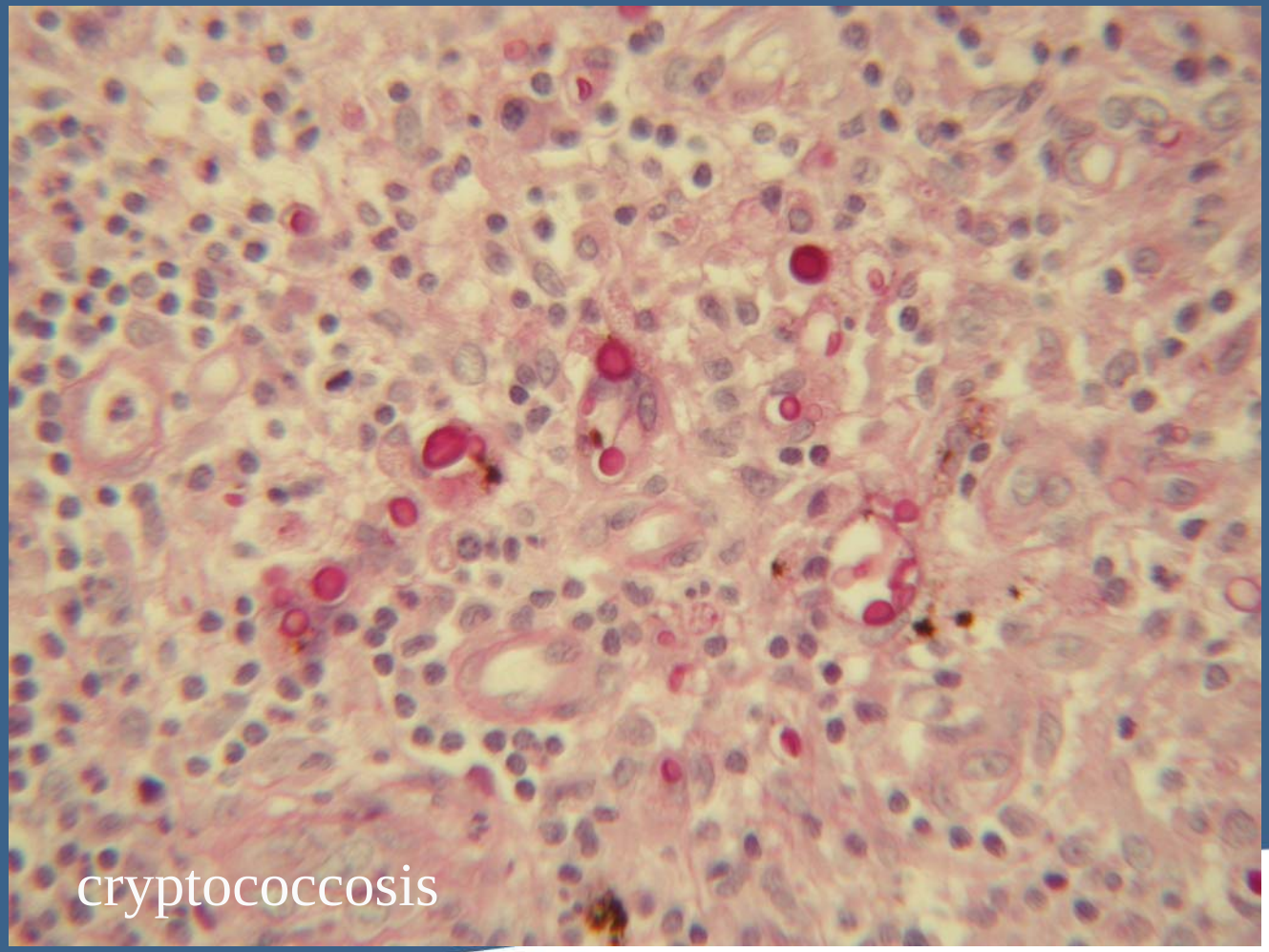
North-American blastomycosis

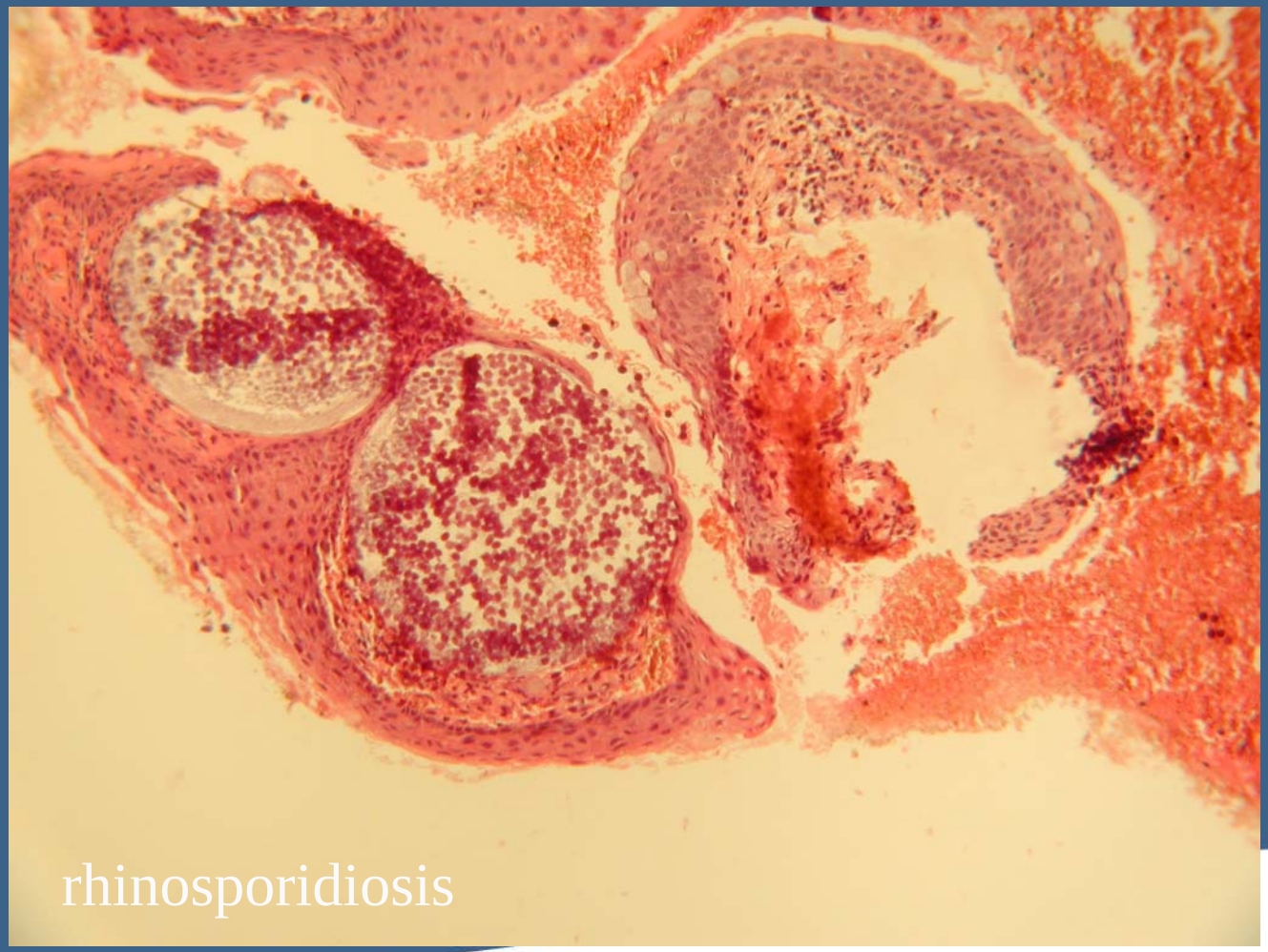

coccidioidomycosis


coccidioidomycosis

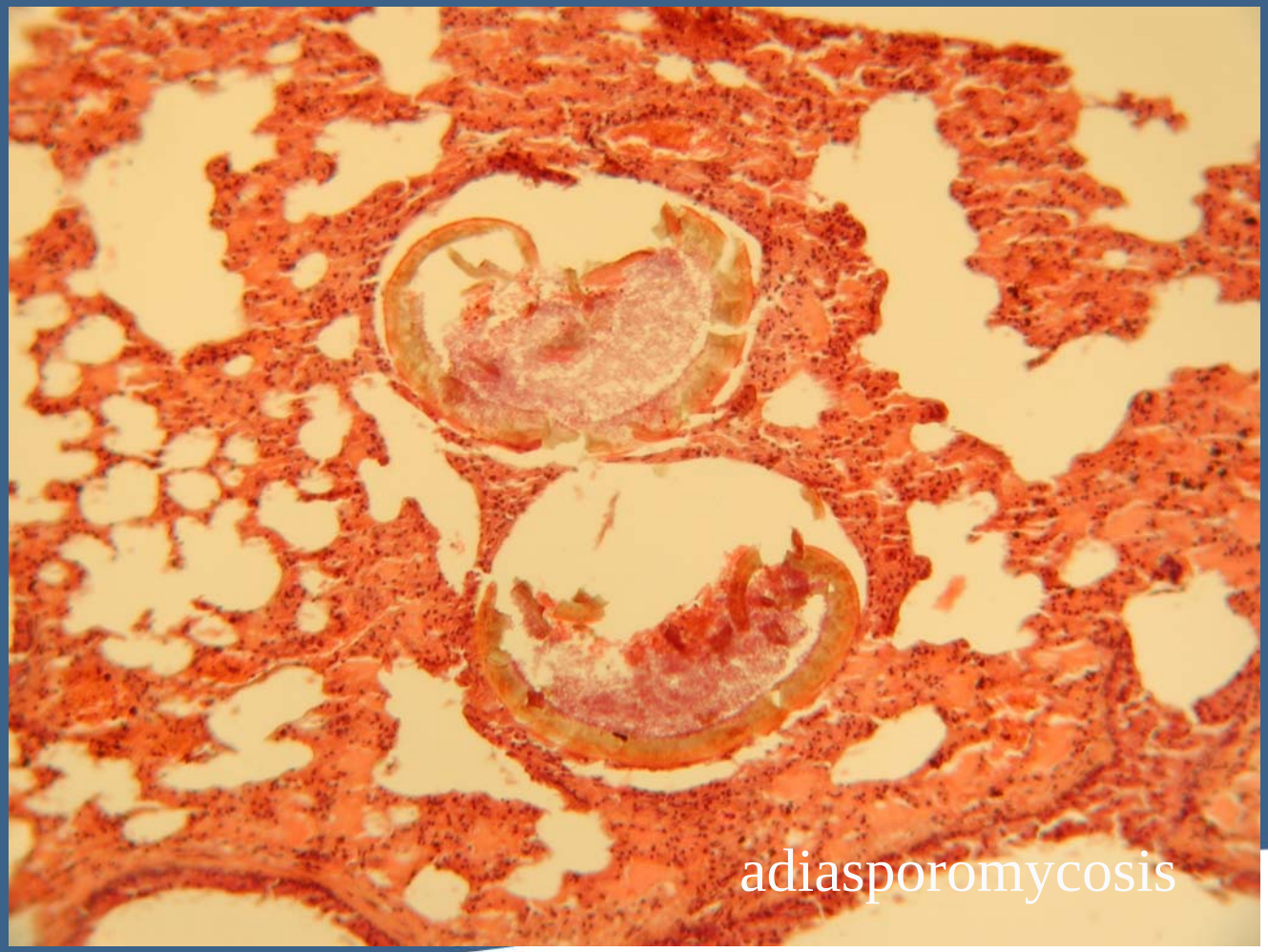


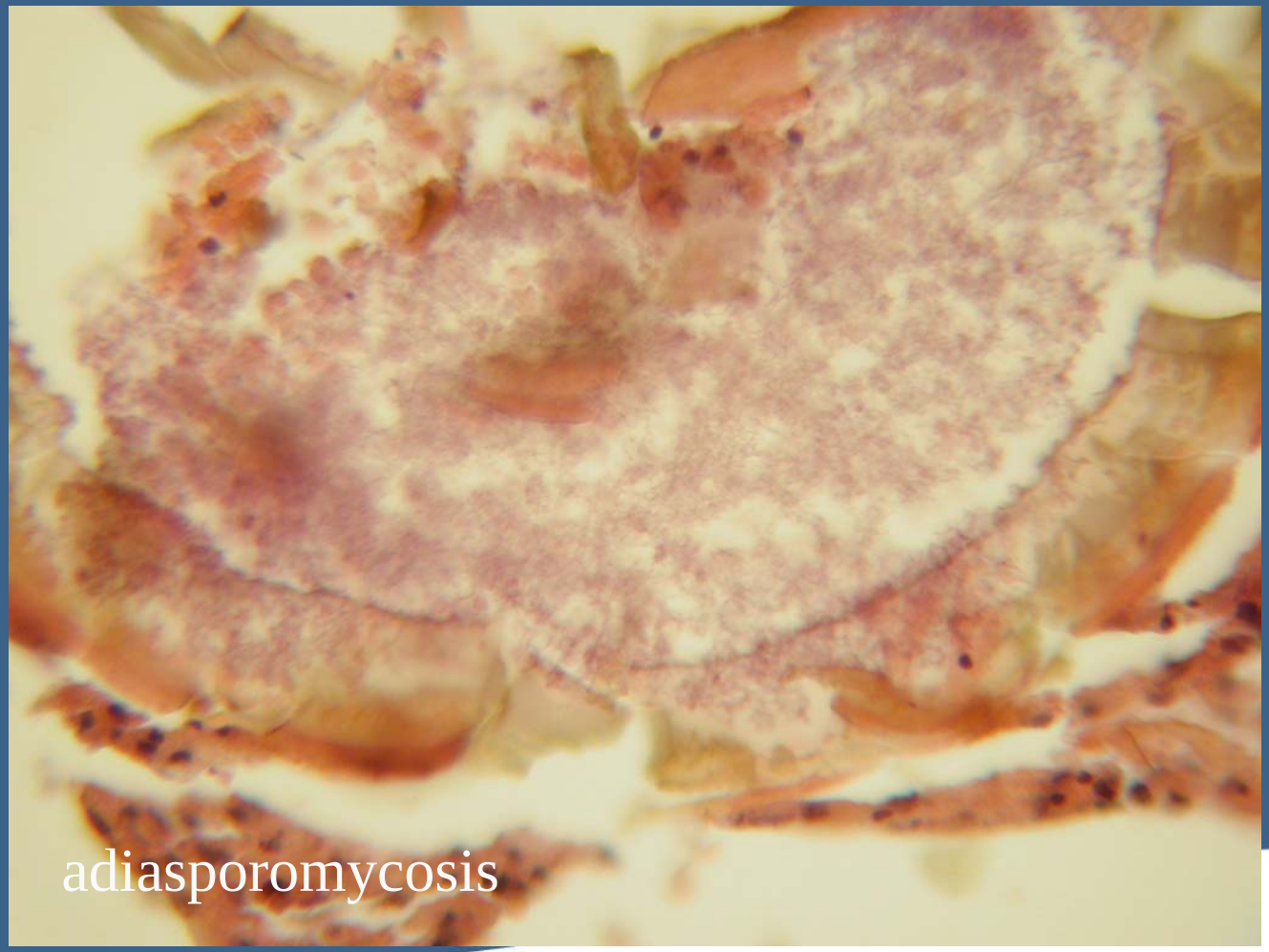
coccidioidomycosis


cryptococcosis



rhinosporidiosis

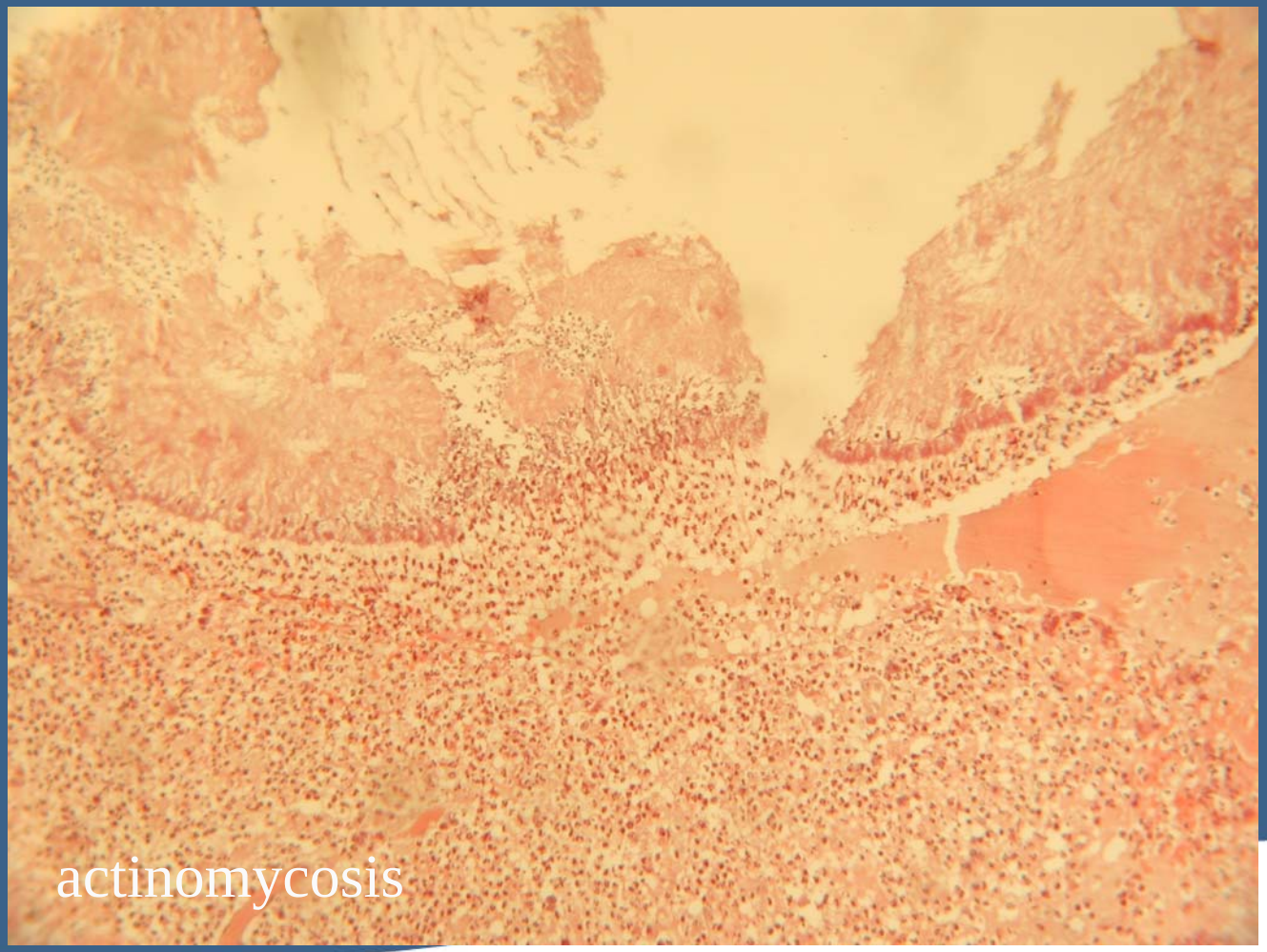

adiasporomycosis

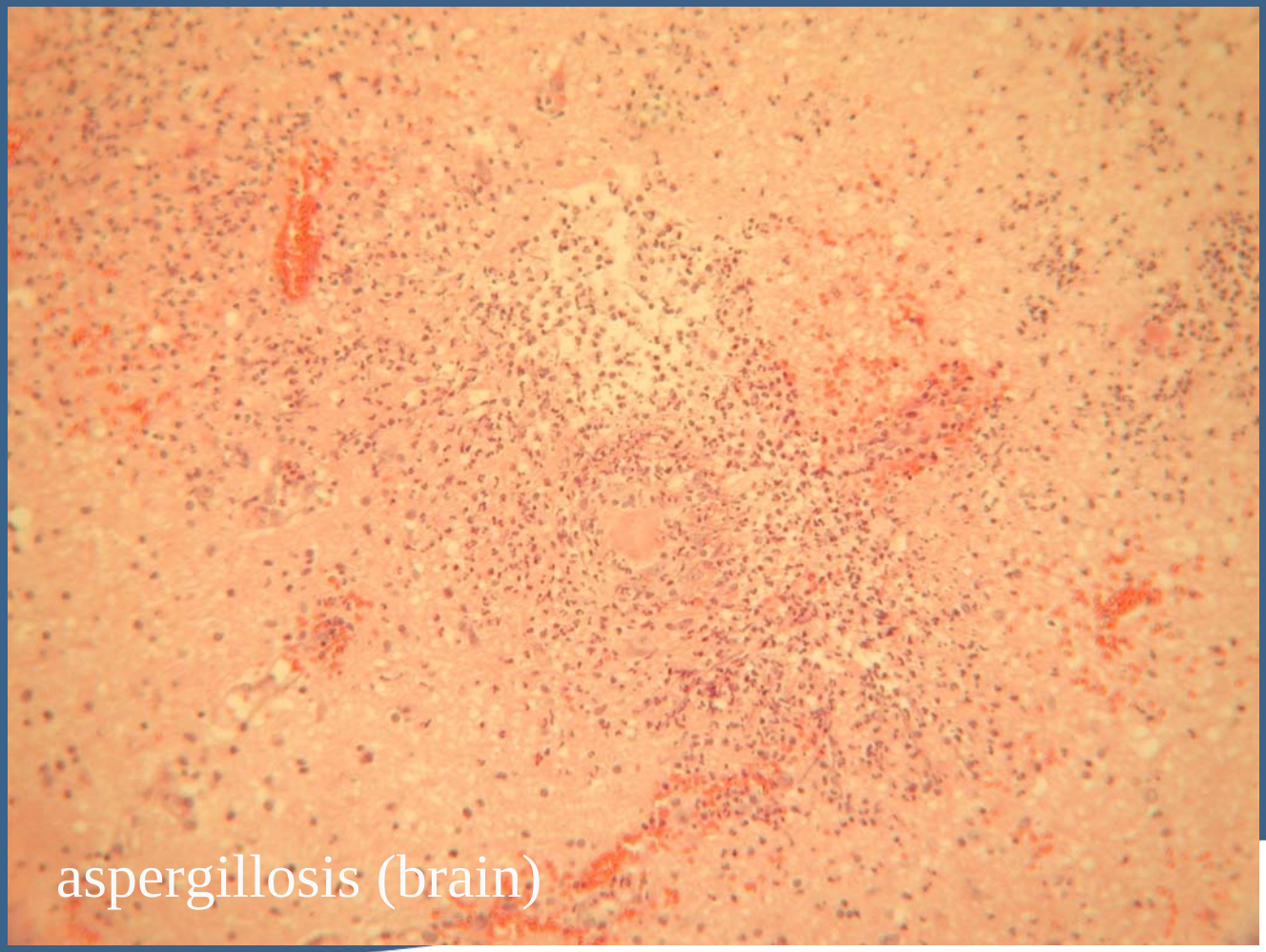

adiasporomycosis

# Accuracy of histopathologic diagnosis (2)

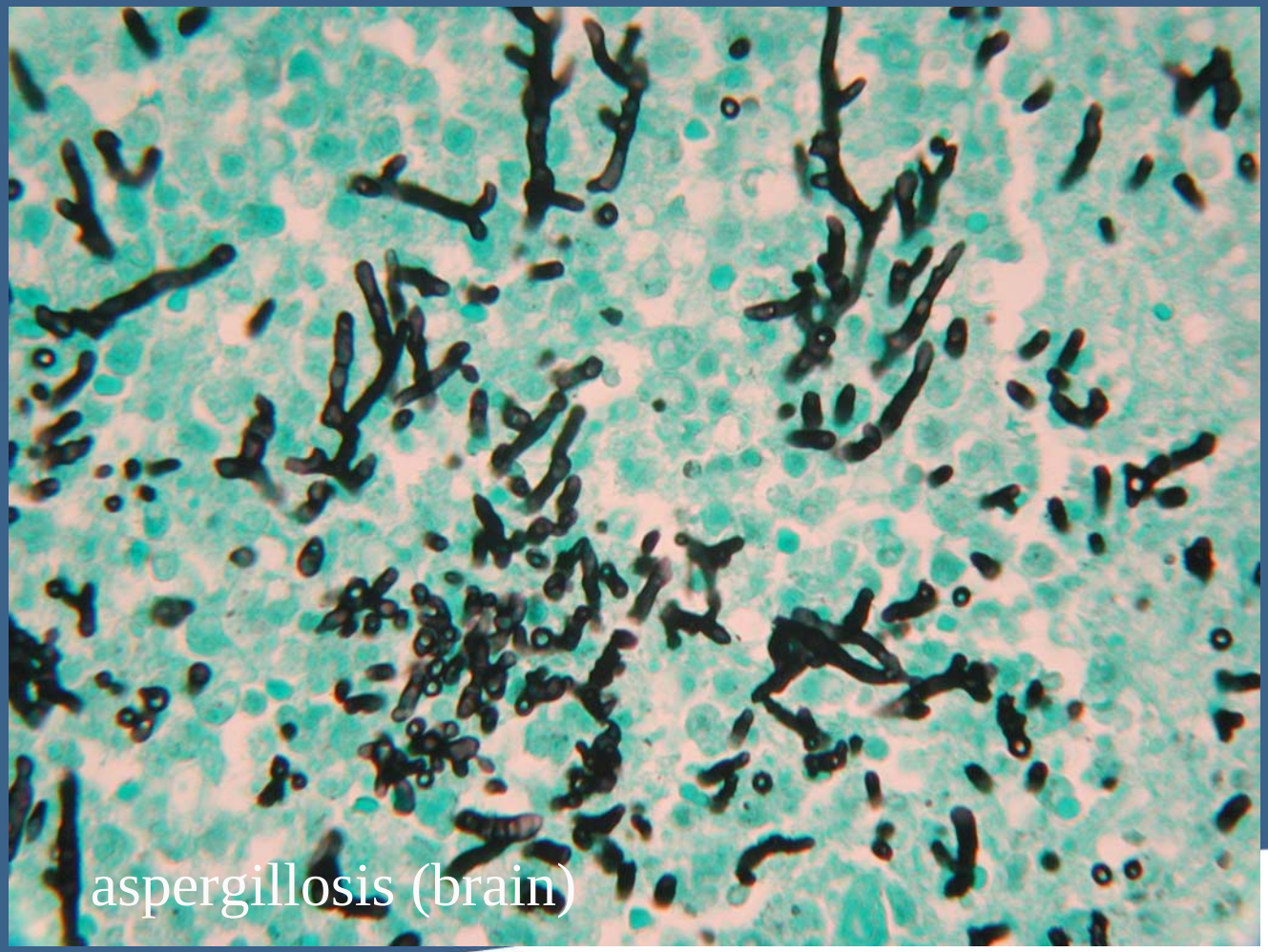
- other organisms can be identified at the genus level and the disease can be named:
  - actinomycosis (bacterial agent!)
  - nocardiosis (bacterial agent!)
  - protothecosis (algae !)
  - chlorellosis (algae !)
  - candidiasis
  - aspergillosis

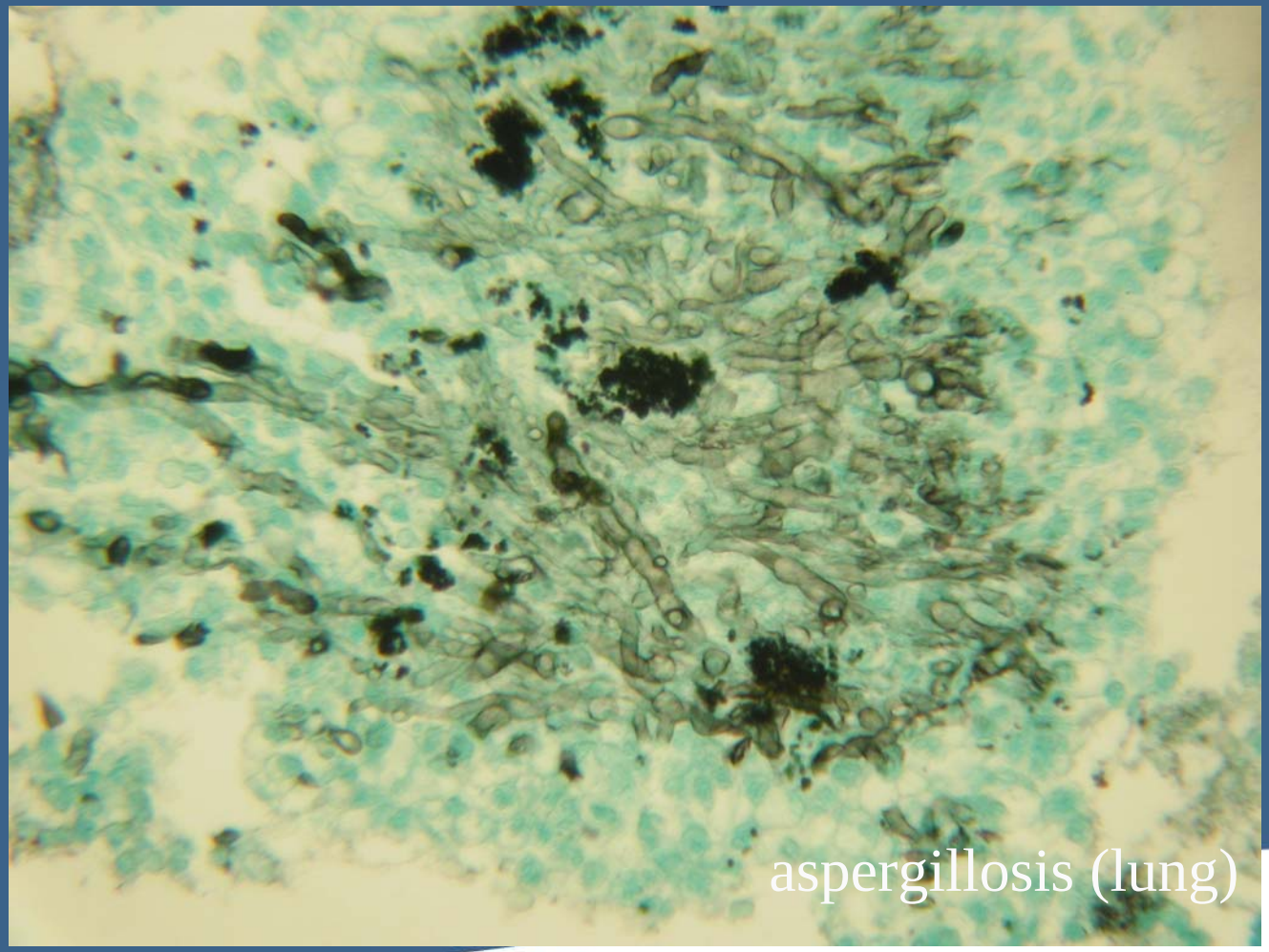

actinomycosis


actinomycosis



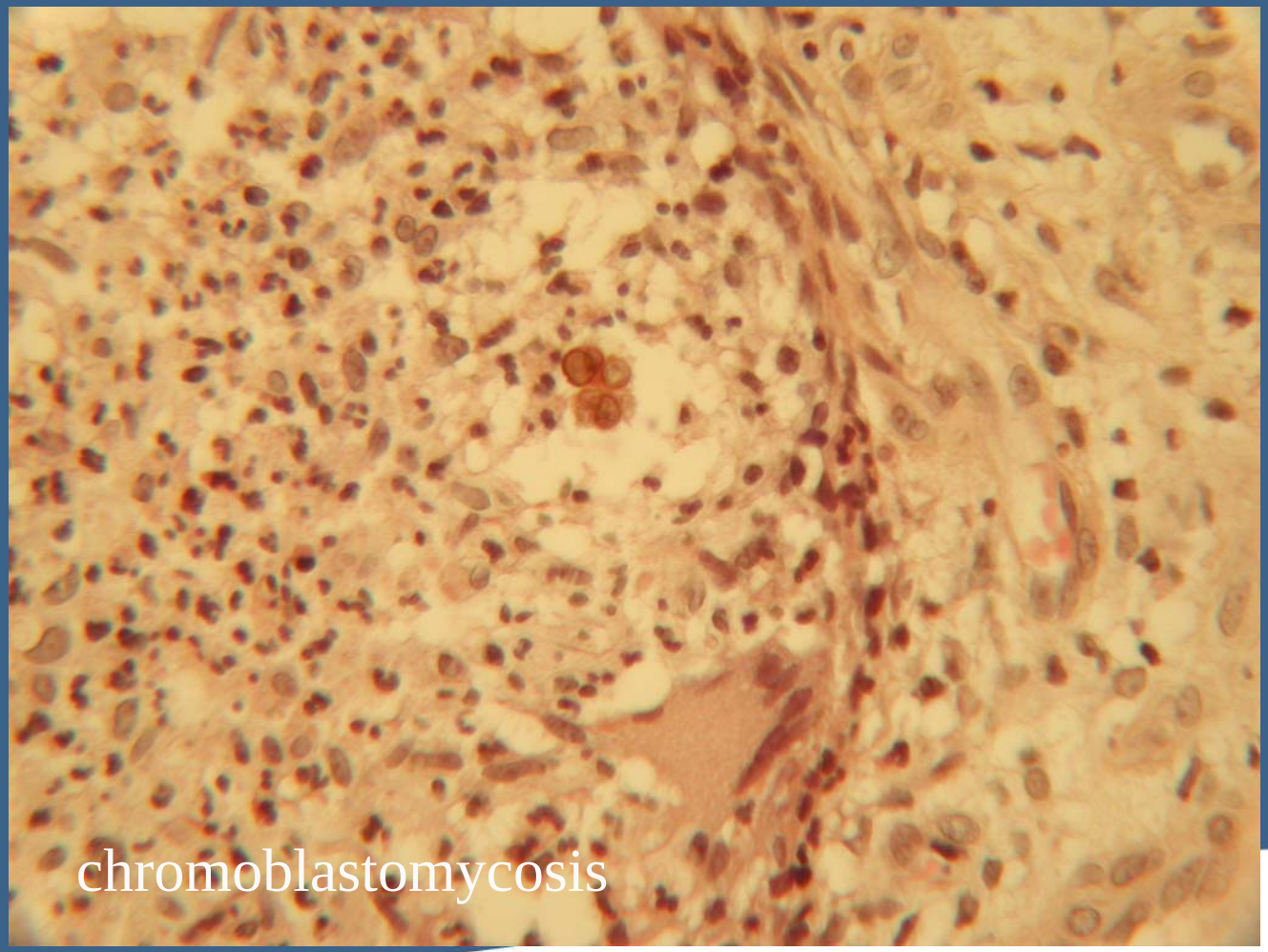
aspergillosis (brain)

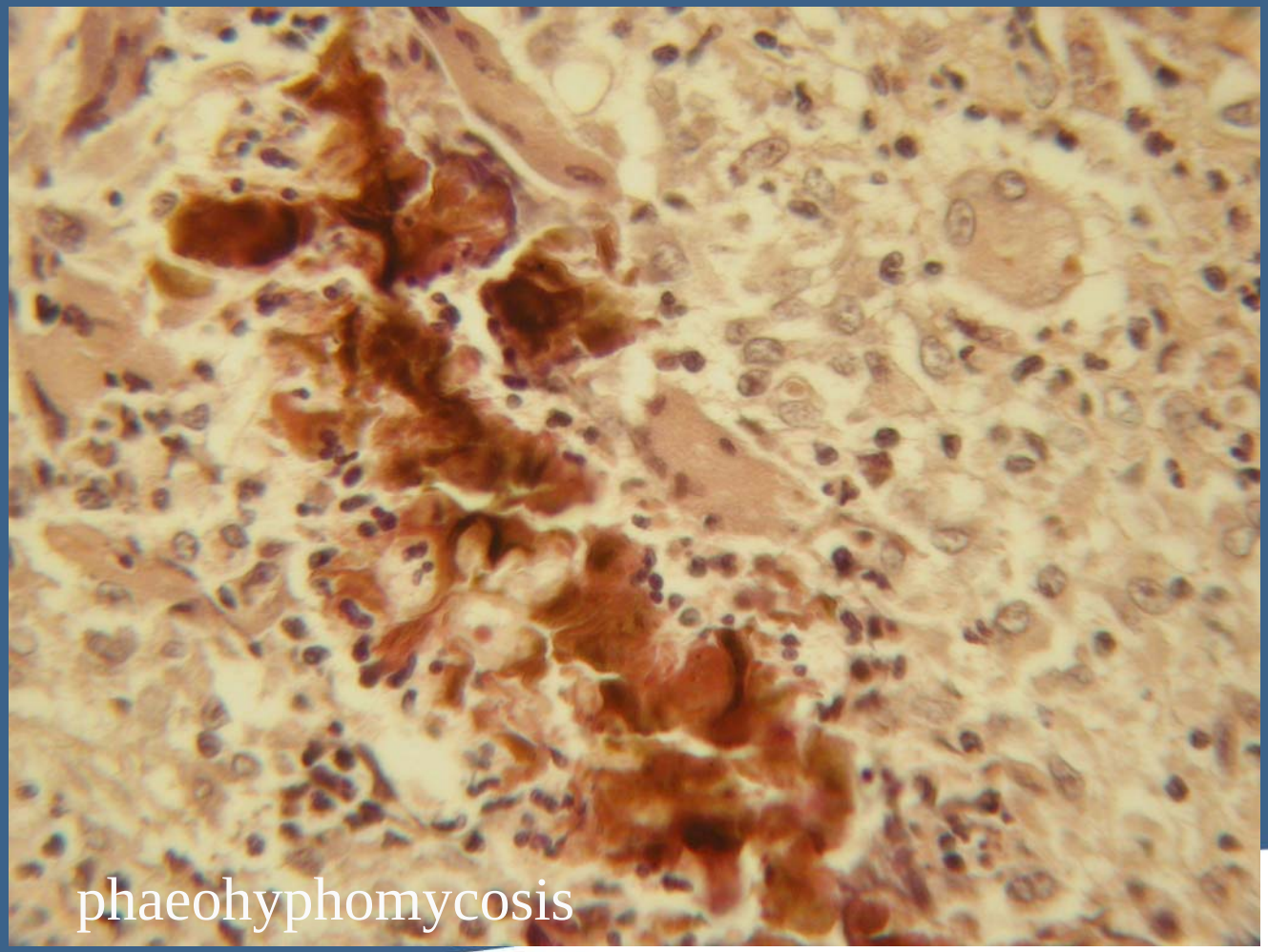

aspergillosis (brain)

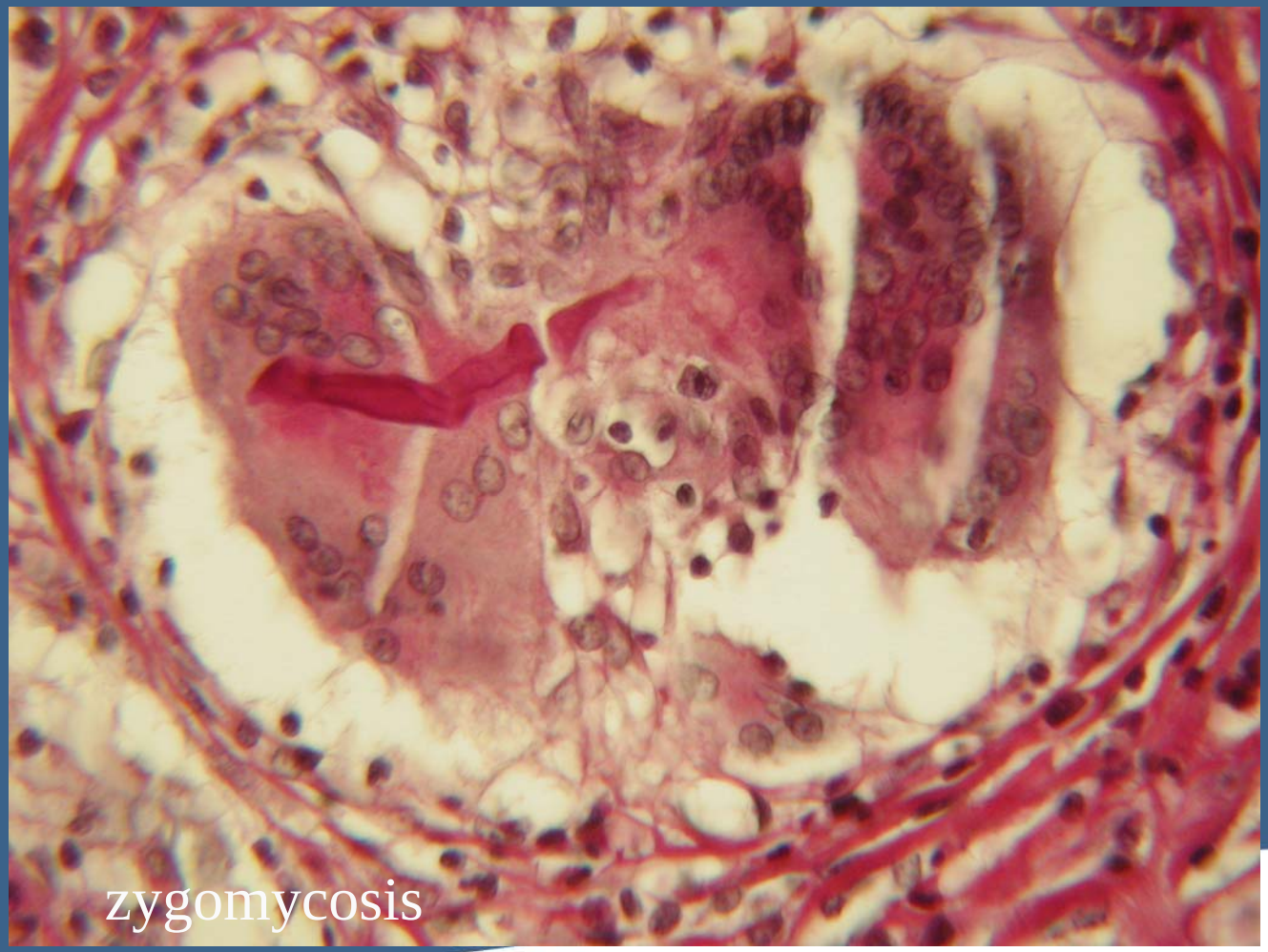

aspergillosis (lung)

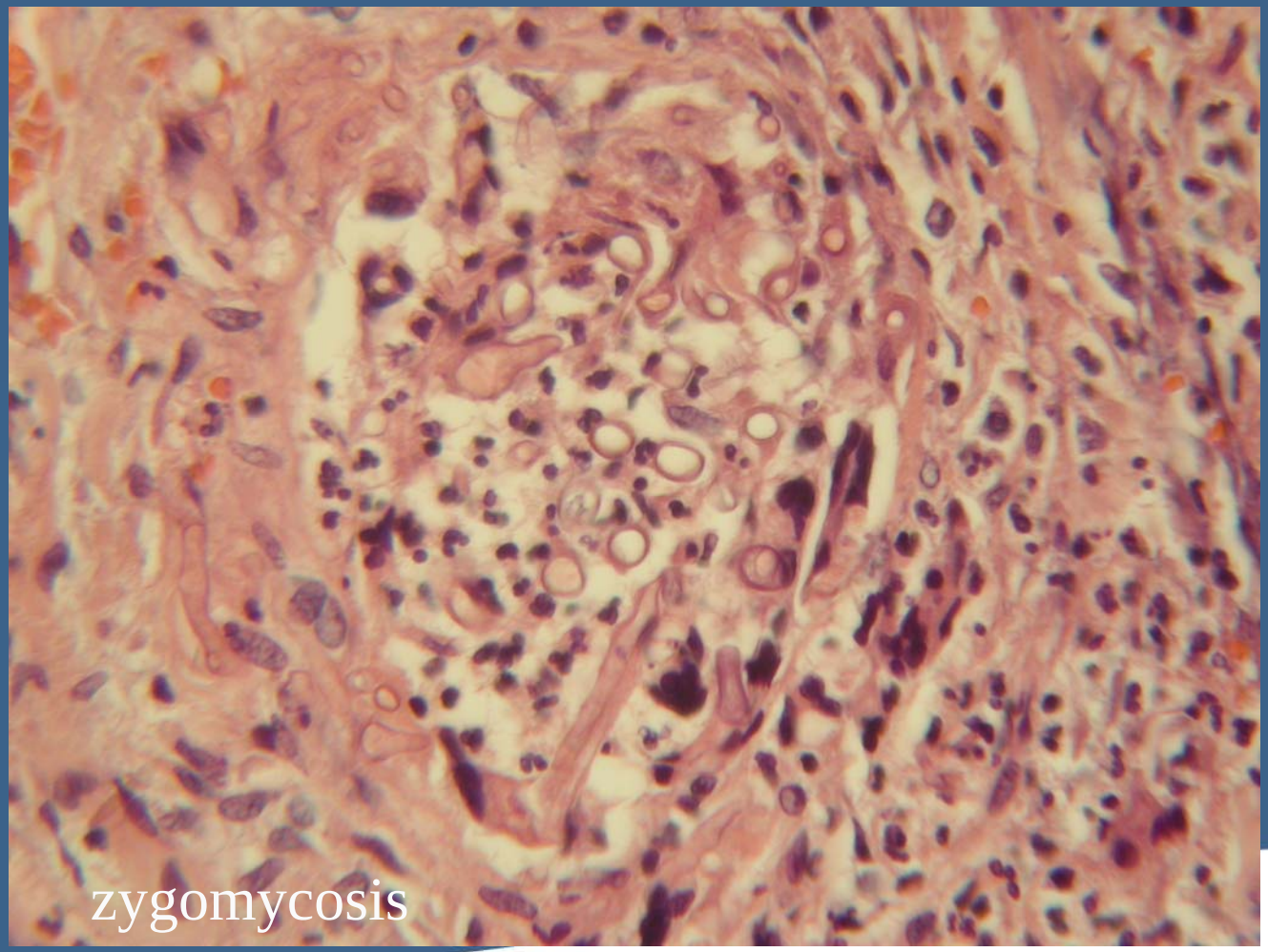
# Accuracy of histopathologic diagnosis (3)

