

Laboratorijska dijagnostika parazitarnih infekcija Zagreb, 11. i 12. lipanj 2026.

Organizatori:

Hrvatsko društvo za kliničku mikrobiologiju HLZ-a -Sekcija za parazitologiju

Nacionalni referentni laboratorij za humane parazite

Hrvatski zavod za javno zdravstvo

Knjiga sažetaka

Laboratorijska dijagnostika parazitarnih infekcija

Voditelj radionice:

- Izv.prof.dr.sc. Mario Sviben, prim.dr.med.

Suradnici na radionici:

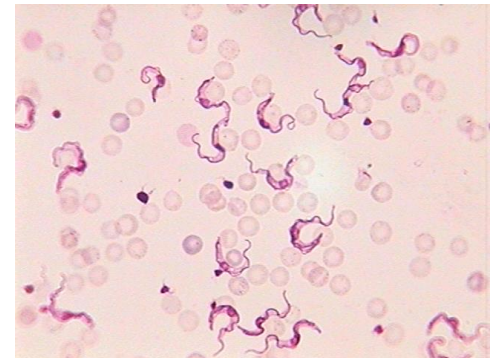
- Djelatnici Odjela za parazitologiju HZJZ-a i Katedre za mikrobiologiju i parazitologiju Medicinskog fakulteta Sveučilišta u Zagrebu



Epidemiologija i značaj parazitarnih infekcija
Principi pravilnog skupljanja, transporta i obrade uzoraka za
parazitološku dijagnostiku
Reagensi, bojenja i mediji u parazitologiji

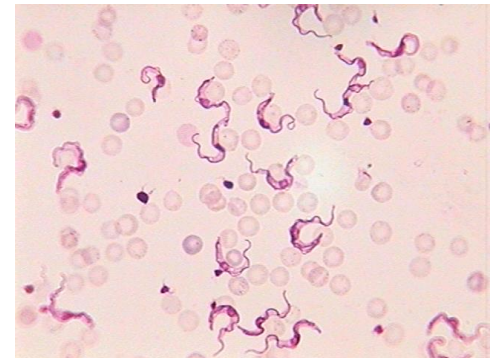
Paraziti

- Parazit - organizam čiji život u potpunosti ovisi o drugom živom biću (domaćinu)
- Parazitizam - biološka asocijacija u kojoj parazit ima koristi, a domaćin biva iskorišten
- Obligatni parazit - za održanje života mora biti uvijek u kontaktu sa svojim domaćinom
- Slobodno-živući parazit - može postojati i samostalno



Paraziti

- Domaćin - konačni ili prijelazni
- Konačni domaćin - spolno razmnožavanje parazita, najrazvijeniji oblik parazita
- Prijelazni domaćin - nespolni ili larvalni oblici parazita
- Neki paraziti za svoj kompletni razvoj trebaju jednog, a neki i više prijelaznih domaćina



Paraziti

Jednostanični

- Protozoi
- Mikrosporidije

Višestanični

Helminti - crvi

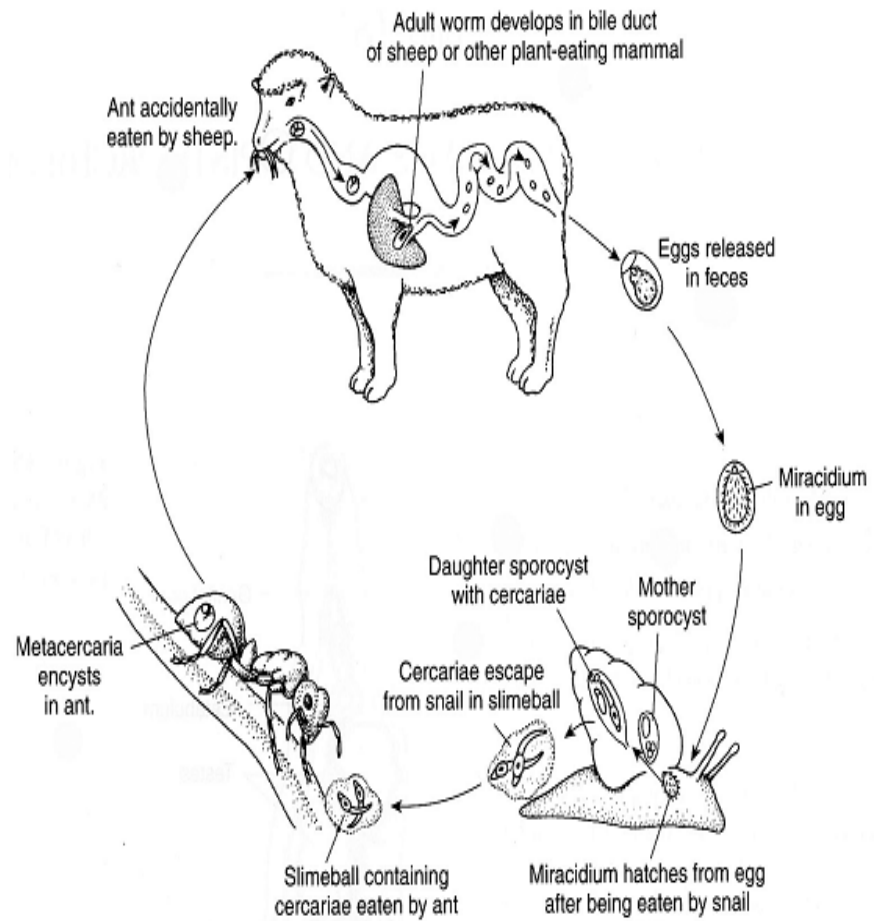
- Valjkasti crvi
 - Plosnati crvi
metilji i
trakavice
- Člankonošci

Humana parazitologija

- 200 vrsta helminta
- 80 vrsta jednostaničnih parazita
- Artropoda - ?

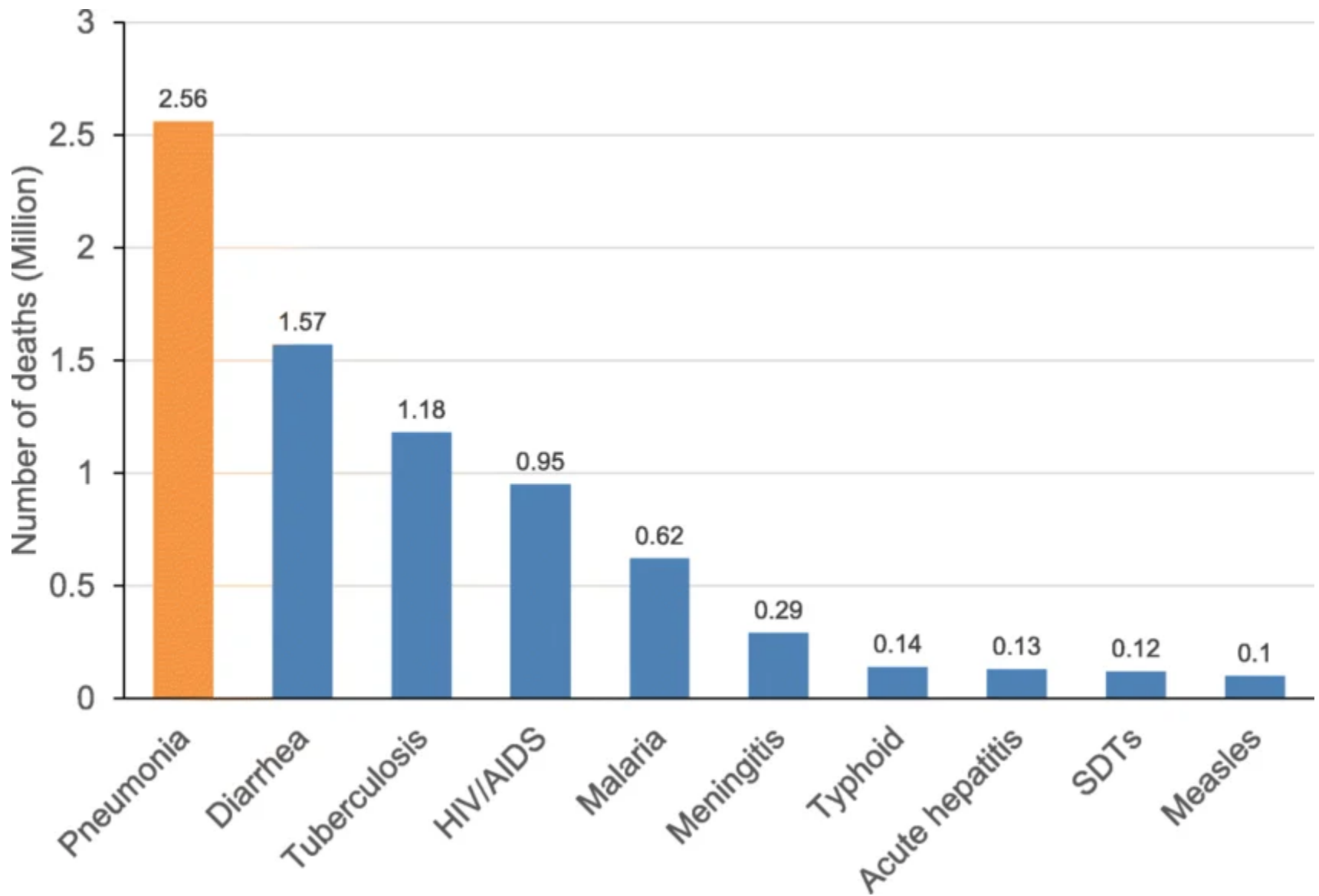


Životni ciklus



Učestalost

Askarioza	1,38 milijardi
Trihurioza	1 milijarda
Ankilostomoza i nekatoroza	1,25 milijardi
Strongiloidoza	100 milijuna
Tenioza	60 milijuna
Amebijaza	50 milijuna
Kriptosporidioza	10 % proljeva infektivne etiologije

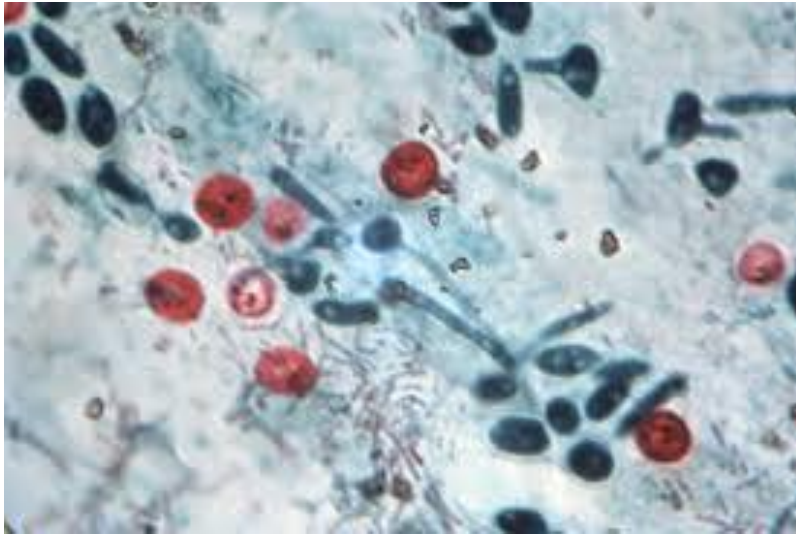


World Health Organisation. WHO Weekly Epidemiologic Record 2025.

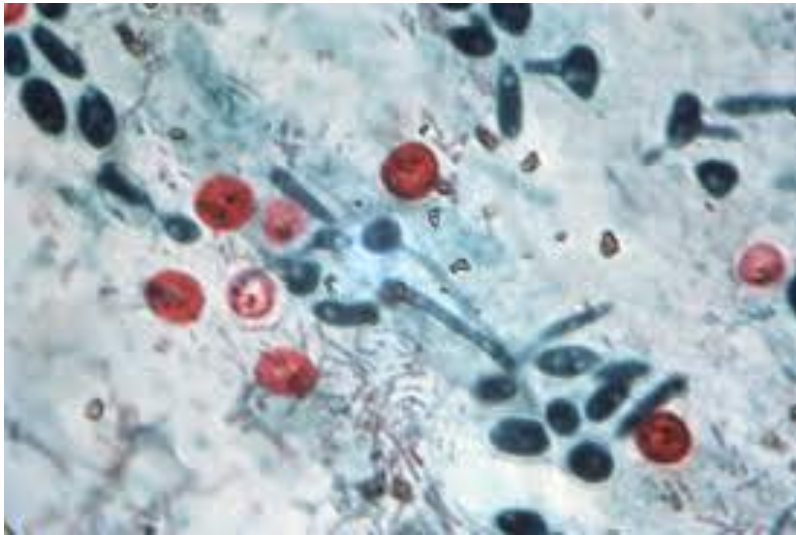
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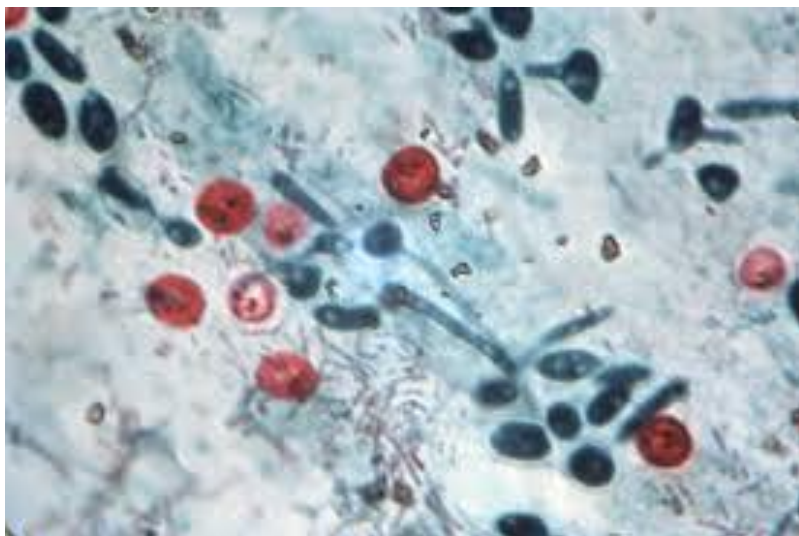
Prijenos preko kontaminirane vode



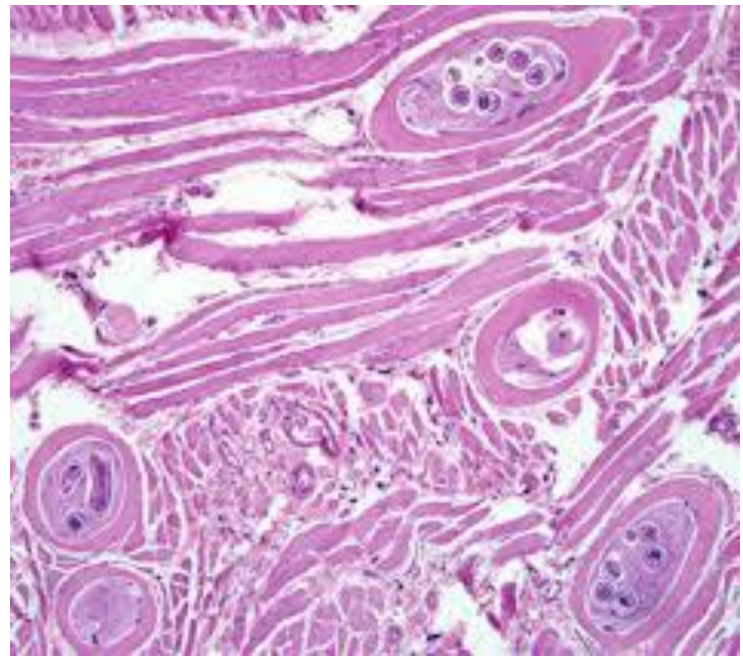
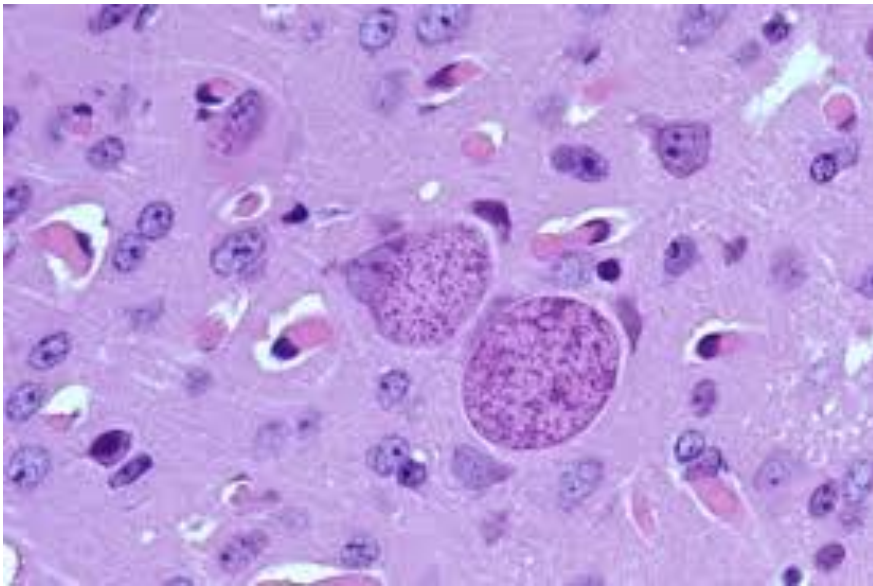
Prijenos preko kontaminirane vode



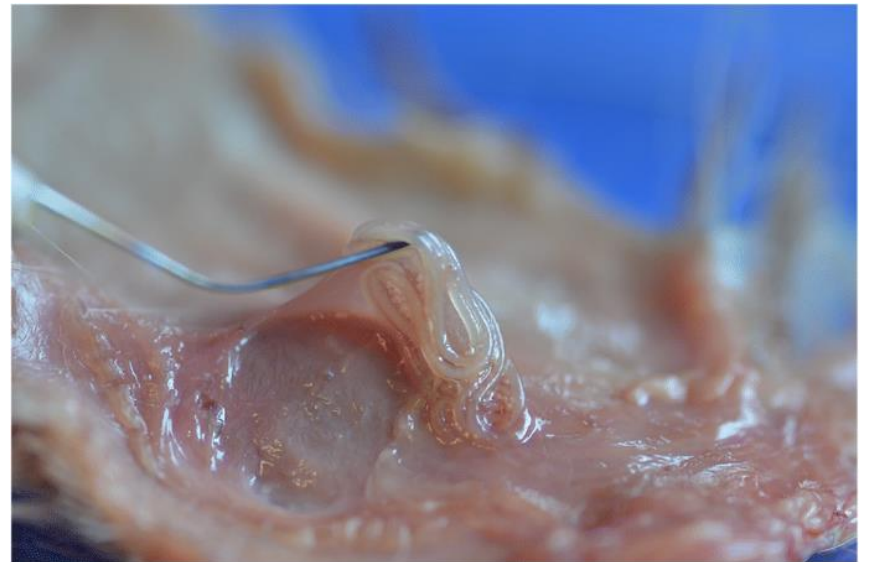
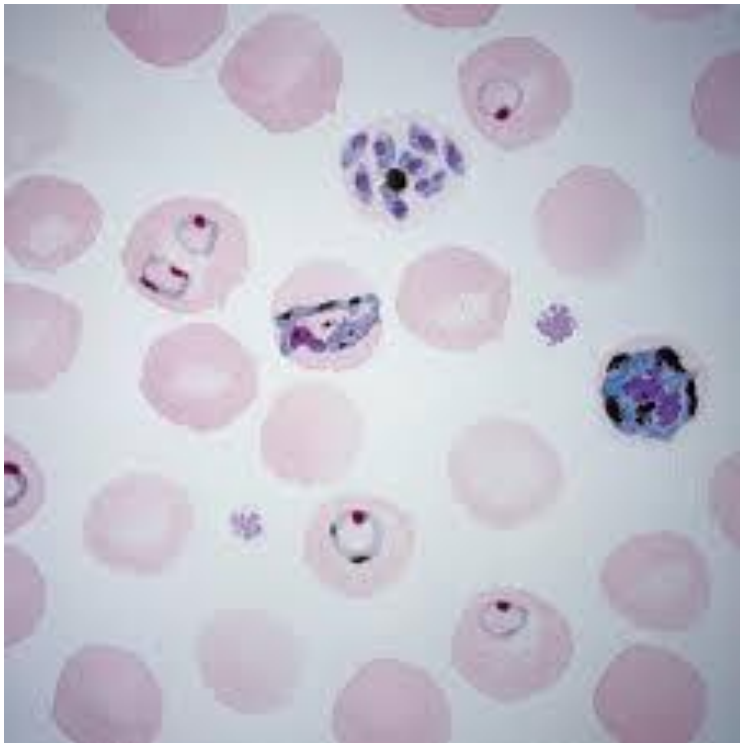
Prijenos preko kontaminirane vode