- several mycoses are caused by organisms belonging to various genera:
  - chromoblastomycosis
  - phaeohyphomycosis
  - zygomycosis
  - dermatophytosis

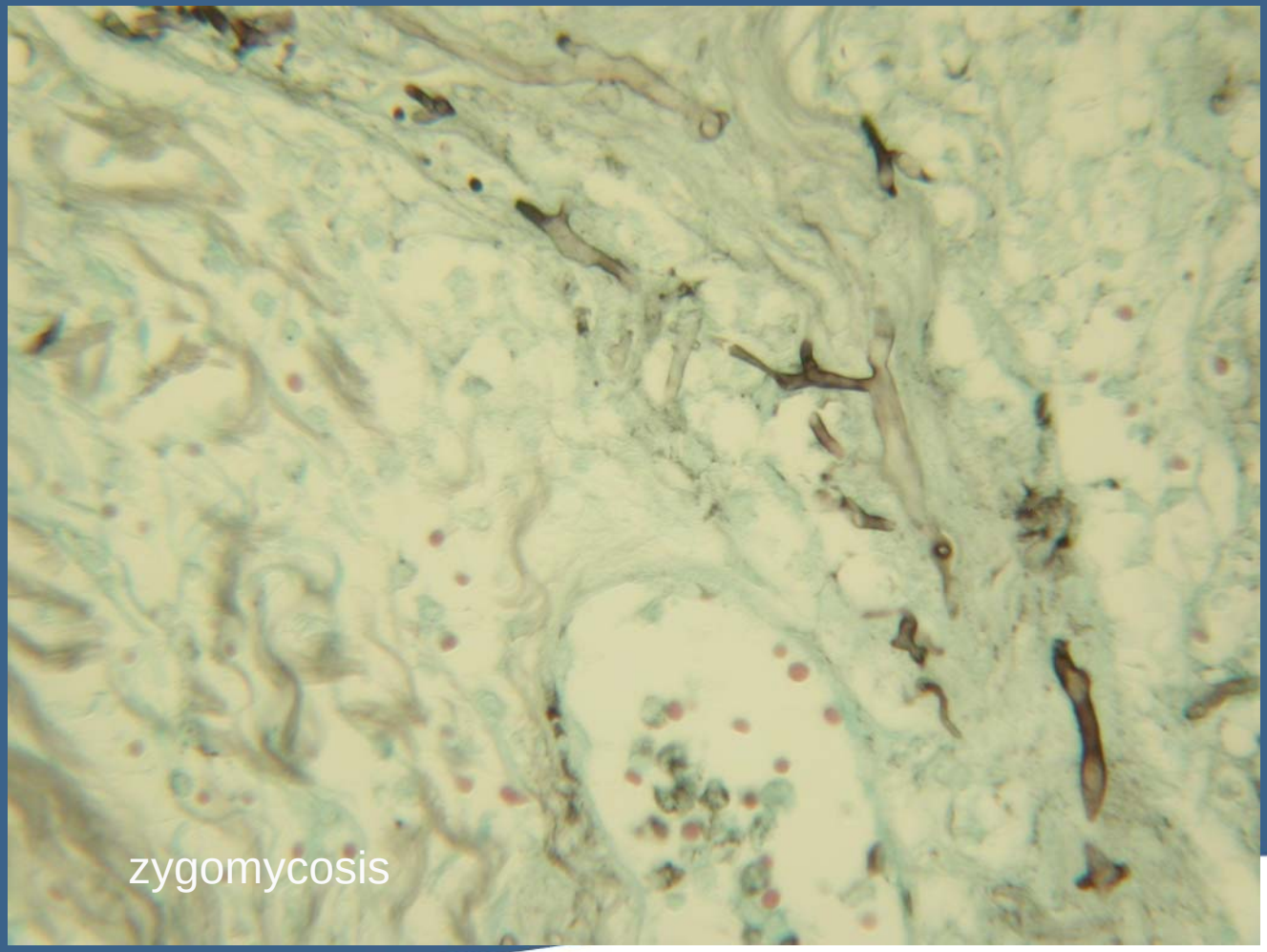

chromoblastomycosis

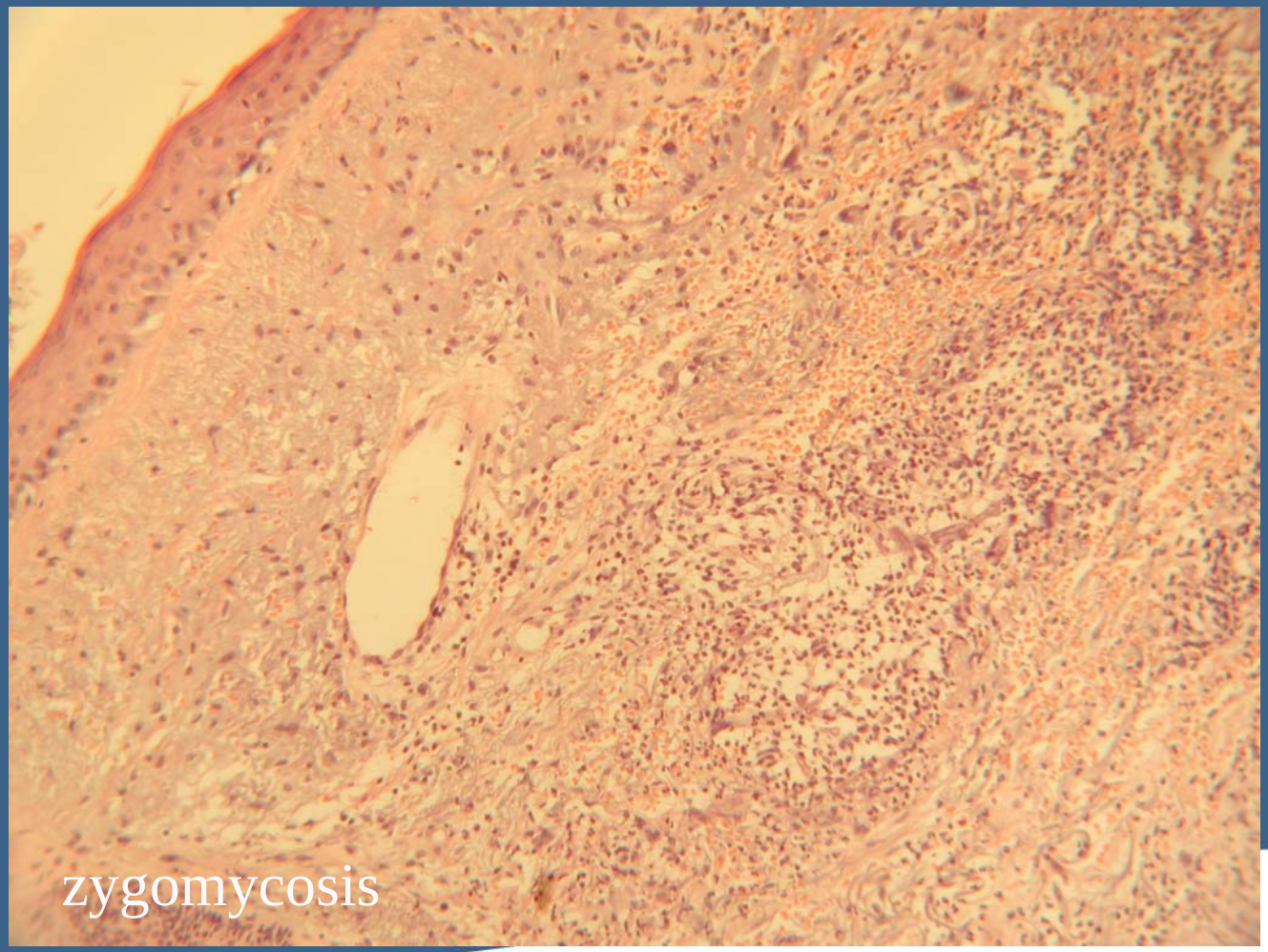

phaeohyphomycosis

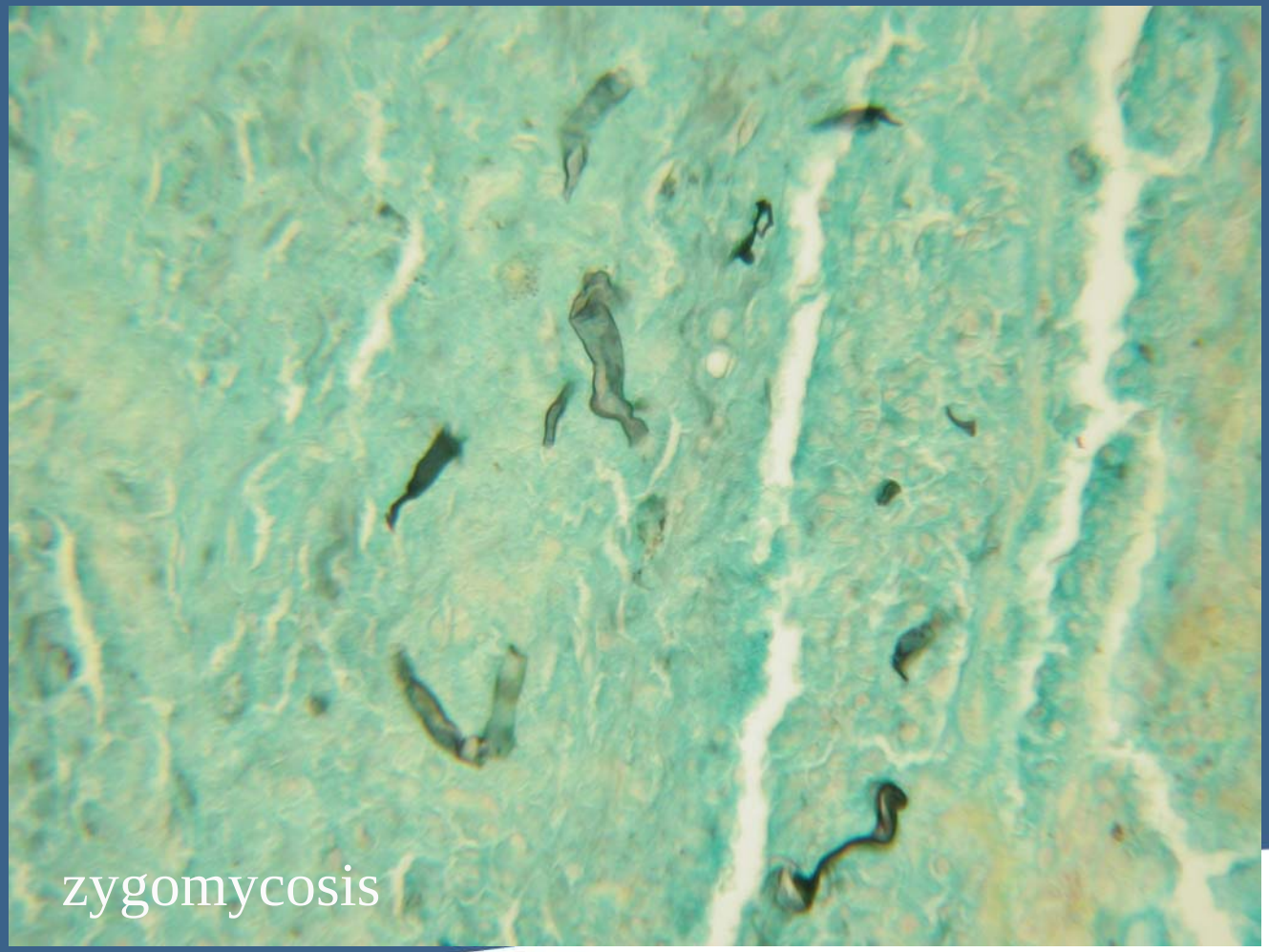

zygomycosis

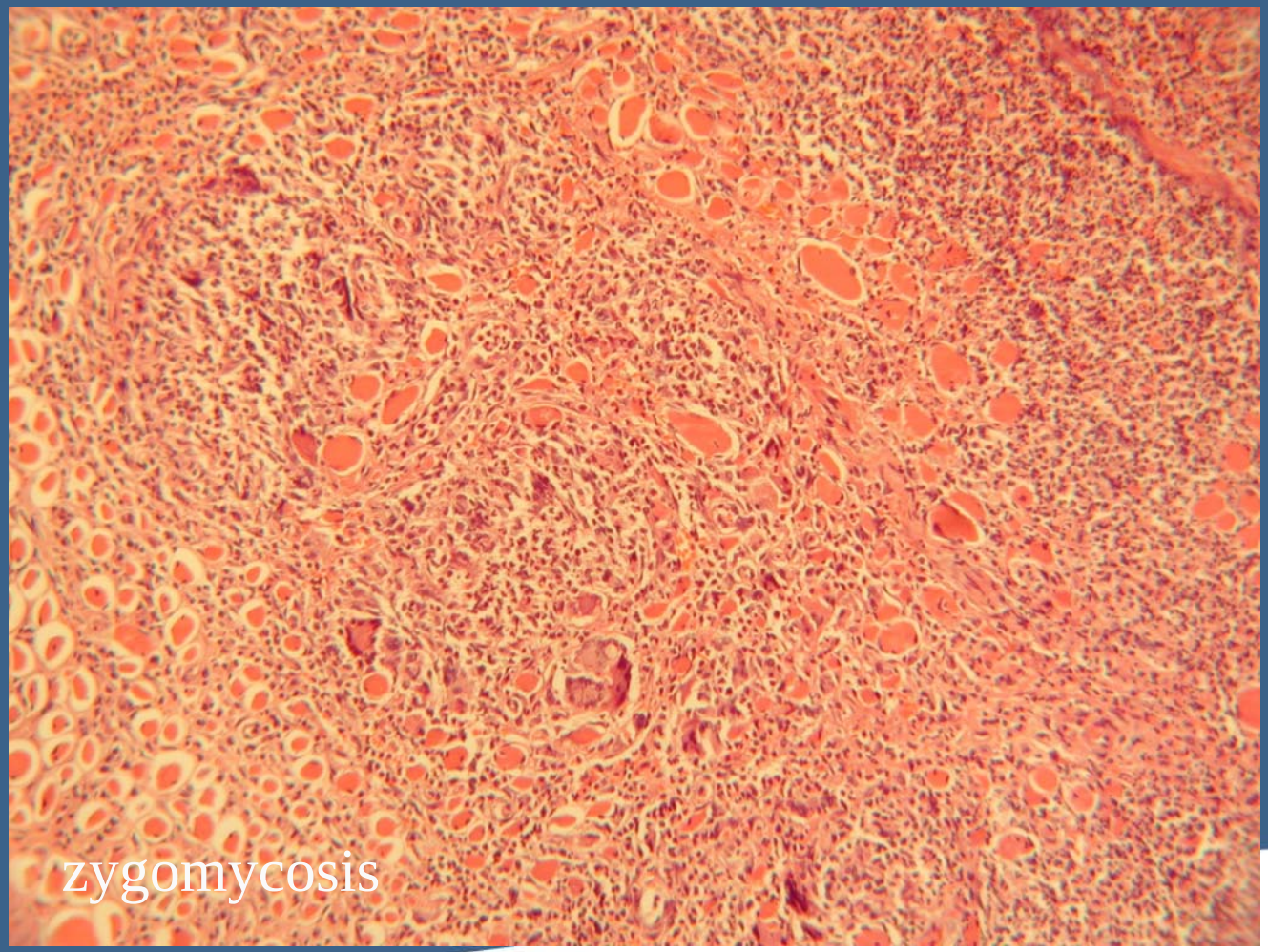


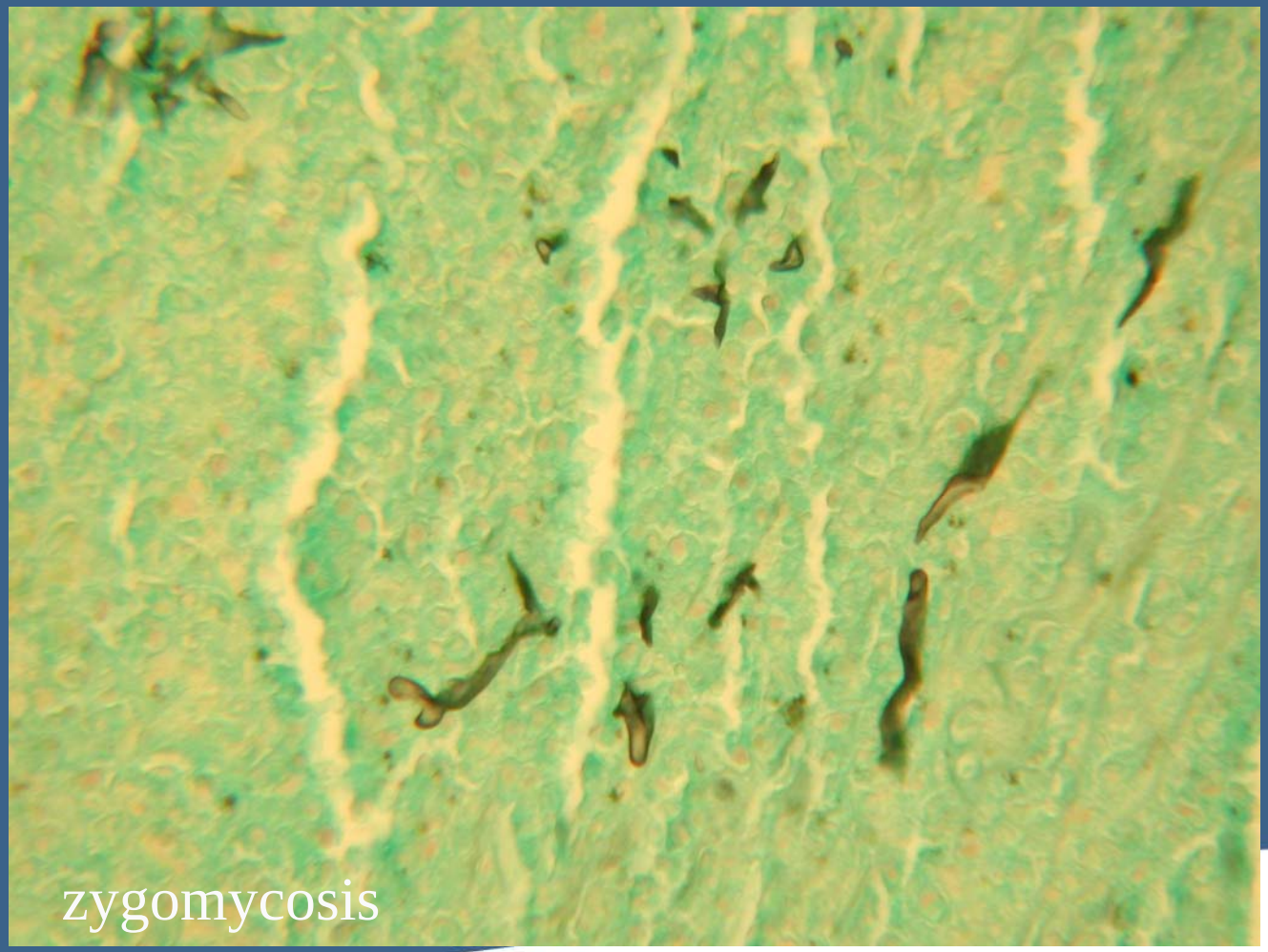
zygomycosis


zygomycosis


zygomycosis


zygomycosis

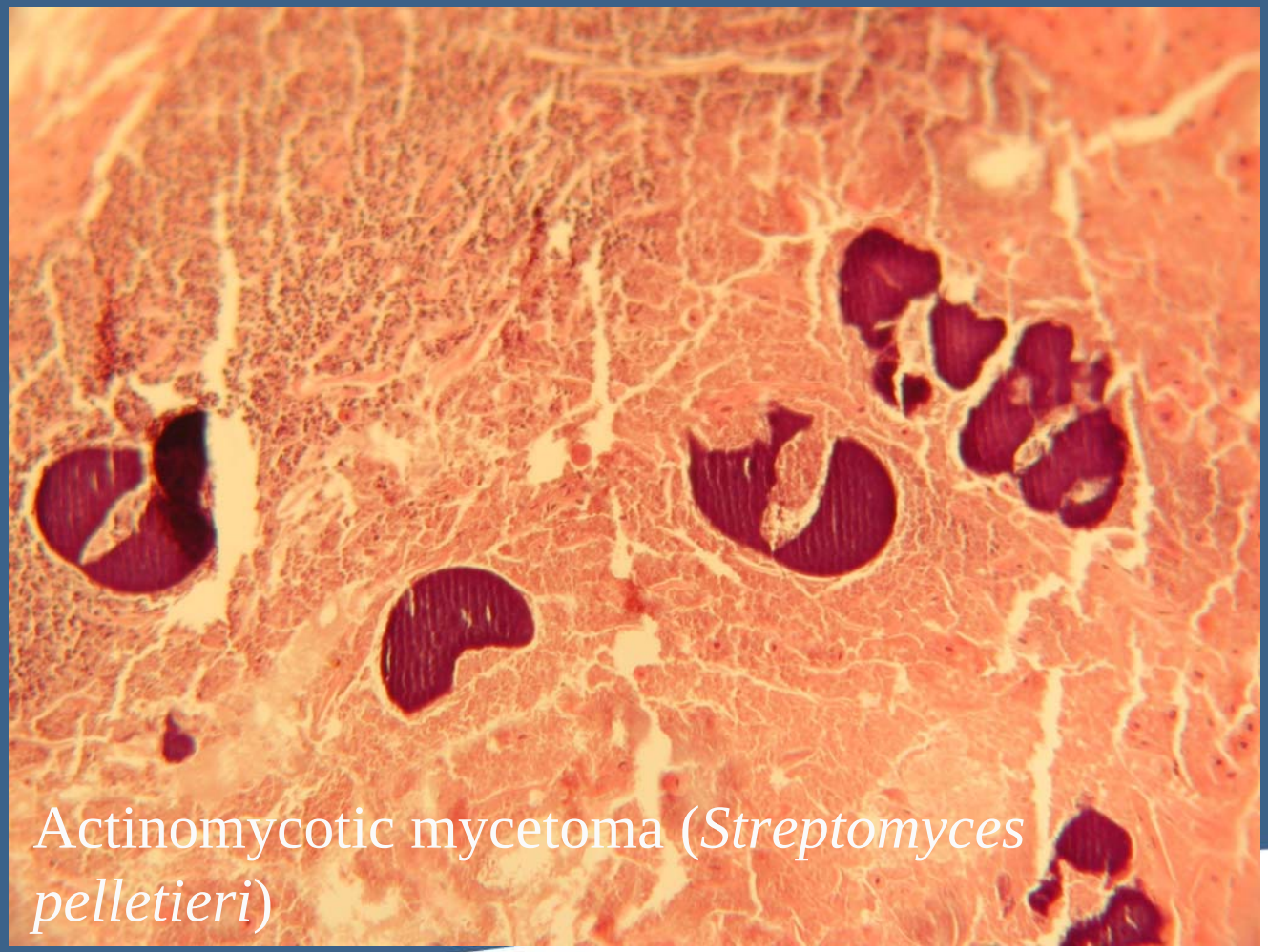

zygomycosis


zygomycosis

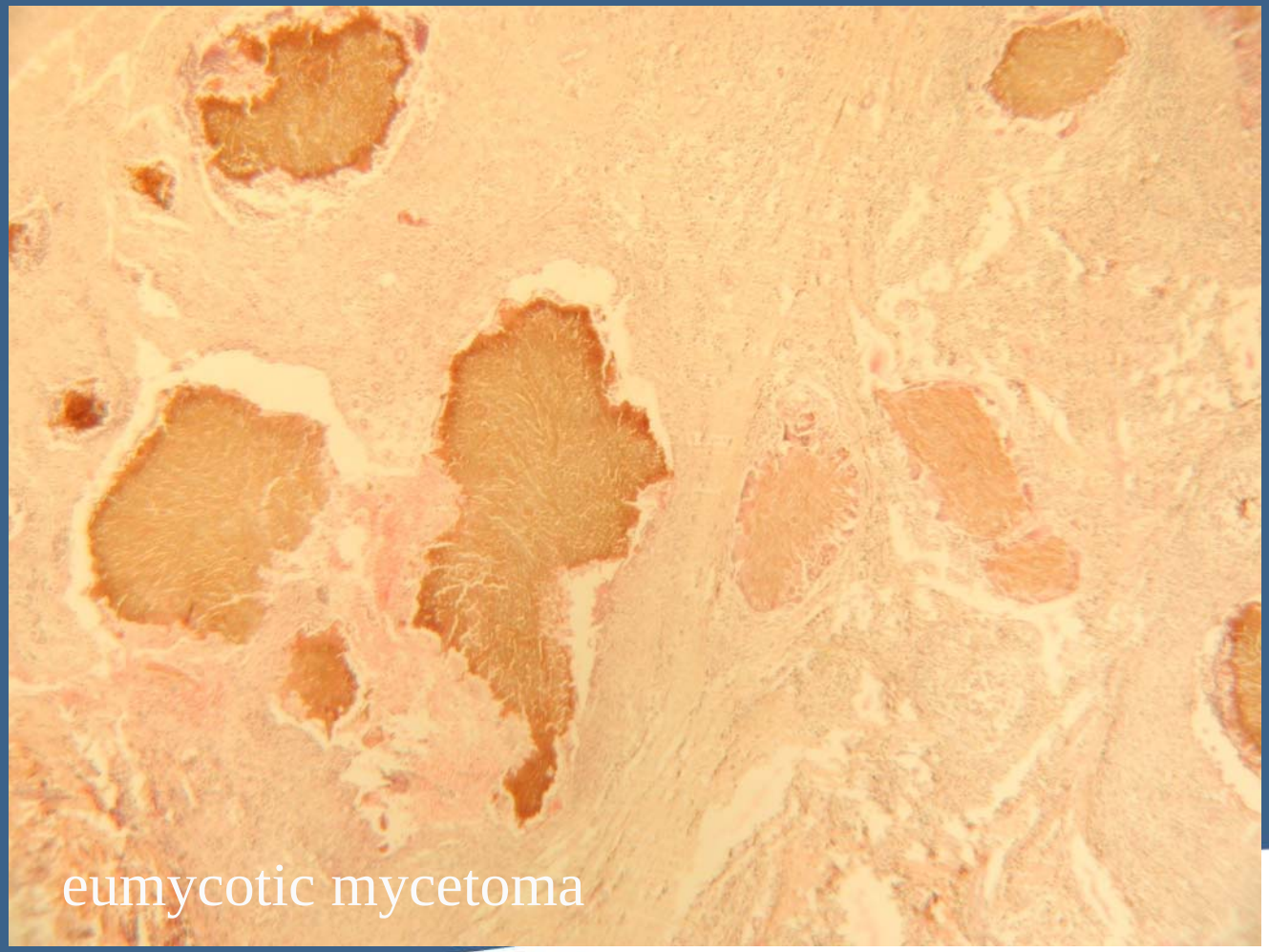
# Mycetomas

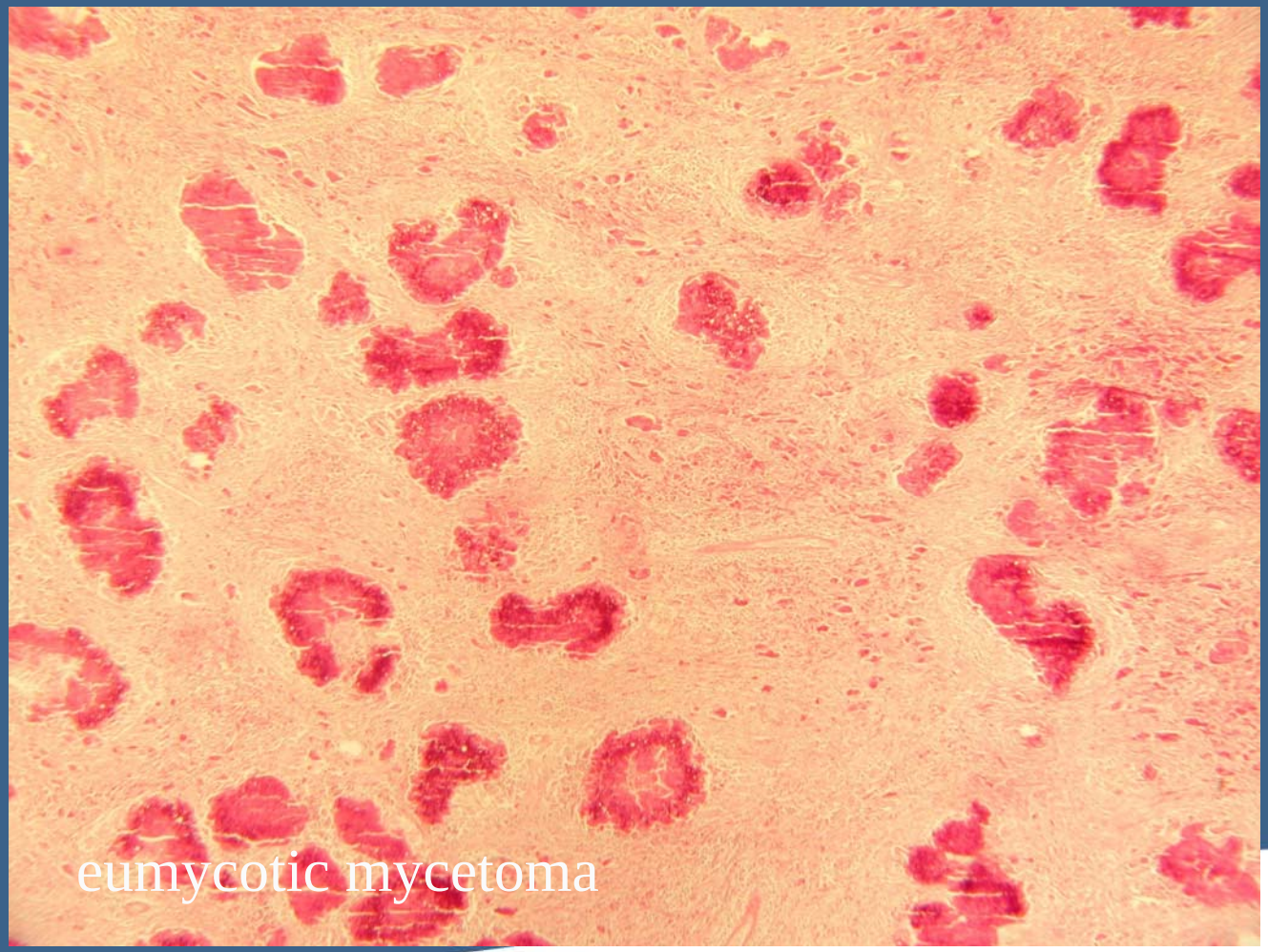
- Pathologists, by studying the granules, can make a major contribution in differentiating between:
  - actinomycotic mycetomas
  - eumycotic mycetomas
    - hyaline eumycotic mycetomas
    - dematiaceous eumycotic mycetomas

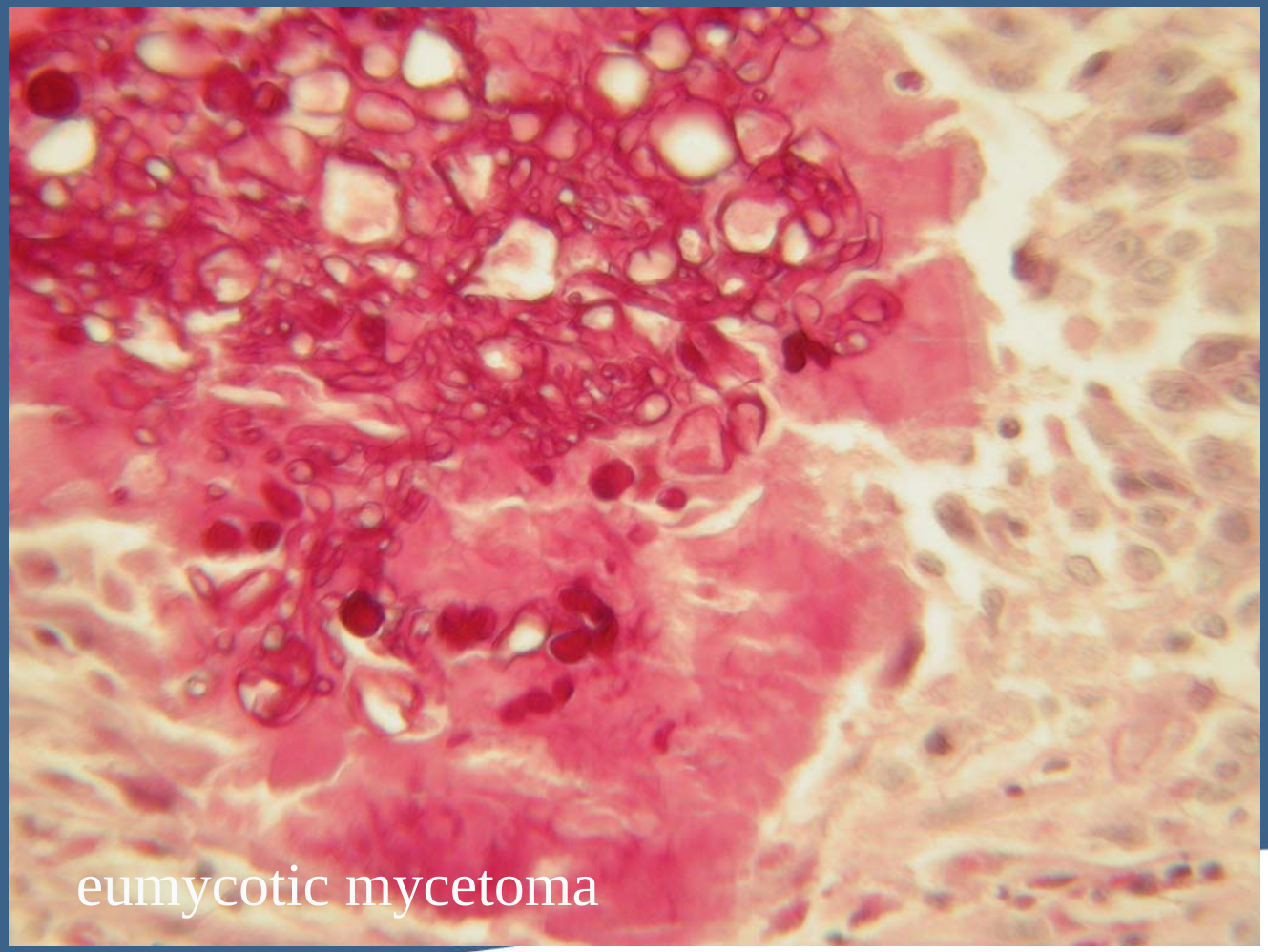




Actinomycotic mycetoma (*Streptomyces pelletieri*)

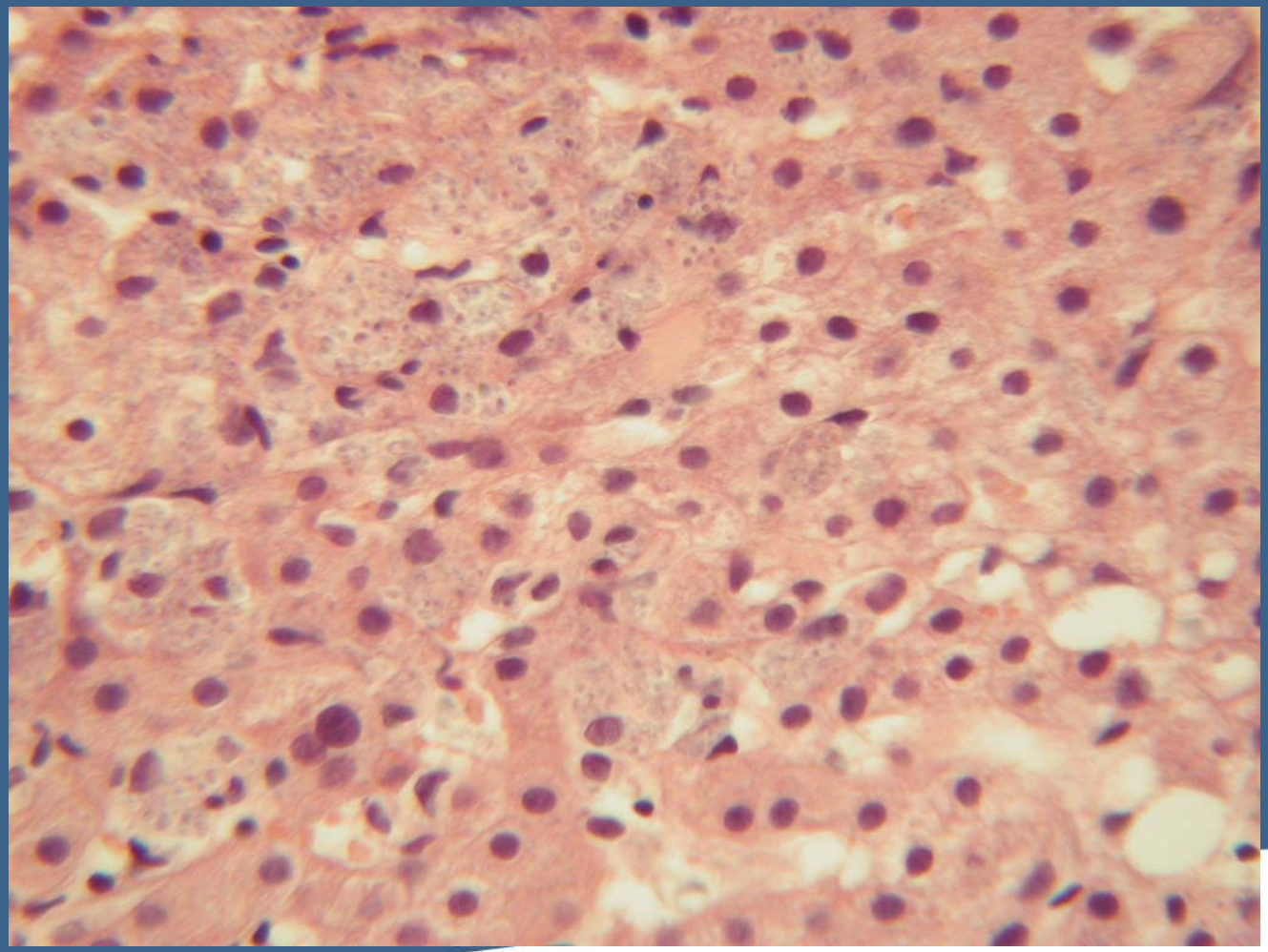

eumycotic mycetoma

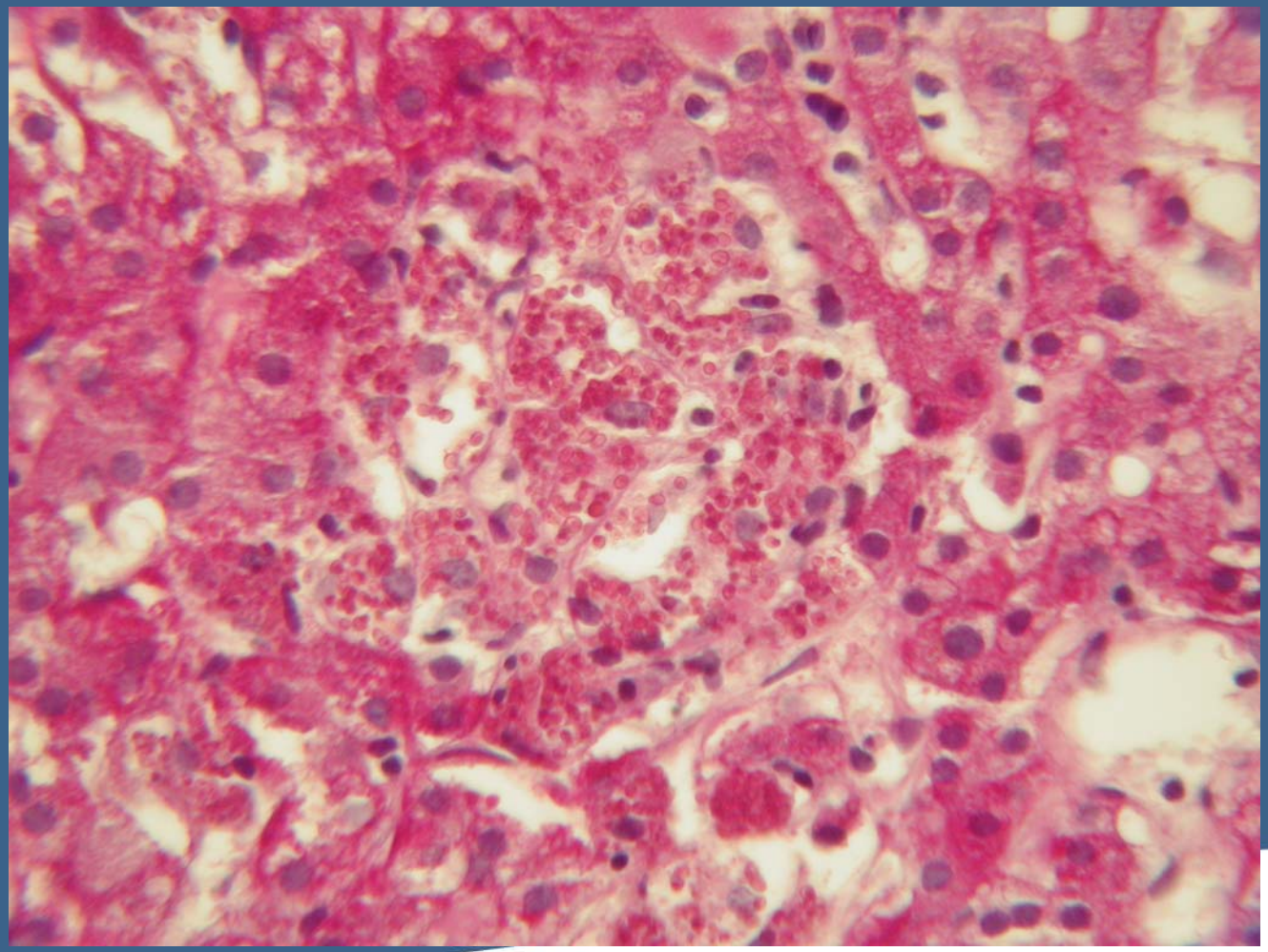

eumycotic mycetoma


eumycotic mycetoma

# Quiz

## Question 1





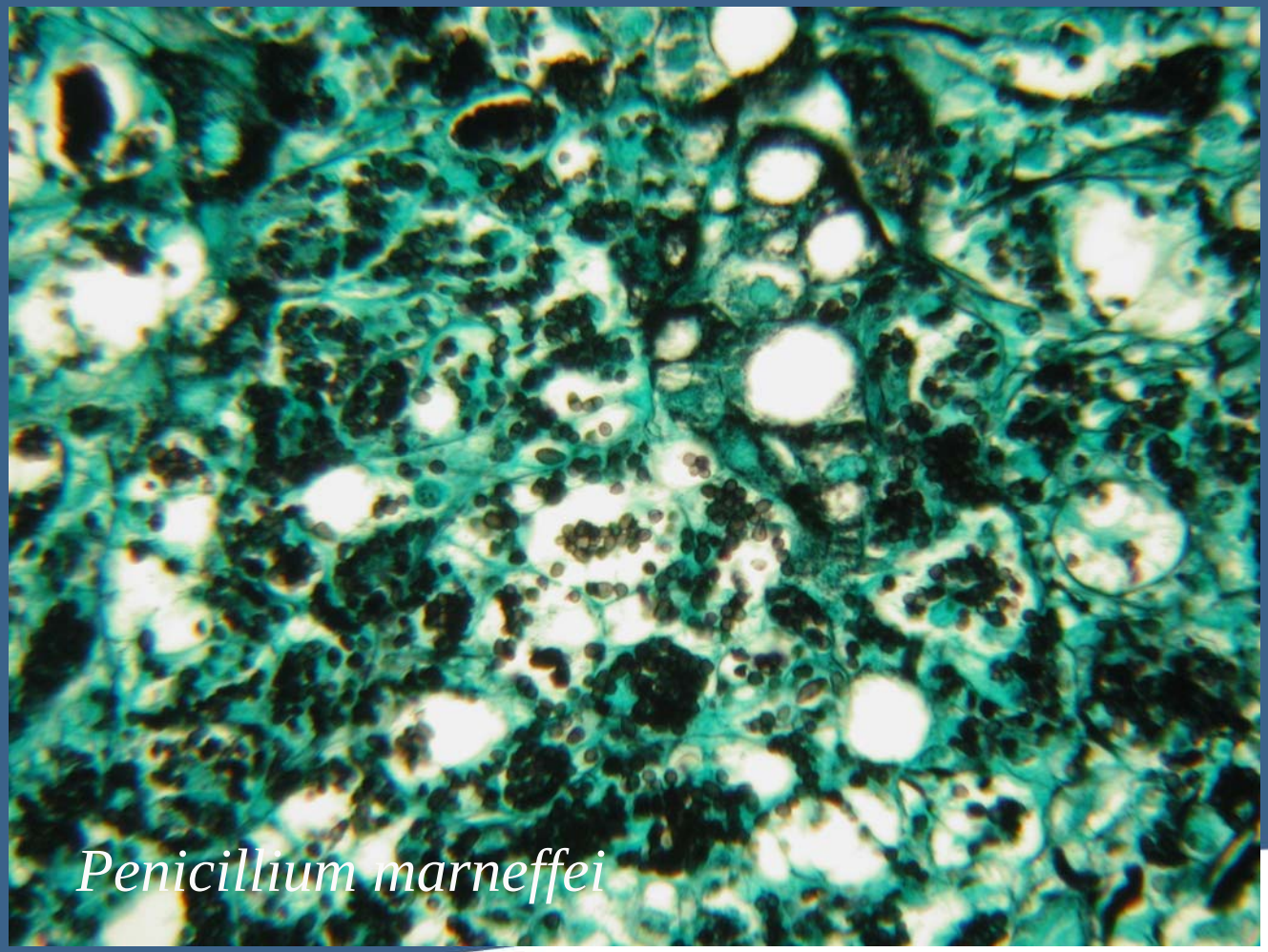
# One answer is correct

- *1) Candida sp.*
- *2) Histoplasma capsulatum*
- *3) Histoplasma duboisii*
- *4) Blastomyces dermatitidis*
- *5) Other yeast or fungus*

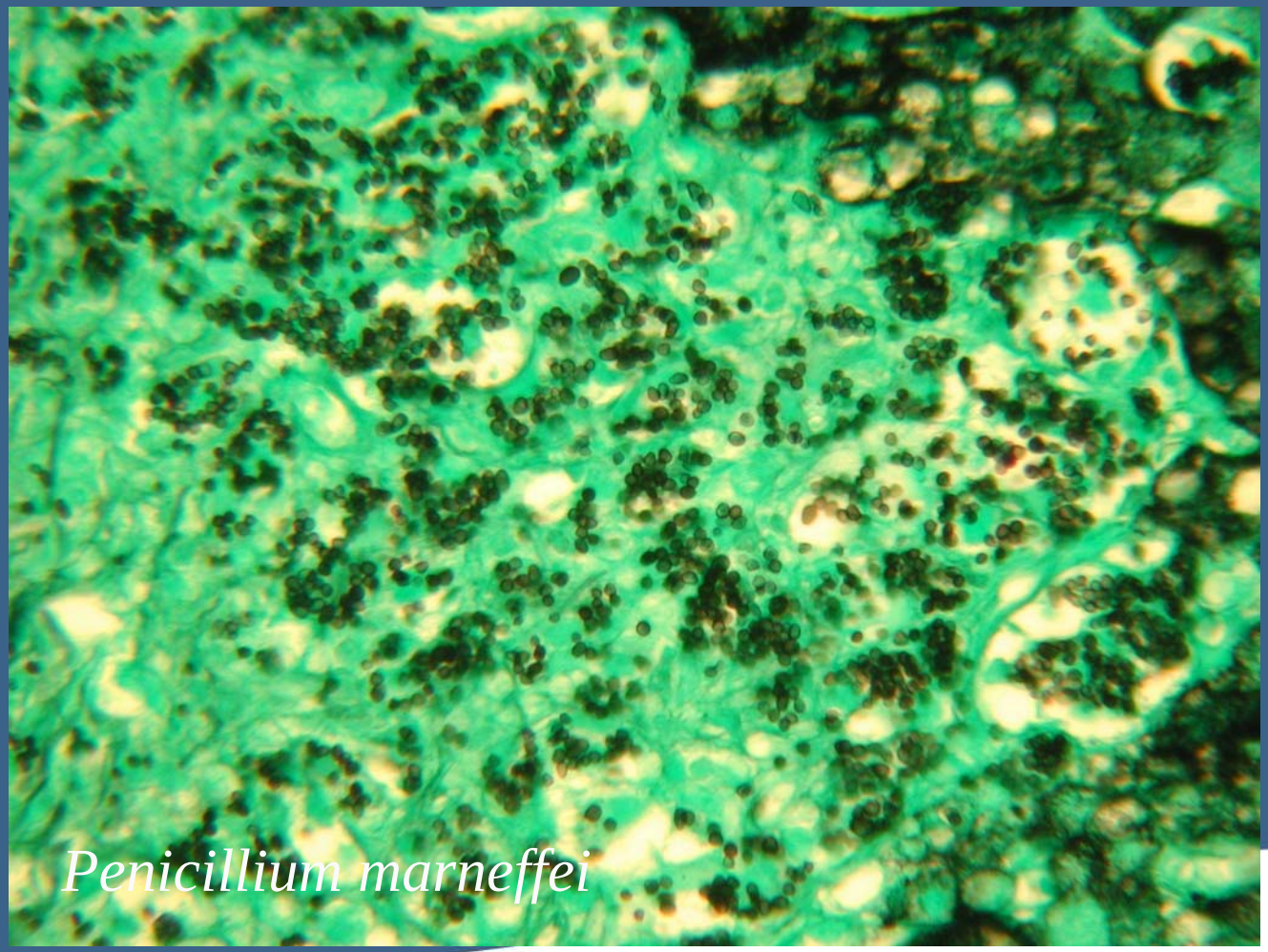
# Answer to question 1

- Correct answer: 5

- Systemic involvement in an AIDS patient with numerous *Penicillium marneffei* organisms in the liver (and in other organs)



*Penicillium marneffei*



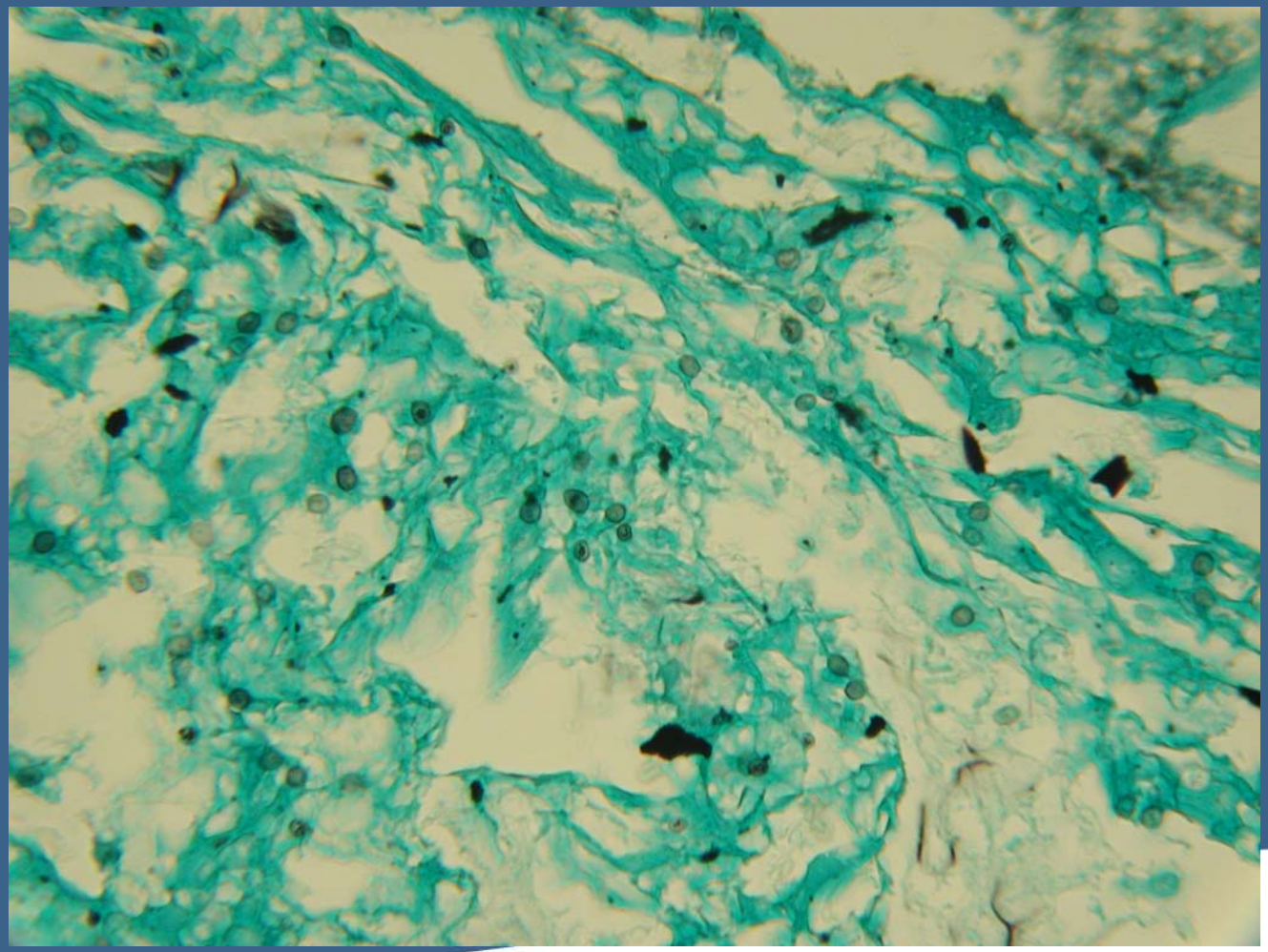
*Penicillium marneffei*

# Take-home lesson (1)

- do not rely on just one stain and always ask for a silver impregnation stain when suspecting a fungus or yeast
- a travel anamnesis is of utmost importance

# Quiz

## Question 2

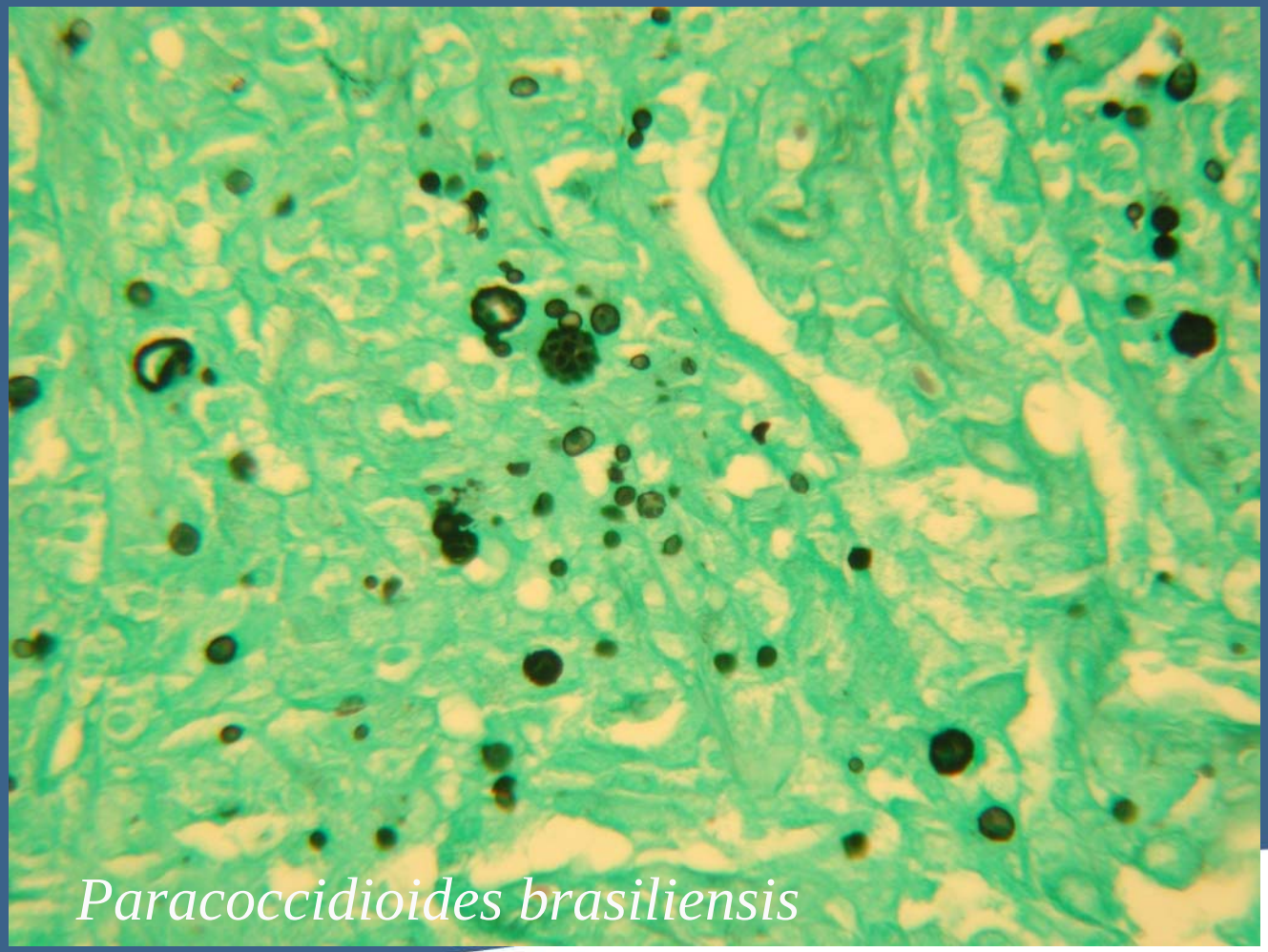


# One answer is correct

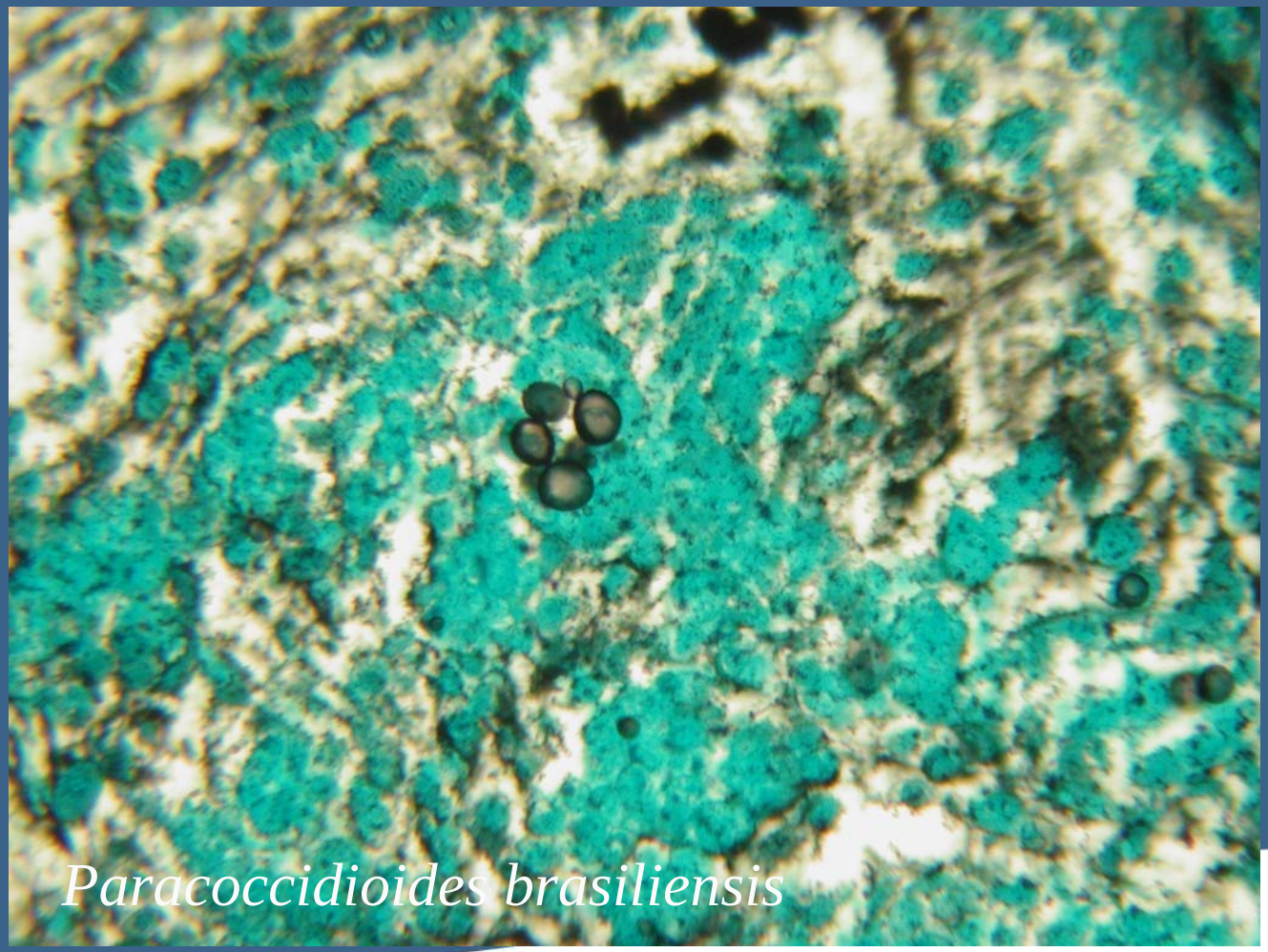
- *1) Candida sp.*
- *2) Pneumocystis jirovecii*
- *3) Paracoccidioides brasiliensis*
- *4) Coccidioides immitis*
- *5) Cryptococcus neoformans*

# Answer to question 2

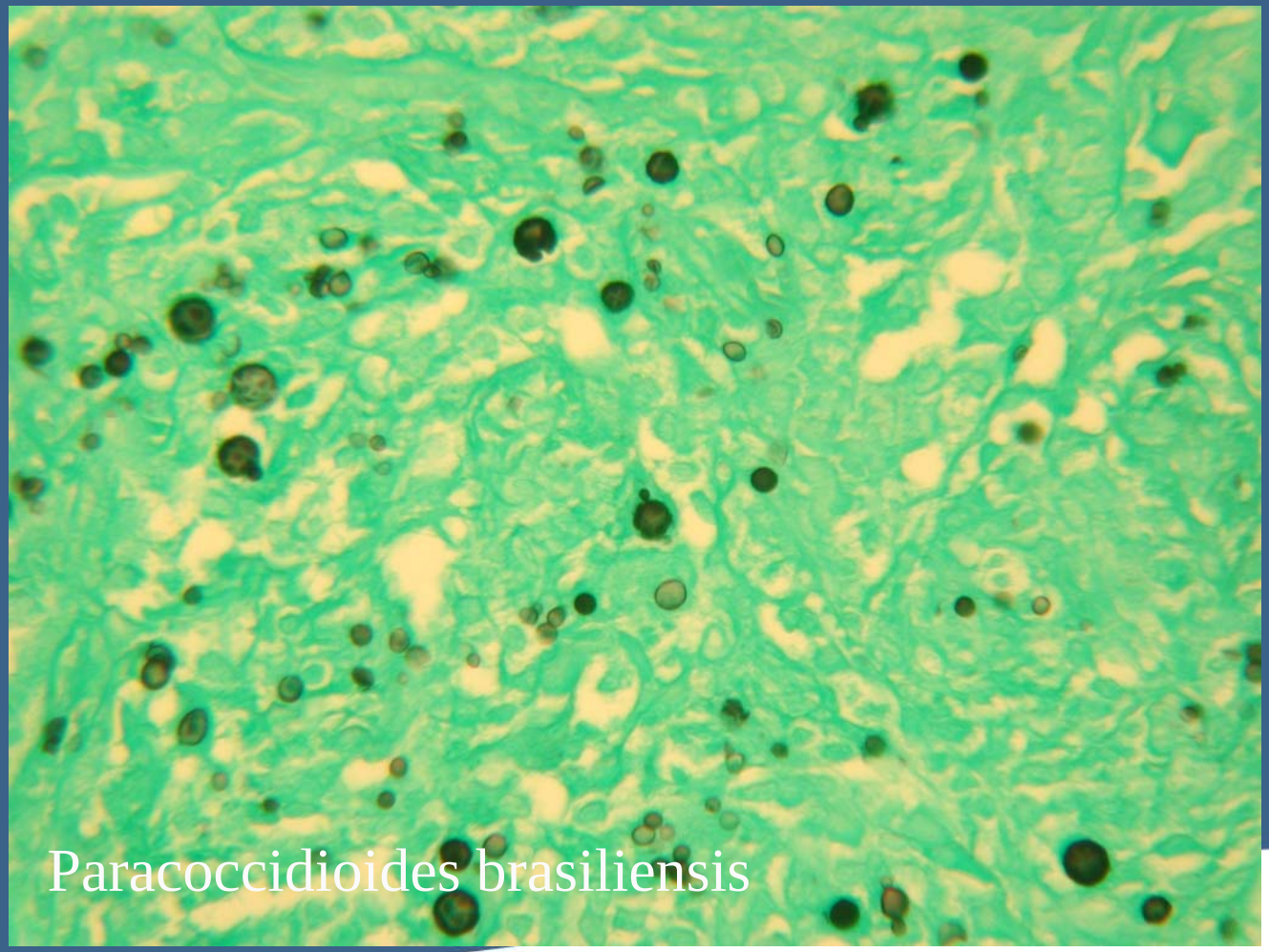
- Correct answer: 3
- South-American blastomycosis due to *Paracoccidioides brasiliensis* infection



*Paracoccidioides brasiliensis*



*Paracoccidioides brasiliensis*



Paracoccidioides brasiliensis

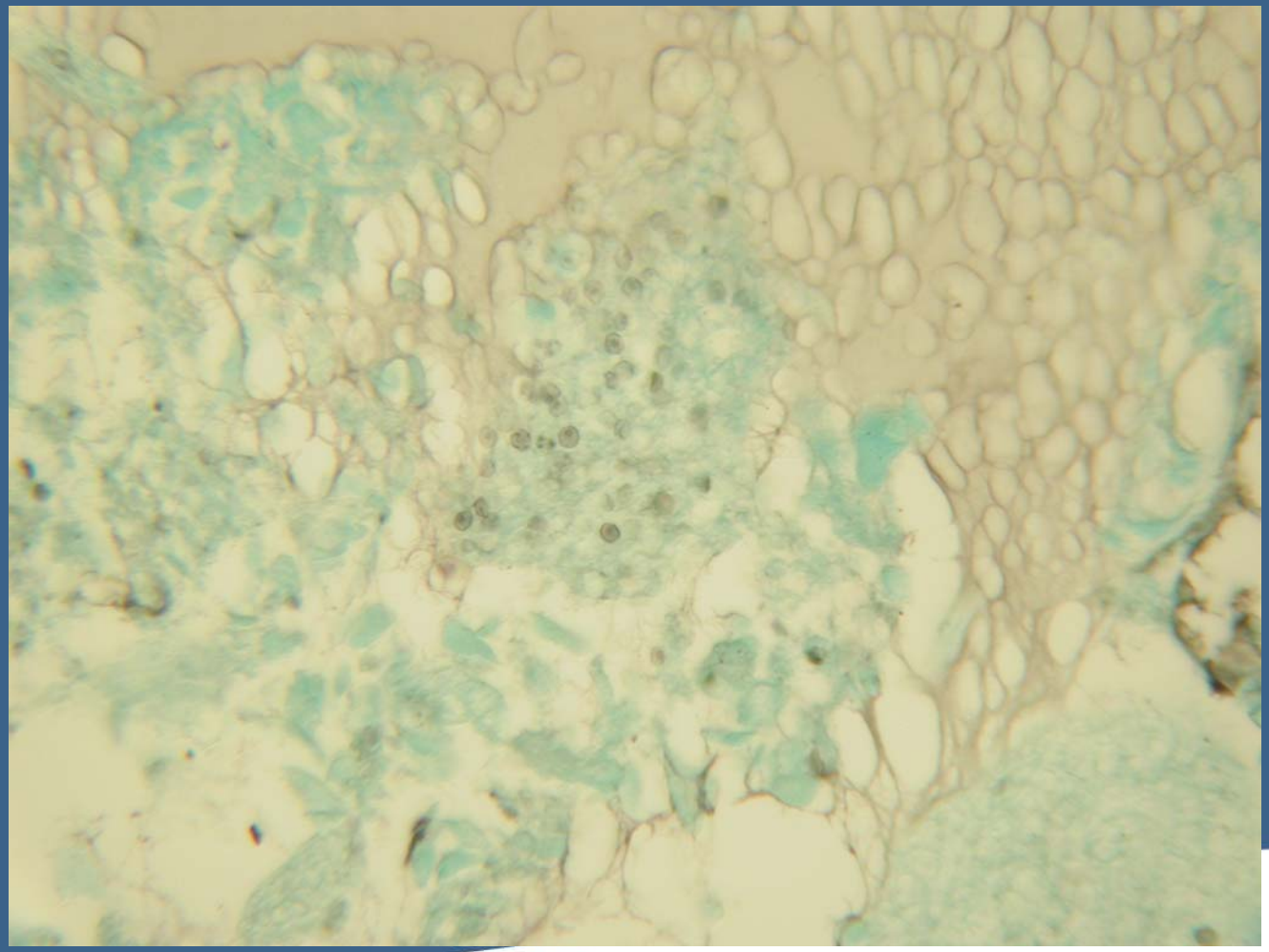
# Take-home lesson (2)

- when fungal elements are scanty or rare, try to find more e.g. by cutting deeper sections
- do not rely on these few elements to give a specific diagnosis

# Quiz

## Question 3

Universiteit Antwerpen



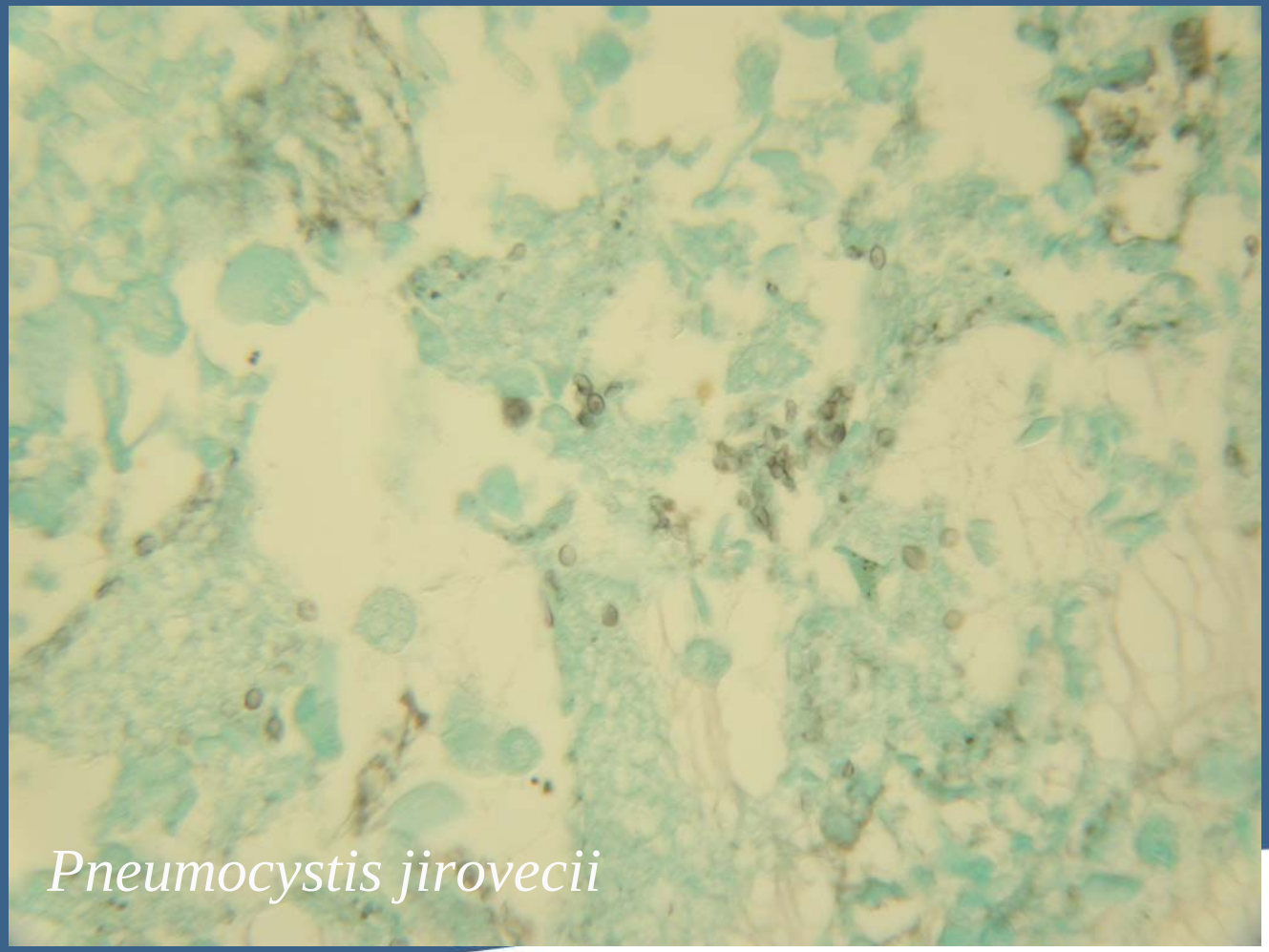
# One answer is correct

- 1) *Pneumocystis jirovecii*
- 2) *Blastomyces dermatitidis*
- 3) *Paracoccidioides brasiliensis*
- *4) Cryptococcus neoformans*
- 5) *Coccidioides immitis*

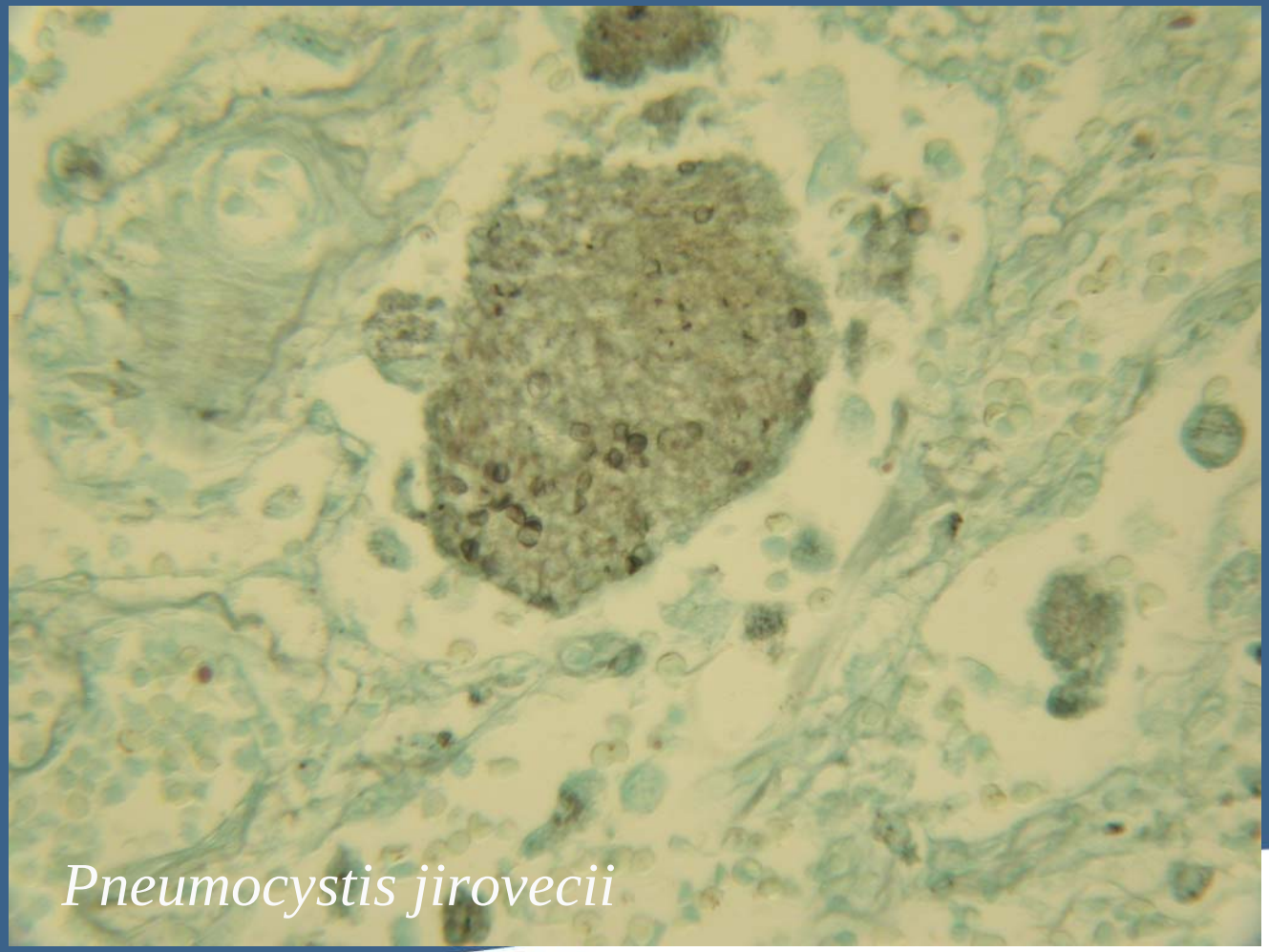
# Answer to question 3

- Correct answer: 1

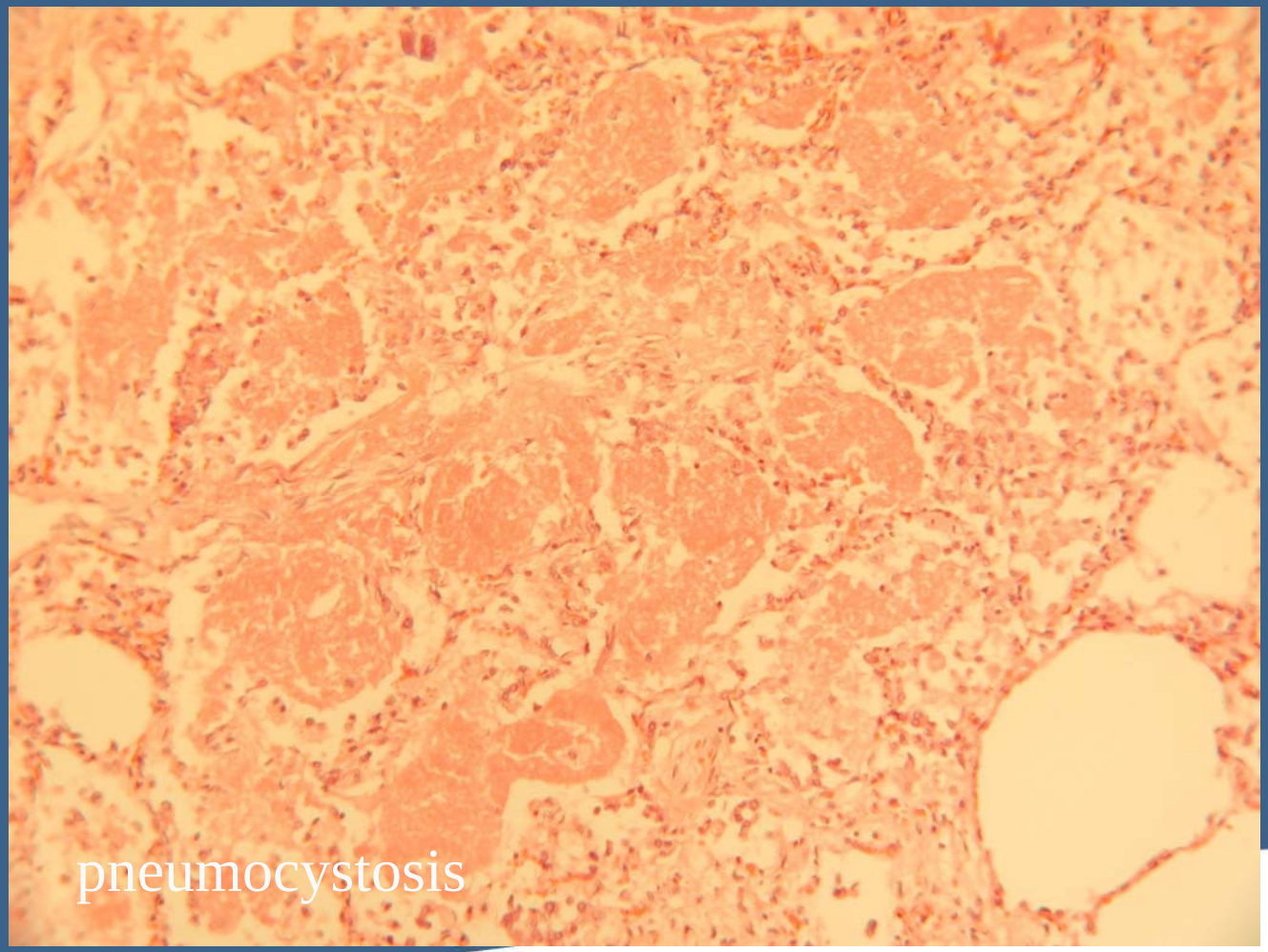
- *Pneumocystis jirovecii* infection



*Pneumocystis jirovecii*



*Pneumocystis jirovecii*

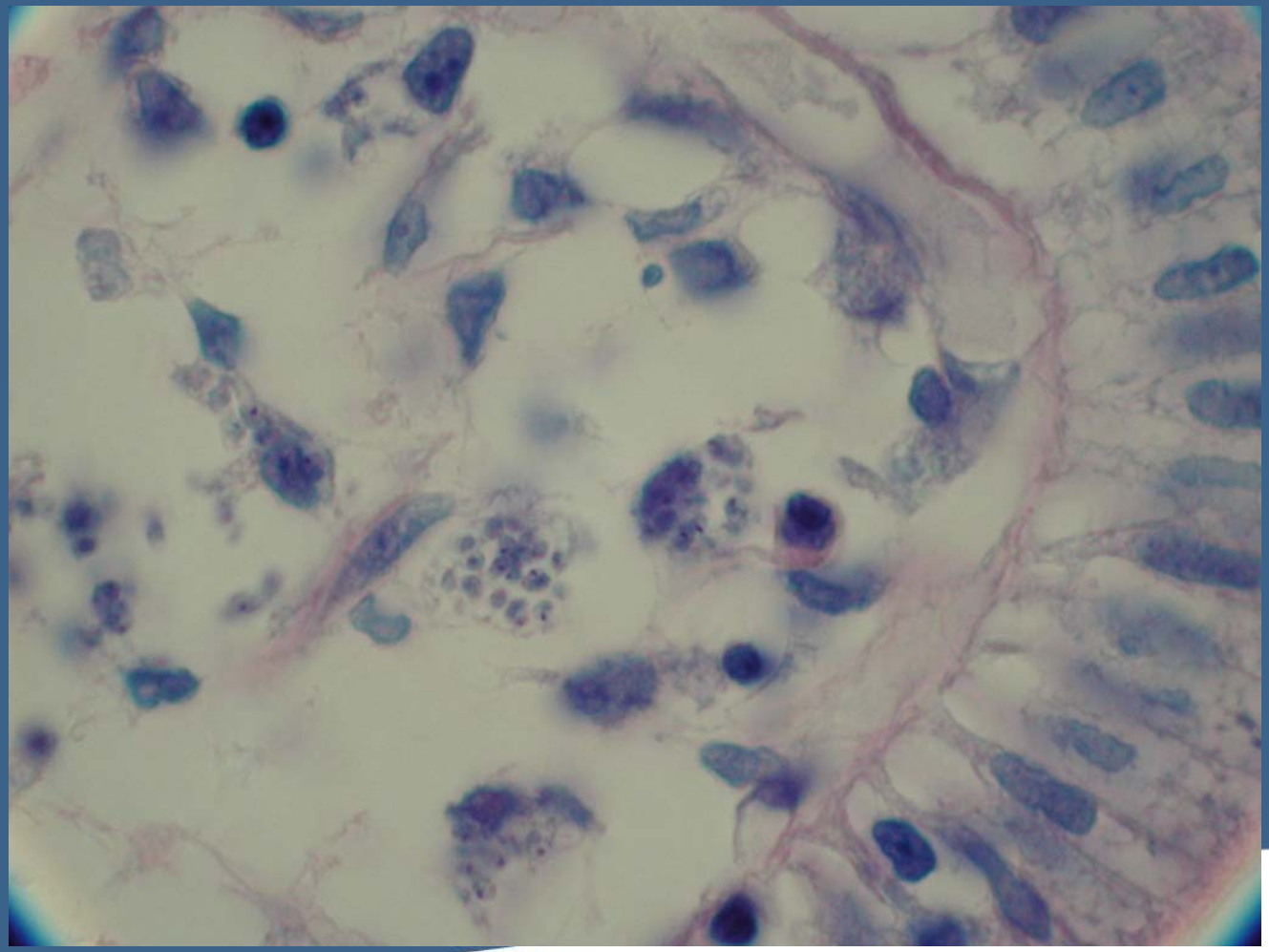

pneumocystosis

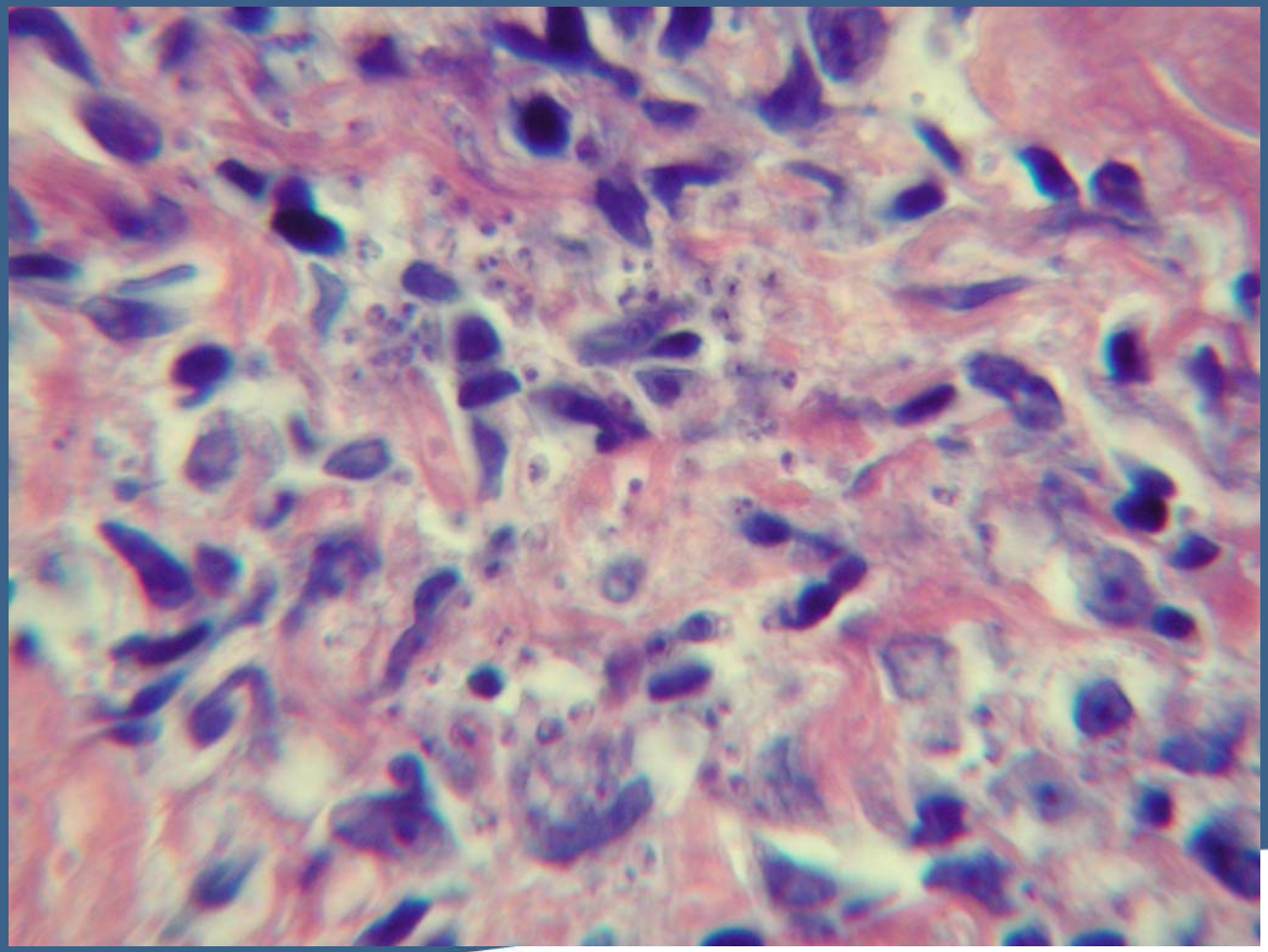
# What can the pathologist contribute to the diagnosis of parasitic diseases?