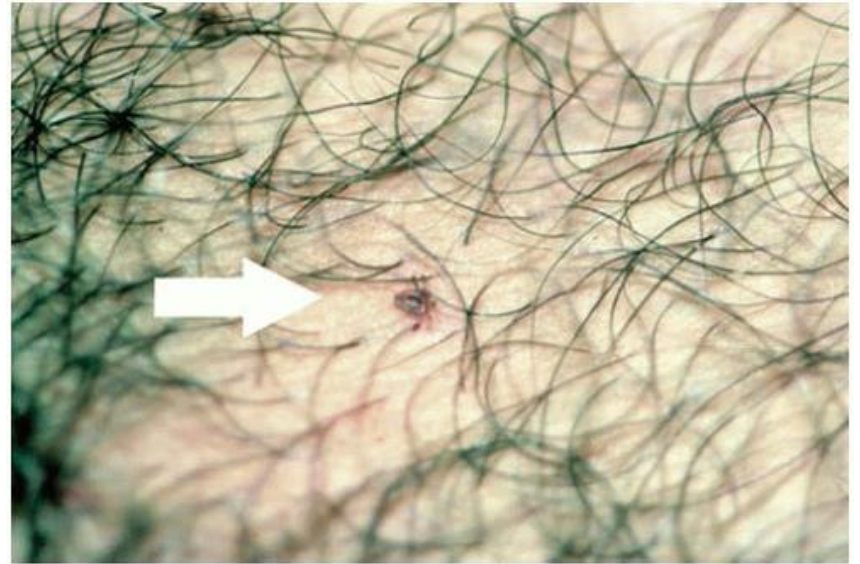
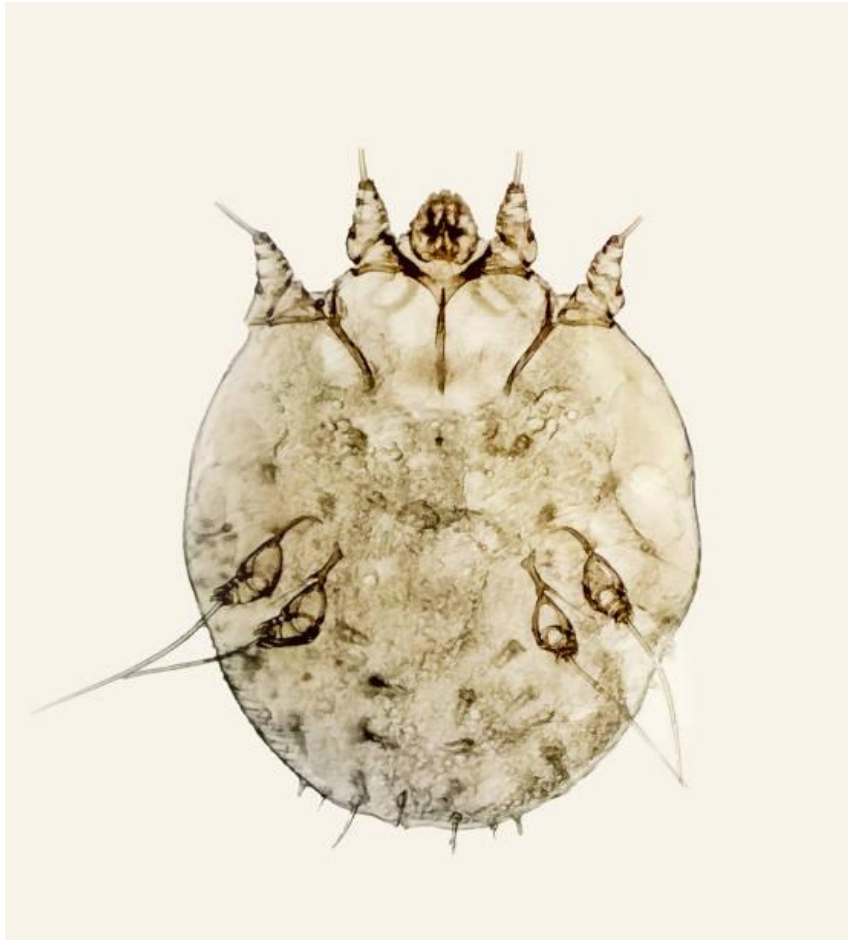
Prijenos preko kontaminirane hrane



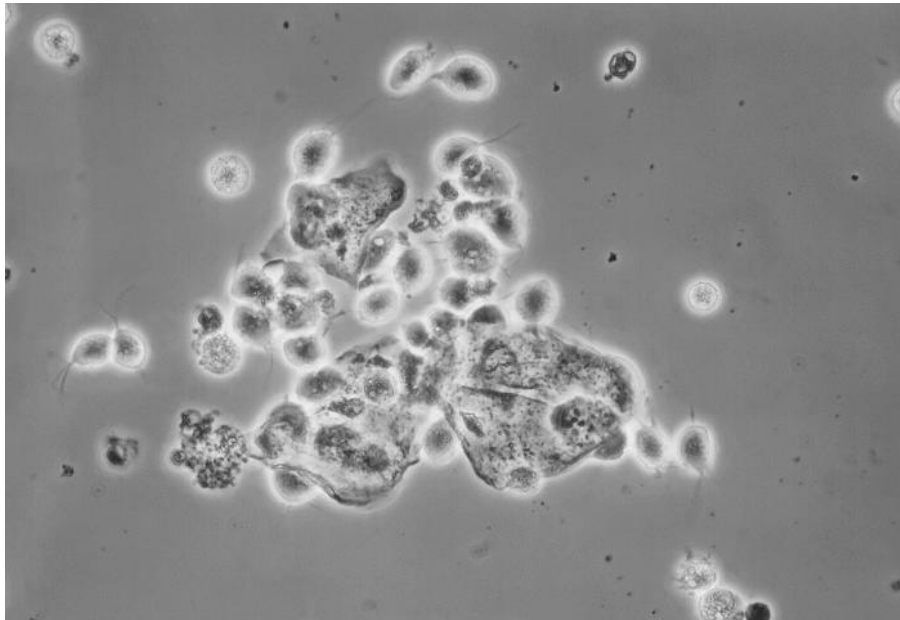
Prijenos preko člankonošca



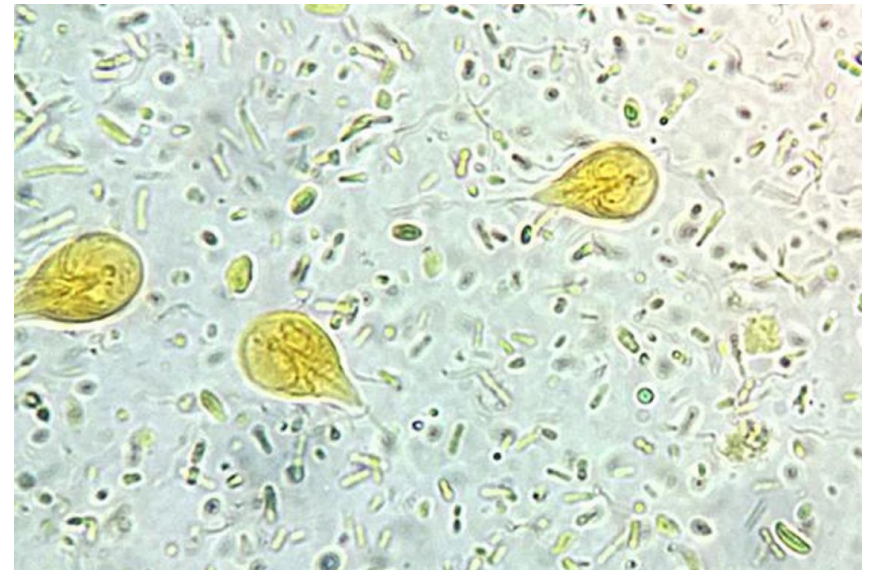
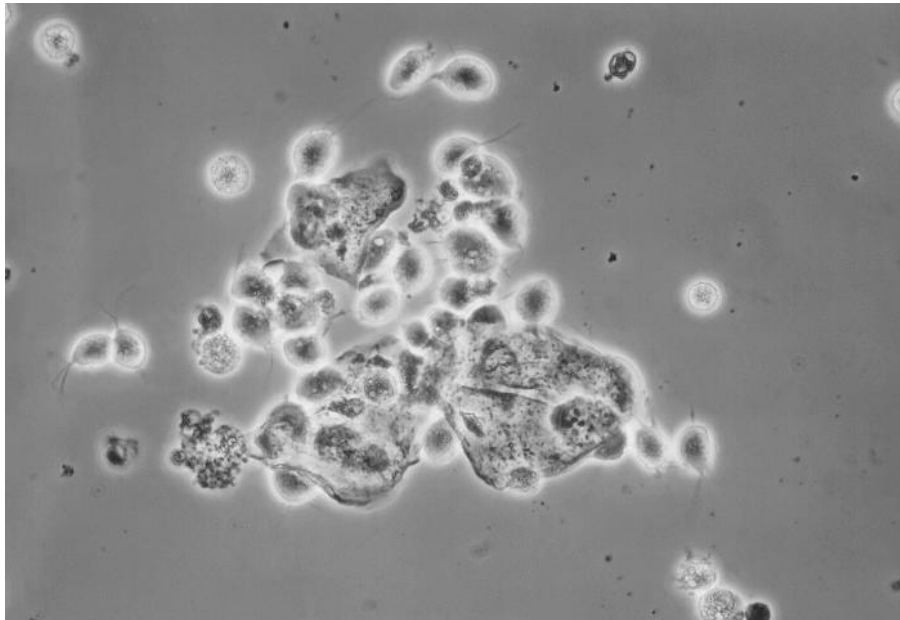
Direktni kontakt