# Brevia of parasitology…

# Parasites

- ectoparasites
  - residents (e.g. lice)
  - temporary (e.g. mosquitoes)
  - partly residents (e.g. fleas, ticks)
- endoparasites
  - derived from ectoparasites (e.g. mites in scabies, fly larvae in myiasis)
  - derived from commensals (possibly in all organs)
    - special case: intracellular parasites (small parasites)





# Hosts

- one host: **monoxenic parasites**
  - obligatory monoxenic
  - facultative monoxenic
- more than one host: **heteroxenic parasites**
  - obligatory heteroxenic
  - facultative heteroxenic (di- or polyheteroxenic)

# Hosts (2)

- in endoparasites of separate sex and with different hosts: **definitive host** is the host harbouring the **sexually mature parasite**, the other host(s) being the **intermediate host(s)**
- **main host**: complete parasitic cycle
- **occasional host**: parasitic cycle incomplete (often man for animal parasites)

# Vectors

- ectoparasites transmitting the agent
- some vectors are definitive host (e.g. mosquitoes for malaria)
- other vectors are intermediate hosts (e.g. for larvae of filariae)

# Developmental cycle

- usually one **definitive host** (e.g. carnivore)
- one or more **intermediate hosts** (crabs, fishes)
- sometimes two definitive hosts: one **primary definitive host** (e.g. snake) and a **secondary definitive host** (e.g. rodent)
- in some parasites definitive host is also intermediate host (e.g. *Toxoplasma gondii*, *Trichinella spiralis*)

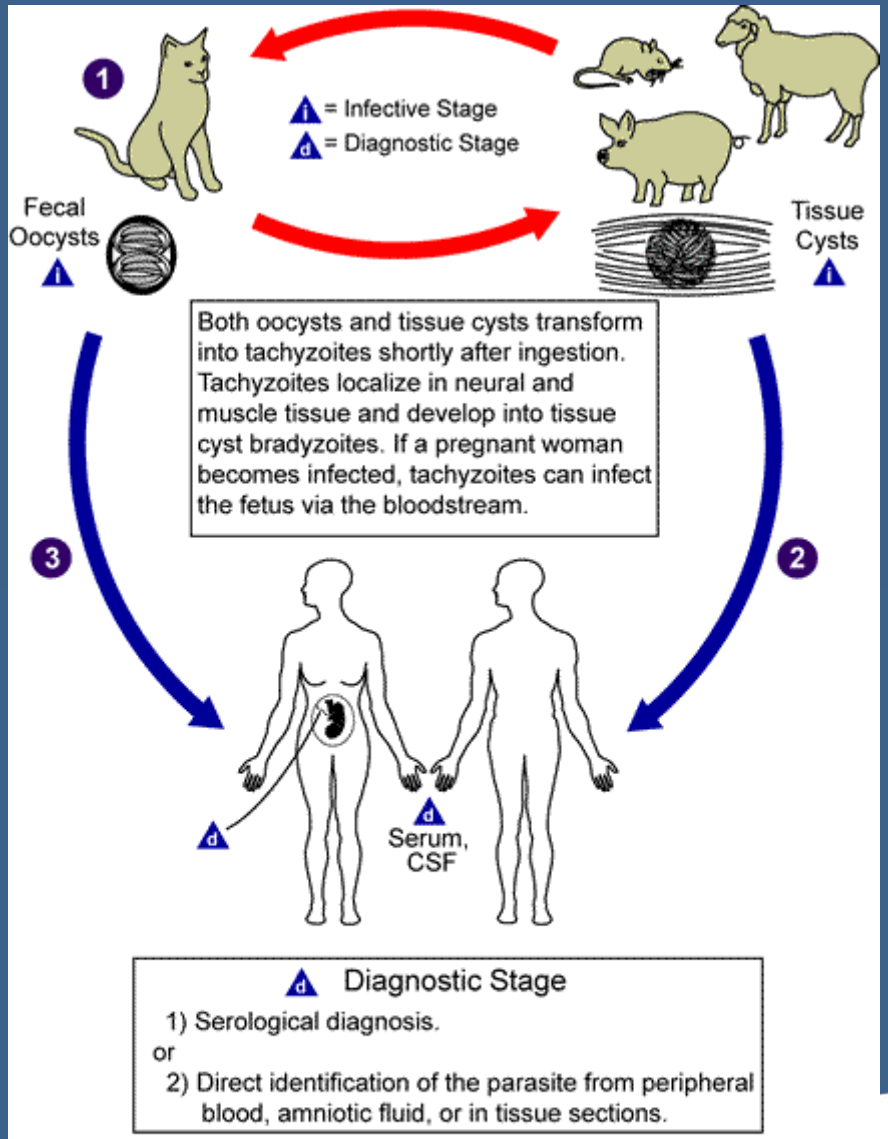
# Other host types

- reservoir
  - dogs and rodents for leishmaniae; man for malaria parasites
- transporter host (“paratenic host”)
  - only maturation, no multiplication
- accidental host
  - man in toxoplasmosis
  - man or animal in some tapeworms (sparganosis), roundworms (*Toxocara*) or flukes (schistosomes of birds)

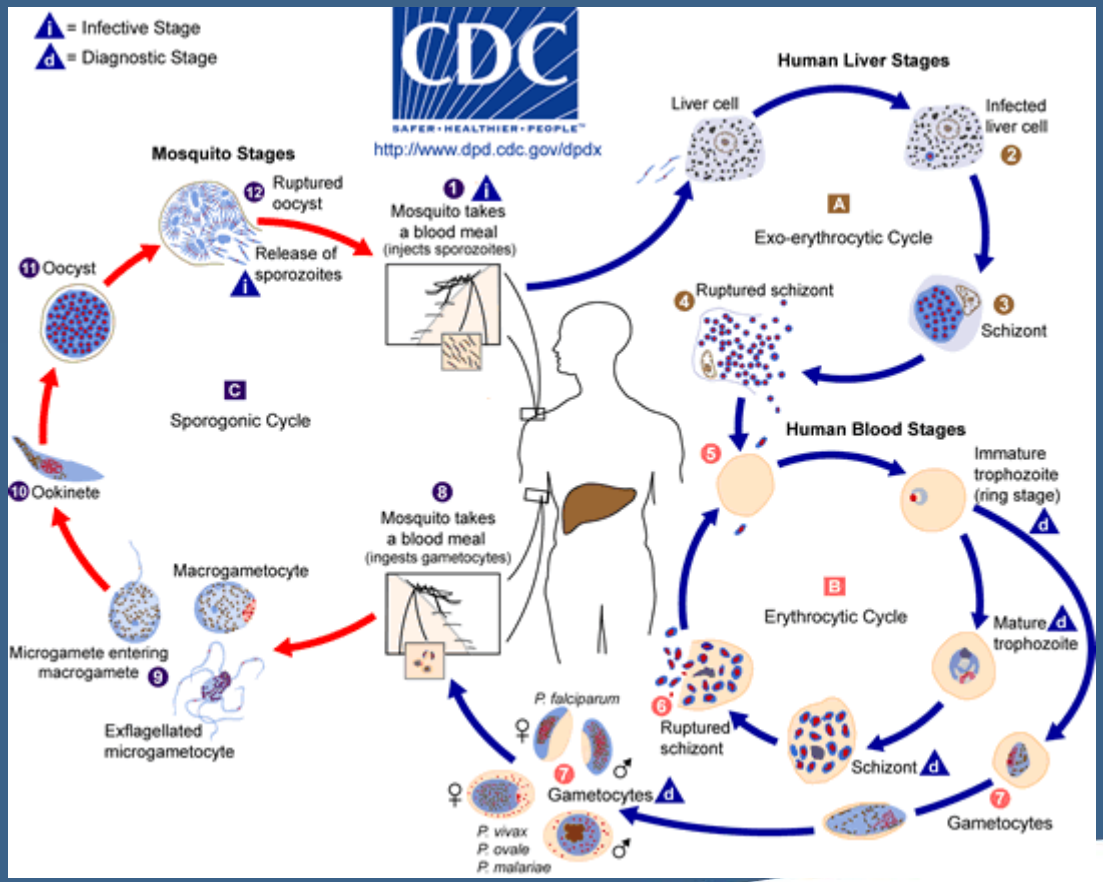


# Host specificity

- variations possible in both definitive and intermediate host
- specificity can be very **strict**: *Taenia solium* (man), *Eimeria*-species
- specificity can be very **loose**: many trematodes, *Cryptosporidium*, blood-sucking ectoparasites
- sometimes many intermediate hosts, but only one definitive host: *Toxoplasma gondii* (definitive host cat, intermediate hosts many mammal species and birds)
- sometimes many definitive hosts and only one intermediate host (malaria: man intermediate host, *Anopheles*-mosquitoes definitive host)



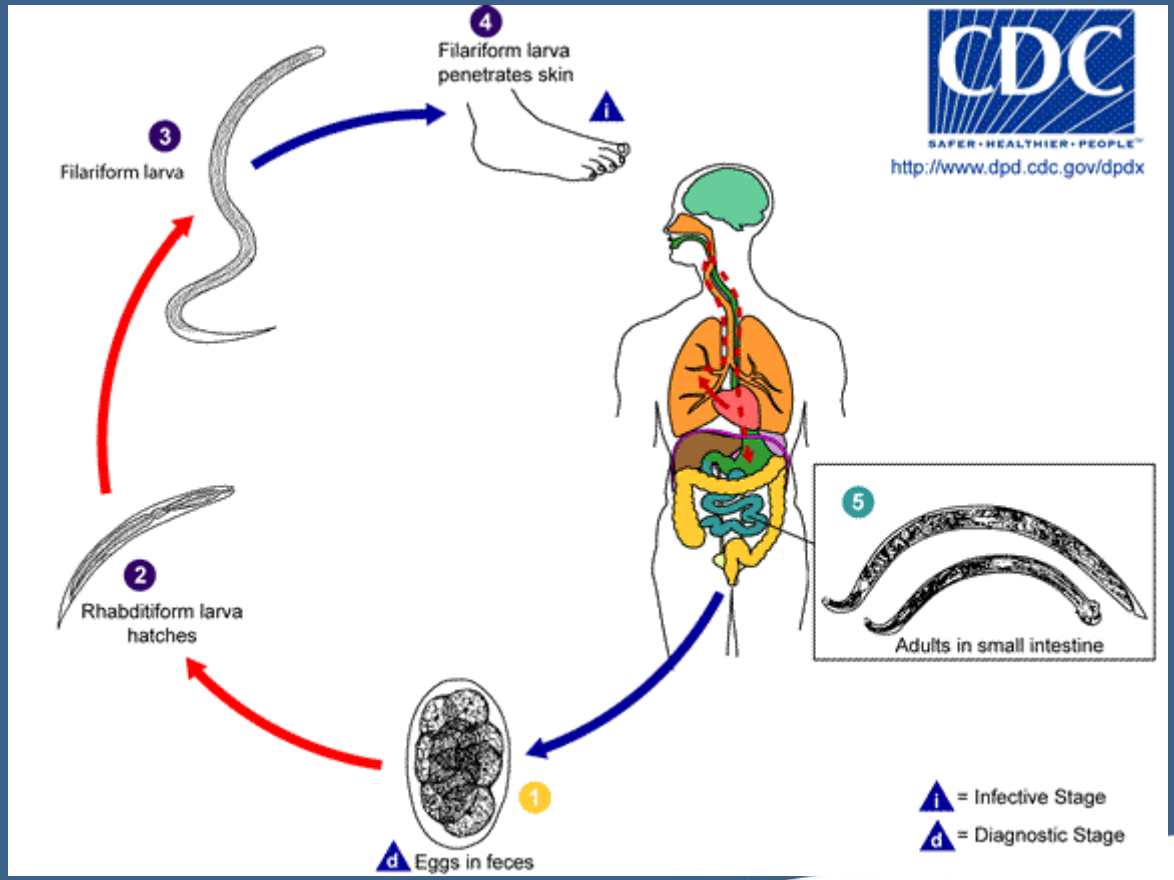

CDC
SAFER · HEALTHIER · PEOPLE™
http://www.dpd.cdc.gov/dpdx

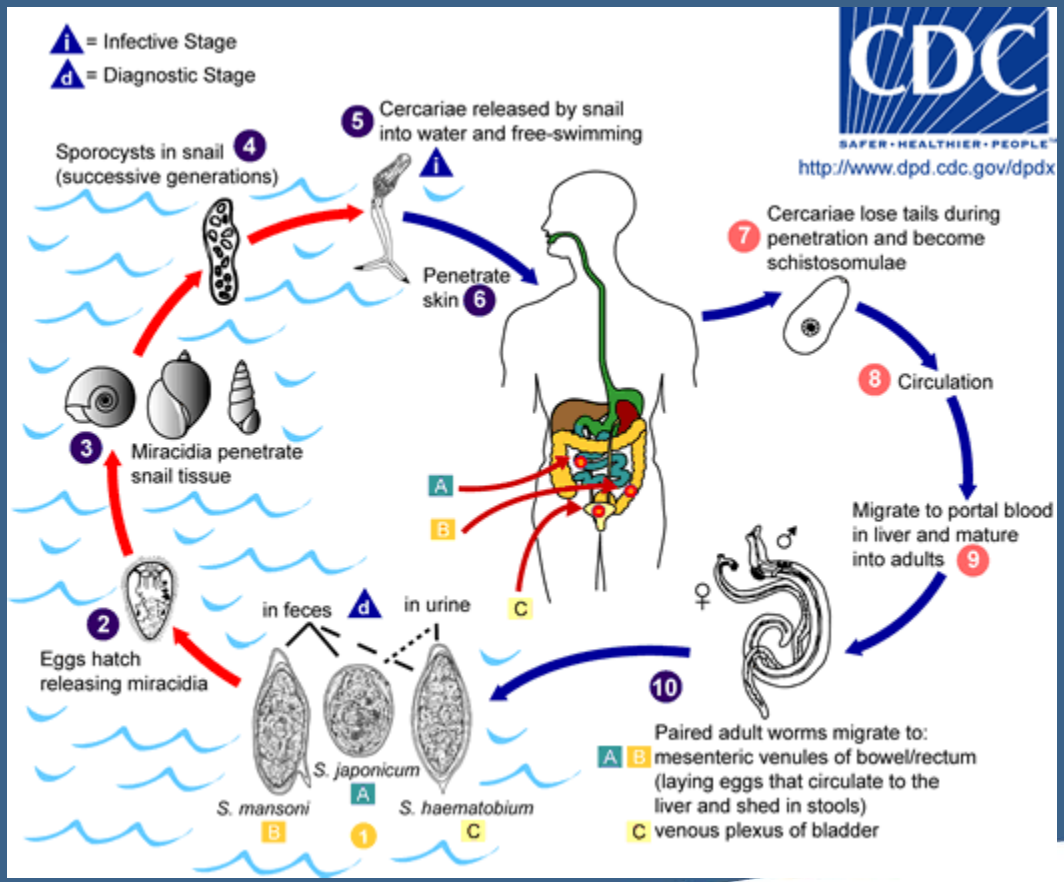



Universiteit Antwerpen

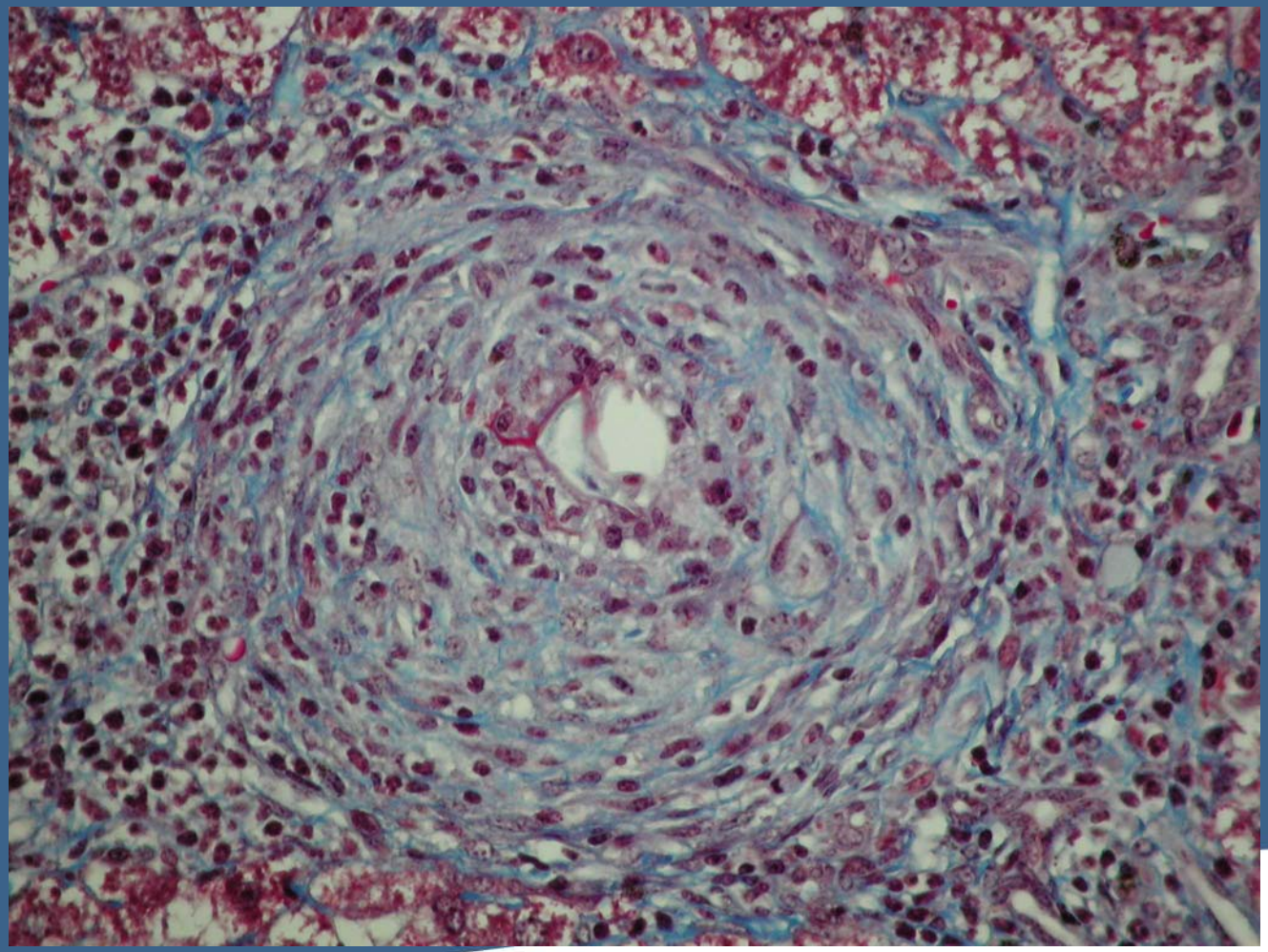
# Ontogenesis of parasites

- **direct development** (without multiplication) of larvae resembling the adult stage (metamorphosis of insects, nematodes)
- **indirect development** with a multiplication stage (coccidia, trematodes) with successive generations (obligatory in trematodes, in *Sarcocystis,* facultative in *Strongyloides*)


4
Filariform larva
penetrates skin
i
3
Filariform larva
CDC
SAFER · HEALTHIER · PEOPLE™
http://www.dpd.cdc.gov/dpdx
5
Adults in small intestine
2
Rhabditiform larva
hatches
1
d Eggs in feces
i = Infective Stage
d = Diagnostic Stage

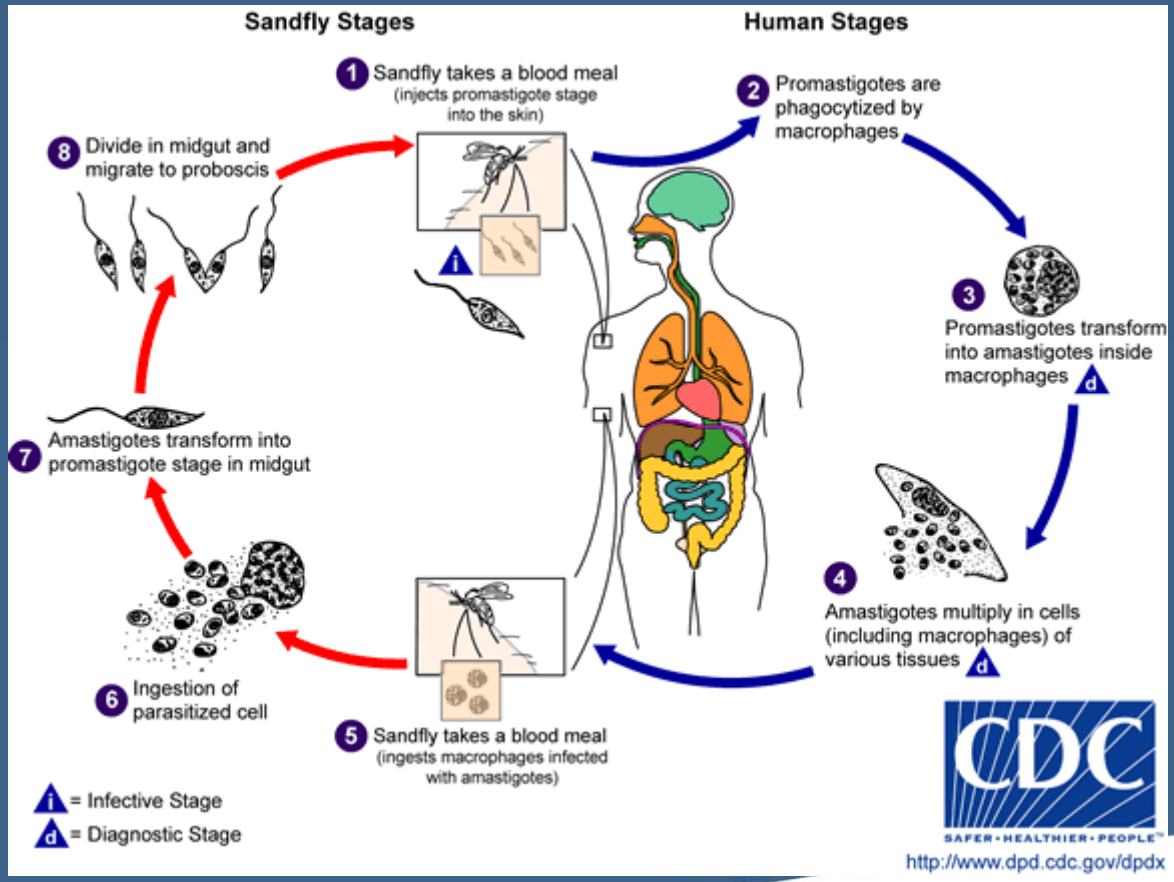



Voorbeeld van indirecte ontwikkeling: schistosomencyclus

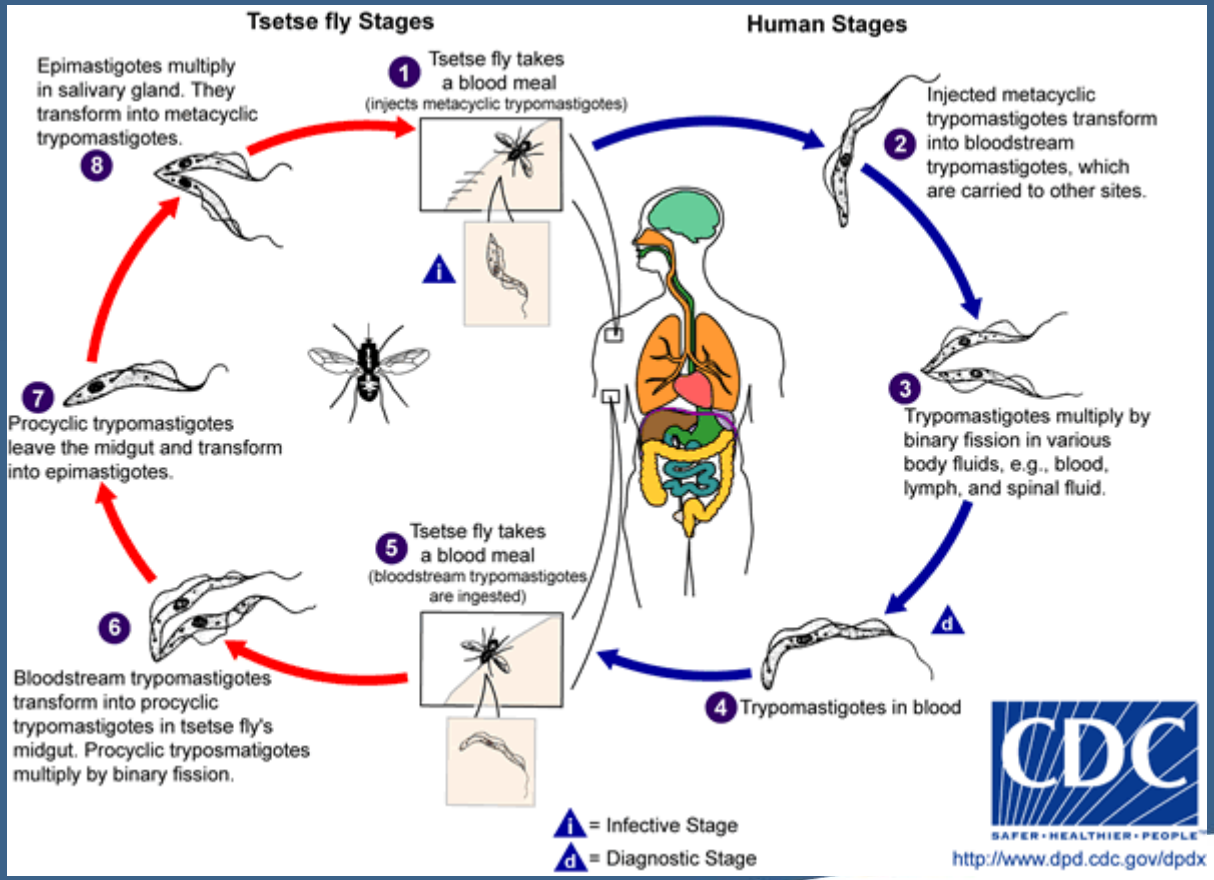


# Generation switch

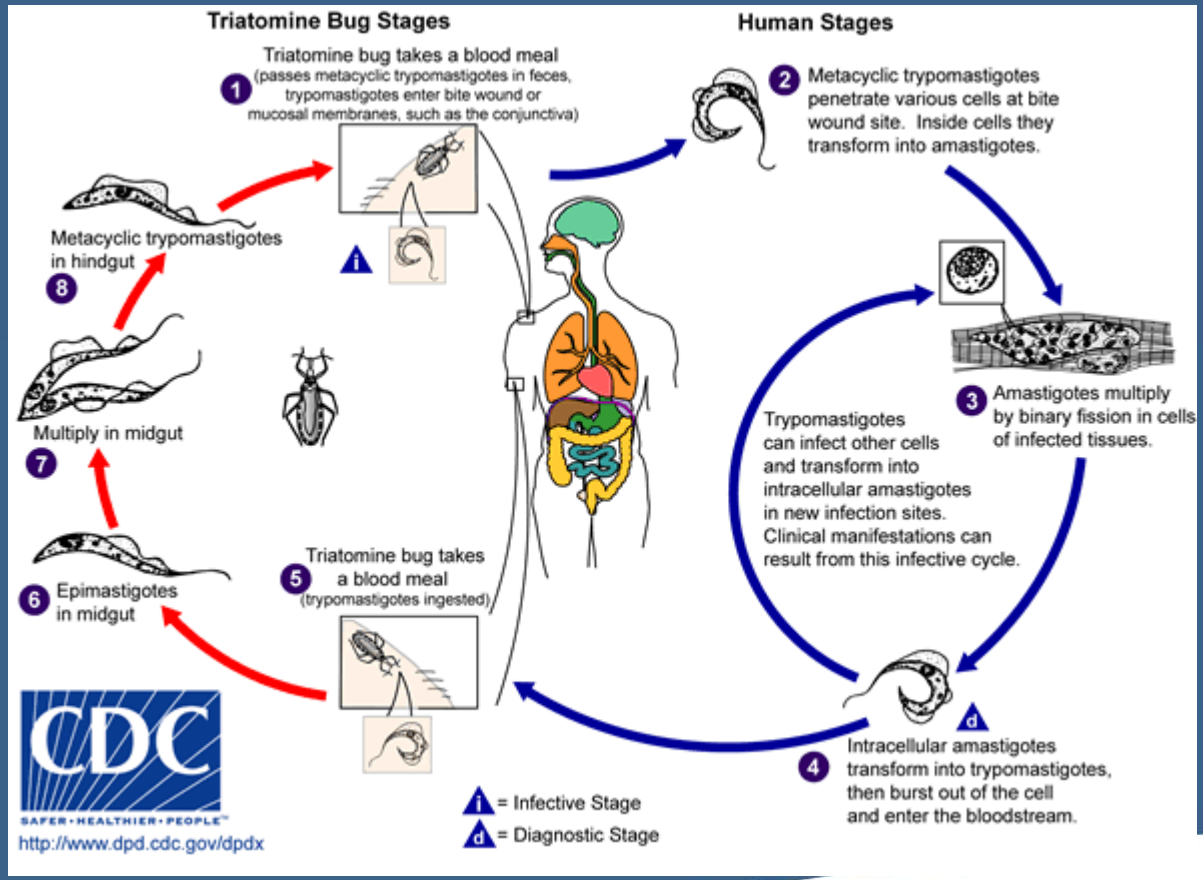
- **primary generation switch**: multiplication through cell division in protozoa
- **secondary generation switch**:
  - switch between sexual and asexual generations in protozoa like coccidia
  - in metazoa: **metagenesis in** *Echinococcus;* **heterogony in** *Strongyloides stercoralis*




Primaire generatiewisseling: cyclus van leishmaniae

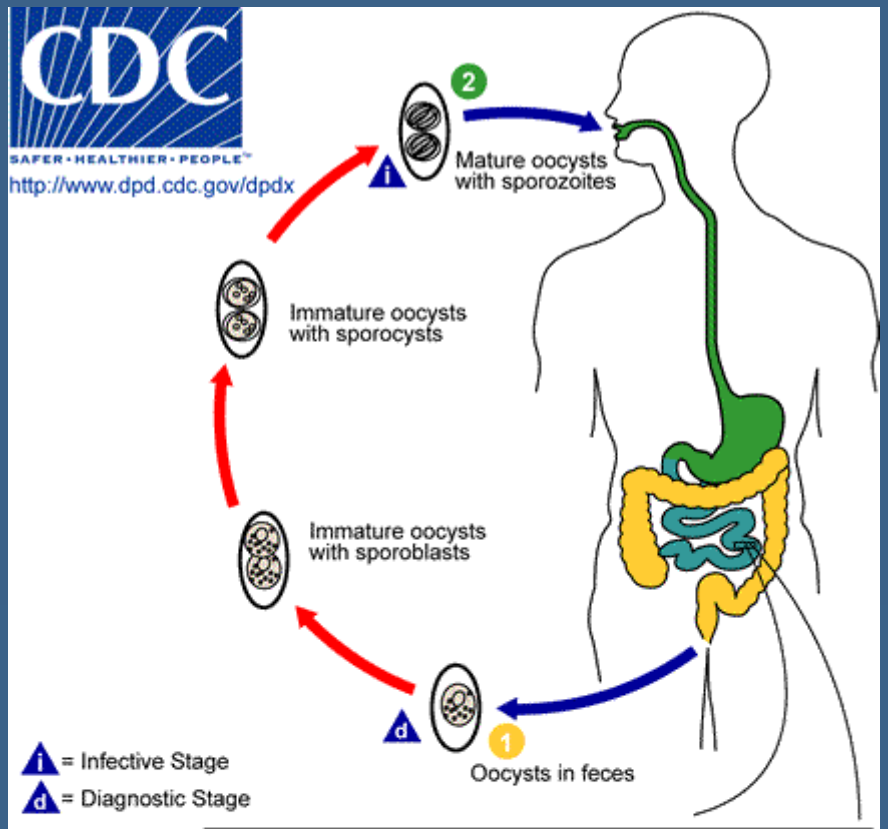


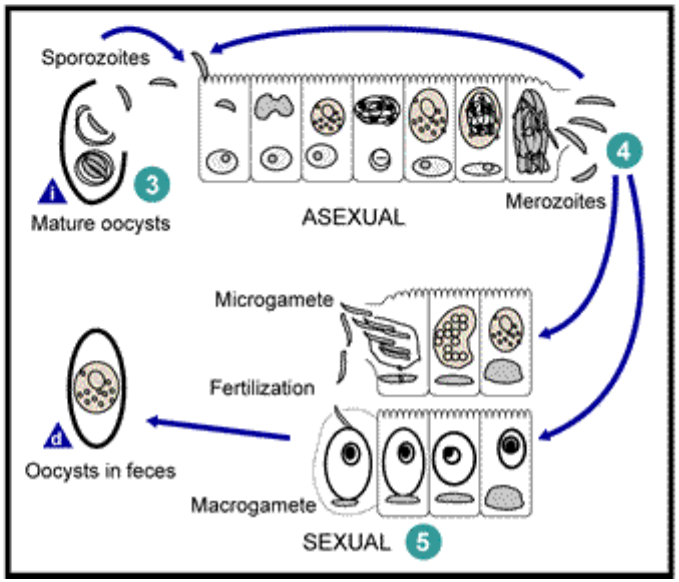

Primaire generatiewisseling: cyclus van Afrikaanse trypanosomen

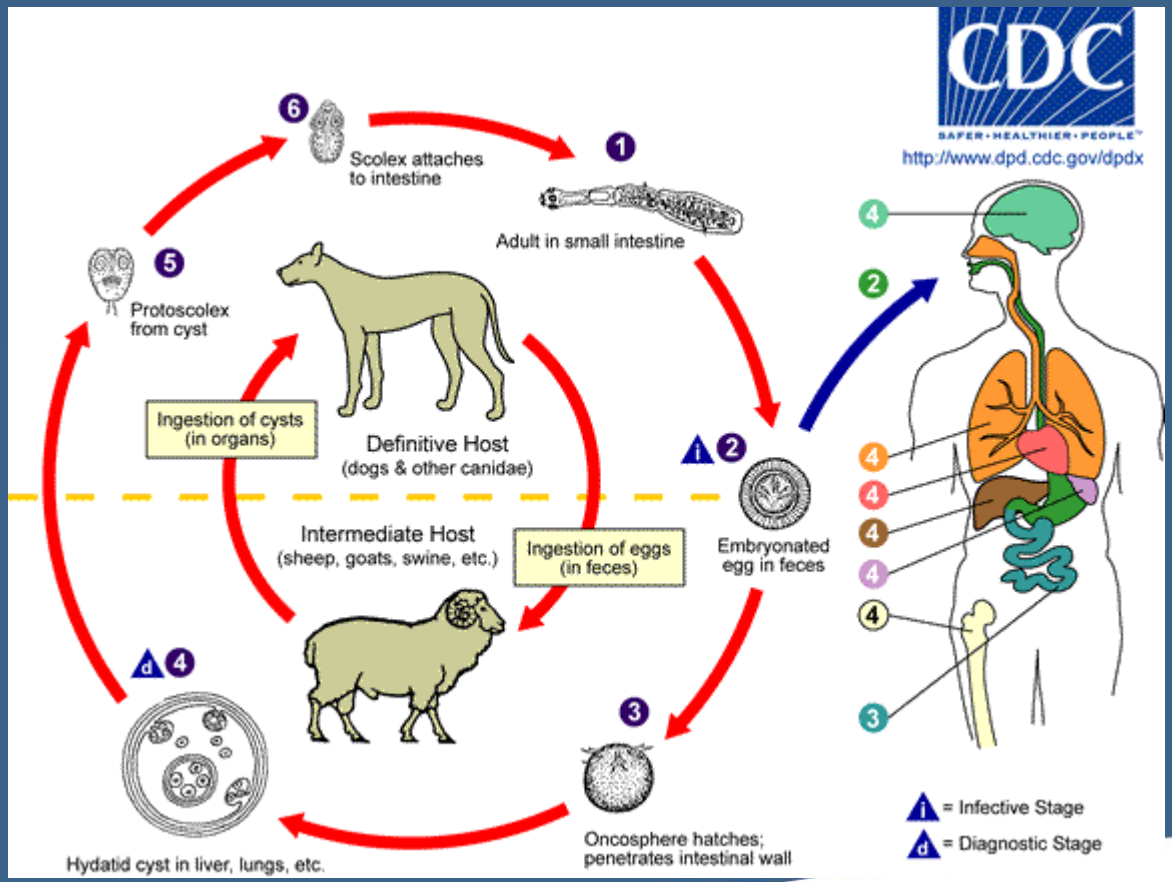



Primaire vermenwisseling: cyclus van Amerikaanse trypanosomen

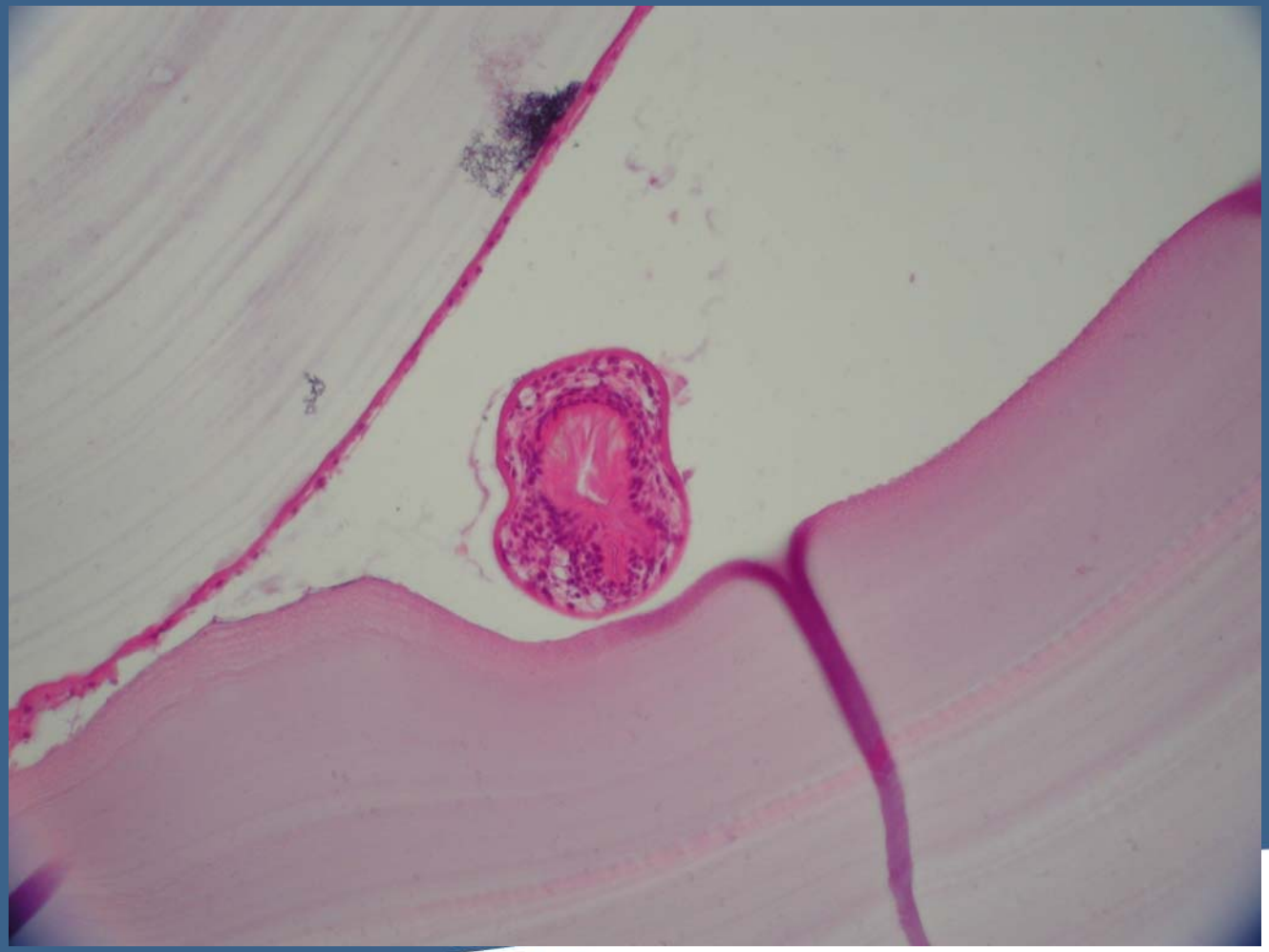
# Secondary generation switch in *Isospora*



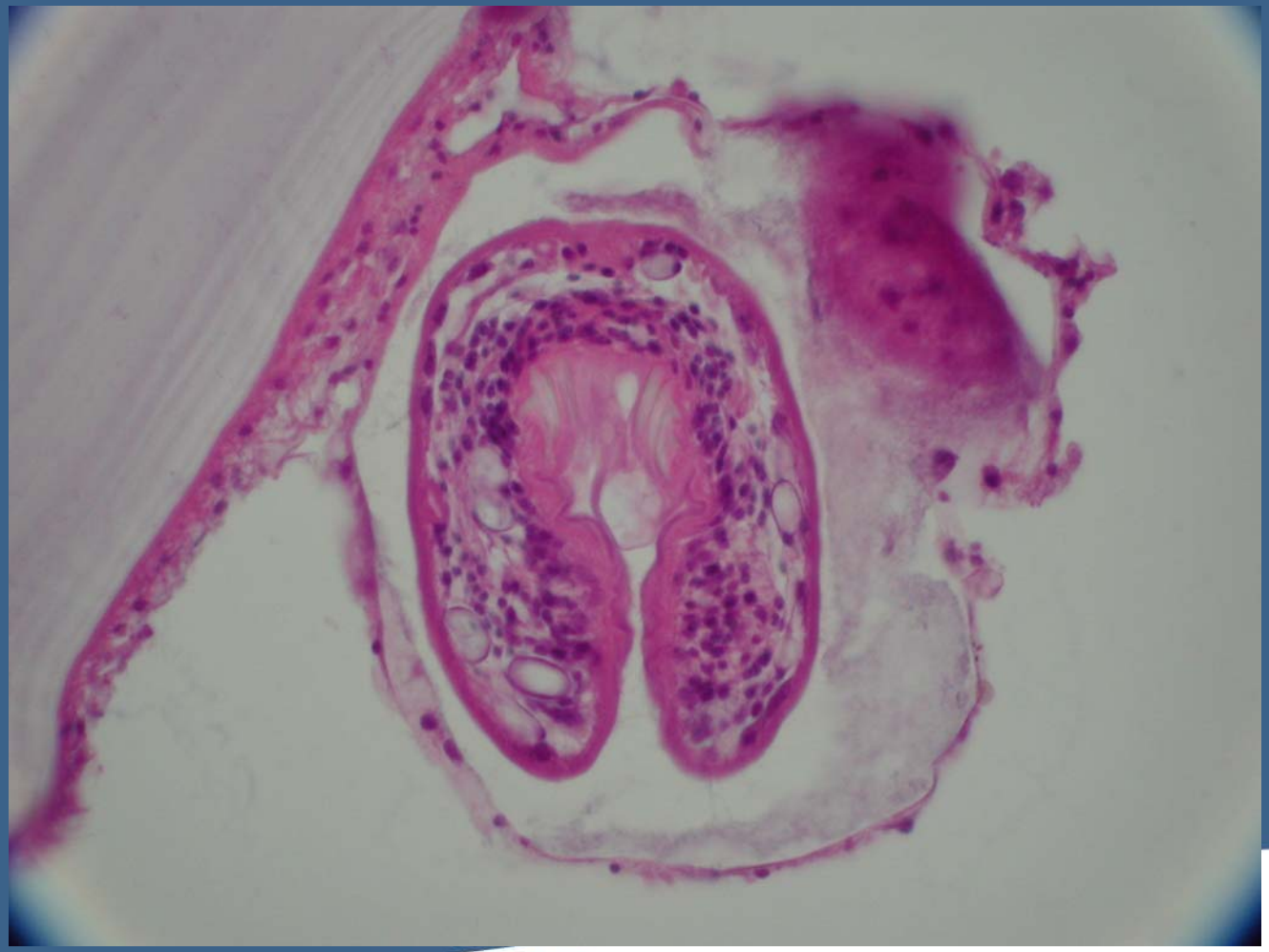





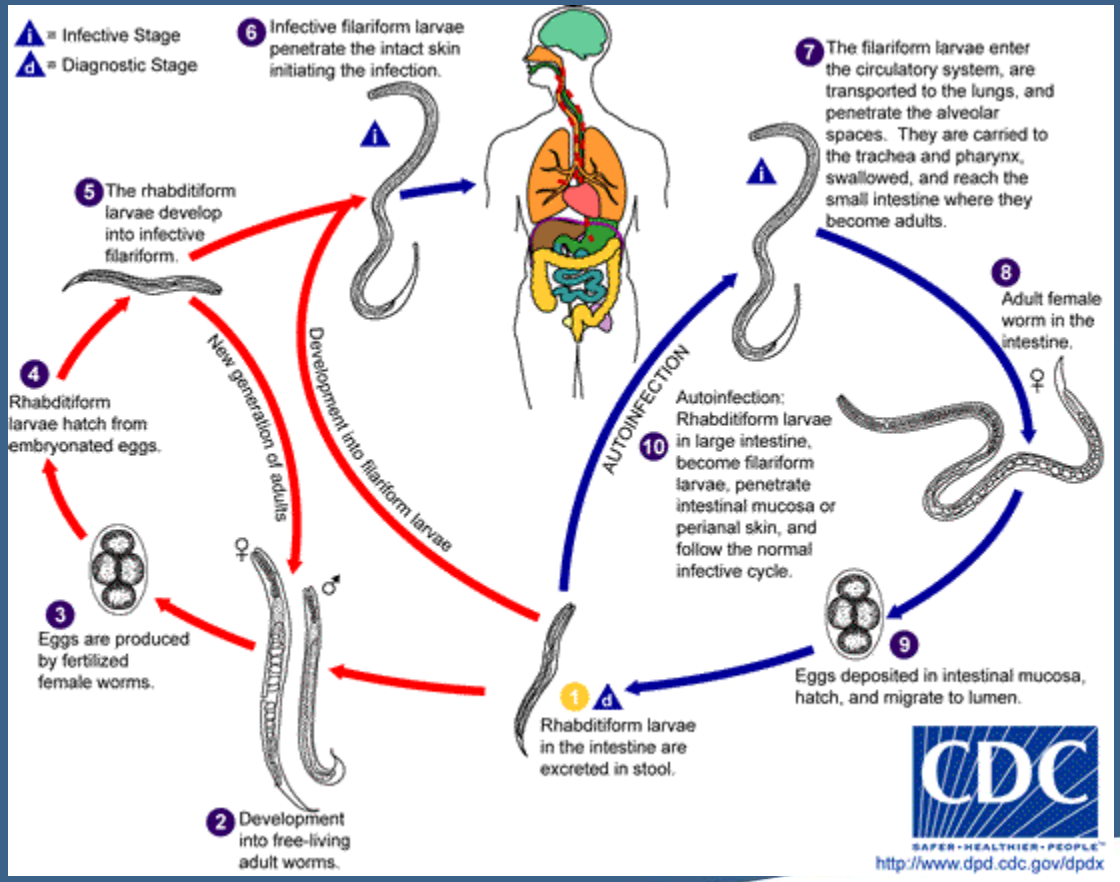

Metacestoses: cyclus van *Echinococcus granulosus*



Laminaire membraan en scolex van *E. granulosus*



Laminaire membraan en scolex van *E. granulosus*




Heterogonie: cyclus van *Strongyloides stercoralis*

# Adaptations

- invasion mechanisms
- attachment and food intake
- resistance to host defence mechanisms