STI infekcije



STI infekcije



Potential sexual transmission of *Giardia* in an endemic region: a case series

Angel A Escobedo ¹, Gustavo Acosta-Ballester ², Pedro Almirall ³, Alfonso J Rodriguez-Morales ⁴, Cecilia Ortíz ⁵, Alfredo Laffita ⁶, Elaine Chirino ⁷

Affiliations + expand

PMID: 29932093

Free article

Abstract

We present four cases in which probable sexual transmission of *Giardia lamblia* was suspected. Diagnosing this mode of transmission in endemic areas is often difficult and should be considered only as possible, because exposure to poor sanitation and a potentially contaminated environment are always latent. However, as patients reported, there was no history of drinking tap water, exposure to recreational water, eating contaminated food, or other potential sources of infection but anilingus with an infected partner. We consider that in endemic countries, even when other more frequent modes of transmission could be playing the main role, the possibility of (re)infection due to sexual transmission should not be forgotten. Talking openly with patients, strengthening patient-specific preventive measures and counselling appear to be needed to reduce risks of *Giardia* infection transmission due to this often neglected route.

Skupljanje uzoraka, transport i obrada

- Rutinska parazitološka dijagnostika – procedure za detekciju parazita u kliničkim uzorcima na osnovi morfoloških kriterija
- Svjetlosni mikroskop – mikroskopija nativnih, koncentriranih ili obojenih preparata
- Kultivacija, detekcija antigena, detekcija nukleinske kiseline, elektronska mikroskopija, serološka dijagnostika

Skupljanje uzoraka, transport i obrada

- Jedan od najznačajnijih preuvjeta za točnu i pouzdanu dijagnostiku – adekvatno sakupljen, obrađen i pregledan uzorak
- Uzorak – životni ciklus parazita!!!
- Laboratorij – striktni kriteriji za prijem i odbijanje uzoraka
- Uzorci – potencijalno infektivni – mjere predostrožnosti



Skupljanje uzoraka, transport i obrada

Stolica

- Barijeva kaša, mineralno ulje, bizmut, antibiotici, antimalarijski lijekovi – do nekoliko tjedana stolica negativna!
- Upozorenje: “Pojedini antibiotici (metronidazol, tetraciklini) mogu utjecati na detekciju parazita iz uzorka stolice”
- Posudica – čista, širokog grla, adekvatnog poklopca, bez vode ili urina

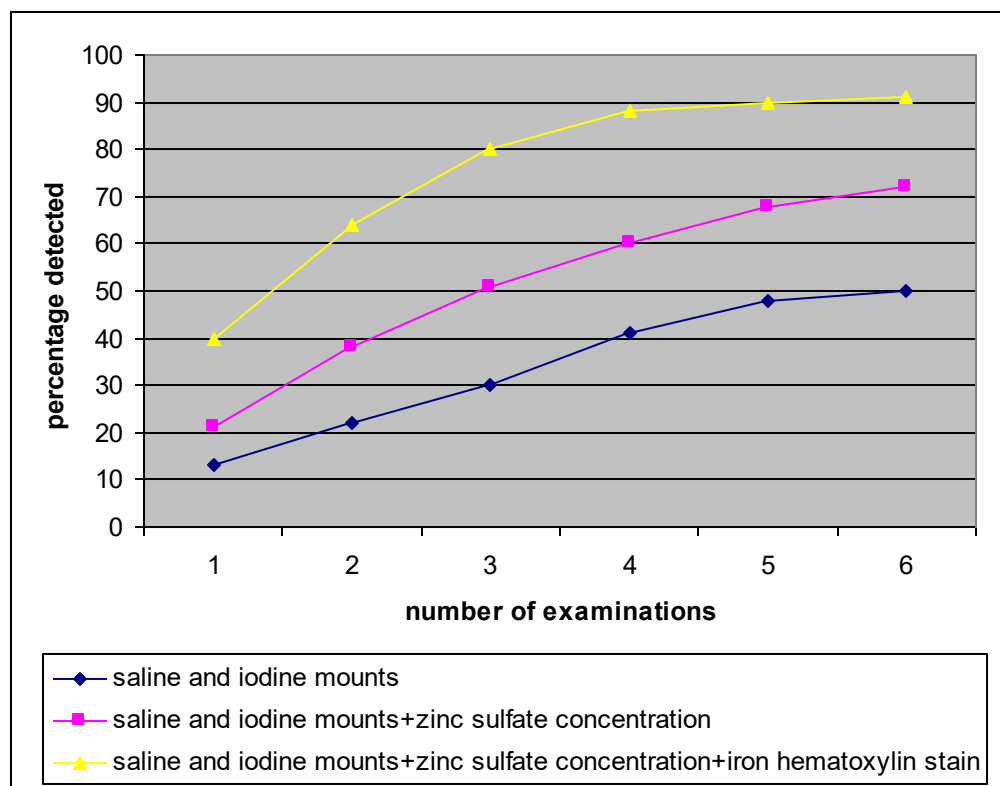


Skupljanje uzoraka, transport i obrada

Stolica

- Uzorak – ime i prezime, identifikacijski broj, ime liječnika, datum i vrijeme skupljanja uzorka
- Uputnica – naziv pretrage koja se traži, epidemiološki podaci (putovanja)

Skupljanje uzoraka, transport i obrada



Skupljanje uzoraka, transport i obrada

Stolica

- Preporuke
- Serija od tri uzorka stolice
- Svaki drugi dan, ne više od 10 dana
- Ne svaki dan!!!
- Nakon terapije
- Tri uzorka stolice
- 1-2 tjedna nakon završetka terapije kod infekcije protistima, 3-4 tjedana nakon završetka terapije helmintnih infekcija



Skupljanje uzoraka, transport i obrada

Stolica

- Svježa stolica, proljevasta stolica – trofozoiti
- Pregled unutar pola sata
- Jaja, ličinke, kokcidije, mikrosporidije – nisu jako osjetljivi – pregled unutar jednog dana - frižider
- Primjena fiksativa
- Detekcija antigena – pregled unutar 24 sata, ledenica spremanje više od jednog dana

Skupljanje uzoraka, transport i obrada

Stolica

- Fiksativi
- Čuvanje morfologije parazita i sprječavanje daljnjeg razvoja jaja i ličinki
- Direktno stavljanje stolice u bočicu sa fiksativom ili naknadno doljevanje fiksativa
- Adekvatno mješanje
- Interferencija – detekcija antigena, molekularne metode

Skupljanje uzoraka, transport i obrada

Stolica - fiksativi

- Formalin – 5, 10 %
- Neutralni puferirani formalin
- Zagrijani formalin 60 stupnjeva – Taenia
- SAF
- Shaudinn
- PVA

Skupljanje uzoraka, transport i obrada

Krv

- Plasmodium, Babesia, Trypanosoma, Leishmania, Toxoplasma, mikrofilarije
- Puna krv, buffy coat, koncentрати, krvni razmazi, gusta kap
- Razmaz, gusta kap – svježa krv, krv s antikoagulansom (EDTA), koncentрати
- Vrijeme uzimanja krvi!!!
- Hitne pretrage – javljanje direktno kliničaru svakog pozitivnog nalaza



Skupljanje uzoraka, transport i obrada

Krv

- Imunodijagnostika - detekcija protutijela
- Serum – spremanje do tjedan dana na $+4^{\circ}\text{C}$ ili kod dužeg spremanja na -20°C
- Transport seruma na temperaturi okoline (put < 1 tjedan; temperatura okoline < 30°C)
- Ako je nužna usporedba dva seruma – istovremeno testiranje oba seruma
- Testiranje likvora/očne vodice + seruma – uzorci uzeti isti dan i testirani isti dan

Skupljanje uzoraka, transport i obrada

Ostali uzorci

- Duodenalni sok
- Sputum
- Bioptati
- Punktati
- Amnionska tekućina
- Uzorci urogenitalnog sustava

Reagensi, mediji i bojenja

- Kultivacija – ograničeno primjenljiva u parazitologiji
- Mikroskopija - nezamjenjiva
- Nativni preparat – živi organizam
- Priprema, bojenje preparata, čuvanje – reagensi, mediji, boje
- Laboratorij – odabir, dobra laboratorijska praksa, tržište
- Toksičnost (formalin, živini spojevi, fenol, ksilen)

Reagensi, mediji i bojenja

Formalin

- Česta upotreba (čuvanje, za koncentracijske metode)
- Dobro čuva morfologiju jaja, ličinki, cisti, oocisti i spora
- Dugo čuvanje moguće
- 5% protisti, 10% jaja

Rcp (10%)

- Formaldehid 37-40%100 ml
- 0,85% NaCL ili destilirana voda.. 900 ml

Reagensi, mediji i bojenja

Puferirani formaldehid

- Za dugotrajno spremanje i čuvanje morfologije organizama
- Sadrži fosfatne soli natrija i kalija
- Rcp
- Na_2HPO_46.1 g
- KH_2PO_40,15 g
- 0,8 g ad 1 L 10% ili 5% formalina

Reagensi, mediji i bojenja

Merthiolate-jod-formalin (MIF)

- Fiksacija
- Bojenje
- Dvije otopine – svježa otopina
- Komercijalno dostupan

Reagensi, mediji i bojenja

PVA

- Adheziv – omogućuje stolici da prione za predmetno stakalce – izrada preparata

Schaudinn otopina/fiksativ

- Sadrži živu, izbjegava se

Sodium acetate-acetic acid-formalin (SAF)