# Invasion mechanisms

- passive ingestion of eggs, cysts, tissue cysts
- injection by ectoparasites during blood meal

# Attachment and food intake

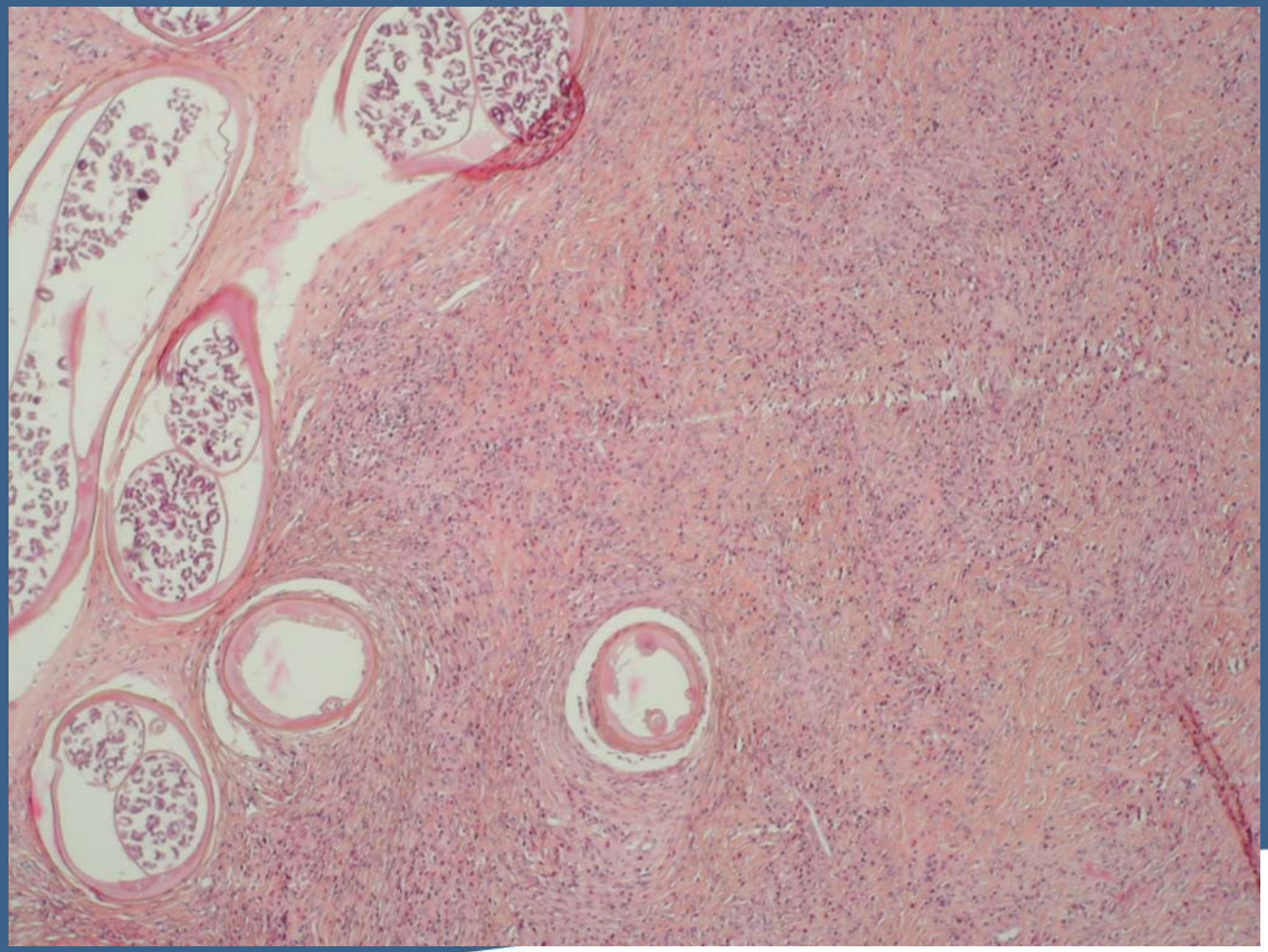
- by means of hooklets, thorns, suckers, folds of the cuticle
- food intake generally with intestinal organ but often through the cuticle (e.g. in tapeworms without intestinal tract)

# Resistance against defence mechanisms of the host ("immune evasion")

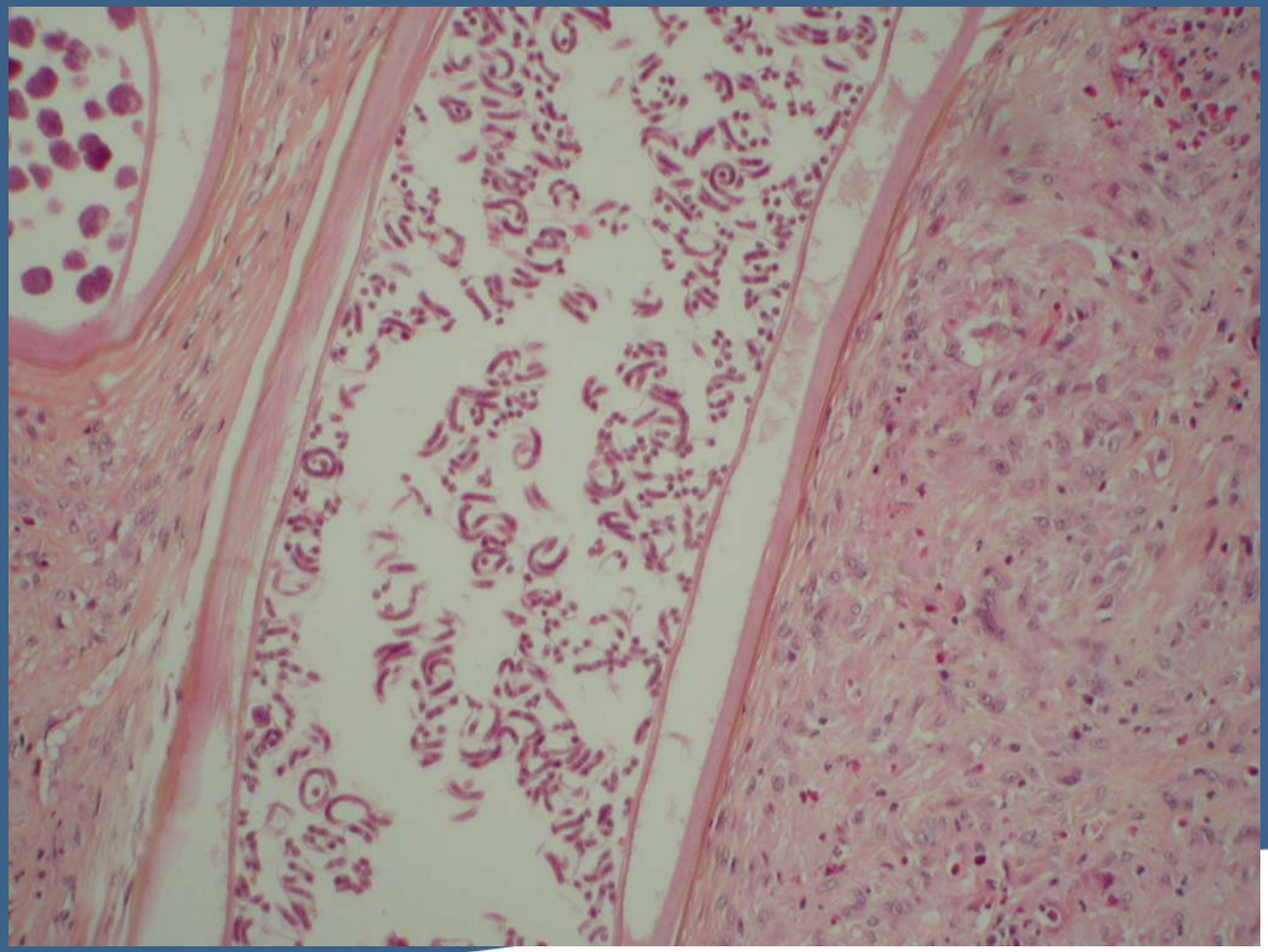
- endoparasites
- "surface coat" (mucopolysaccharides and antigenic variation in trypanosomes)
- lack of recognition by the host
  - molecular mimicry (schistosomes, *Fasciola*)
  - masking through antibodies (*Fasciola*)
  - sequestering in areas with little immune reactivity (e.g. brain)

# Resistance against defence mechanisms of the host

- cuticle of nematodes (barrier against host antibodies)
- some protozoa find shelter in an intracytoplasmic parasitophoric vacuole
- tissue cysts (*Sarcocystis*, *Trichinella*, *Onchocerca*)



Onchocerca nodule



# Pathogenicity

- mechanical disruption of cells and organs (*Plasmodium*, *Onchocerca*, *Ancylostoma)*
- growth inhibition  (liver flukes)
- food derivement (*Diphyllobotrium*, blood sucking nematodes)
- intoxication by metabolites (*Trypanosoma cruzi*, malaria parasites, ticks)
- secondary bacterial infections (*Entamoeba*, *Ascaris*-larvae in the lungs)
- transmission of other causative organisms (protozoa, worms, also bacteria and viruses)

# Virulence

- the evolutionary best adapted parasite from the parasite's viewpoint is the parasite that causes minimal damage to the host and is often able to live for 20 or more years with its host (tapeworms, some filariae)
- disease symptoms can be acute, or chronic, or a latent infection can ensue

# Zoönosis and anthroponosis

- zoönosis: infections in both man and vertebrates (toxoplasmosis, trichinosis)
- anthroponosis: infections confined to man and transmitted from one person to the other (e.g. infection with *Enterobius vermicularis*)

# Parasites of man

- protista/protozoa: unicellular organisms
- helminths: worms like Plathelminthes, Nemathelminthes, Acanthocephala, Annelida, Pentastomida varying between a few mm to 30 metesr long
- arthropodes: Chelicera, Insecta of Crustacea

# Diagnosis of parasitic diseases

- Demonstration of the parasite or of one of its stages (eggs, larvae)
  - blood film or thik-drop
  - bone marrow (mainly for *Leishmania*)
  - body flouids (dermal fluid, sputum, urine, bile)
  - faecal specimen (fresh for larvae, cysts and eggs)
  - histopathologic specimen of affected organ (skin, liver, lymph nodes and bone)

# Outline of this chapter

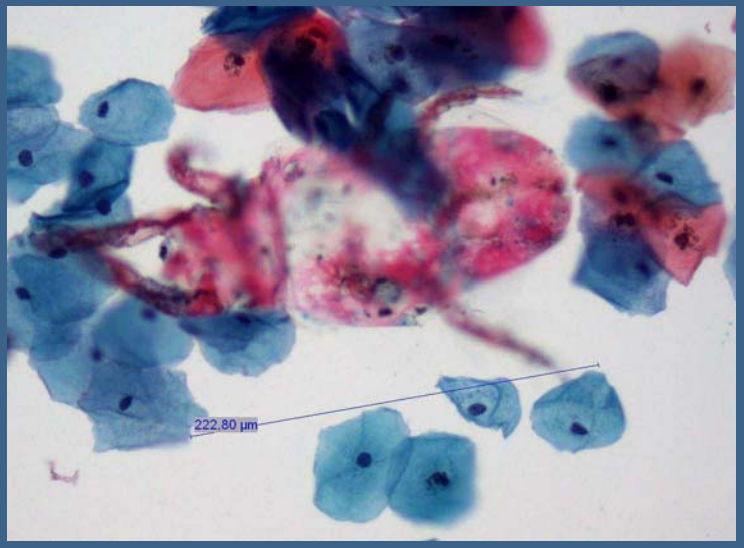
- What is pathology?
- Prerequisites for adequate pathological examination of specimens
- Methods in pathology
- Reaction patterns in parasitic infections
- Identification of parasites
- Limitations of pathological identification of parasites
- Prospects

# What is pathology?

- Investigates the morphological basis of disease
- Pathology is a biomedical science
- Pathology is a clinical discipline

# Pathology as a clinical discipline

- Materials studied are:
  - Cellular samples (fluids, needle aspiration, smears)
  - Surgical resection specimens
  - Bodies (post-mortem examination)


222,80 µm

- bodies: cool as soon as possible
- tissue: rapid fixation (formalin 10%), or else, keep cool (+4°C)
- fluids: keep cool (+4°C) and transport to laboratory, or add fixative (e.g. Saccomano)

# Prerequisites for adequate pathological examination of specimens

- identification of sample
- age of patient
- nature of the sample
- type of fixation

# Necessary clinical information

- history of immune depression (immune suppressive medication in transplant patients or patients with autoimmune diseases, use of cytotoxic drugs, congenital immune deficiency, HIV infection)
- occupational history (work and hobbies)
- travel history

# Tissue specimens

- embed in paraffin after adequate fixation
- prepare paraffin sections
- stain
  - H&E
  - special stains (Grocott, PAS, Giemsa, Ziehl-Neelsen, ...)

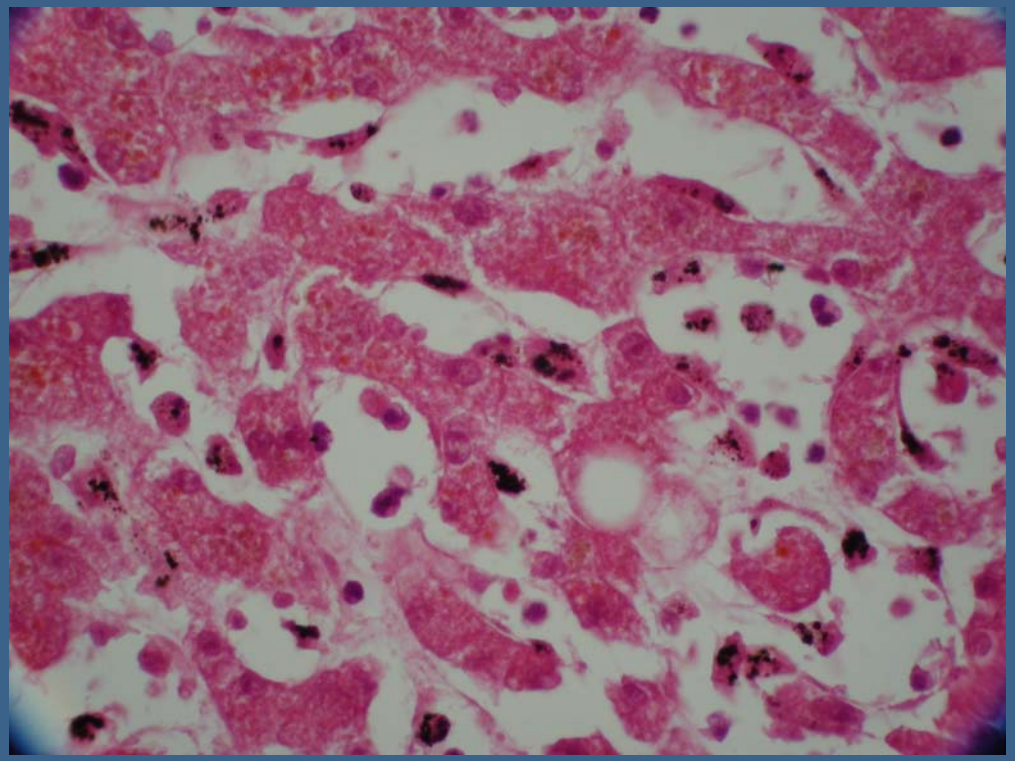
# Pathological diagnosis of parasitic diseases

- Is there a specific reaction pattern in relation to parasitic infections?

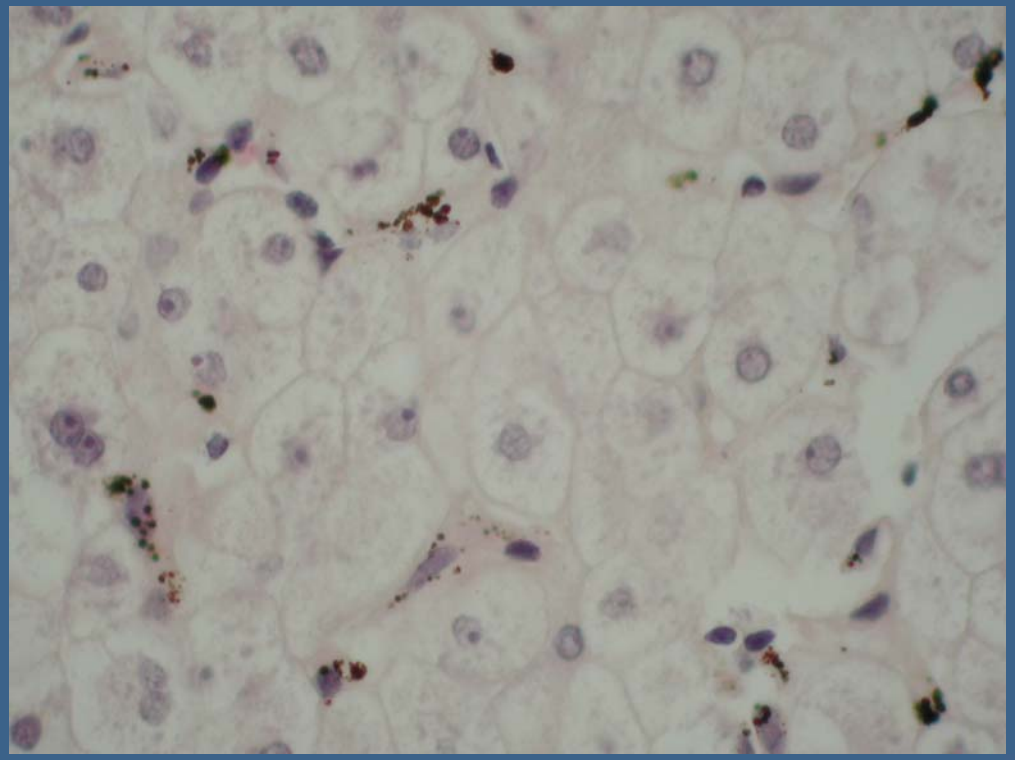
# Reaction patterns in parasitic infections

- Live parasites, especially metazoan parasites, often do not induce any inflammatory reaction
- Secretion products can cause a granulomatous reaction
- Dead (metazoan) parasites cause usually a vehement acute inflammatory reaction
- Some parasites elicit an almost specific reaction pattern
- Some parasites will manifest themselves by metabolic products

# Indirect evidence of a parasitic disease



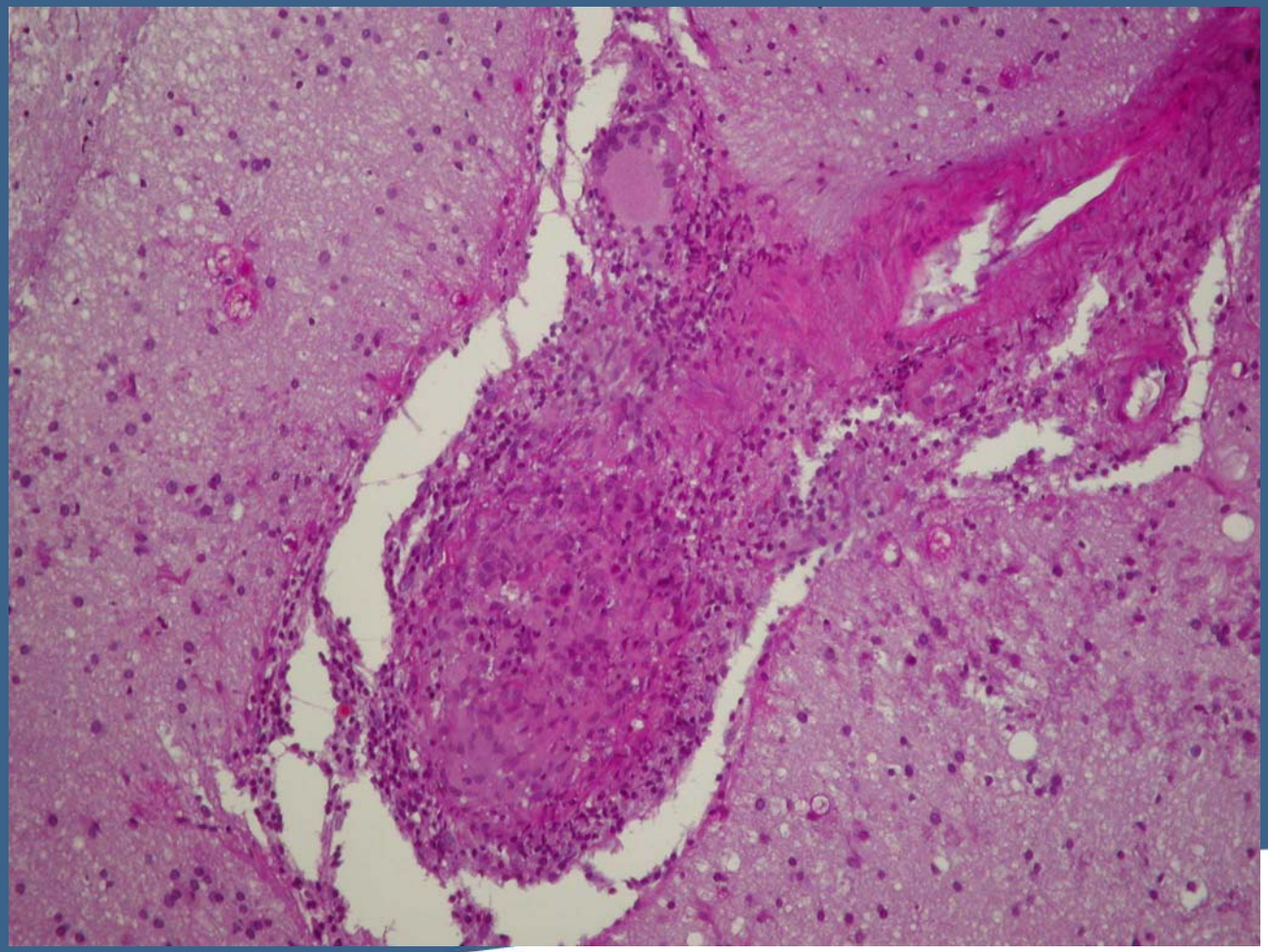
# Indirect evidence of a parasitic disease

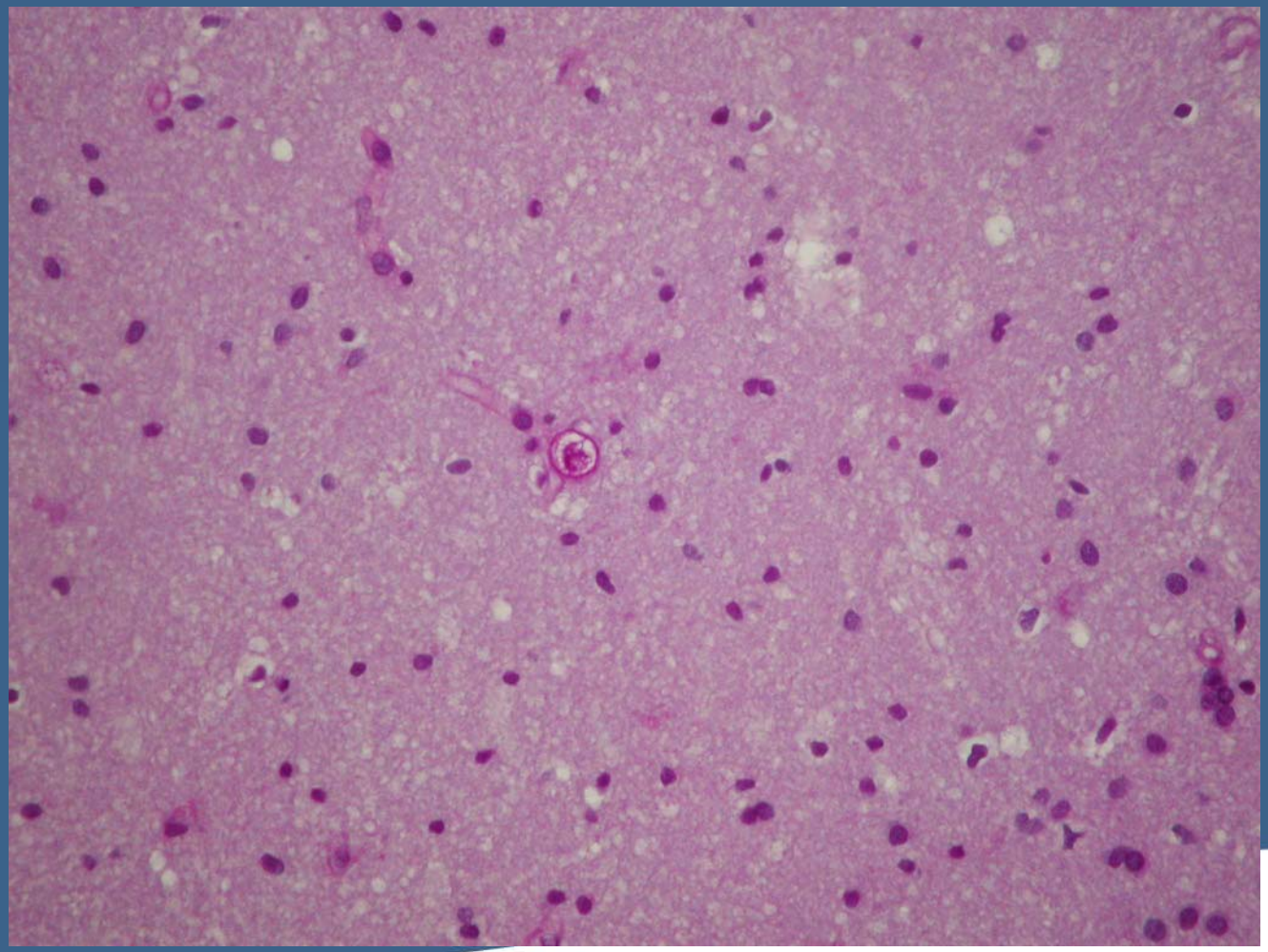


# Pathological diagnosis of parasitic diseases

- Diagnosis will rest on the identification of the parasite, although the inflammatory reaction in tissues will quite often lead to a more thorough search for (small) parasites

# Reaction patterns as a lead to a specific diagnosis



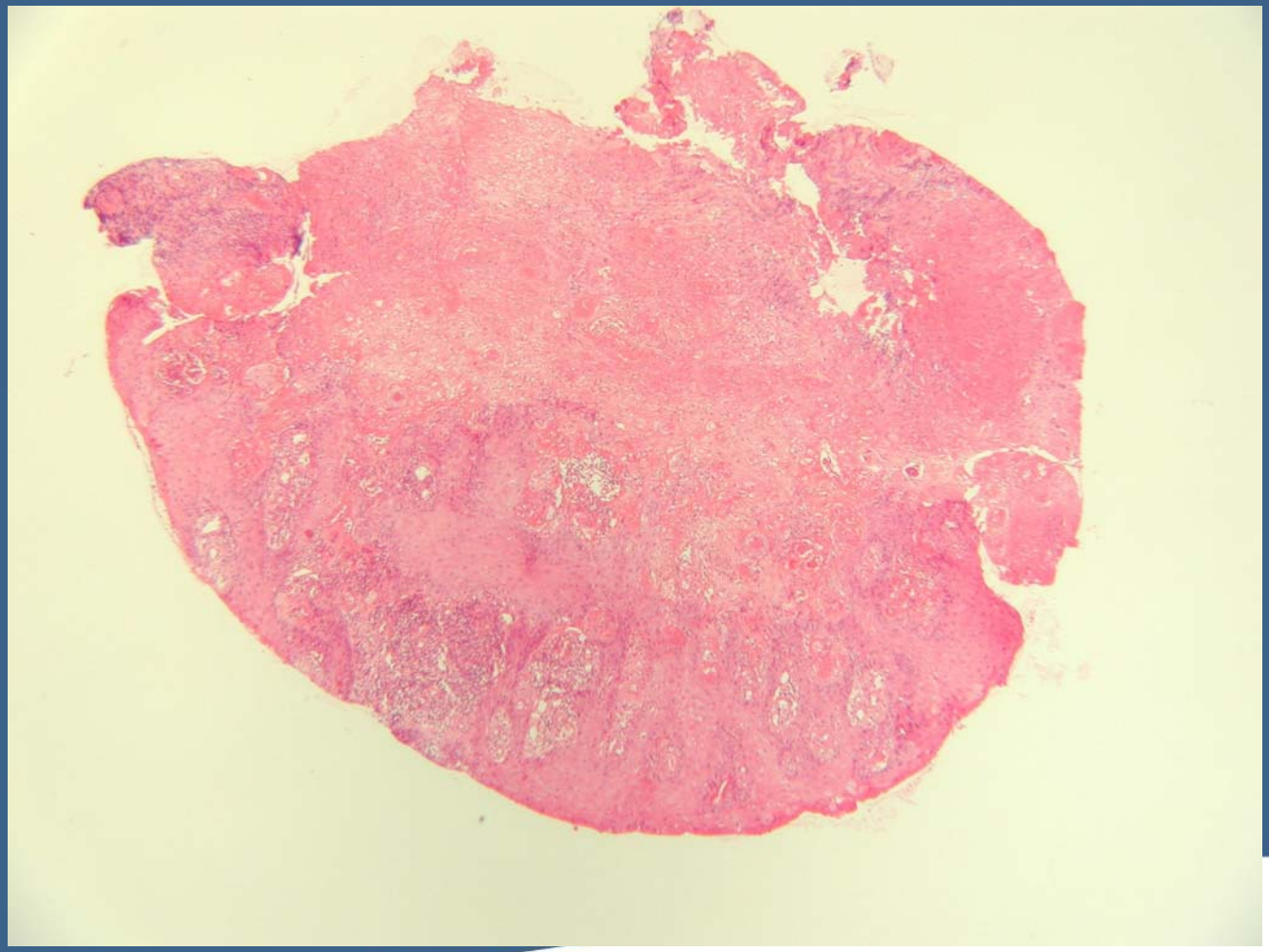


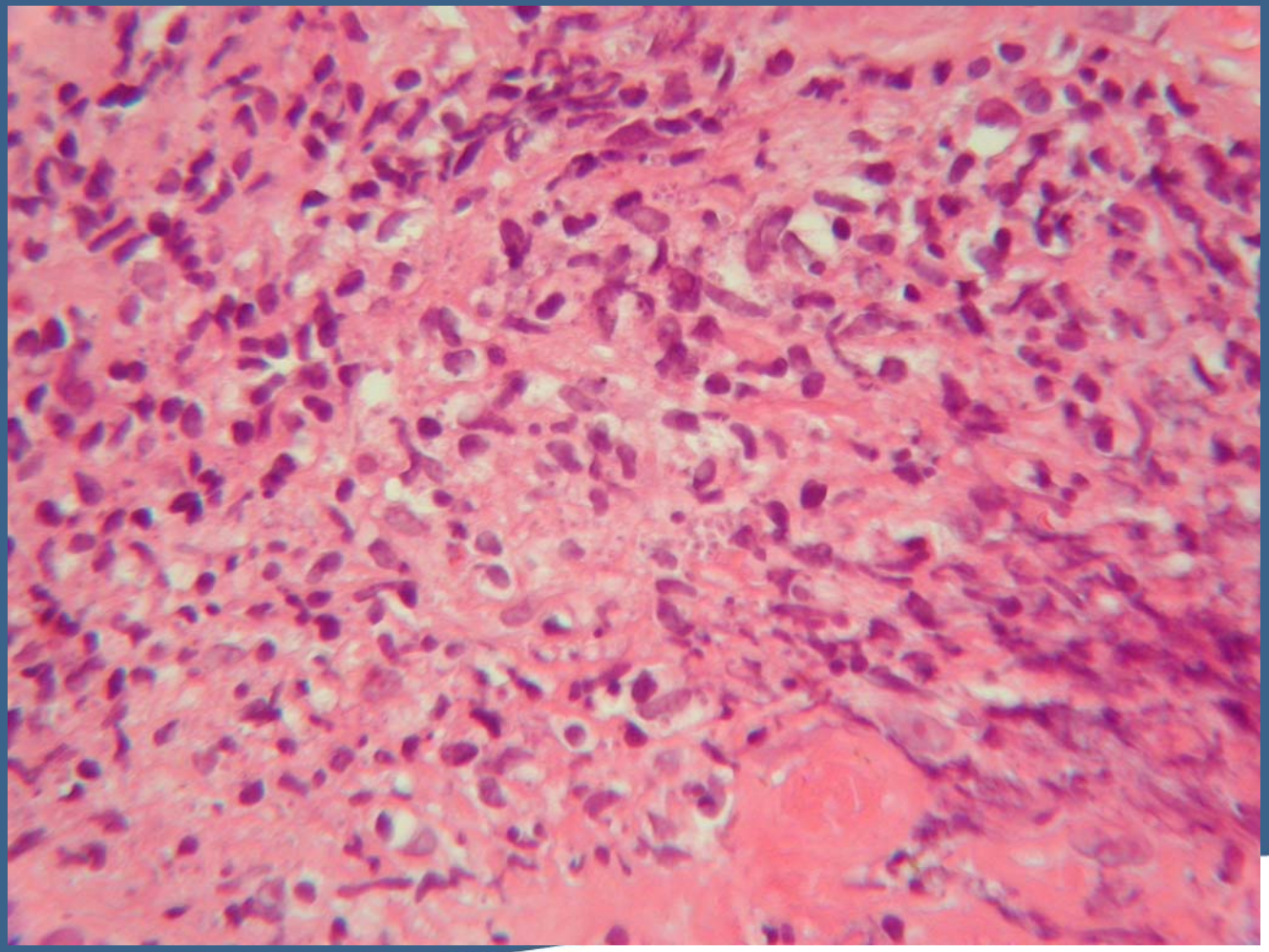
# Reaction patterns as a lead to a specific diagnosis

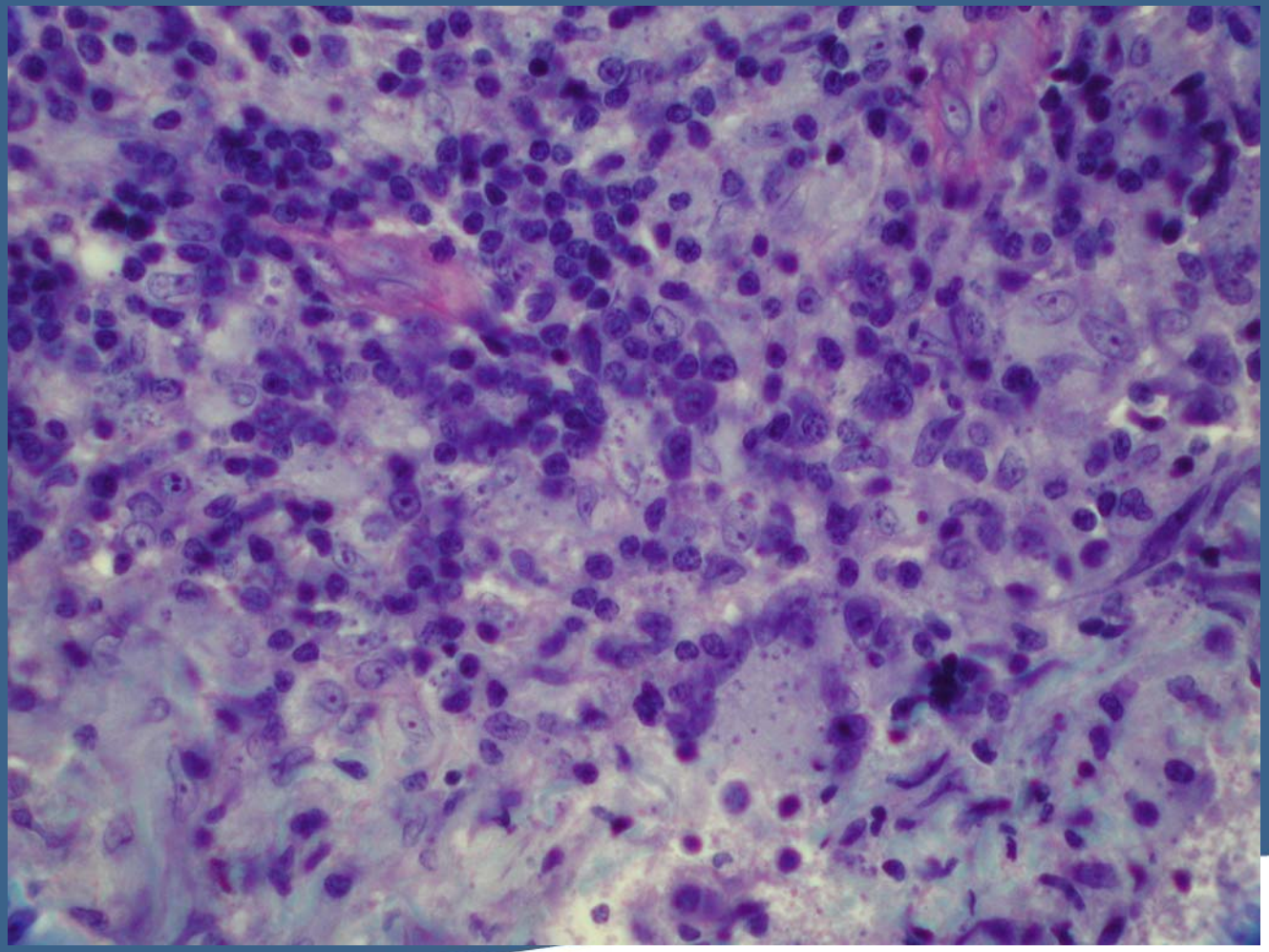
- The case of leishmaniasis

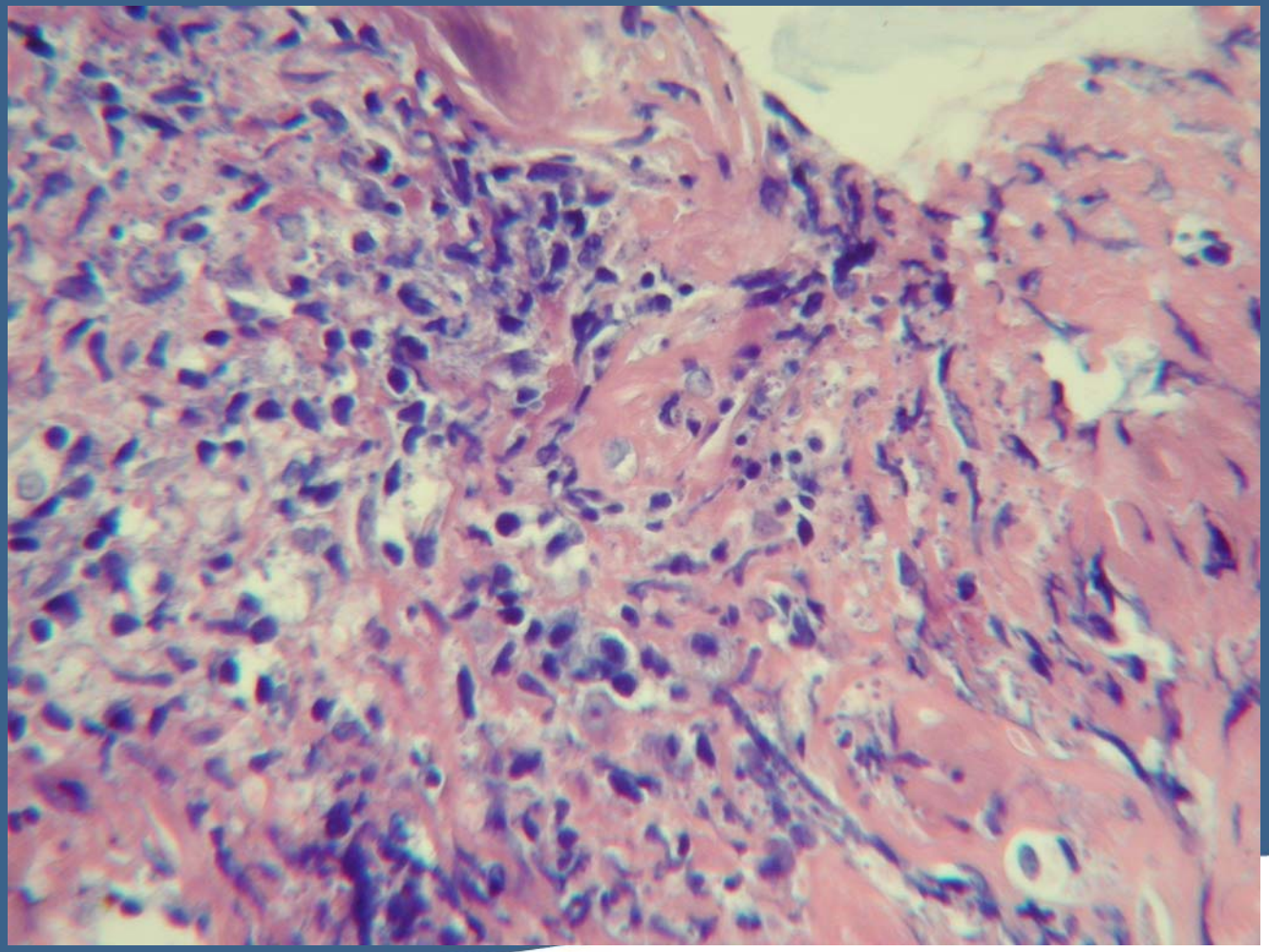
# Cutaneous leishmaniasis

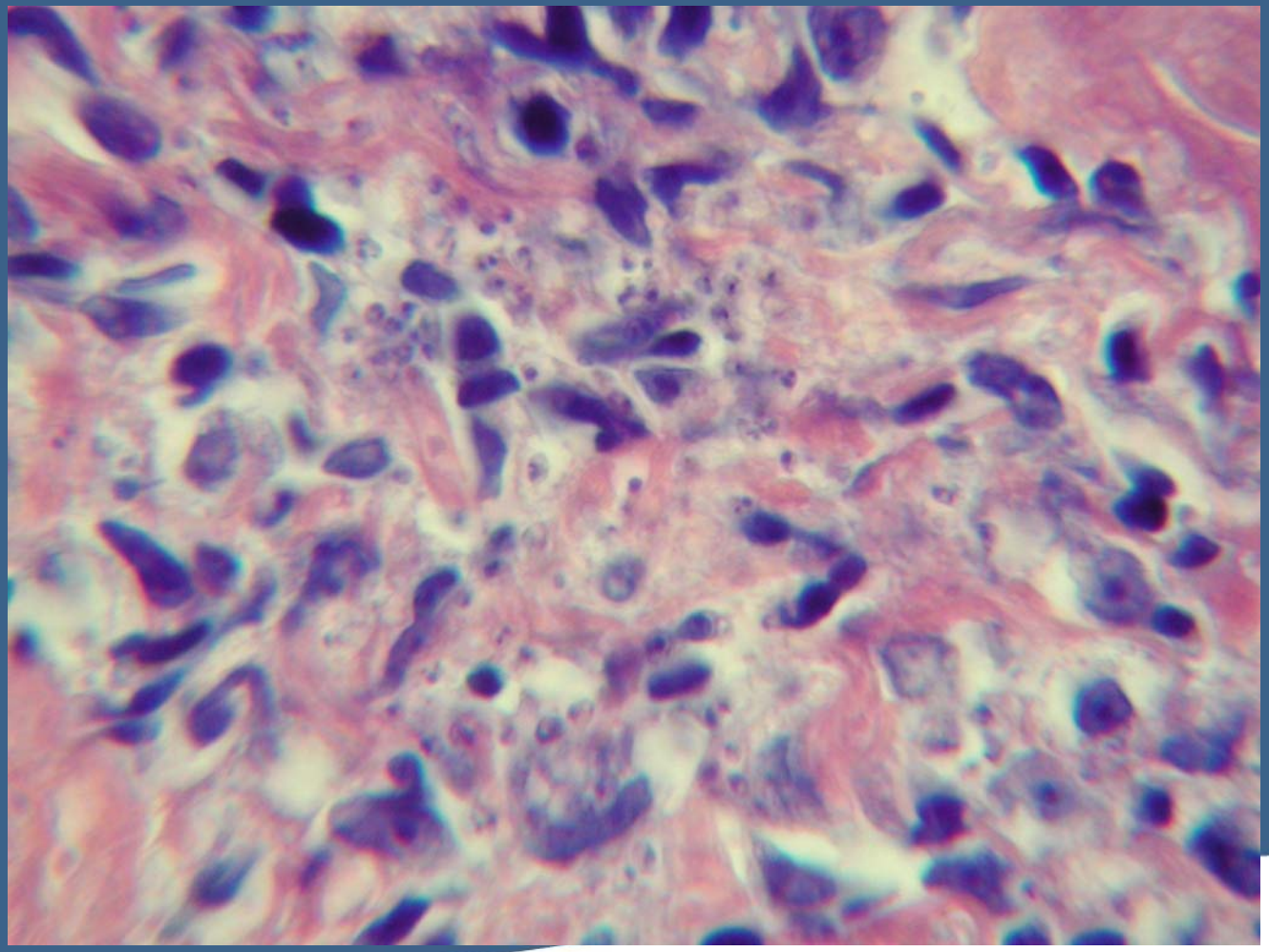
- cutaneous ulceration
- ulceration border: pseudoepitheliomatous hyperplasia, hyperkeratosis
- dermal inflammatory infiltrate varied (mixture of lymphocytes, plasma cells, histiocytes, some Langhans giant cells)
- in histiocytes: amastigotes

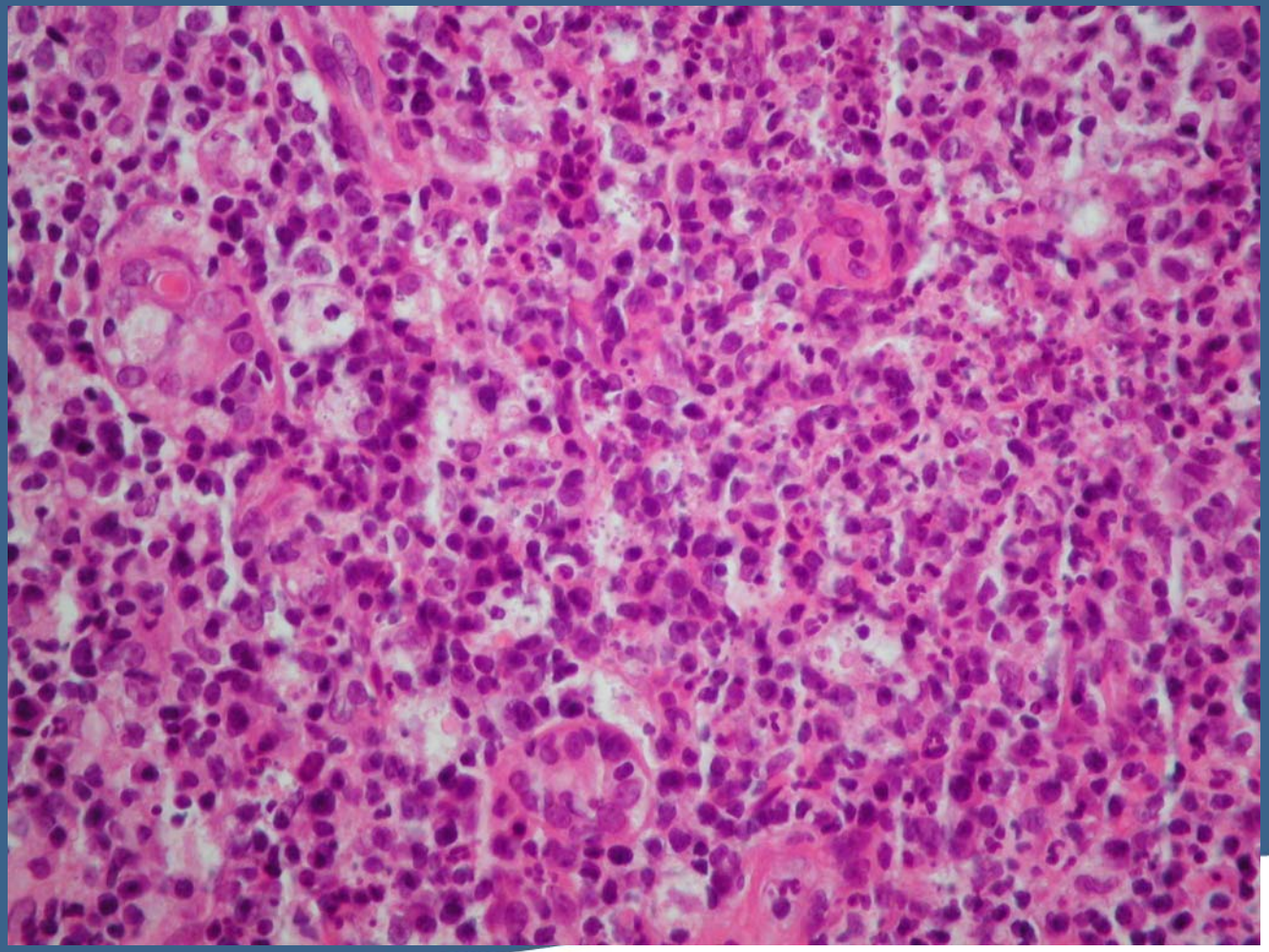


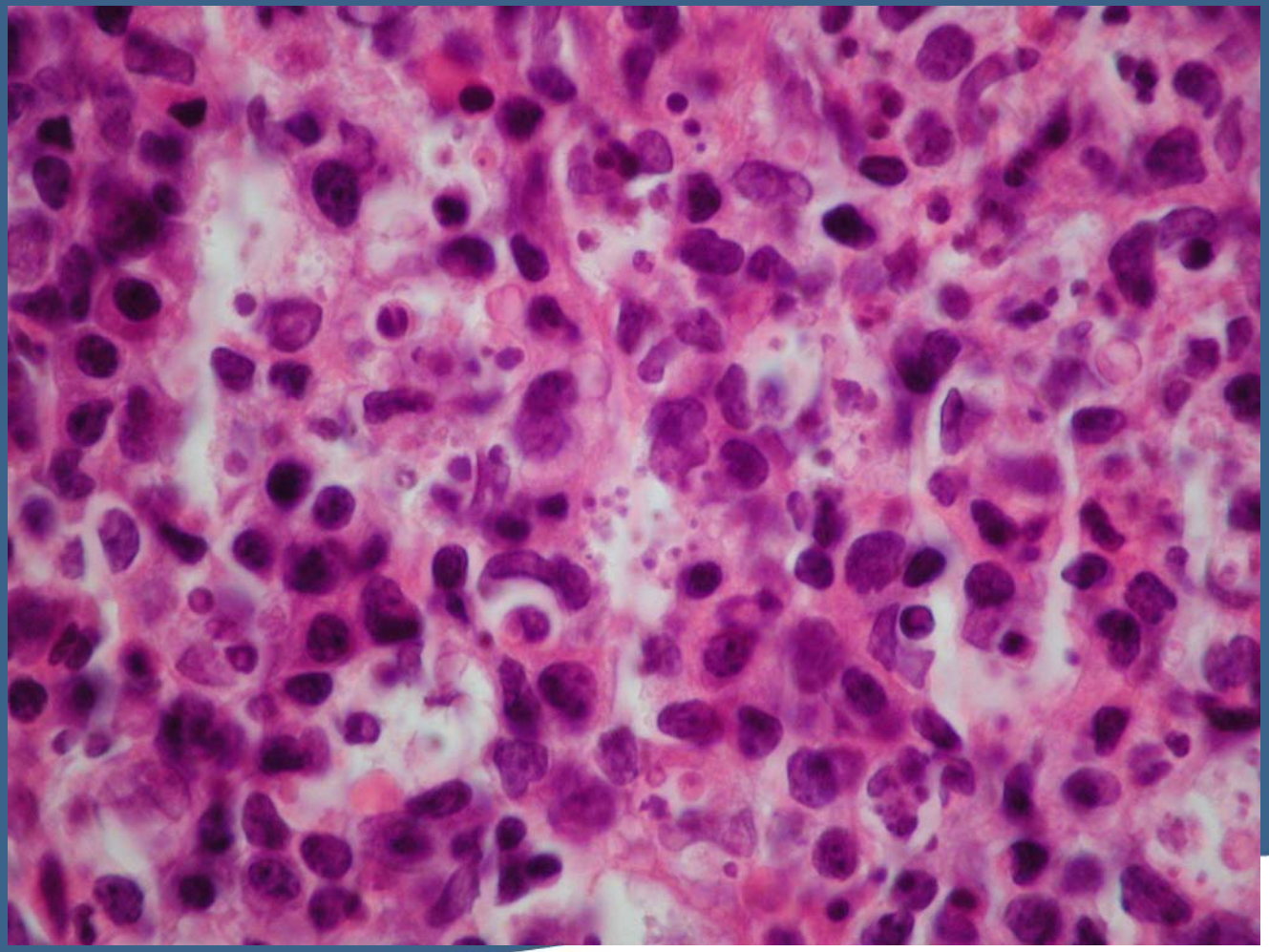


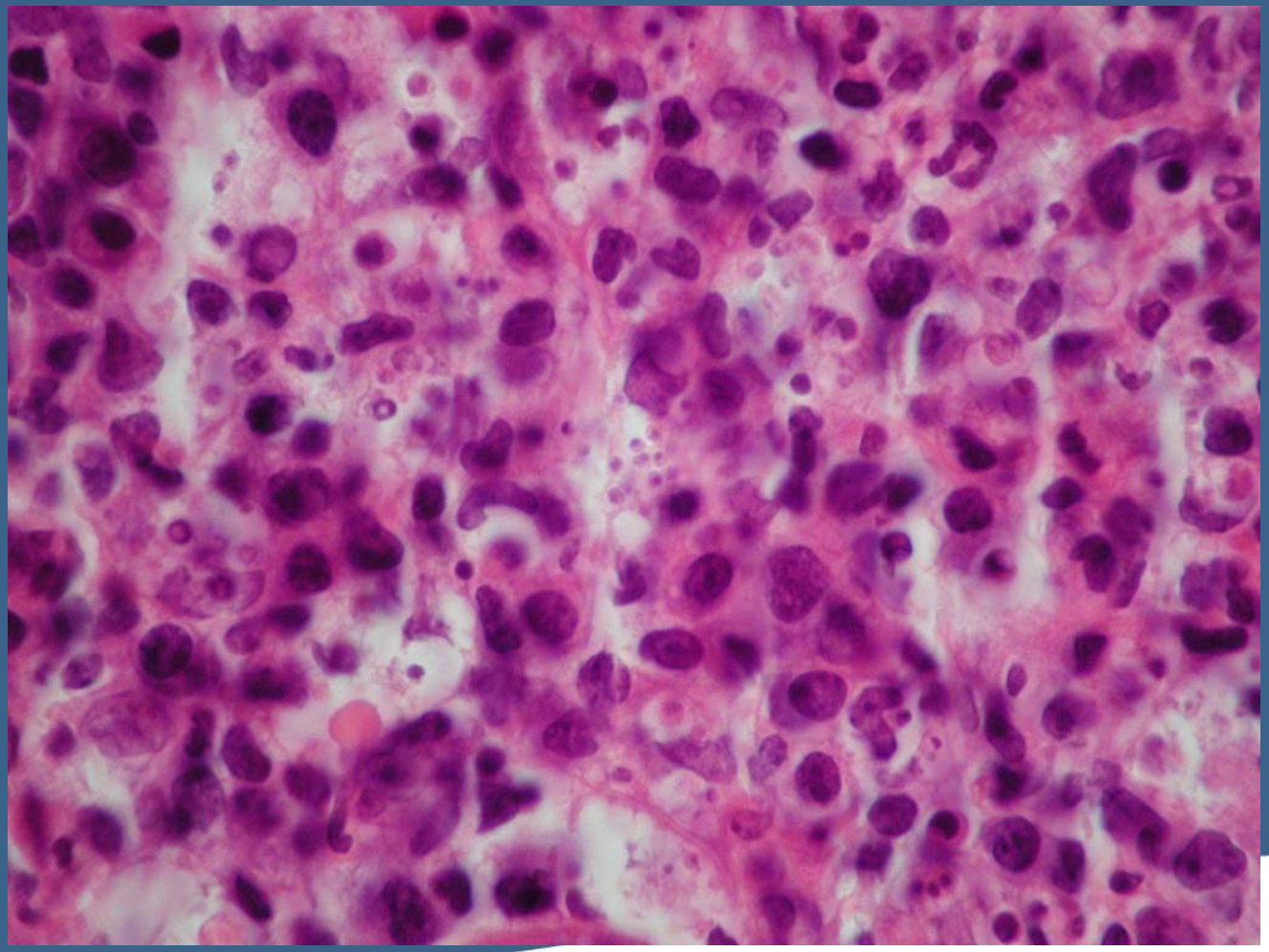


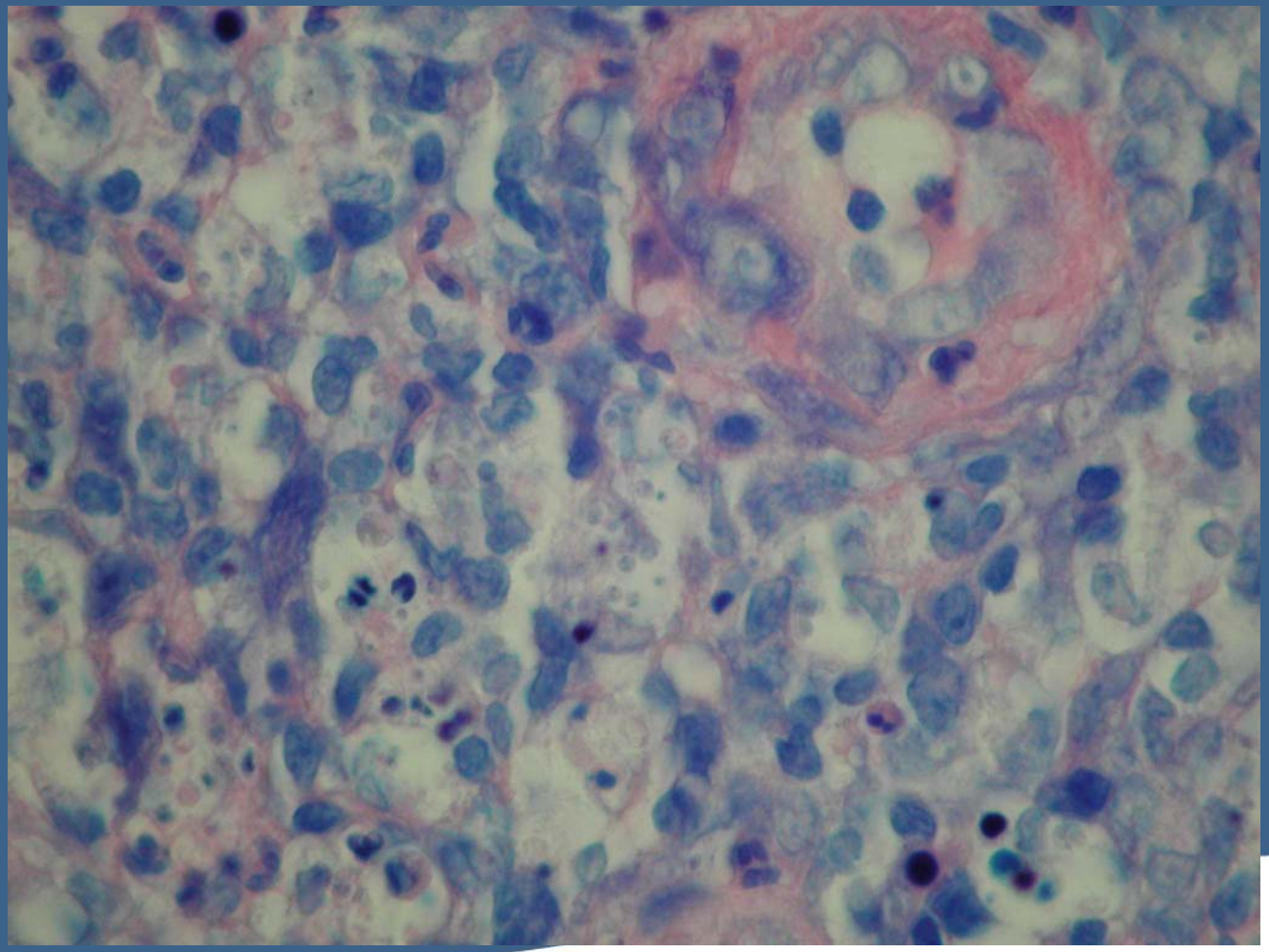


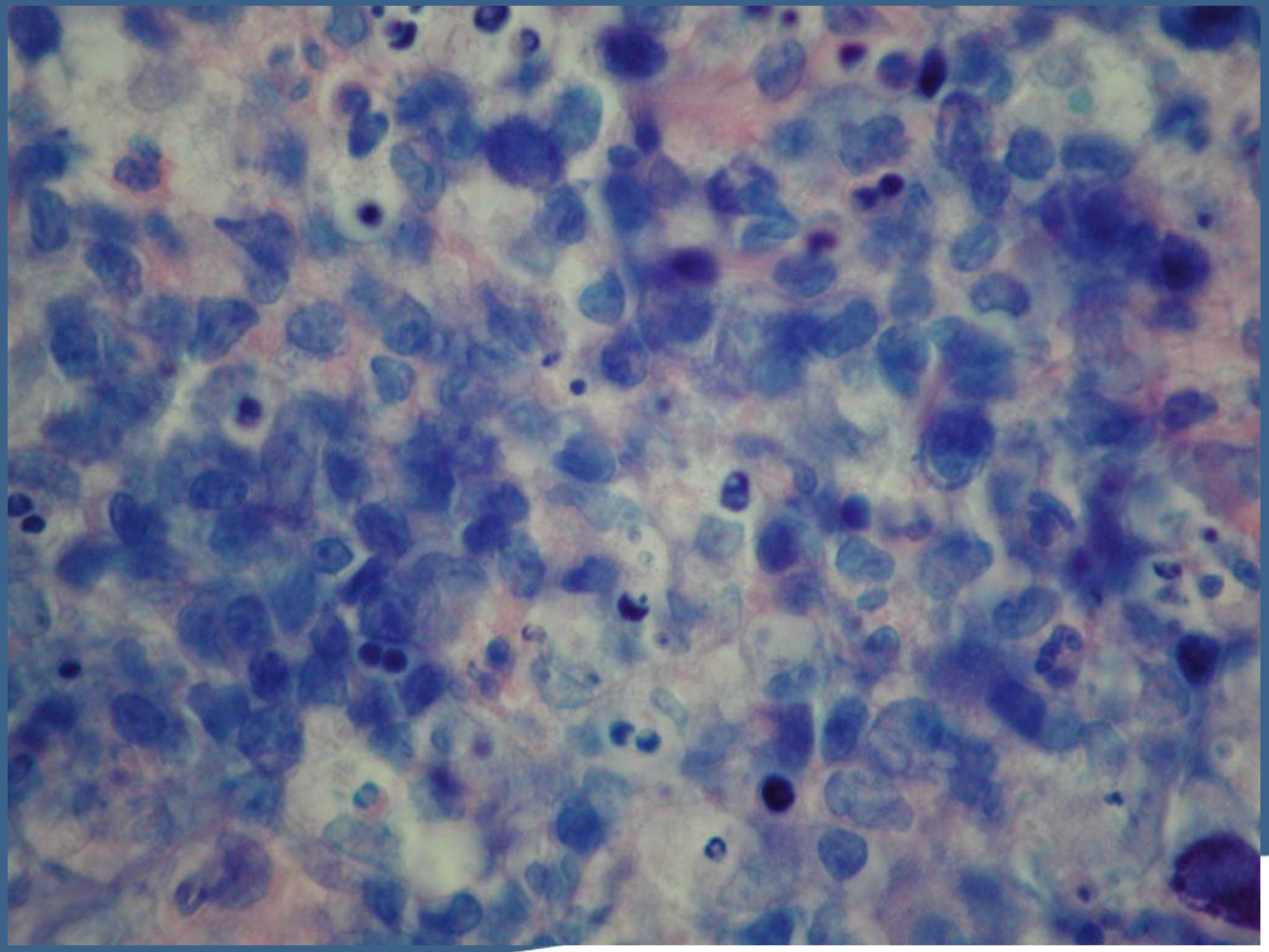


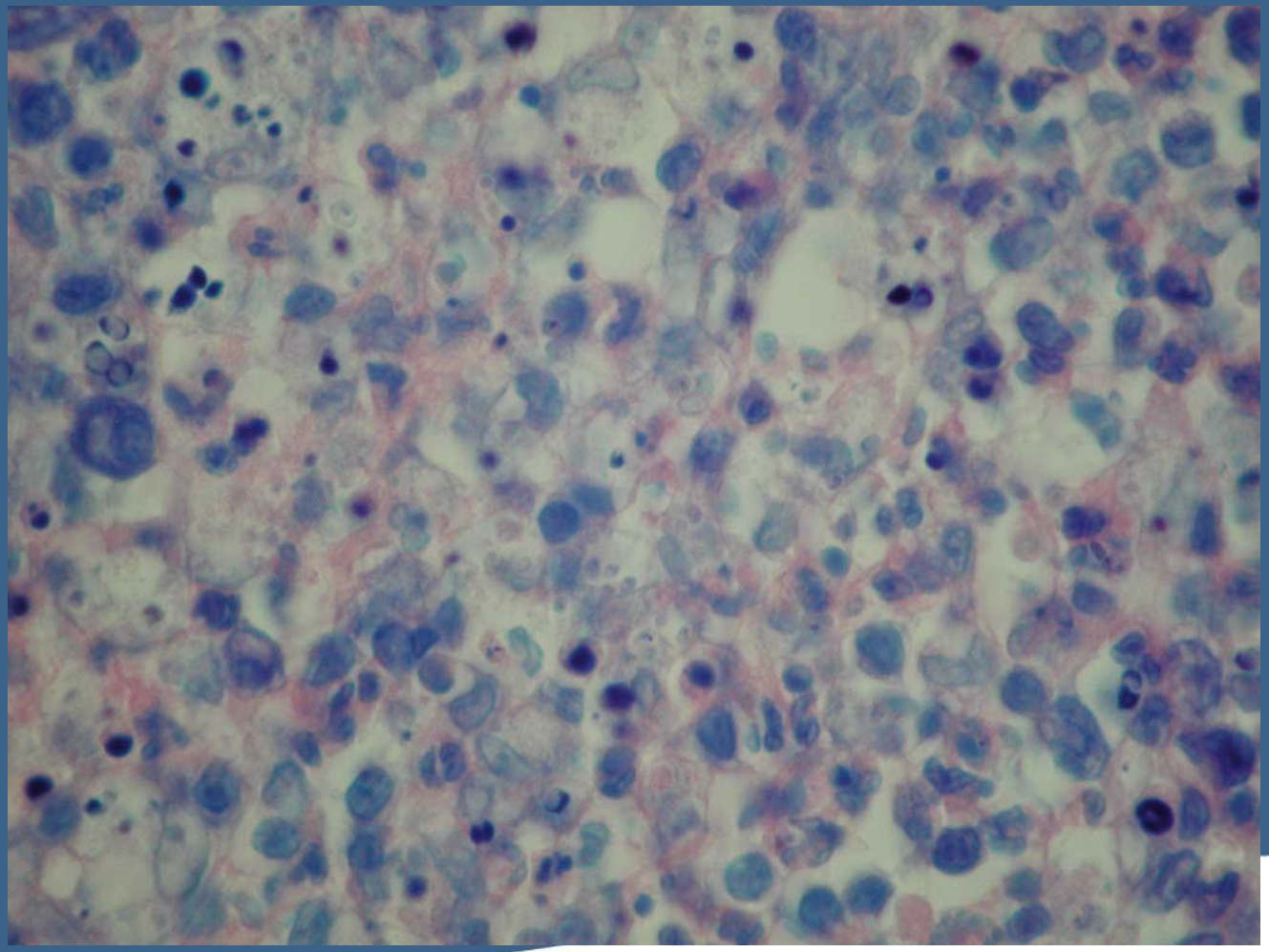


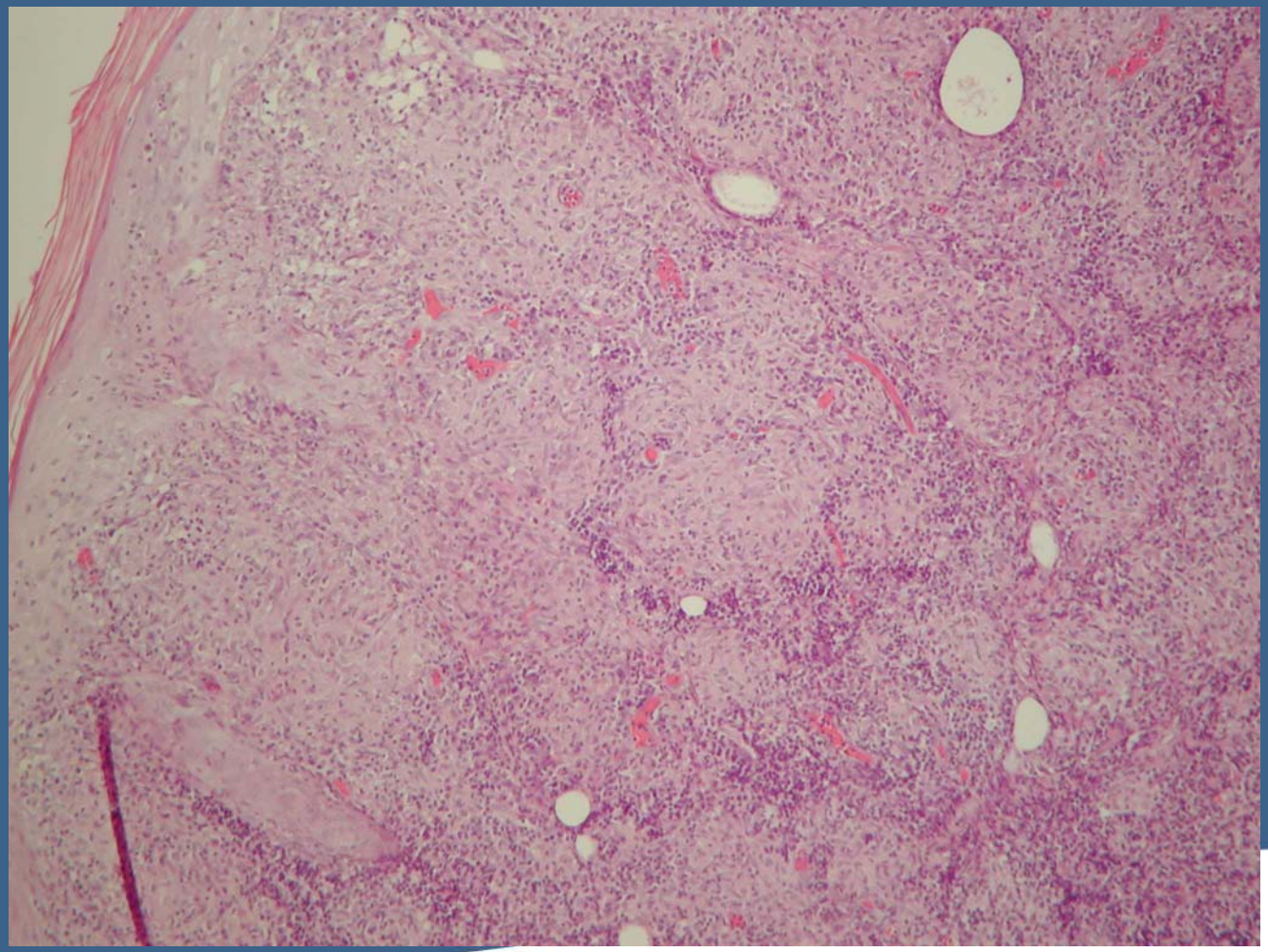


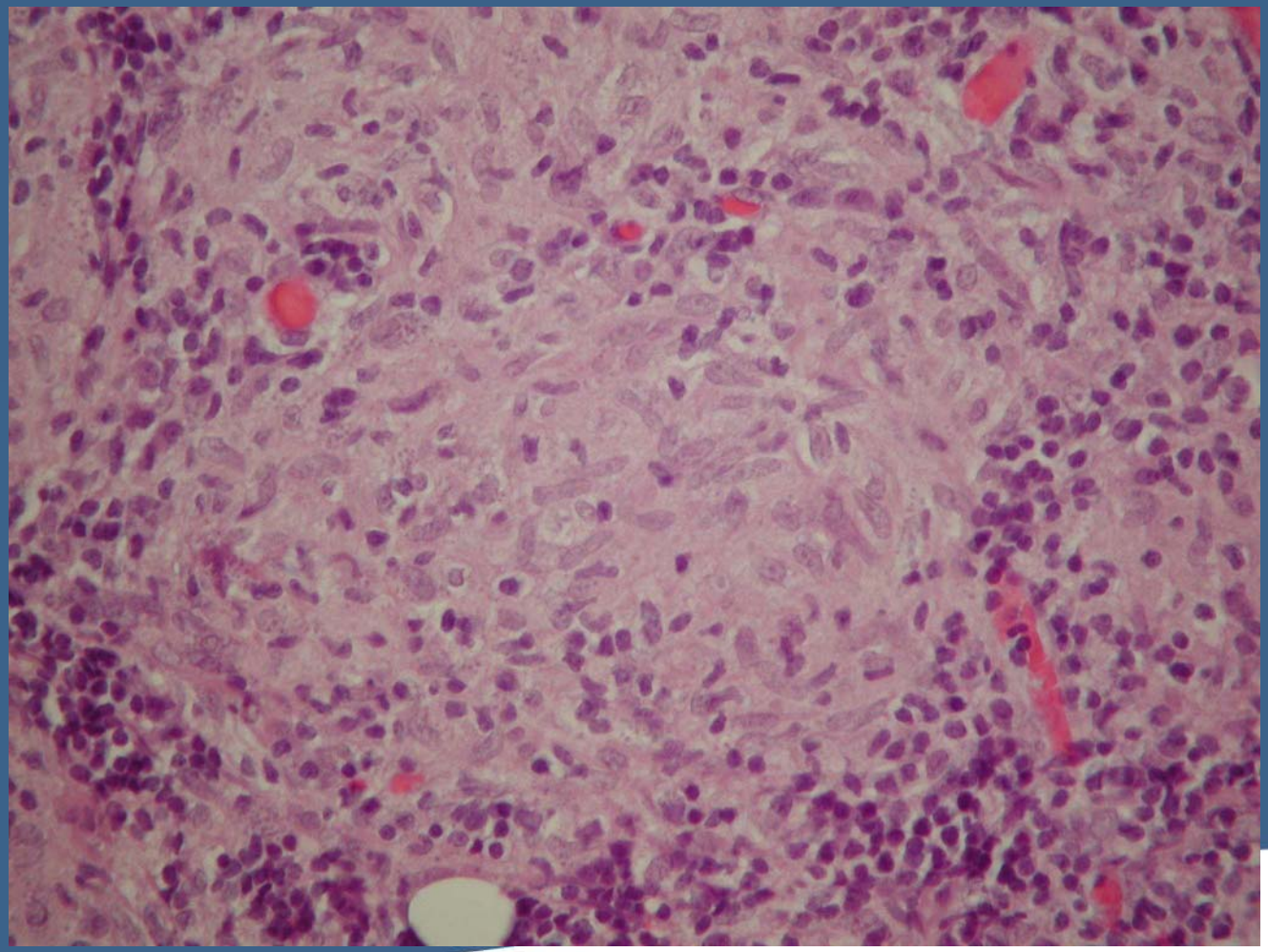


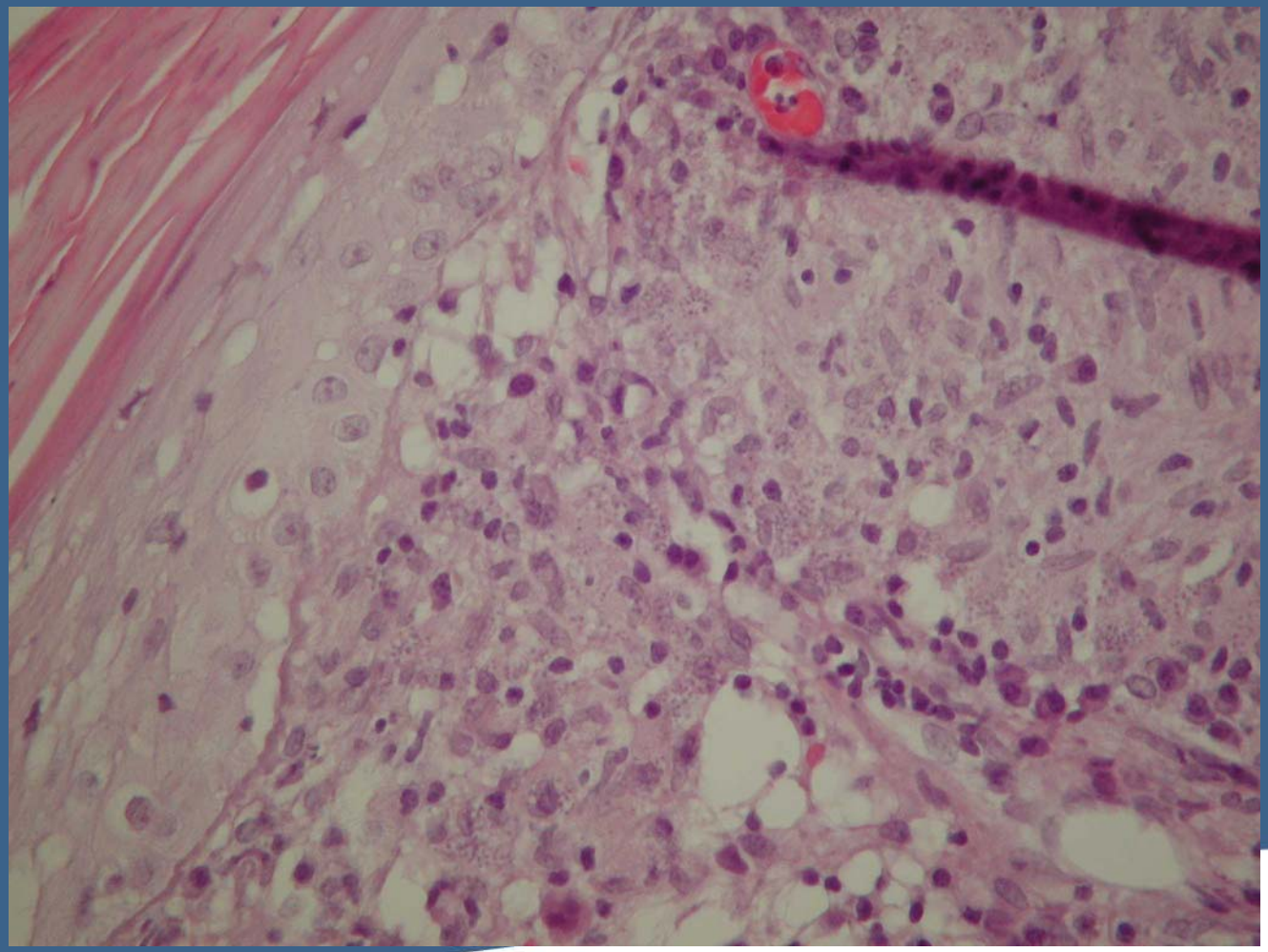


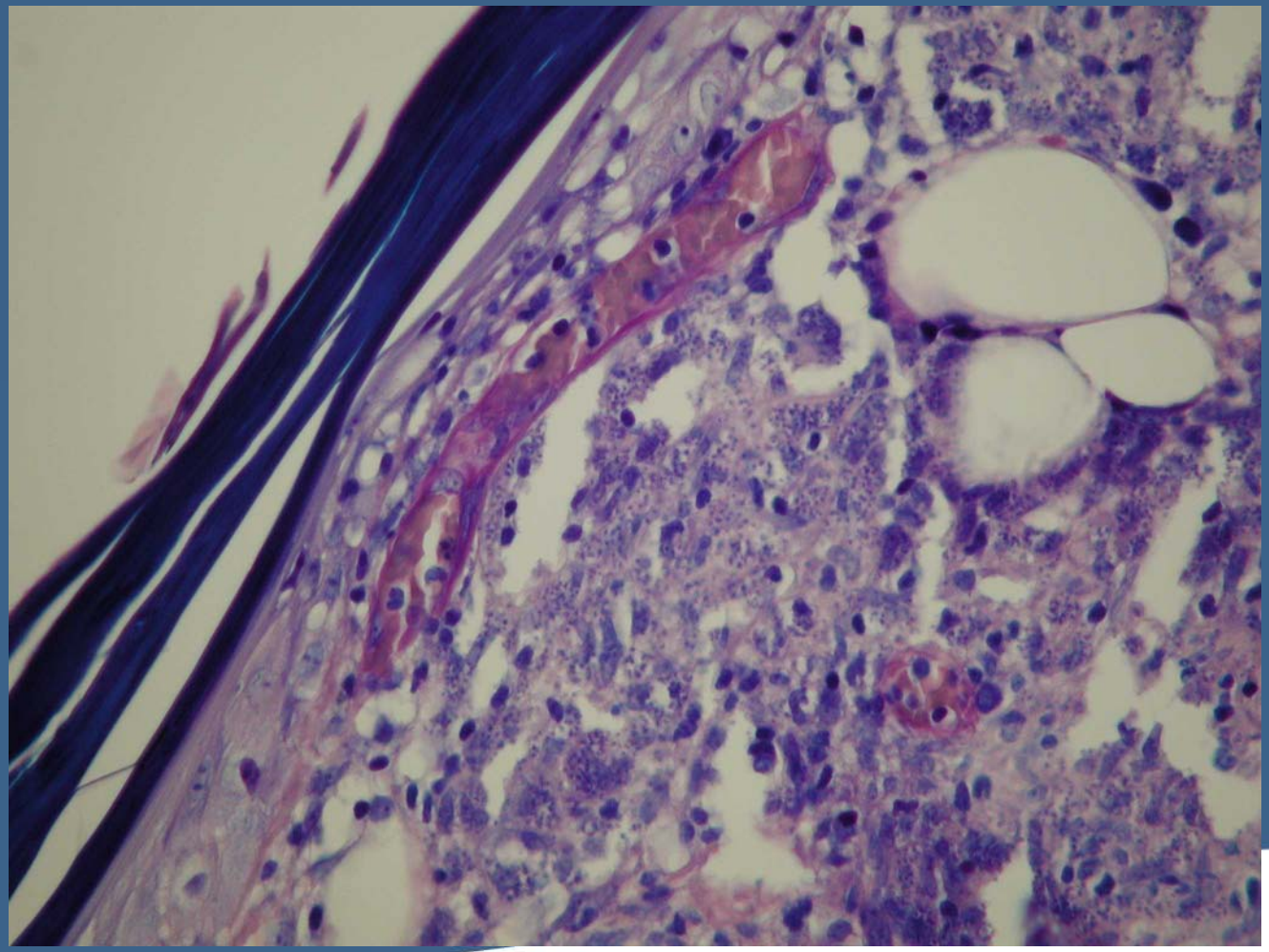




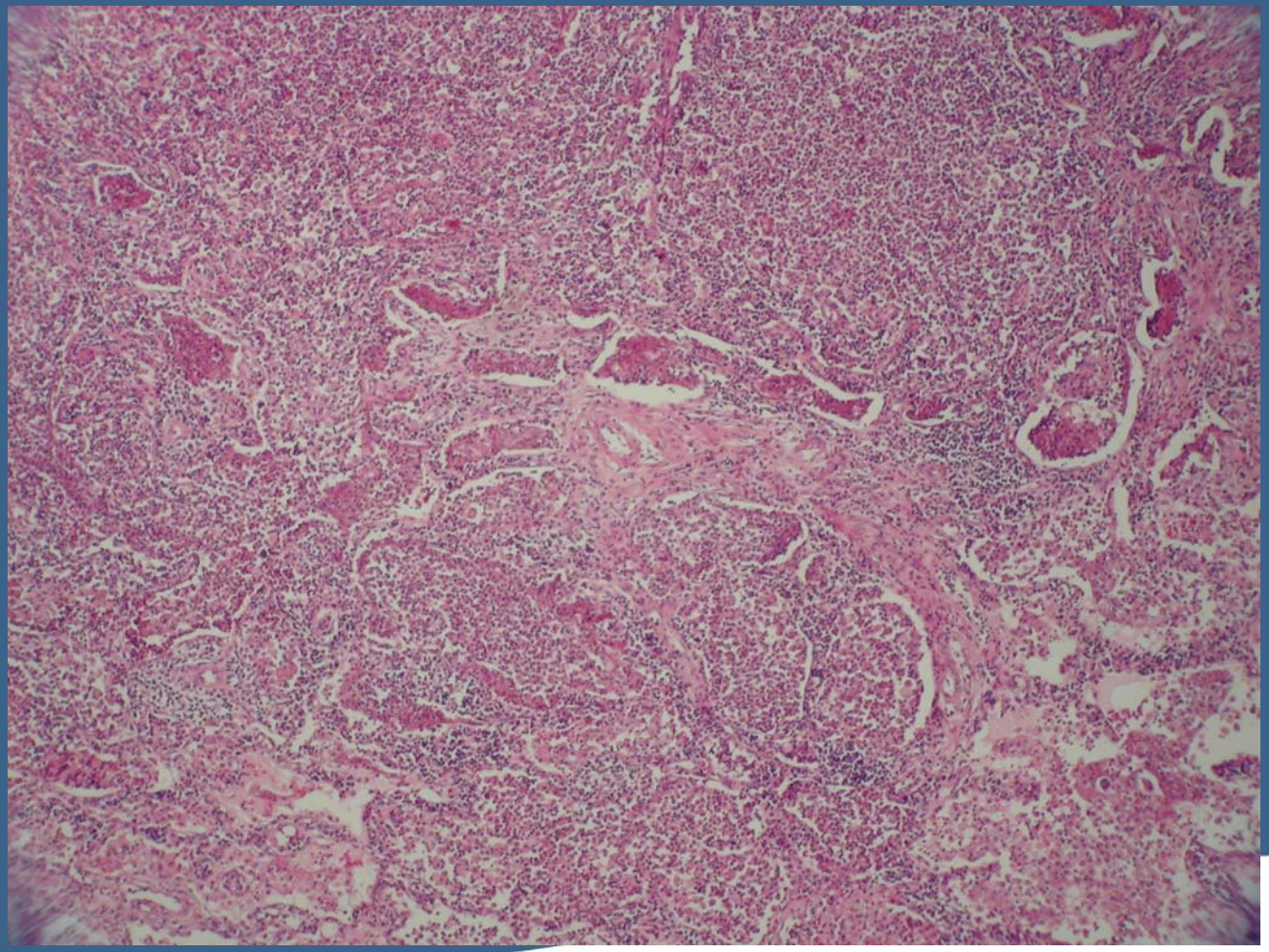


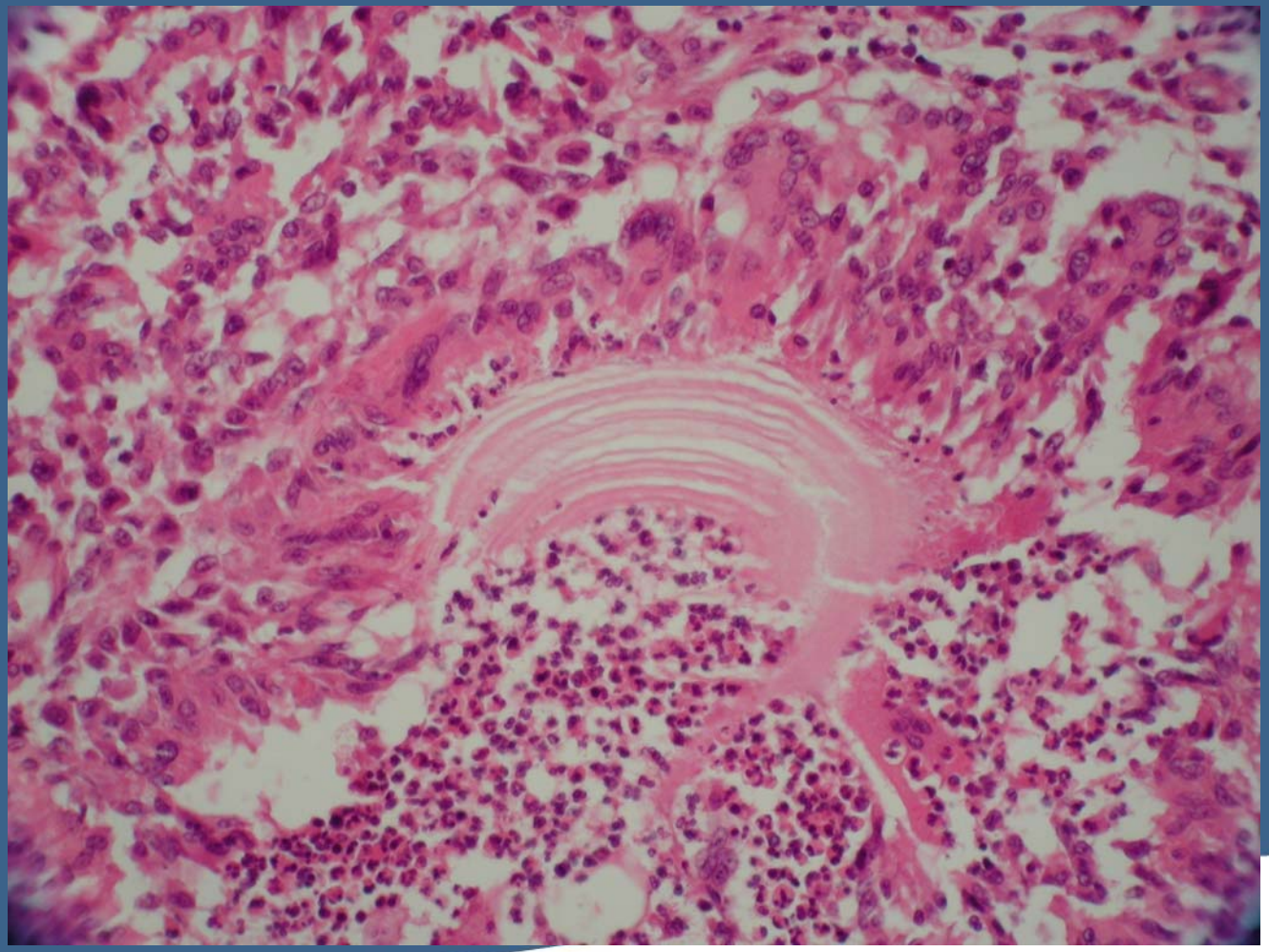


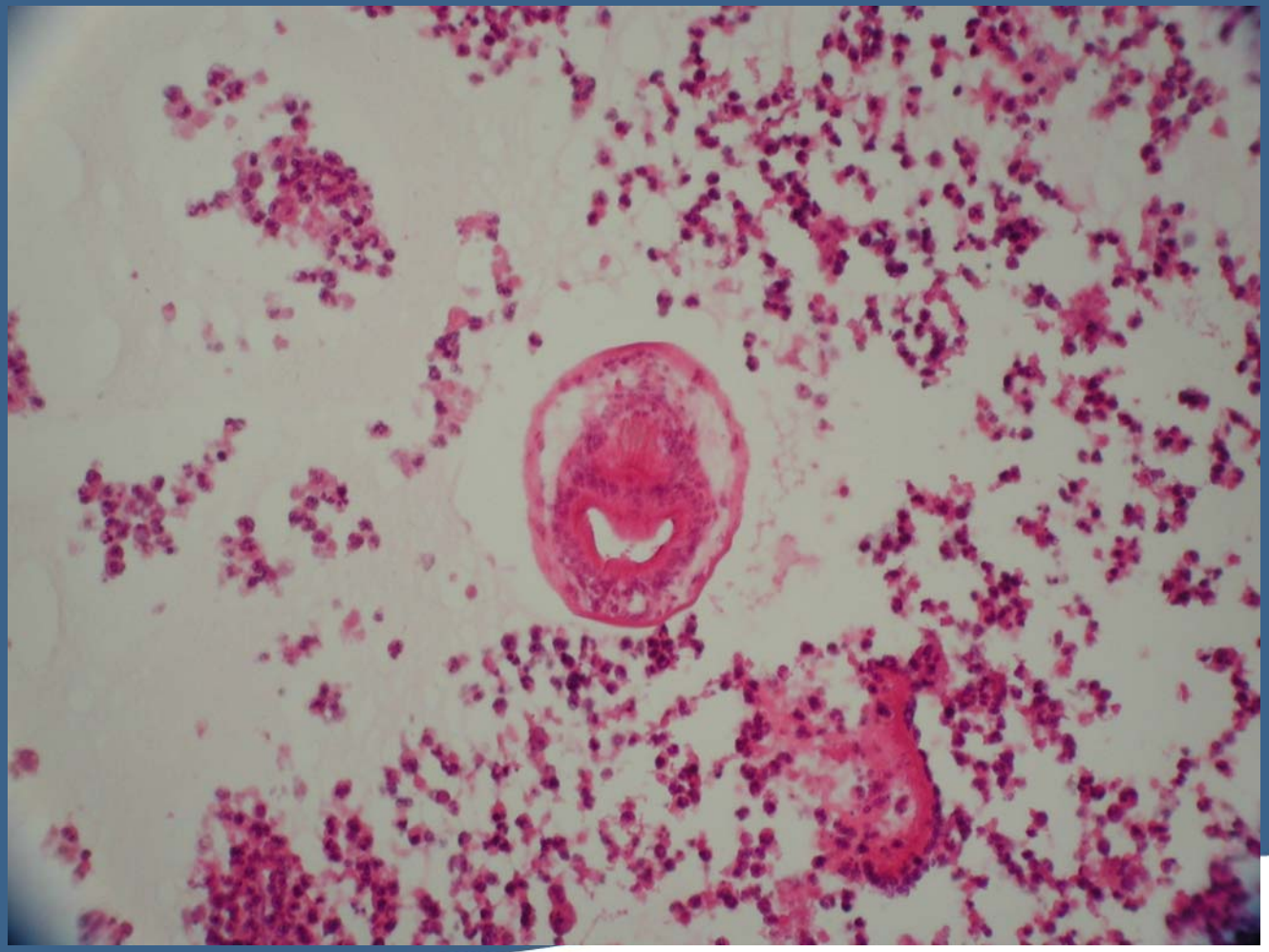


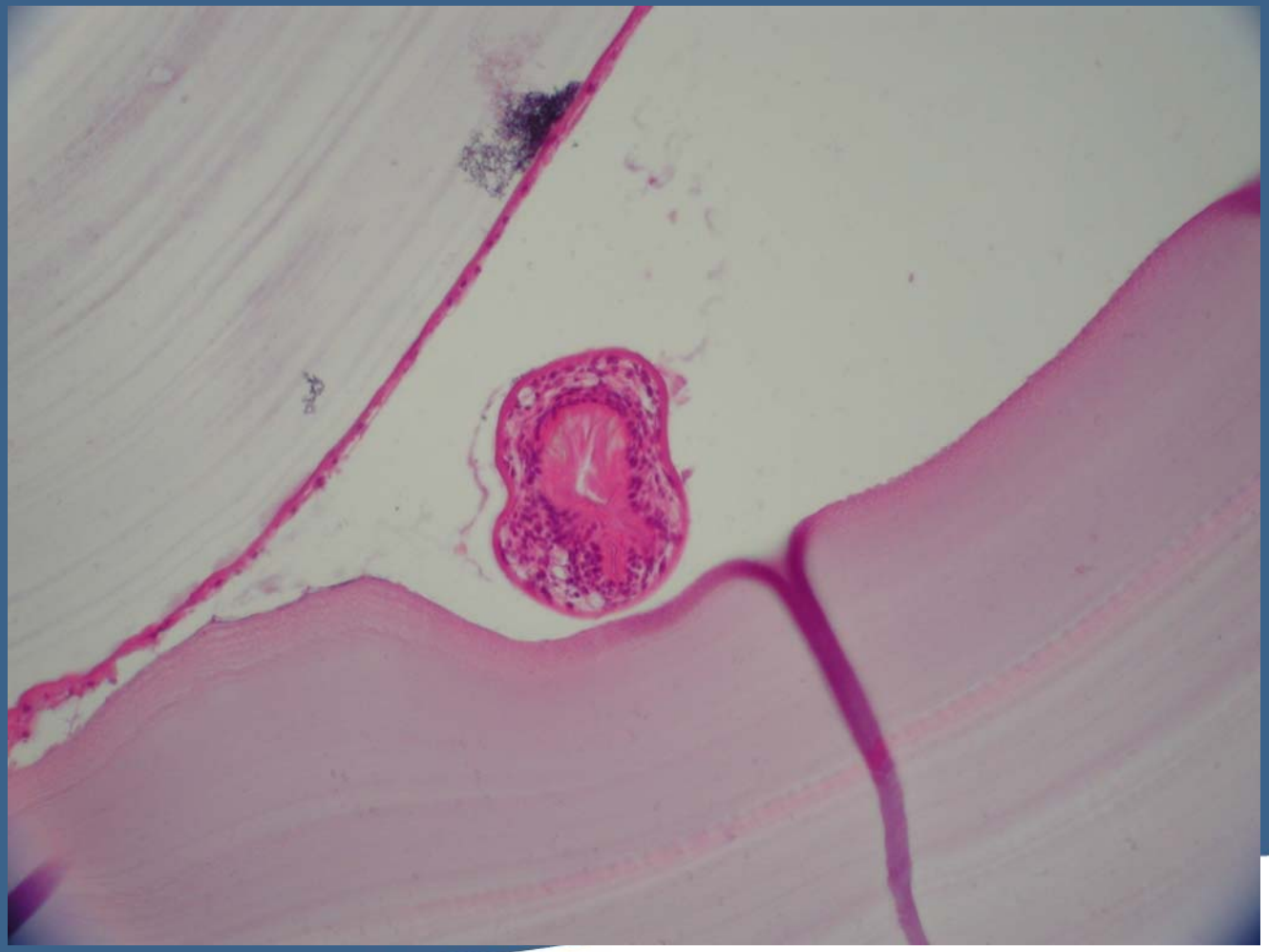