- Fiksativ, ne sadrži živu
- Za koncentracijske metode i za pripremu trajno bojenih preparata
- Komercijalno dostupan
- Rcp

Natrij acetat.....1,5 g

Ledena octena kiselina.....2.0 ml

Formaldehid (37-40%).....4.0 ml

Destilirana voda.....92.0 ml

Reagensi, mediji i bojenja

Otopina joda

- Priprema preparata – dodatak nativnom preparatu, koncentracijske metode
- Nespecifična boja, diferencija parazita od okolnih struktura (ciste, jaja i ličinke zadržavaju boju i postaju svjetlo smeđi)
- Na tamnom, ograničen rok trajanja (posvijetli stajanjem)
- Rcp – 1 kap na stakalce s fecesom, lagano pomješati, pokrovnica

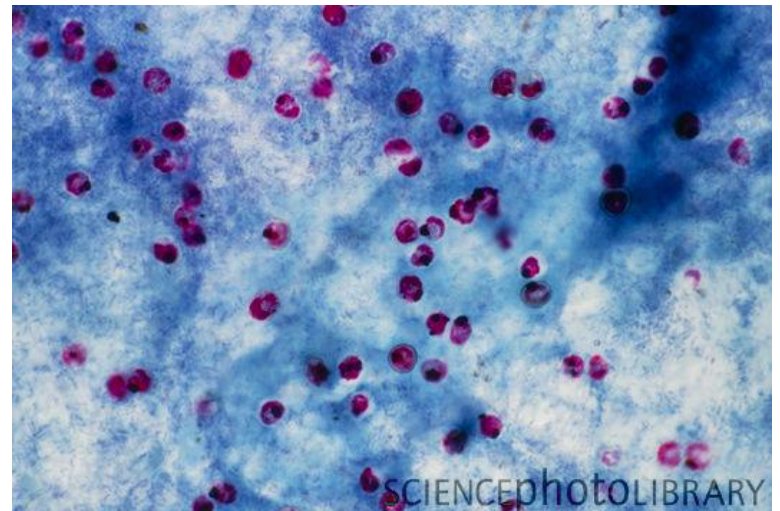
Reagensi, mediji i bojenja

Acid fast stain

- Cryptosporidium, Isospora, Cyclospora

Modificirana Ziehl Neelsen metoda

- “Deblji preparat”, sušenje
- Metanol fiksacija -1 min
- Karbol fuksin – 15-20 min
- 3% HCL u alkoholu 10-15 sek.
- Ispiranje vodom
- Metilensko modrilo – 3 min
- Ispiranje vodom

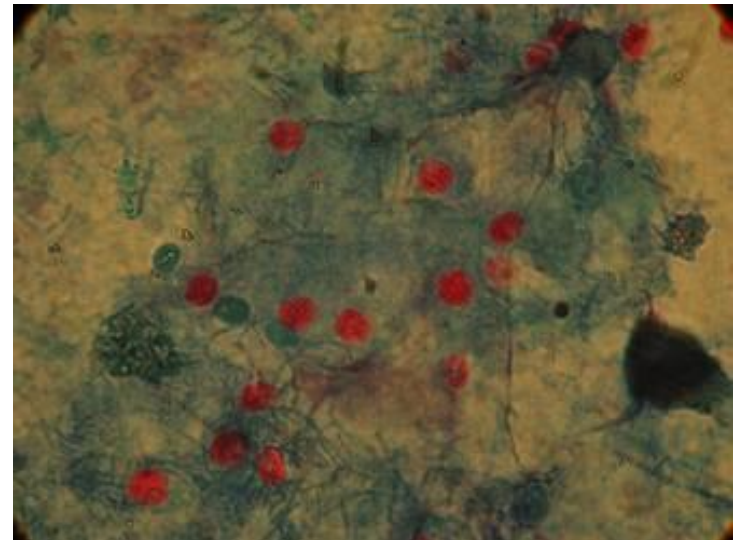


Reagensi, mediji i bojenja

Acid fast stain

Kinyoun

- 1-2 kapi uzorka stolice
- Sušenje, fiksacija metanolom 1 min
- Karbol fuksin 5 min
- 50% etanol ispiranje, voda
- H₂SO₄ 1% 2 min
- Metilensko modrilo 1 min
- Ispiranje voda



Reagensi, mediji i bojenja

Bojenje krvno tkivnih preparata

Malaria, mikrofilarije, Leishmania, babesia, Trypanosoma

- Giemsa, Wright
- Giemsa ne sadrži fiksativ
- Giemsa superiornija za gustu kap

Postupak – krvni razmaz

- Metanol – 1 min
- Otopina Giemse -30-45 min
- Ispiranje vodom



Reagensi, mediji i bojenja

Bojenje krvno tkivnih preparata

- Bojenje hematoksilinom (Delafield)
- Mikrofilarije – bolje prikazuje detalje ovojnice



Reagensi, mediji i bojenja

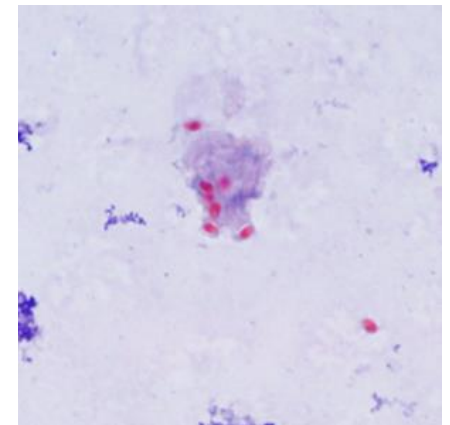
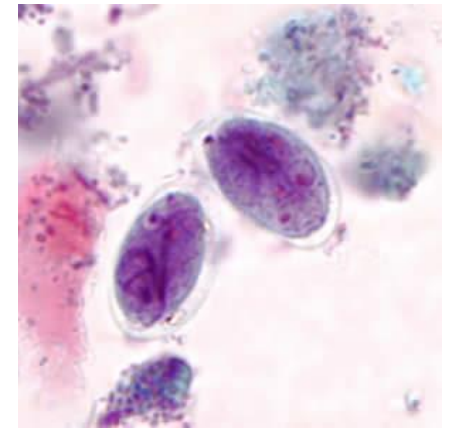
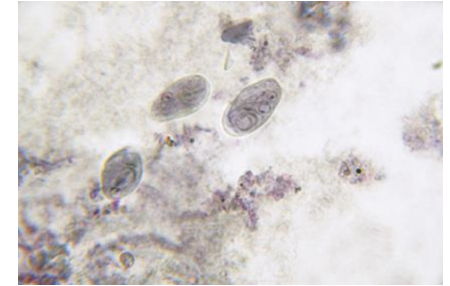
Željezni hematoksilin

Trikrom bojenje

- Za protiste probavnog sustava

Modificirani trikrom

- Za mikrosporidije



Reagensi, mediji i bojenja

Mediji

- Acanthamoeba
- Naegleria
- Amebe probavnog sustava
- Trichomonas
- Leishmania
- Trypanosoma

Algoritmi za detekciju i identifikaciju parazita

Dijagnostičke metode u parazitologiji

Algoritmi za detekciju i identifikaciju parazita

Dijagnostičke metode u parazitologiji

- Dijagnostičke metode

Direktne

- Makroskopija
- Mikroskopija
- Kultivacija
- Detekcija antigena
- Molekularna dijagnostika