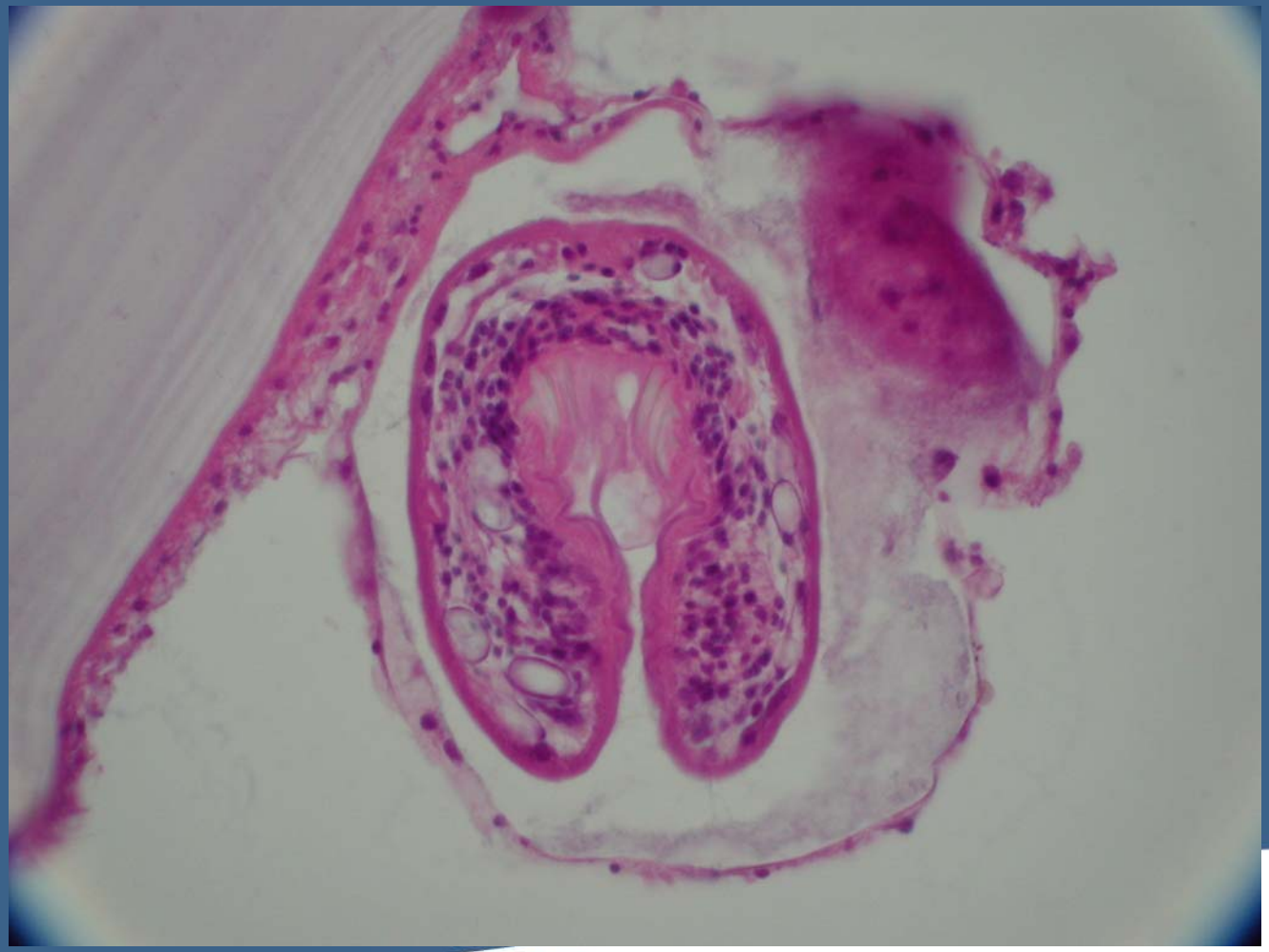
# Reaction patterns as a lead to a specific diagnosis







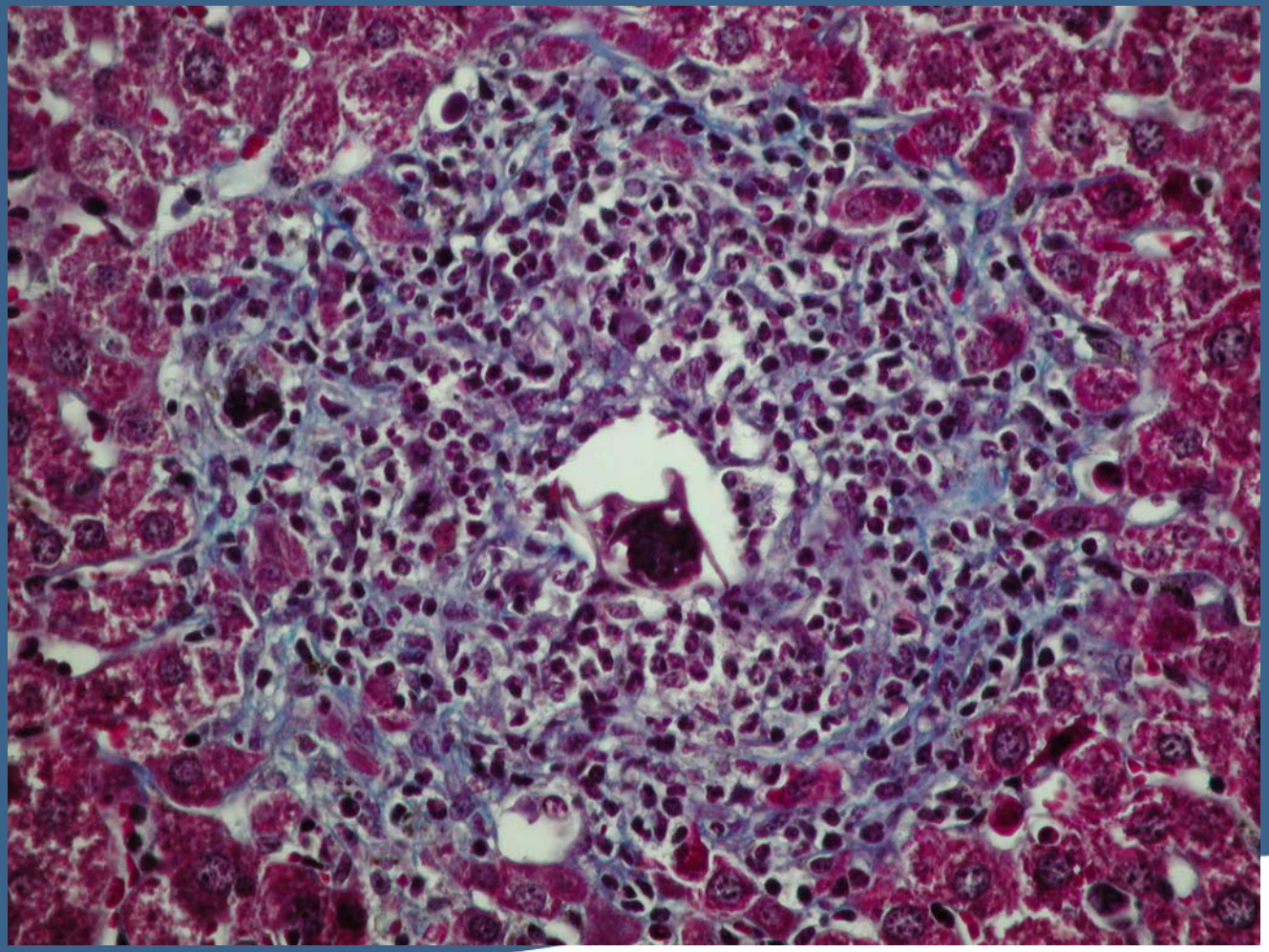


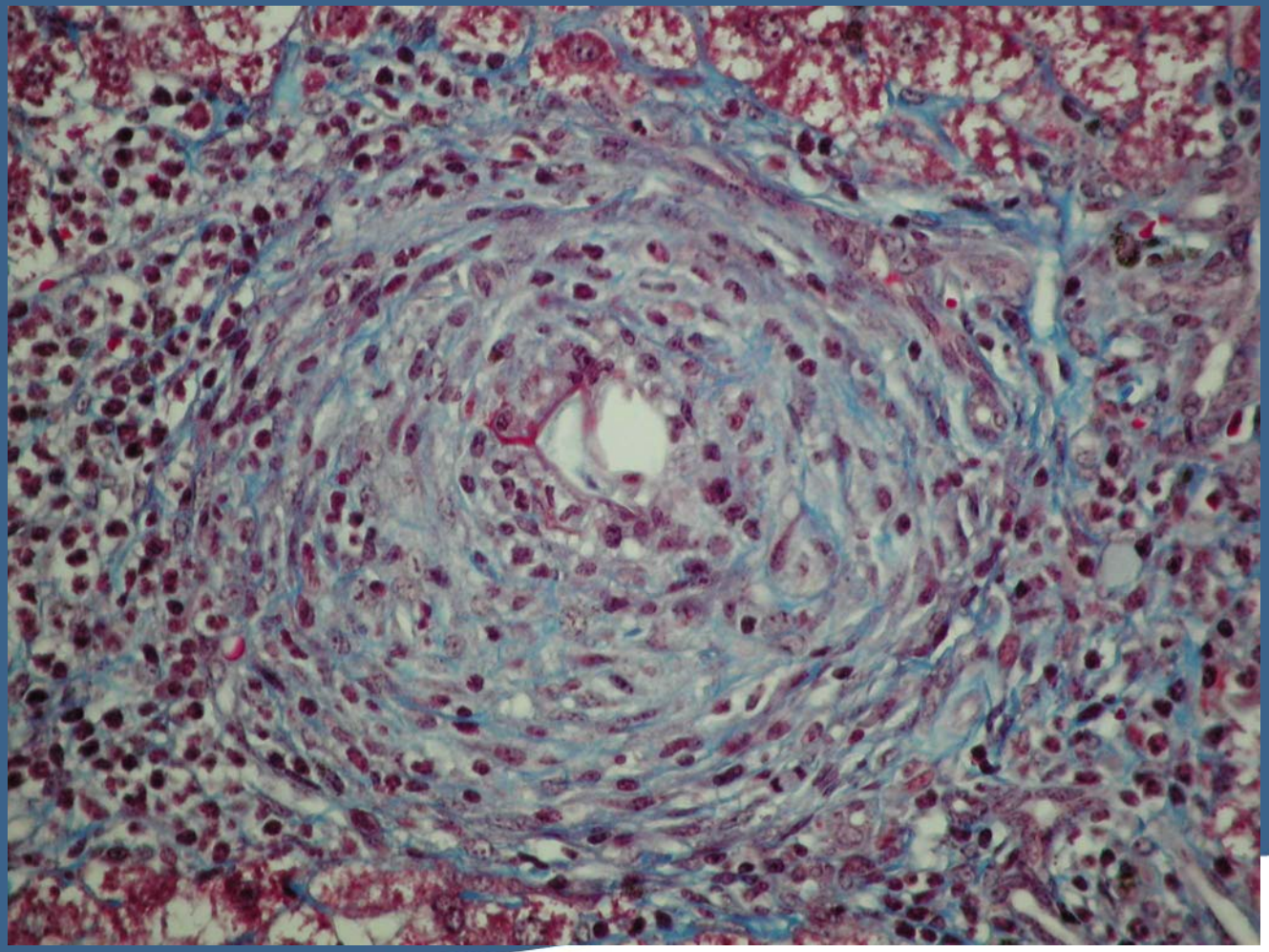


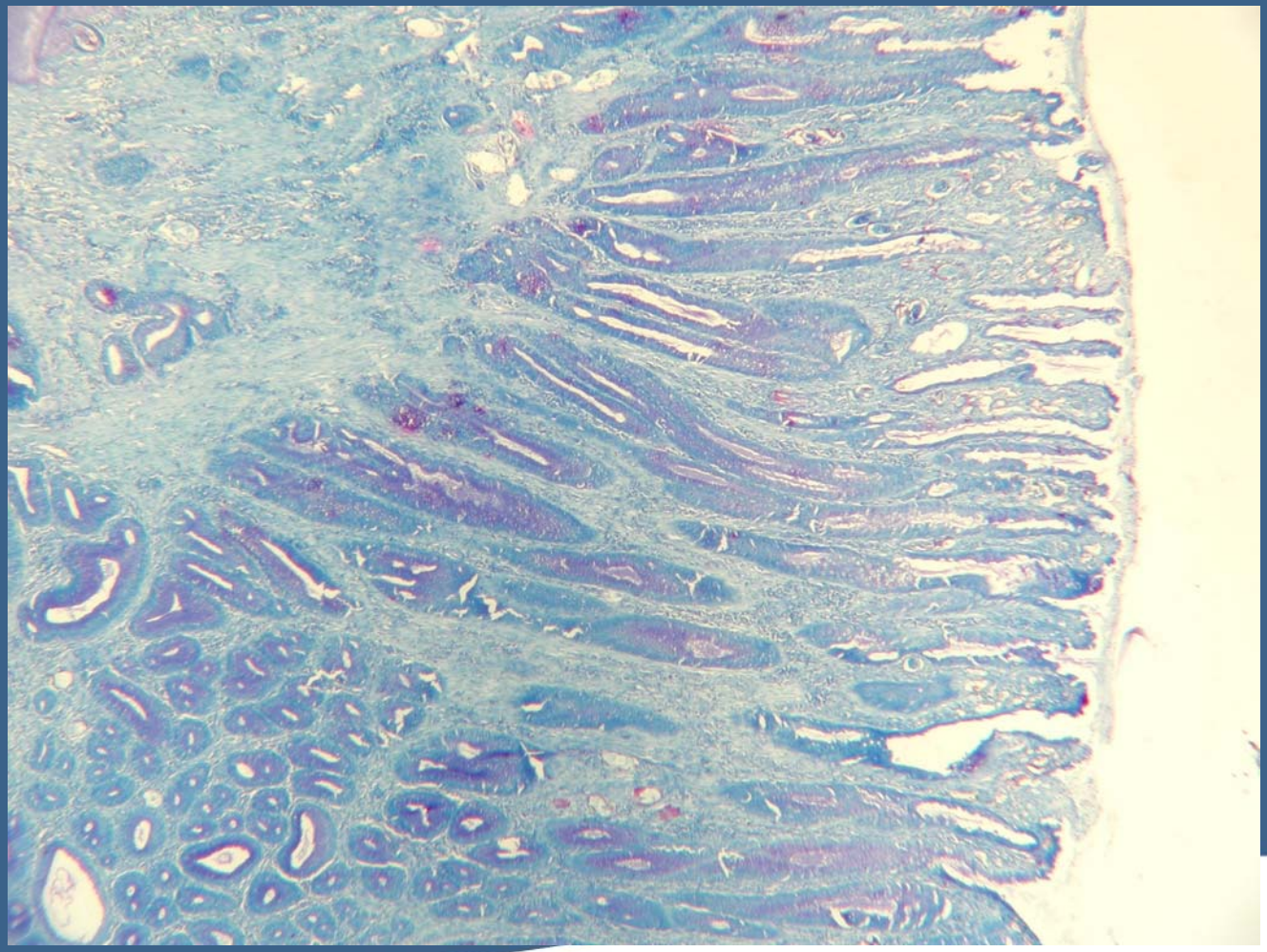
Laminaire membraan en scolex van *E. granulosus*

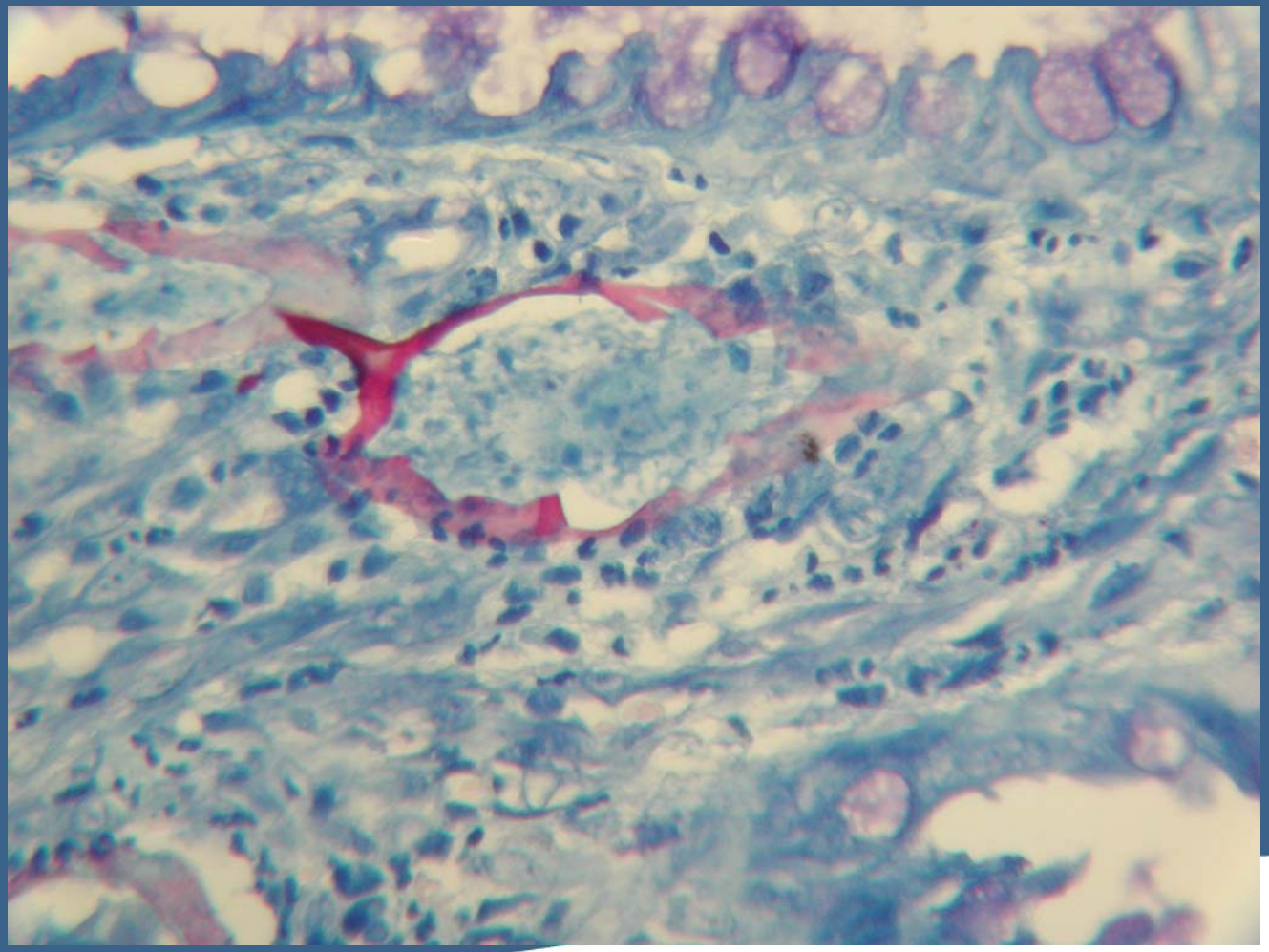
# Granulomas...

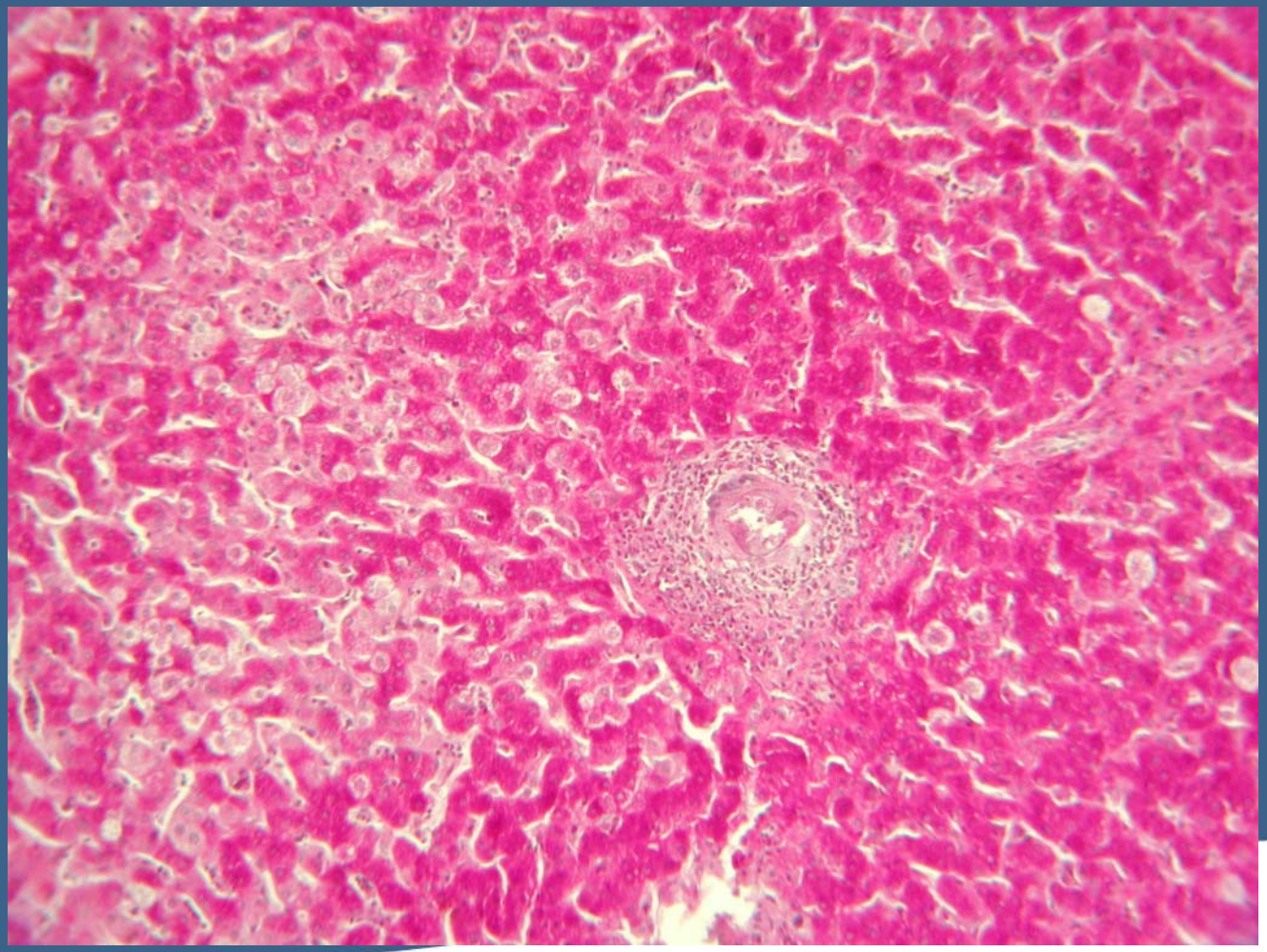
- “rounded structures composed of epithelioid cells (modified histiocytes) admixed with variable numbers of other inflammatory cells, such as lymphocytes, plasma cells, neutrophilic granulocytes, eosinophils, mast cells, giant cells