Indirektne

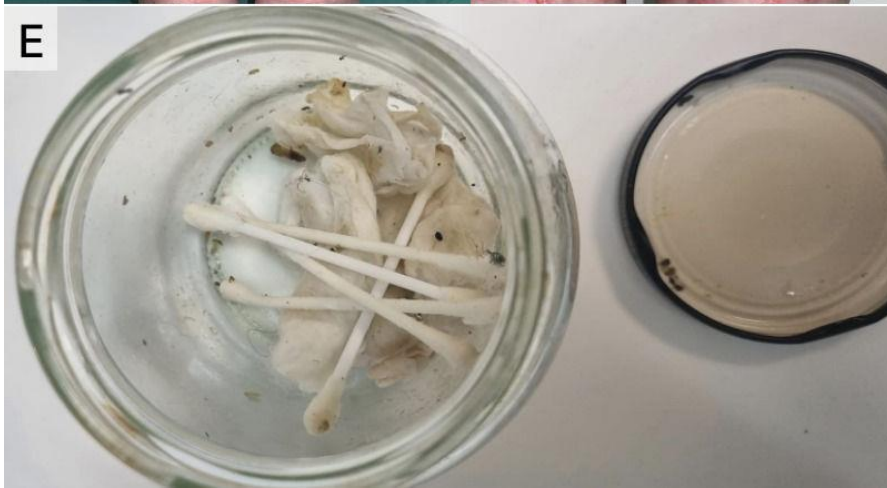
- Serologija

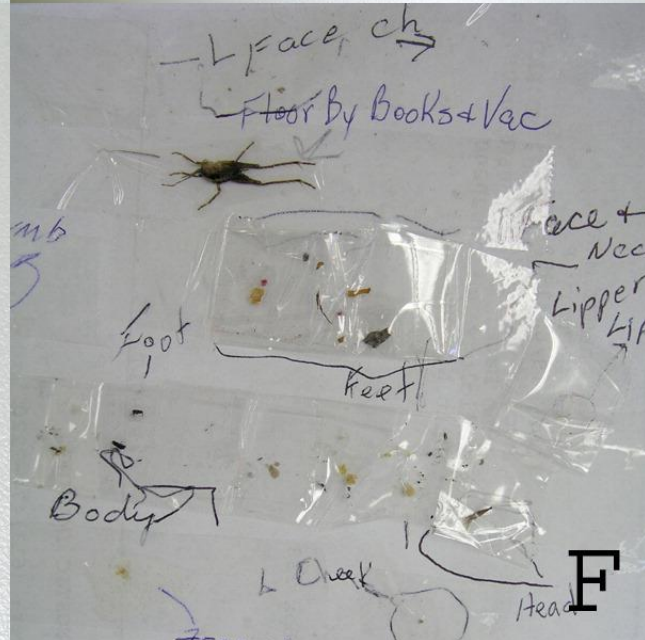
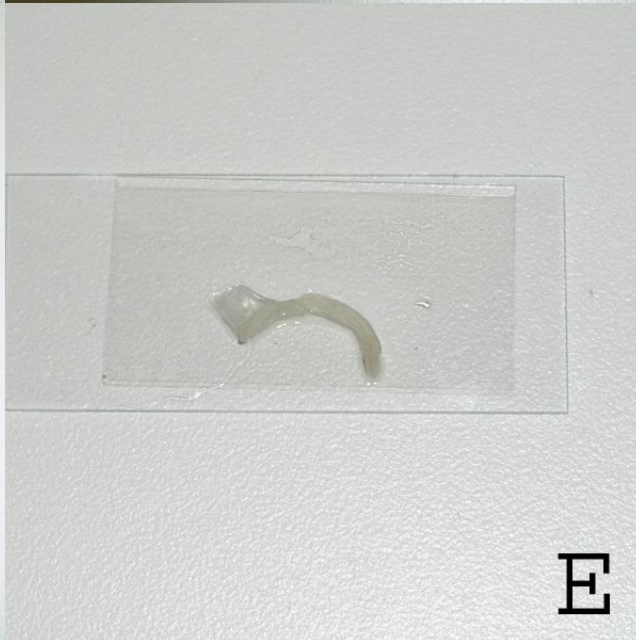
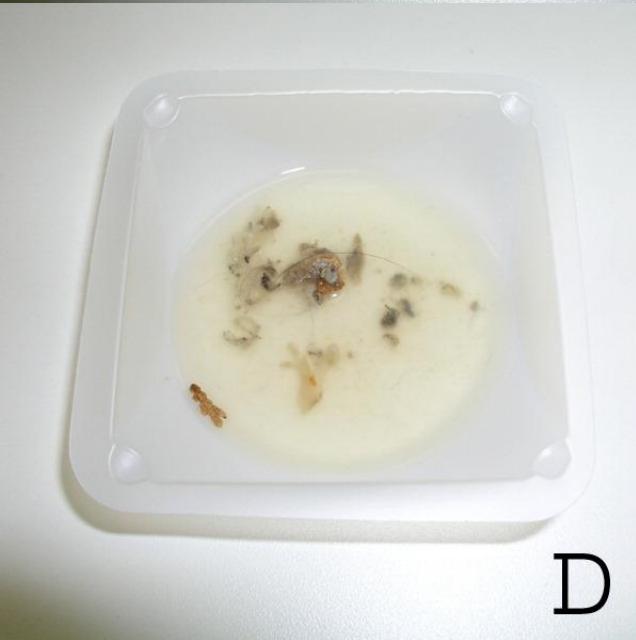










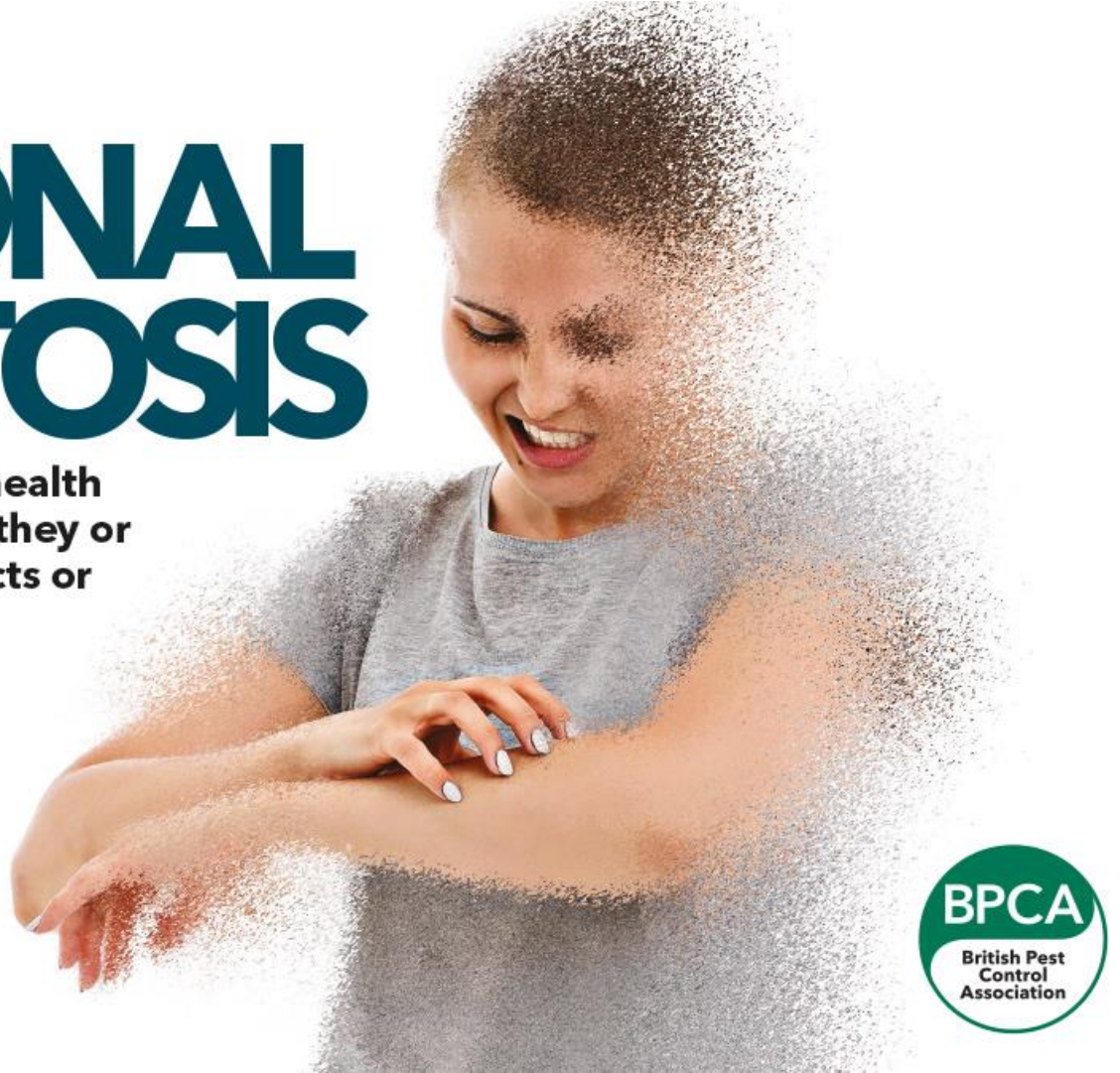


PESTWATCH:

DELUSIONAL PARASITOSIS

Delusional parasitosis is a mental health condition in which people believe they or their homes are infested with insects or parasites

– includes reference to self-harm



American continent – prevalence is 1 – 1.9 per 100,000 person-years

Europe - **prevalence** around 80 cases per million

World-wide: incidence (between 1.9 and 3.7 cases per 100,000); **prevalence** (27.3 per 100,000 person-years)

Dopaminergic system imbalances specific neuroanatomical changes:

1. cortical and/ or subcortical atrophy;
2. striatum and putamen lesions, basal ganglia damages;
3. poor vascularization of temporal-parietal and fronto-temporal areas;
4. dopamine transporter system dysfunction

Epidemiology

Treatment

First line treatment – antipsychotics

The first antipsychotic medication used to treat DP was **pimozide**.

Atypical antipsychotics can be considered: **olanzapine, risperidone, paliperidone, aripiprazole**

Anti-parasitic treatment is not required and may maintain and encourage the patients delusions

Additional psychotherapy methods: cognitive and behavior therapy

Pathophysiology

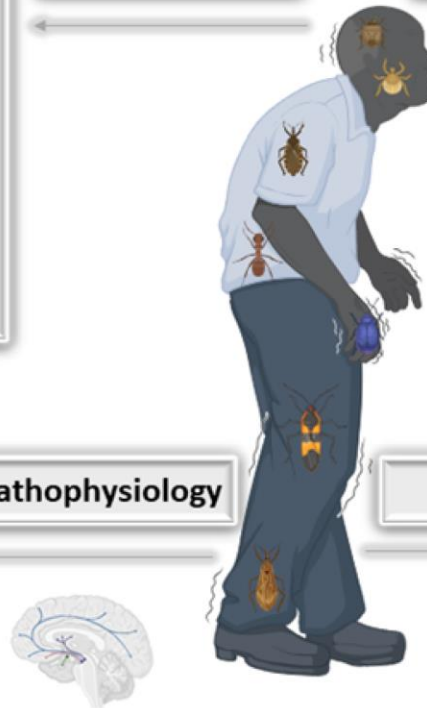
Diagnosis

No standard mental state examination to facilitate the diagnostic

Comparable as a diagnostic with delusional disorder (somatic type) according to DSM V and ICD 10

No conclusive evidence that certain changes in **computed tomography or magnetic resonance imaging** are specific for DP patients

Delusional parasitosis (DP)/infestation



PARAZOL

🔥 UŠTEDITE 75%

✓ Plaćanje pri preuzimanju

NARUČITE SADA

⚡ BRZA PONUDA ~~78 €~~ 20 €

Uklonite parazite prirodnim putem uz PARAZOL

Parazol je prirodan pripravak na bazi biljnih ekstrakata koji pomaže u uklanjanju parazita i toksina, pridonoseći boljoj probavi, jačem imunitetu i općem osjećaju dobrobiti — bez agresivnih kemikalija.

- ✓ Pomaže u borbi protiv parazita i podržava prirodne procese detoksikacije organizma.
- ✓ 100% prirodni sastojci, na bazi biljnih ekstrakata.
- ✓ Doprinosi smanjenju nadutosti i poboljšanju probave već u prvim danima korištenja.
- ✓ Podržava jačanje imunološkog sustava.
- ✓ Sigurno za kontinuiranu upotrebu, s blagom i prirodnom formulom.



Sigurna
kupovina



Brza
dostava



Zadovoljstvo
zajamčeno



Plaćanje
pri dostavi

LIJEČNIČKI PREGLED



ZORAN DRAGIČEVIĆ

SPECIJALIZACIJA: PARAZITOLOG

ISKUSTVO: 8 GODINA

Svi parazitolozi u Hrvatskoj znaju: ako imate tumore na tijelu, onda se isplati provjeriti prisutnost helminta. Često njihove štetne aktivnosti dovode do oštrog pada imuniteta, zbog čega ljudski papiloma virus dobiva potpunu slobodu. Dobro je što se ova sumnjiva veza lako može prekinuti jednim udarcem. Kapsule Toxic OFF nedavno su postale poznate na tržištu lijekova protiv glista, ali su već stekle dobru reputaciju zbog svoje učinkovitosti i prirodnog sastava. Oni neće naštetiti vašem zdravlju i oslobodit će se ne samo parazita, već i papiloma.

Algoritmi za detekciju i identifikaciju parazita

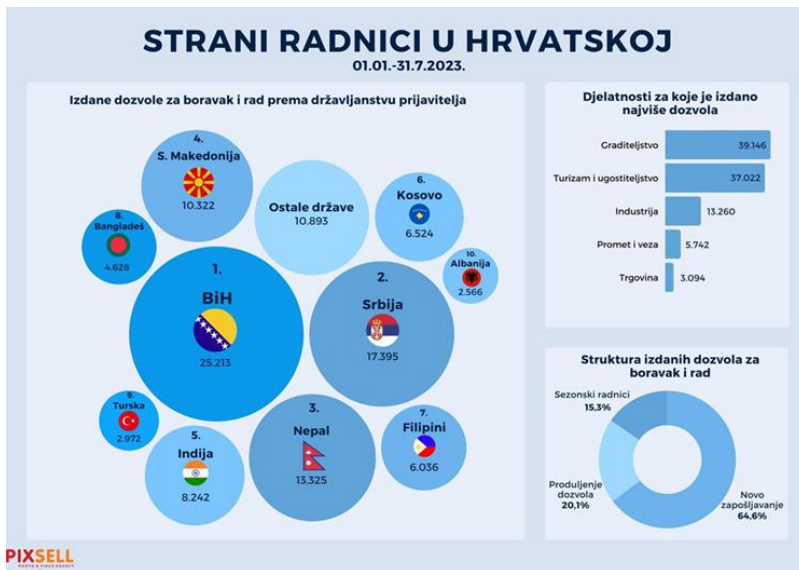
Dijagnostičke metode u parazitologiji

- Mikroskopija i danas najčešće upotrebljavanja dijagnostička metoda u parazitologiji – znanje i vještine laboratorijskog osoblja



Algoritmi za detekciju i identifikaciju parazita

Dijagnostičke metode u parazitologiji



- Mikroskopija i danas najčešće upotrebljavanja dijagnostička metoda u parazitologiji – znanje i vještine laboratorijskog osoblja

- **Izazov**

-putovanja (službena, turistička)

-imunodeficientni pacijenti – širi dijapazon parazitarne organizama

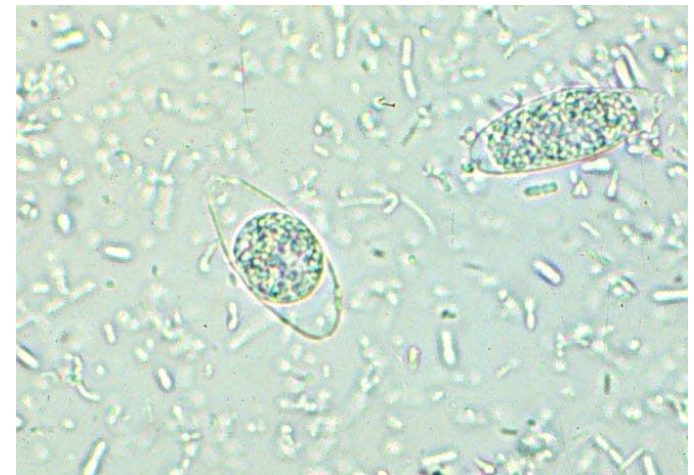
-migracije radnika, migranata



Parazitološke pretrage stolice

Mikroskopija - nativni preparat

- Skrining
- Procjena gustoće parazita, pokretljivost parazita
- Svježa stolica, ne iz frižidera, stigla u laboratoriju u adekvatnom vremenu
- Nativni preparat posebno važan za proljevaste stolice
- 100 i 400 suho povećanje mikroskopa
- Rezultat – preliminaran



Parazitološke pretrage stolice

Mikroskopija – koncentracijske metode

- Sedimentacijske i flotacijske
 - Odvajanje fekalnih elemenata od parazita
 - Sedimentacijske – preparat sadrži više debrisa
 - Flotacijske – teža jajašca i ličinke ne isplivaju na površinu, parazitarne elementi infektivni
-
- MIFC
 - Metoda flotacije s kuhinjskom soli
 - Metoda flotacije s otopinom cinkova sulfata

Parazitološke pretrage stolice

Mikroskopija – MIFC (Merthiolate-Iodine-Formaldehyde-Concentration)

Najjednostavnija metoda za izvođenje

- *MF OTOPINA (OTOPINA I)*

Tct. Merthiolate 1:1000 200,0 ml

Formaldehid 25,0 ml

Glicerin 5,0 ml

Destilirana voda 250,0 ml

- *LUGOLOVA OTOPINA (OTOPINA II)*

Iodum resublimatum 5.0 g

Kalii iodatum (KJ) 10,0 g

Destilirana voda 100,0 ml

Otopine čuvati u smeđoj staklenoj boci sa čepom.

Rok trajanja do 1 godine.

+ Eter ili etil acetat

Parazitološke pretrage stolice

MIF OTOPINA

- Pred samu upotrebu izmiješa se 7,0 ml. otopine I i 0,5 ml otopine II po jednoj stolici

Postupak:

- 1 - 1,5 g svježe stolice (veličine zrna graška) izmiješati - homogenizirati sa 7 ml MIF otopine u prikladnoj posudici
- Dobivenu mješavinu procijediti kroz jedan sloj gaze u koničnu epruvetu
- Dodati u epruvetu 2,0 ml. etera (ili etil acetata), zatvoriti epruvetu gumenim čepom i snažno protresti
- Odčepiti epruvetu i ostaviti da odstoji 2 - 3 minute
- Centrifugirati 10 min. na 1600 - 2000 okretaja u min.
- Poslije centrifugiranja mješavina u epruveti se razdvoji na četiri sloja (eter na površini, sloj detritusa, sloj MIF-a, sediment)

Parazitološke pretrage stolice

- Osloboditi gusti sloj detritusa od stijenki epruvete kružnim pokretom metalnog ili staklenog štapića.
- Brzo ali oprezno dekantirati.
- Preostali sediment pomiješati sa malo tekućine koja se nacijedila po stijenki epruvete nakon dekantacije. Jednu kap sedimenta pomoću pipete prenijeti na predmetno stakalce i staviti pokrovno stakalce veličine 20 X 20 mm.
- Mikroskopirati cijeli preparat pod povećanjem objektiva 40x.

Komercijalni fekalni koncentratori



Advantages

- Enhanced Biosafety:** Being enclosed, one-tube systems, they drastically minimize user exposure to infectious fecal matter and reduced odor
- Reduced Risk from Reagents:** These kits often allow the use of safer, less flammable, or "solvent-free" alternatives to the traditional, dangerous, diethyl ether used in manual methods
- Efficiency and Speed:** They simplify the process of filtration and sedimentation, reducing labor-intensive steps and technical errors
- High Diagnostic Accuracy:** Studies indicate they produce clearer sediments with less debris than conventional methods, and demonstrate high sensitivity for detecting a wide range of parasites, sometimes surpassing the Kato-Katz method
- Ease of Use:** They are often preferred for field studies and in remote laboratories because they are simple, self-contained, and facilitate quick testing

Disadvantages

- Cost:** As disposable, single-use kits, they are significantly more expensive than conventional "homemade" concentration methods
- Variable Sensitivity:** While usually good, some studies suggest that in low-intensity infections, some commercial kits might exhibit lower sensitivity compared to formal-ethyl acetate sedimentation
- Potential for Lower Recovery Rates:** In some studies, commercial systems—particularly "solvent-free" kits—have shown lower efficiency in recovering certain ova (e.g., *Taenia* spp.) compared to traditional methods
- Sediment Size:** Some users report that solvent-free, commercial products can produce a larger amount of debris in the final sediment compared to original solvent-based methods

BIO | SEPAR G
M
B
H
Diagnostics for the future

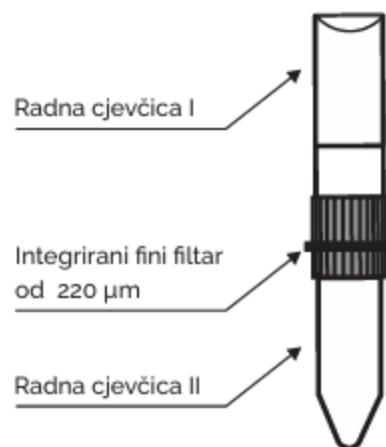
Gesellschaft für Medizin- und Labortechnik mbH

Najbolje rješenje
za vašu dijagnostiku

Parazitološka dijagnostika stolice

Dijagnostički sustav Biosepar ParasiTrap® rezultat je dugogodišnjeg istraživačkog i razvojnog rada u Institutu za medicinsku mikrobiologiju i higijenu Max von Pettenkofer Sveučilišta Ludwig-Maximilians-Universität München. Razvijen je da bi se neugodan i rizičan postupak laboratorijske obrade stolice olakšao, učinio sigurnijim i ekološki prihvatljivijim te kako bi se stopa pogodaka trajno optimizirala.