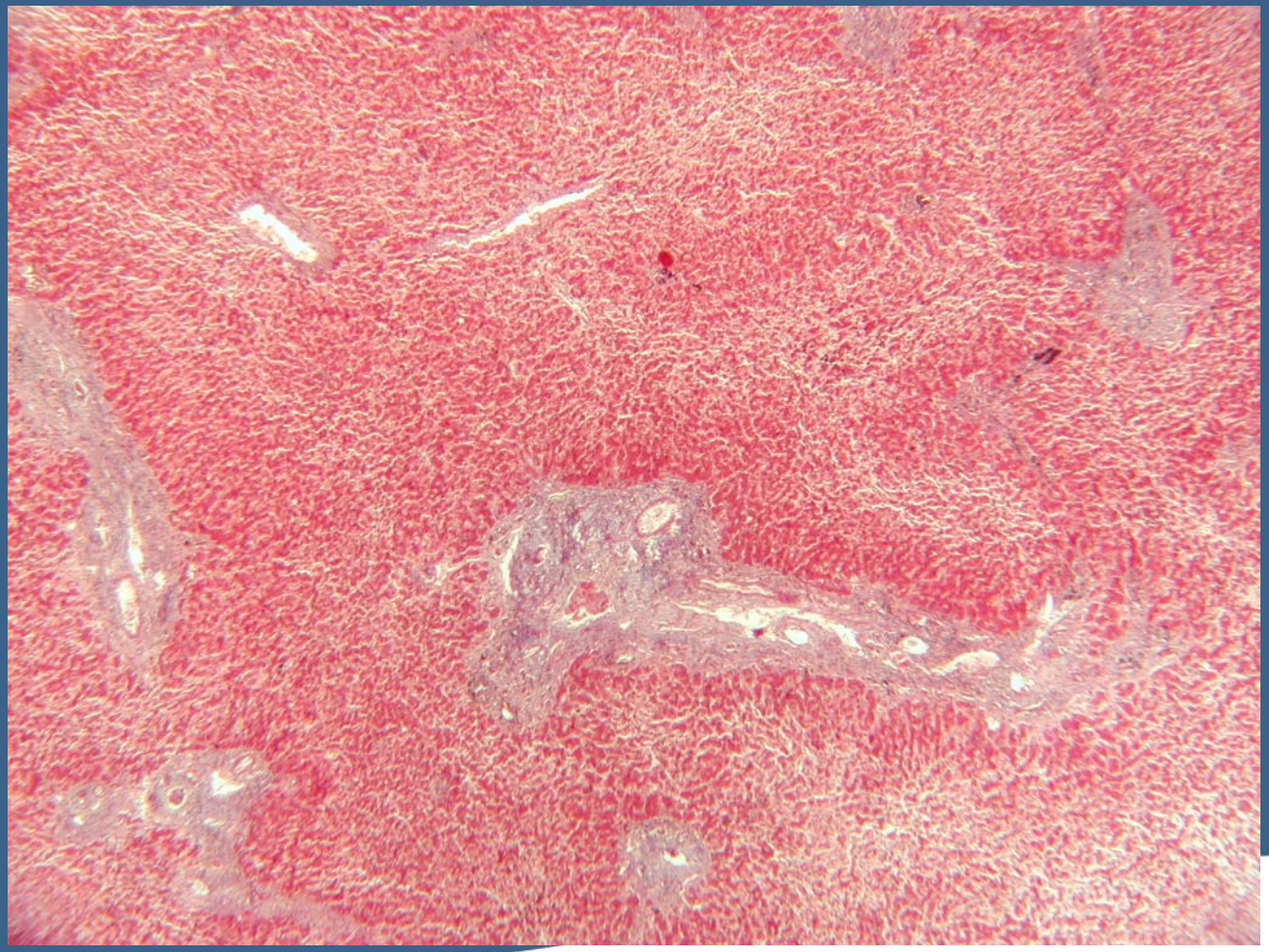


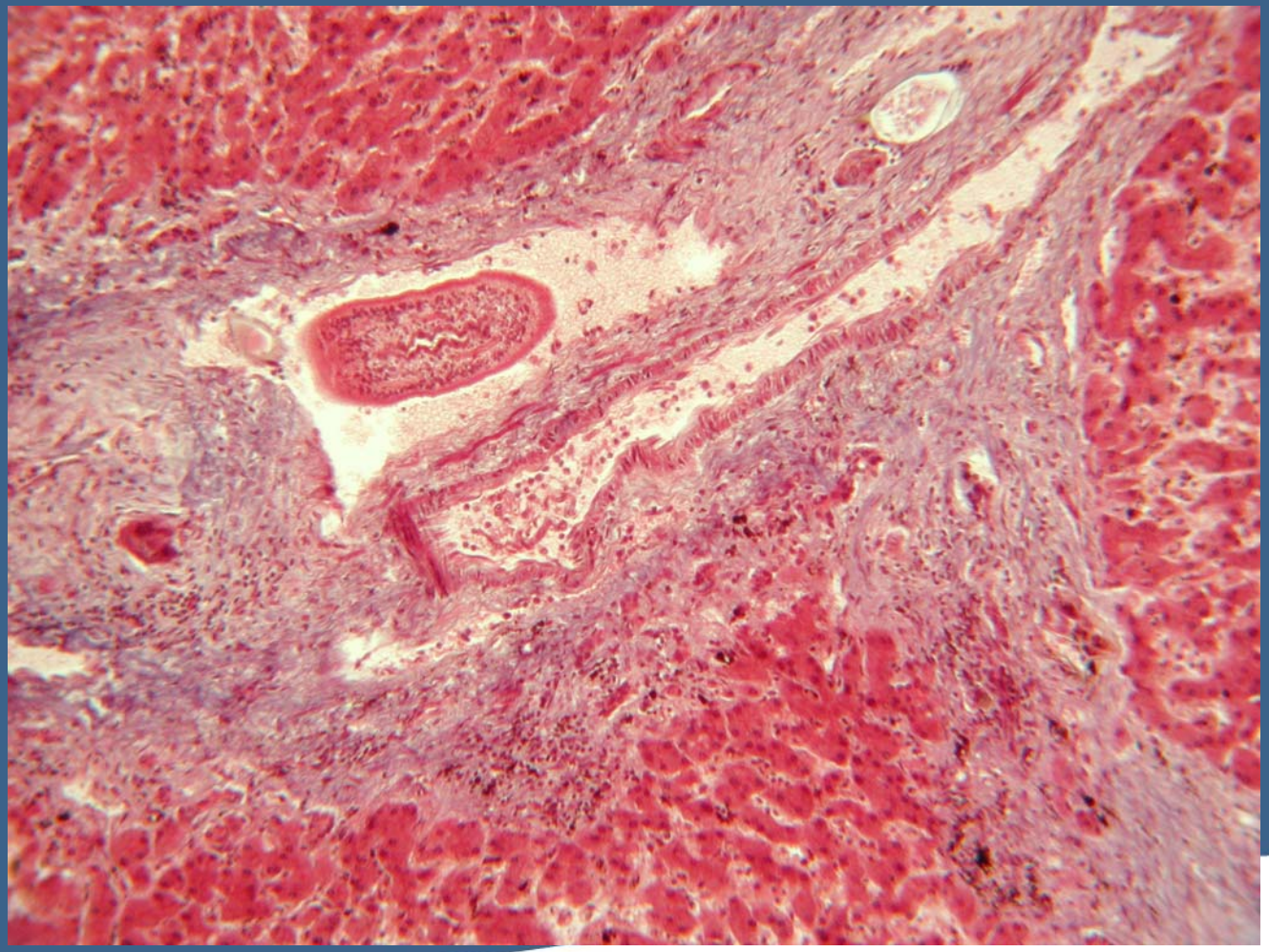


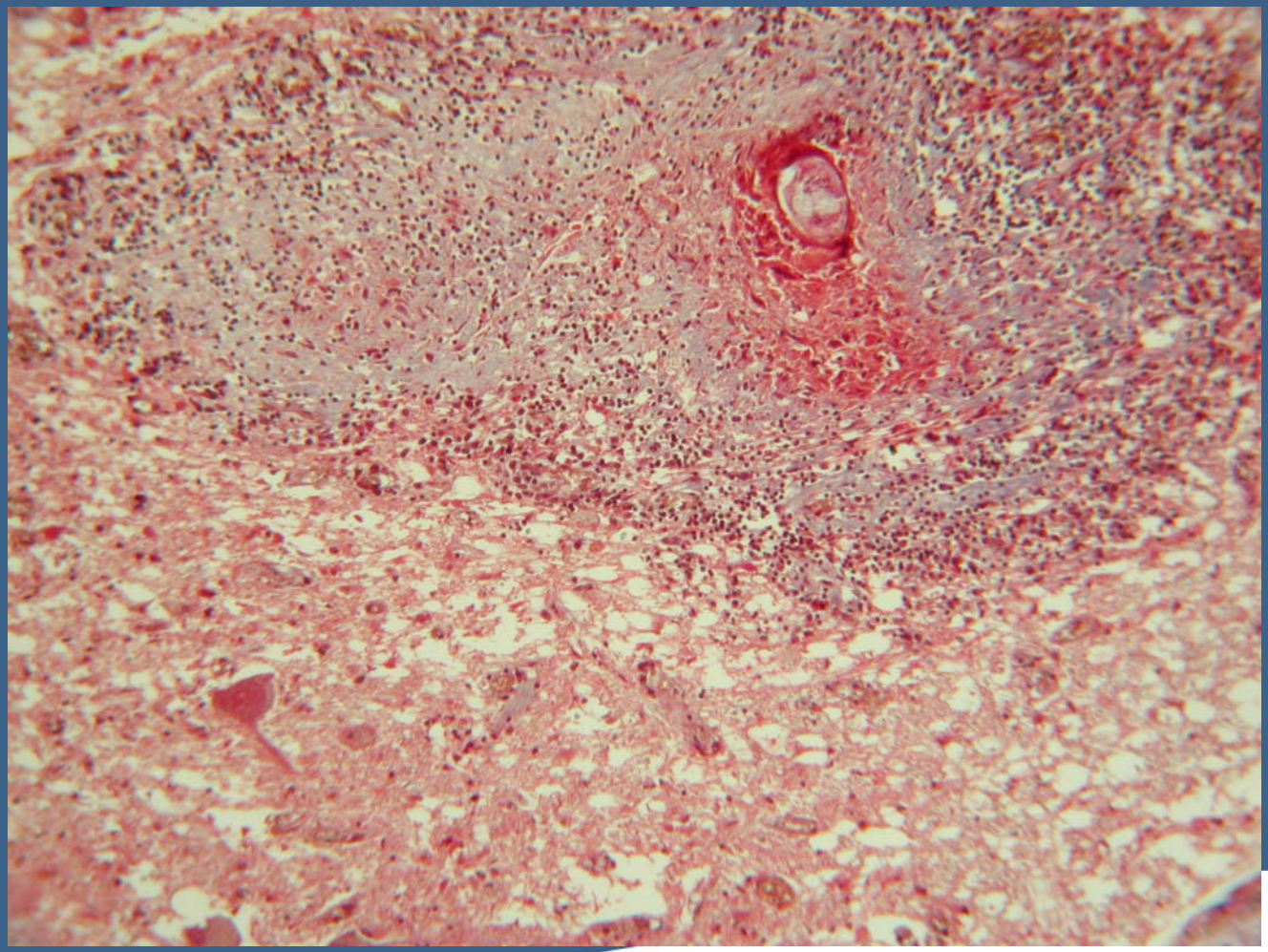




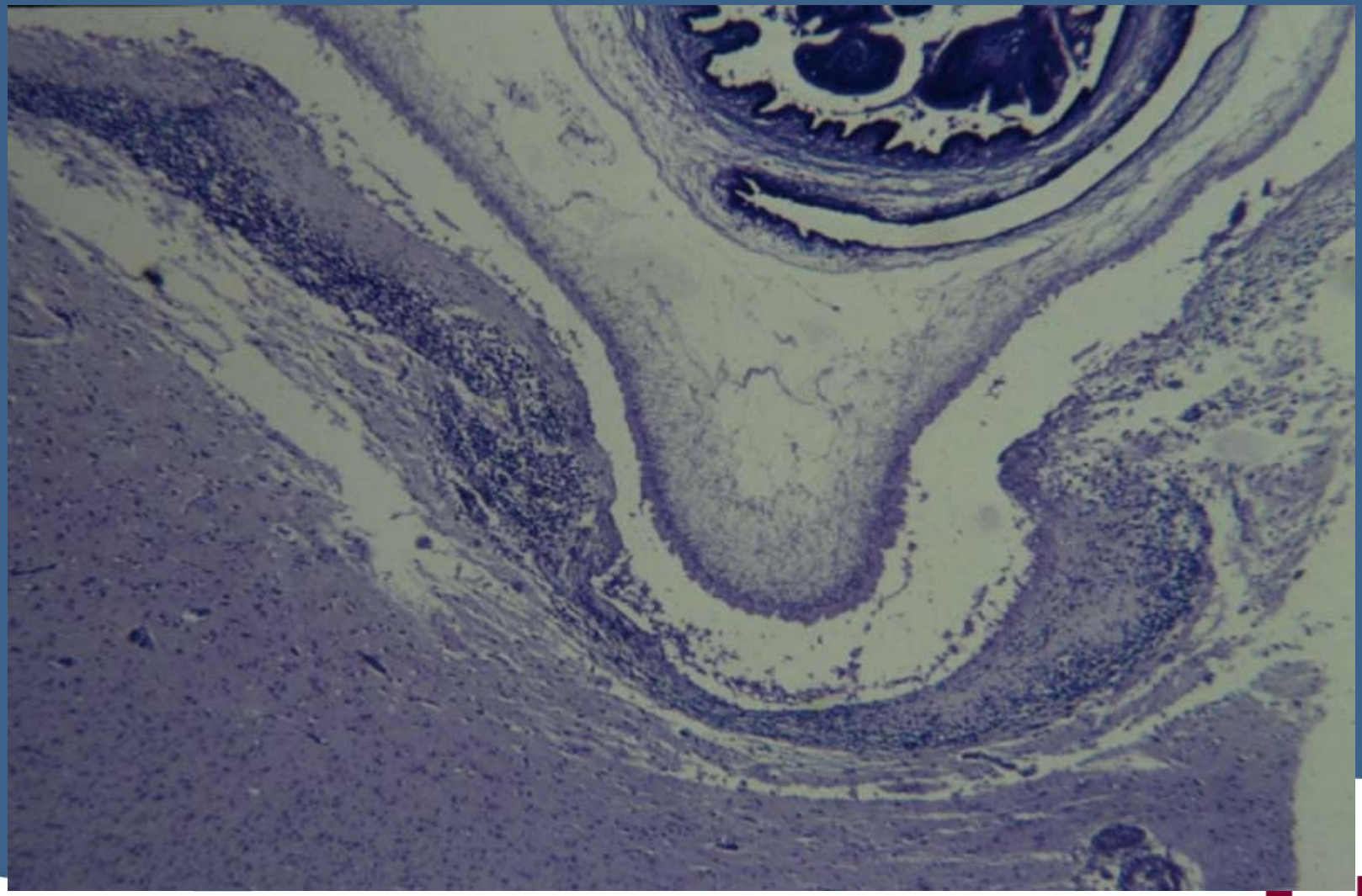


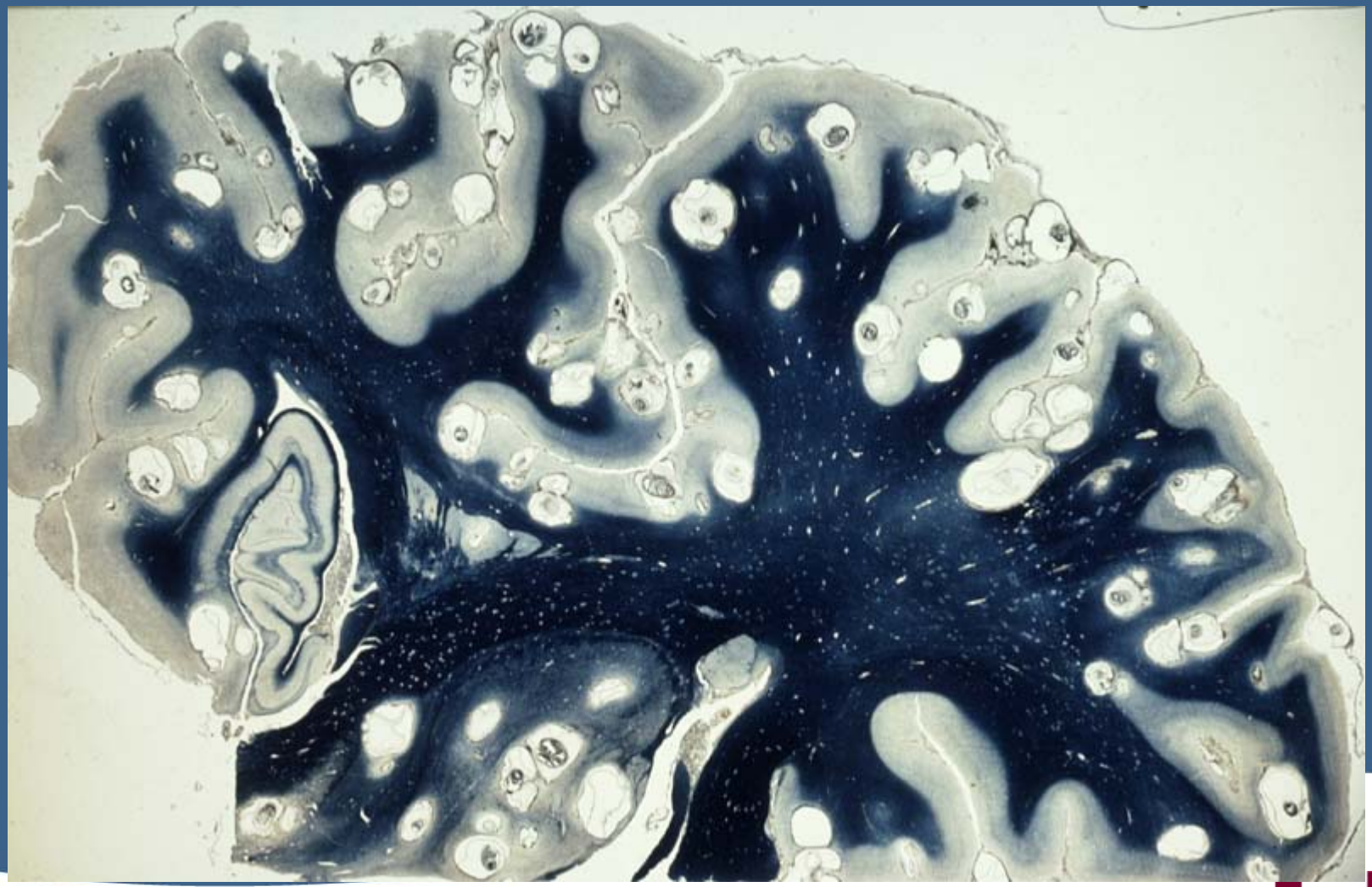


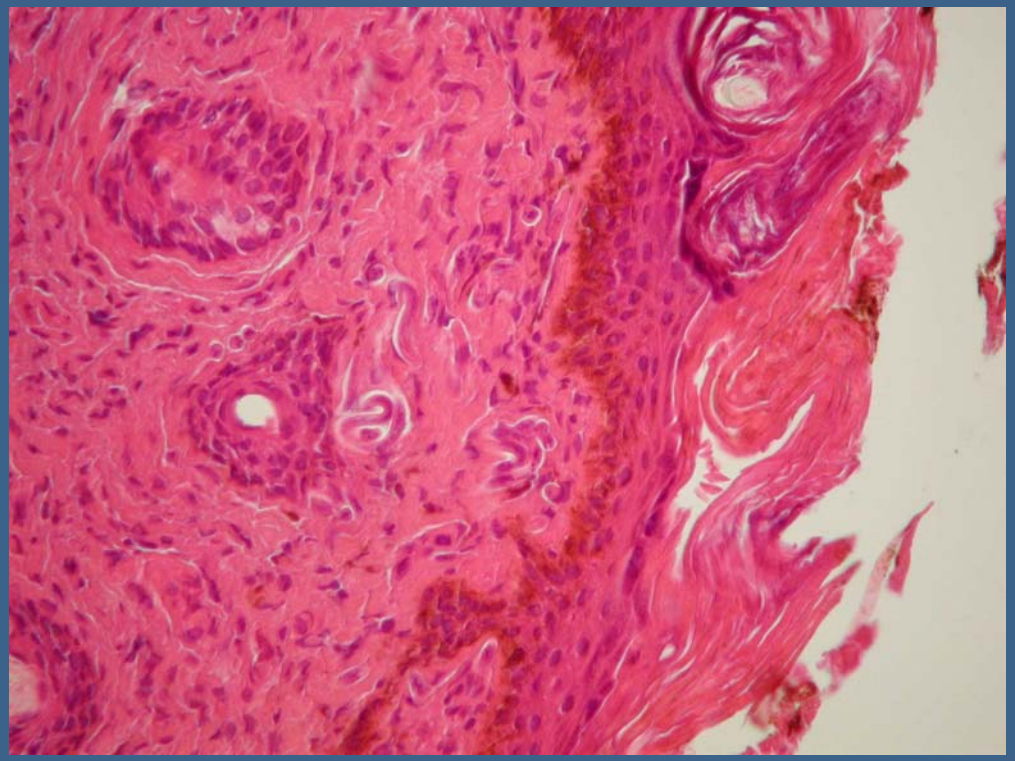


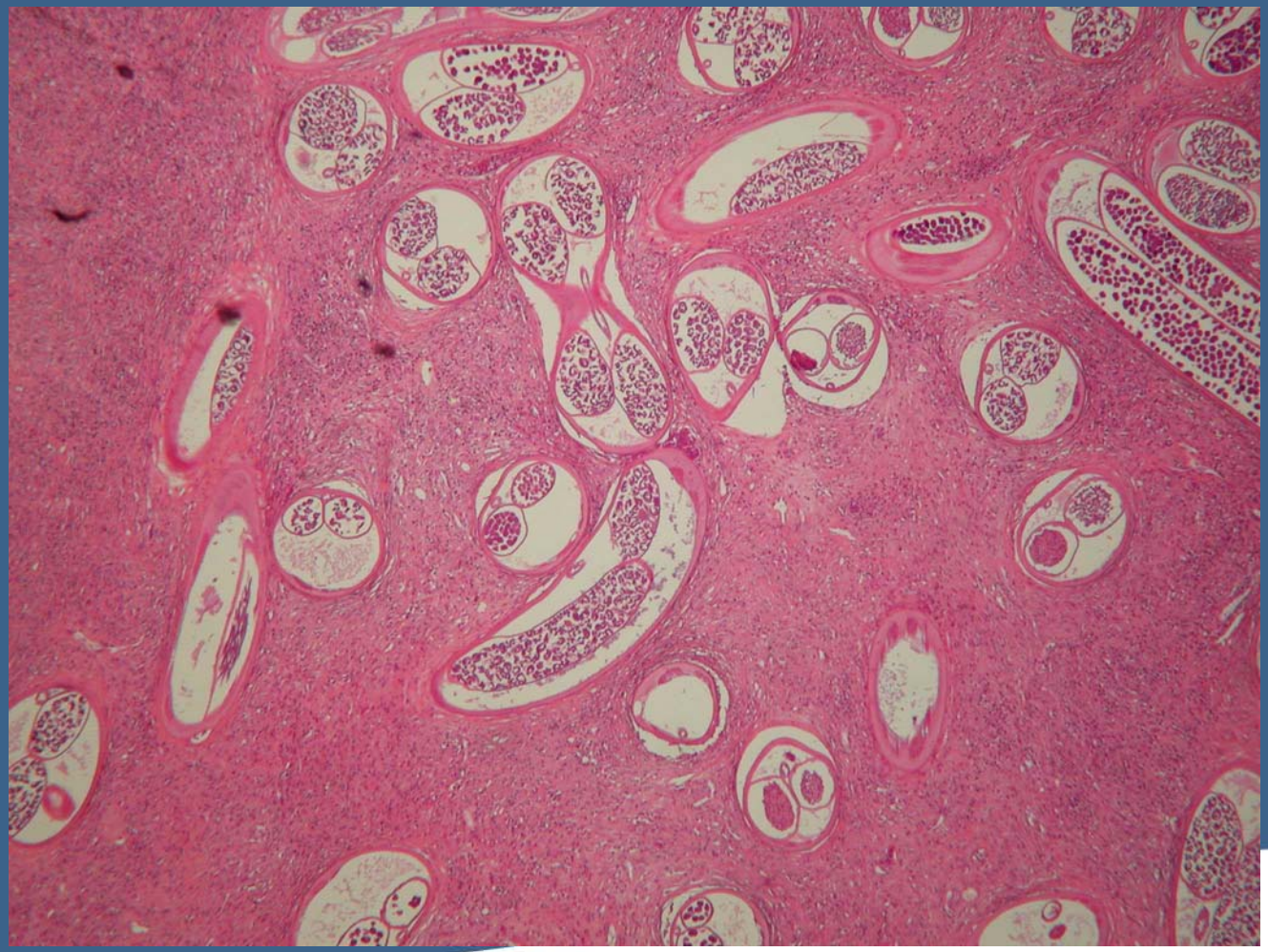


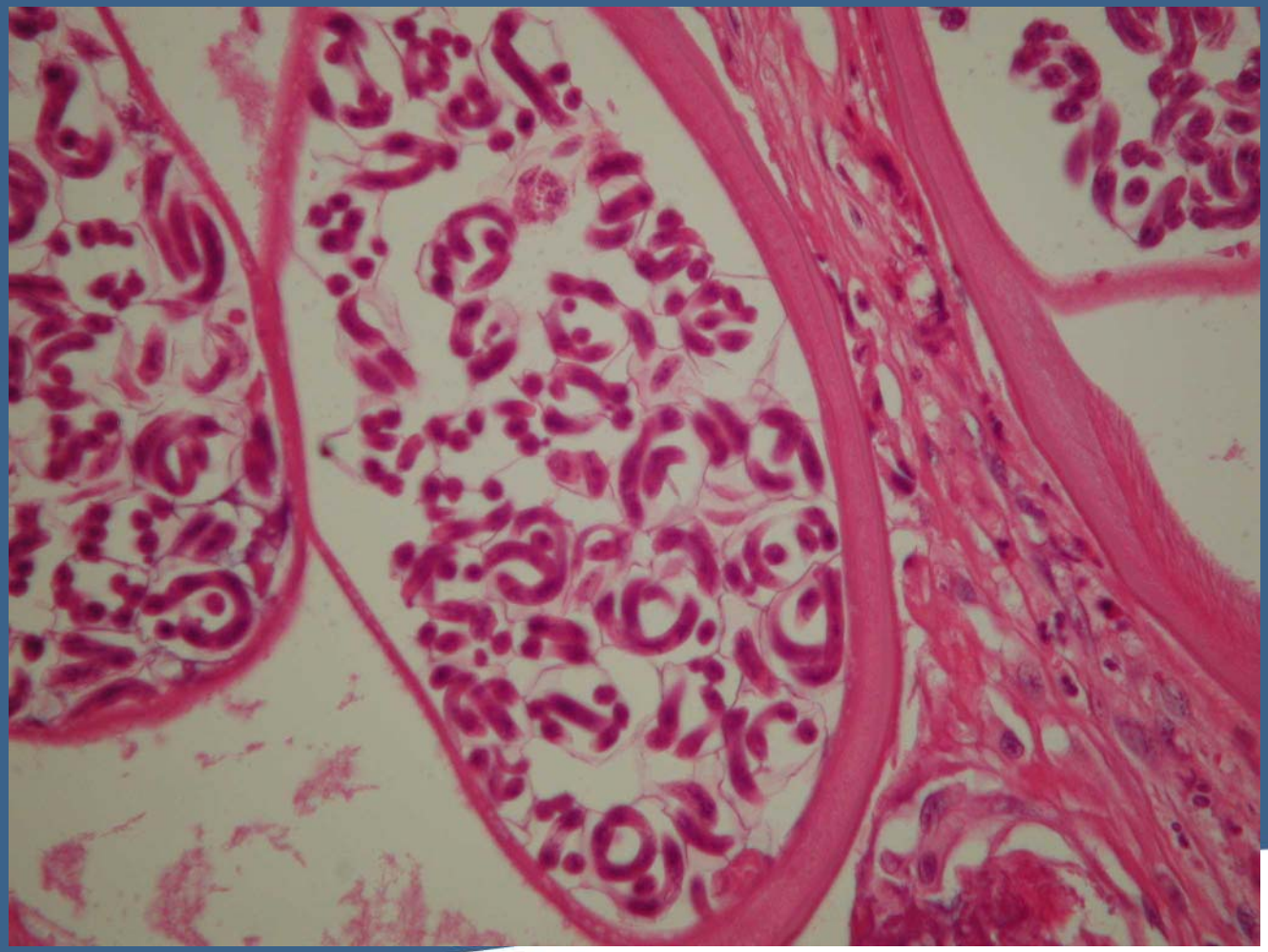
# Live metazoan parasites not leading to an inflammatory reaction (or to almost none)…

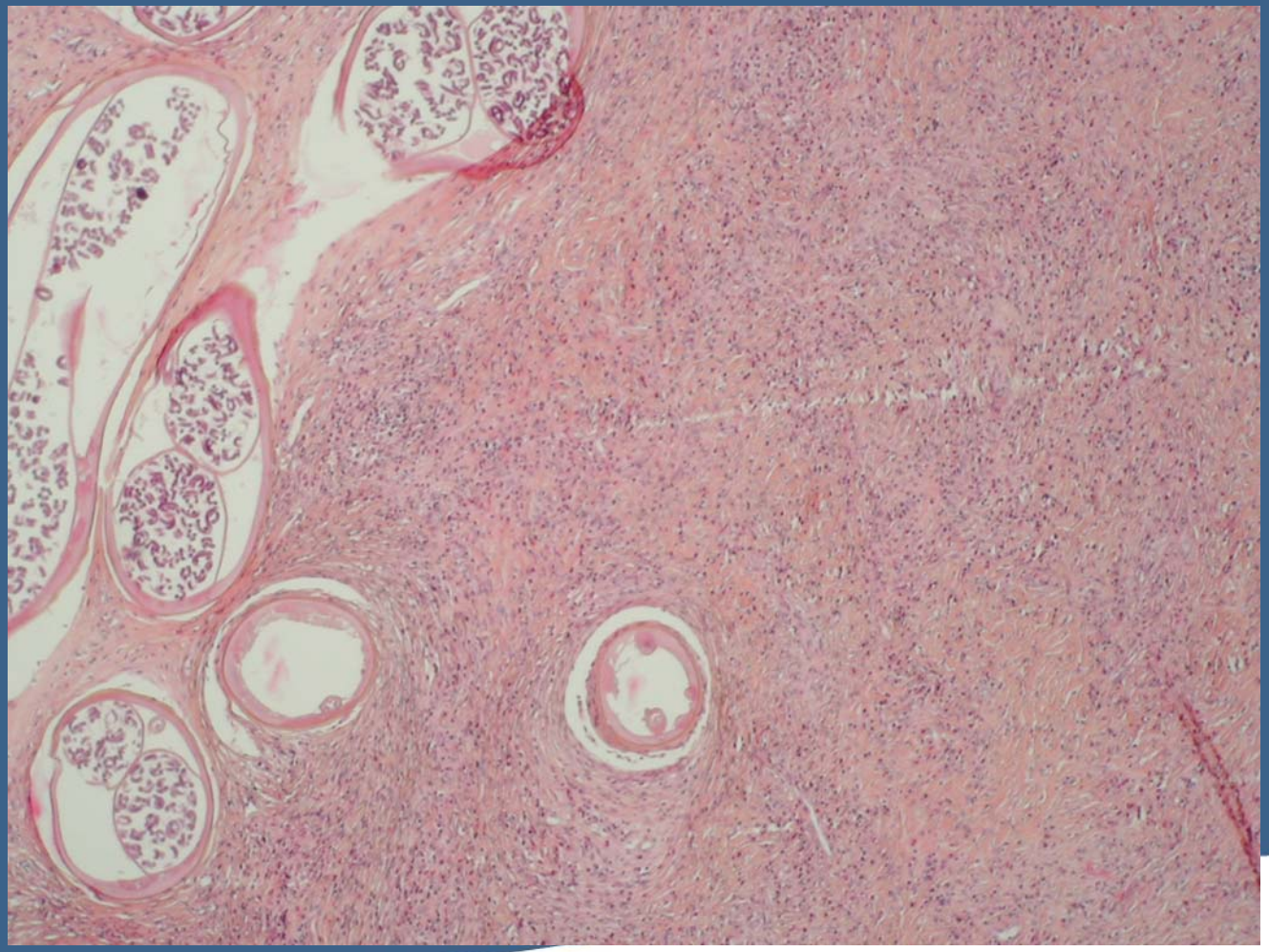


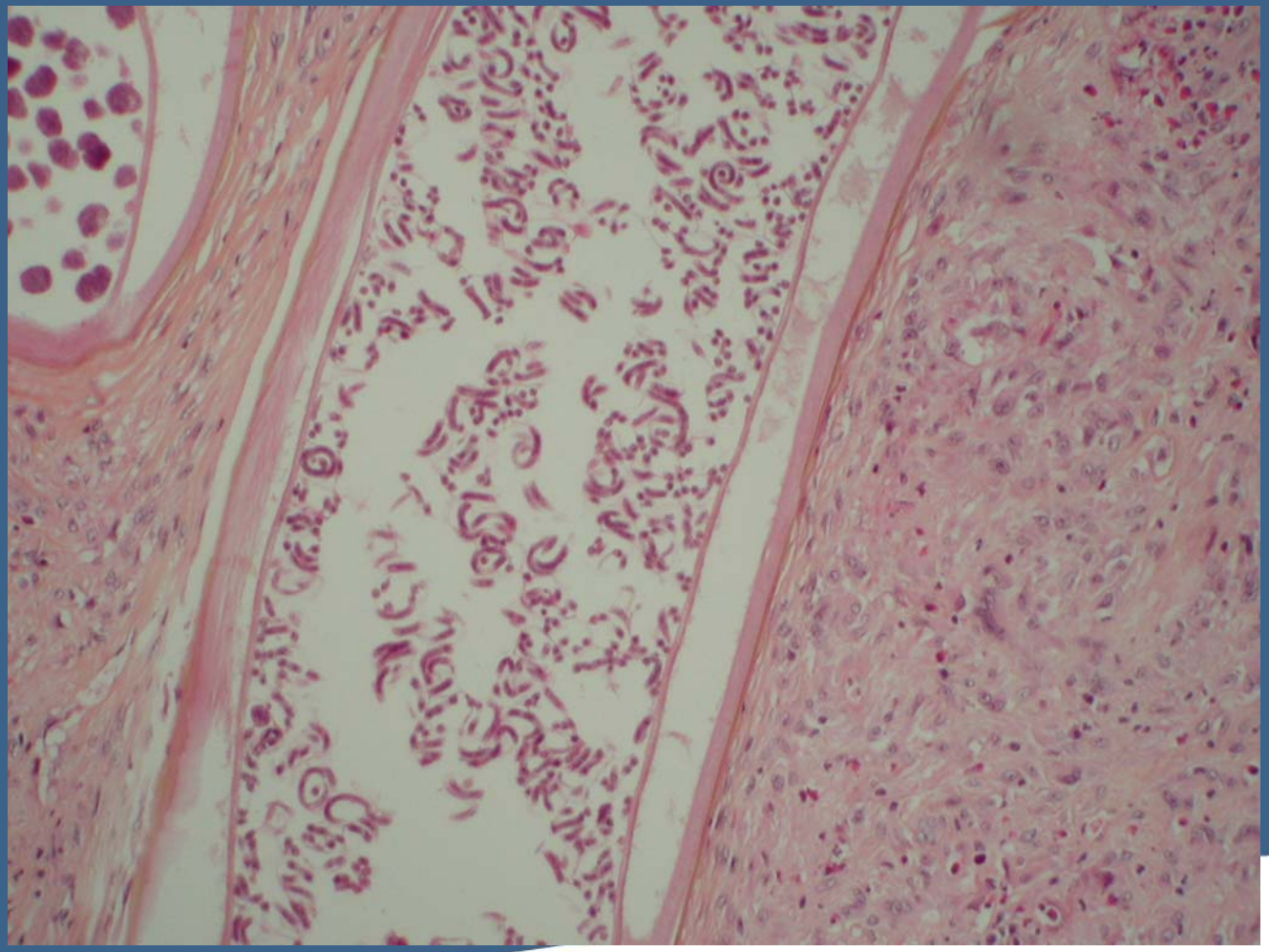


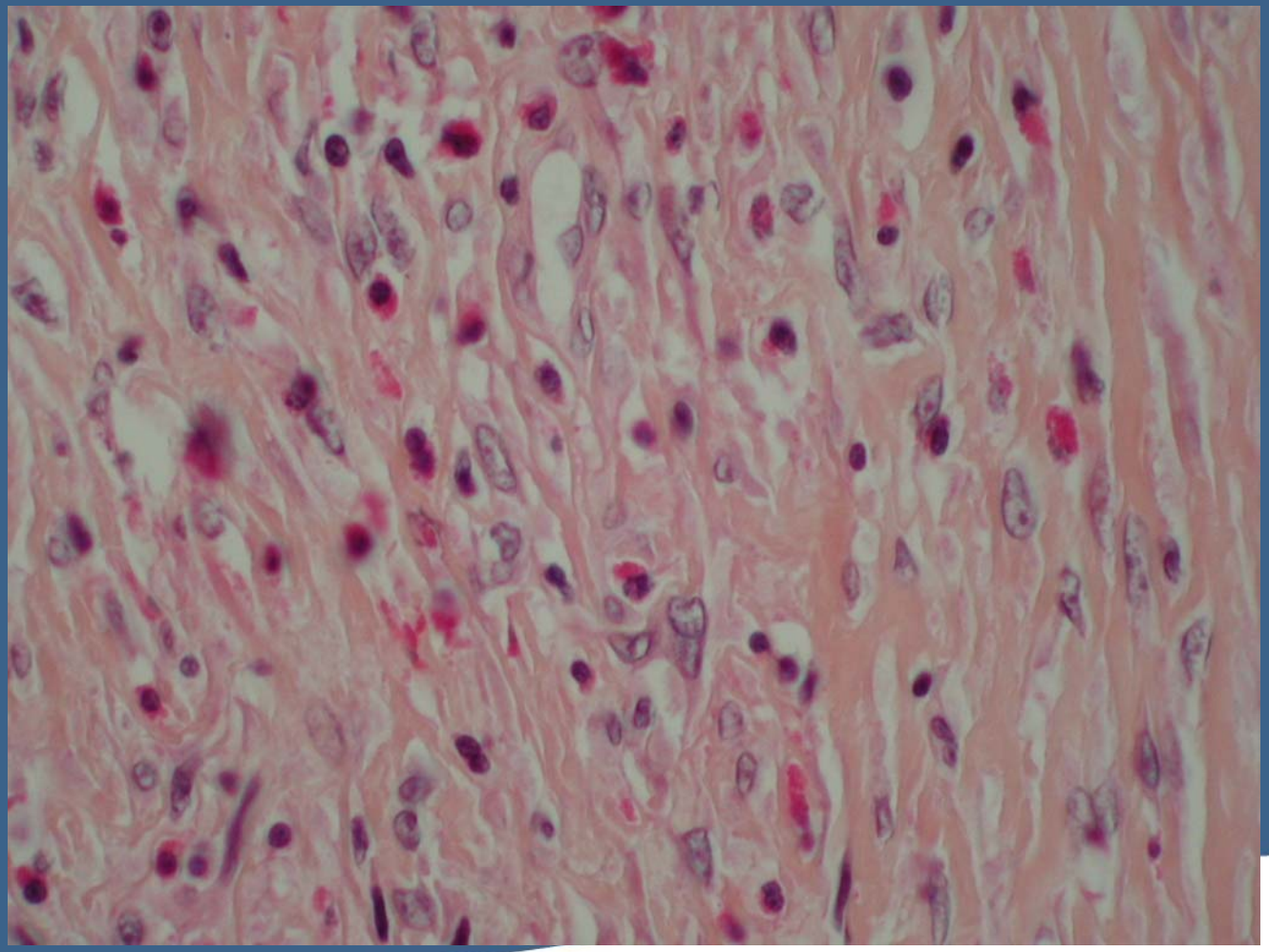


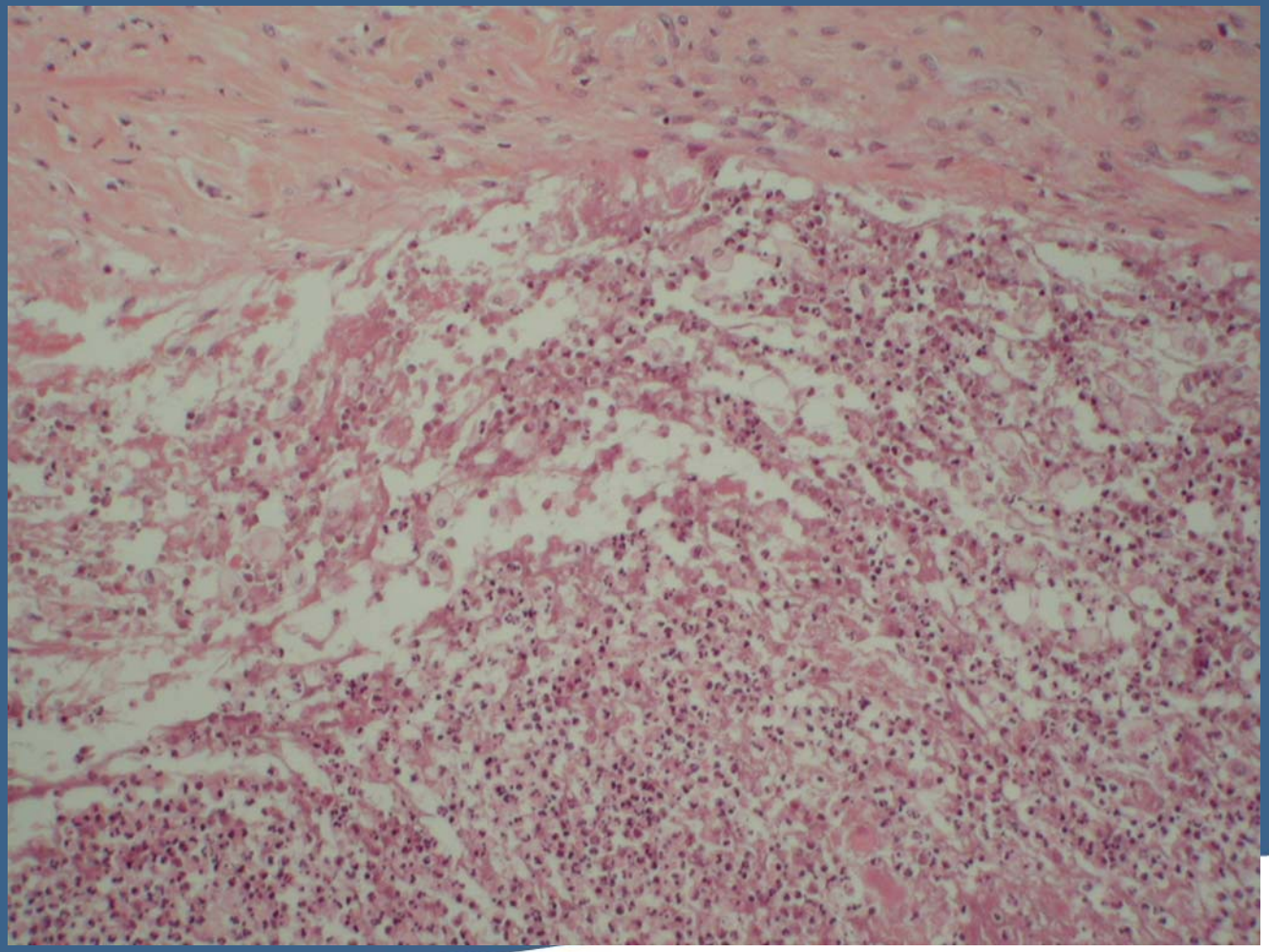




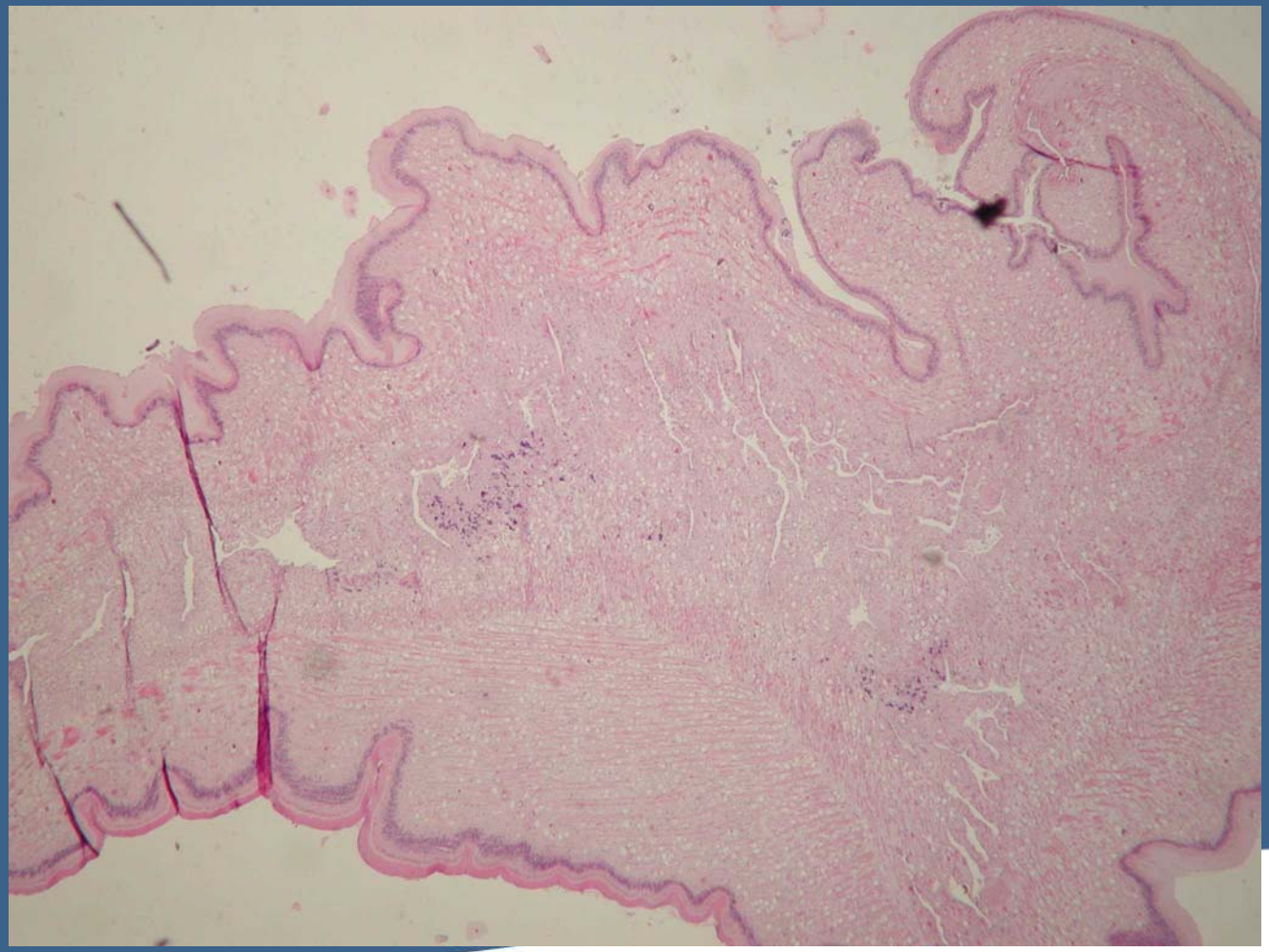


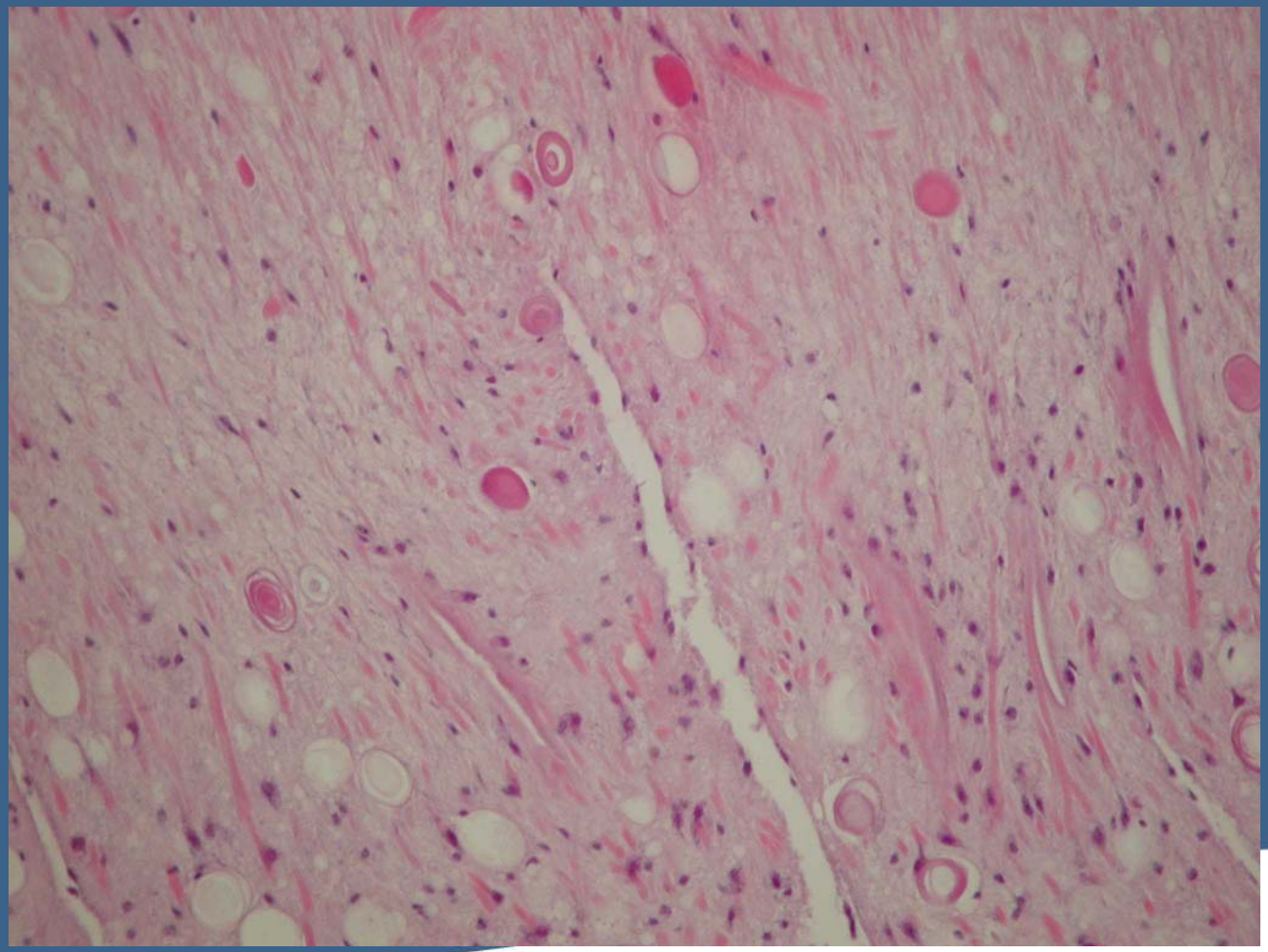


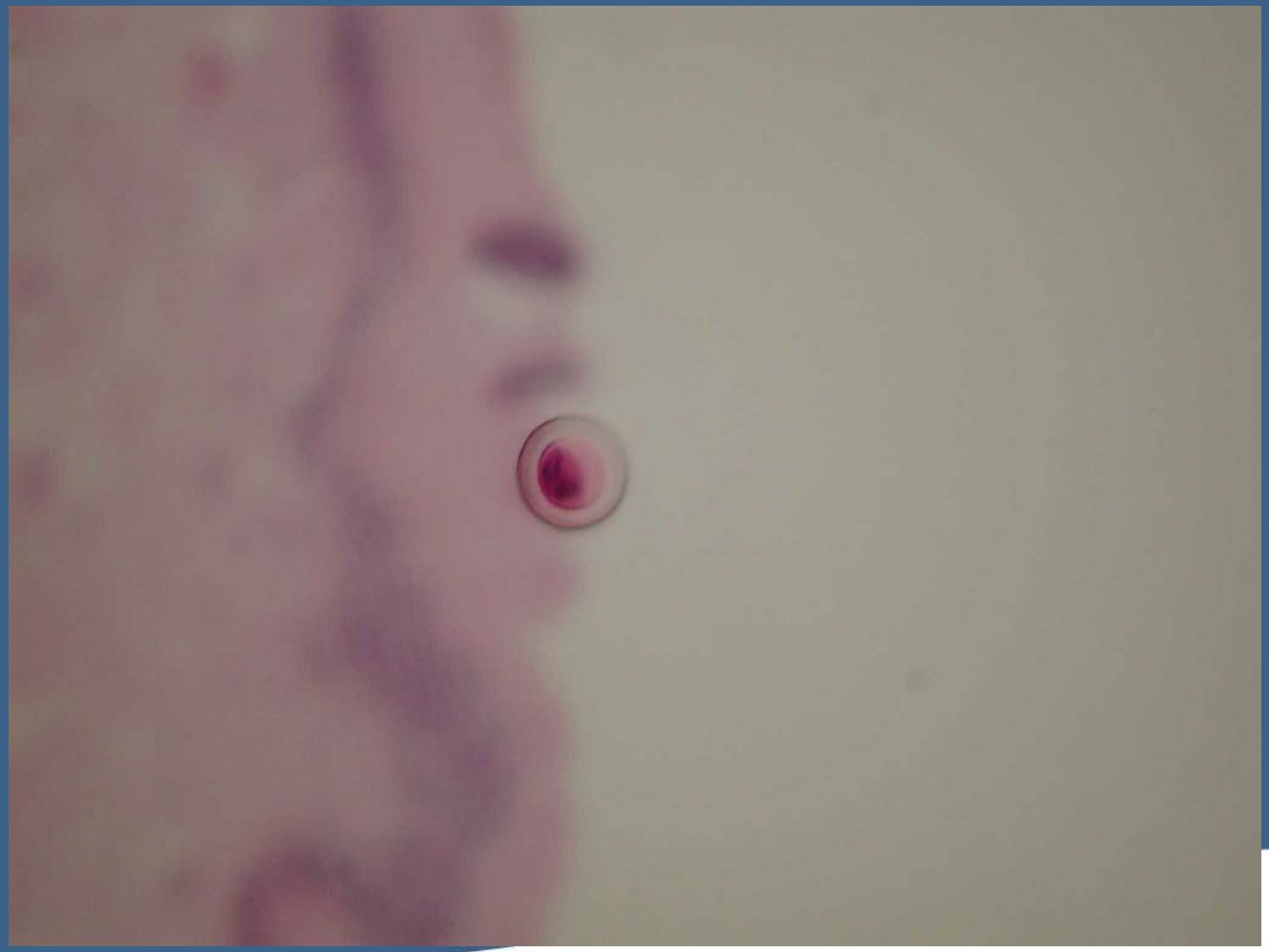




# Limitations of pathology in the identification of parasites







# Conclusions

- In conjunction with other disciplines, pathology is a valuable tool for the diagnosis of parasitic diseases