Struktura sustava ParasiTrap®



Sustav spreman za uporabu

Intenzivan postupak
tresenja i miješanja

Aktivno vertikalno
fino filtriranje = propuštanje
100 % parazita

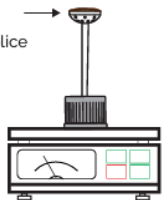
Dijagnostika ParasiTrap® omogućuje vam izbor između postupaka sedimentacije i flotacije.

Sedimentacija	Flotacija**
Nenapunjeni sustav*	Napunjen s BioFlot PLUS Medium
Napunjen s 4 ml Medium A ECO (bez formalina)	Napunjen s medijem magnezijeve soli
Napunjen s 4 ml Medium A AF (6 % formalina)	Napunjen s medijem cinkove soli
Napunjen s 4 ml Medium A SAF (4 % formalina)	
Napunjen s 4 ml Medium A Bailenger (bez formalina)	*Može se naručiti zasebno s postupkom sedimentacije i flotacije
Napunjen s 4 - 4 ml Medium A DUAL	**Može se naručiti s posebnom težinom od 1,28 ili 1,30

Jednostavni koraci za dijagnostiku (sedimentacija)

01. Priprema / uzimanje uzorka

Uvijek definirana
jednaka količina stolice



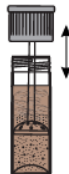
Moguća je
kvantitativna
analiza

02. Obrada uzorka, I. korak

Optimalno miješanje,
ak i tvrdih stolica

Bojenje parazita
medijem Combi-Medium

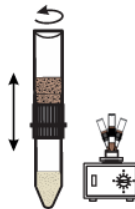
Trenutno fiksiranje
(i bez formalina)



03. Obrada uzorka, II. korak

Intenzivan postupak
tresenja i miješanja

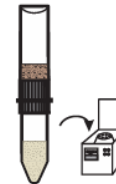
Prvo aktivno vertikalno
fino filtriranje



04. Obrada uzorka, III. korak

Drugo potpuno vertikalno
fino filtriranje: bez mrtvog
prostora, u filtru više
nema parazita

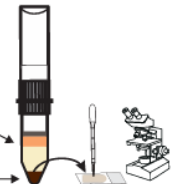
Centrifugiranje ili puštanje
da odstoji 2 – 3 sata



05. Spremno za dijagnostiku

Jasno odvojeni slojevi

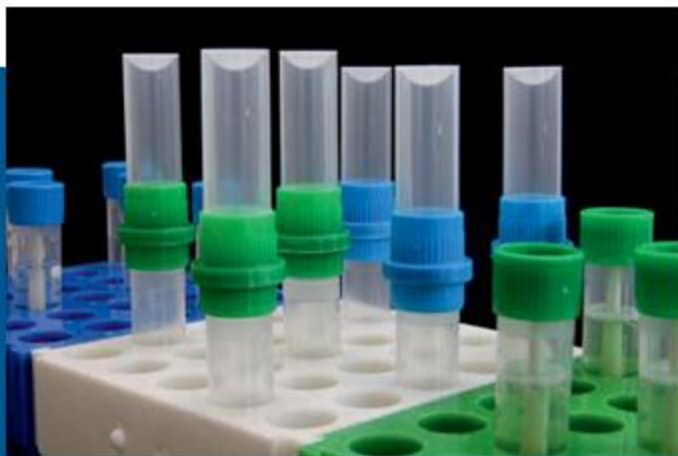
100 % obojeni
koncentrat parazita



Više o našim proizvodima

Dijagnostički sustav Biosepar ParasiTrap® vodeći je na tržištu u pogledu mogućnosti reproduciranja rezultata, mogućnosti standardiziranja i osjetljivosti te se svakodnevno upotrebljava u brojnim laboratorijima u Njemačkoj i inozemstvu s velikim uspjehom. Racionalni radni postupak dopušta obradu i većih serija uzorkovanih materijala u kratko vrijeme,

čime se može uštedjeti na dragocjenom vremenu rada. Dijagnostički sustav ParasiTrap® ispunjava sve kriterije suvremenog upravljanja kvalitetom. Izvrsna stopa pogodaka i kvaliteta mikroskopskih vidnih polja skraćuju vrijeme potrebno za mikroskopsku analizu bez ugrožavanja dijagnostičke sigurnosti.



Zahvaljujući stalnom istraživanju i razvoju sustav za ispitivanje stolice Biosepar ParasiTrap® omogućuje smislene dijagnostičke alternative u suzbijanju parazita.

Značajka	Komercijalni koncentrator	Konvencionalna metoda
Sigurnost	Visoka (zatvoreni sustav)	Niska (otvoreni sustav, aerosol)
Rad	Niski (single use)	Visoki (ručni)
Cijena	Viša	Niža
Efikasnost	Visoka	Visoka

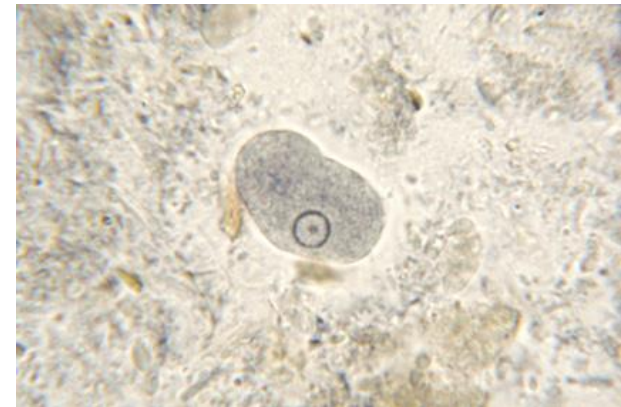
Odabir?

- **Your budget and volume of samples**
- **Available safety equipment**
- **The primary types of parasites you need to detect**

Parazitološke pretrage stolice

Mikroskopija – trajno obojeni preparati

- Trikrom
- Željezo-hematoksilin
- Dozvoljavaju pregled i uočavanje detalja morfologije parazita pod imerzijskim povećanje
- Trebalo bi pregledati minimum 300 vidnih polja
- Konačna determinacija parazita



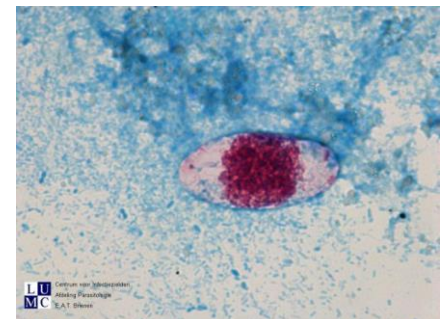
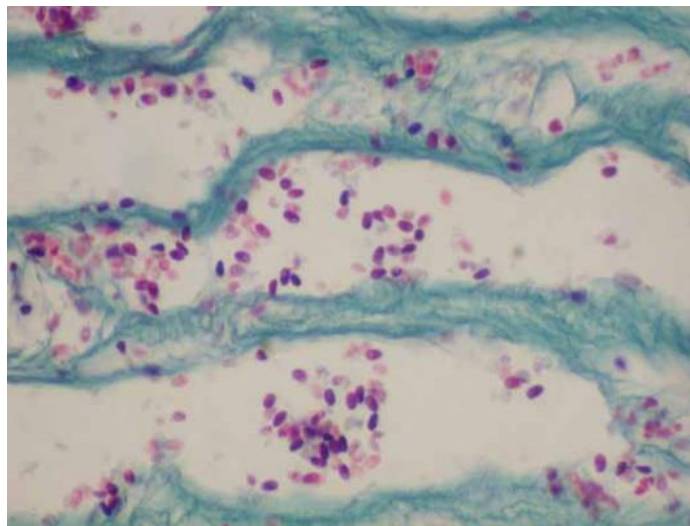
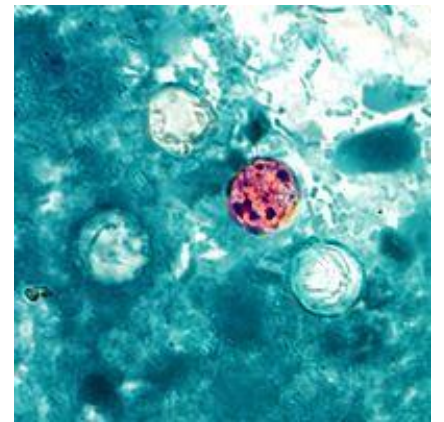
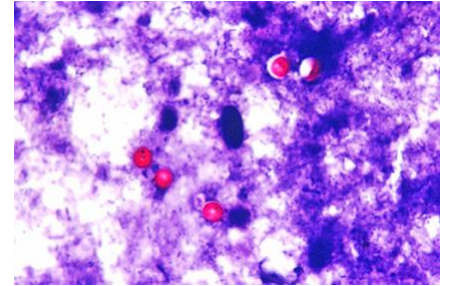
Parazitološke pretrage stolice

“Acid fast bojenje”

- Probavne kokcidije
- 300 imerzijskih vidnih polja

Modificirani trikrom

- Mikrosporidije



Parazitološke pretrage stolice

- Interpretacija rezultata
- Preporuka: izdavanje nalaza svih parazita, kao i izdavanje nalaza apatogenih organizama
- Praksa nije ujednačena!!!

Parazitološke pretrage stolice

Detekcija antigena

- EIA, imunokromatografska metoda, DFA
- *G. lamblia*
- *E. histolytica*/dispar; *E. histolytica*
- *Cryptosporidium* sp.
- Pojedinačni, kombinirani testovi, kazete



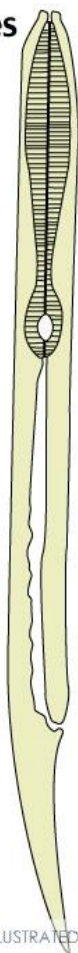
Parazitološke pretrage stolice

Koprokultura

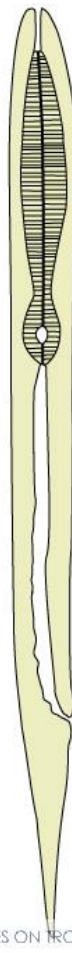
- Diferencijacija infekcija parazitom *Ancylostoma*, *Strongyloides*, *Trichostrongylus*

Parazitološke pretrage stolice

Strongyloides



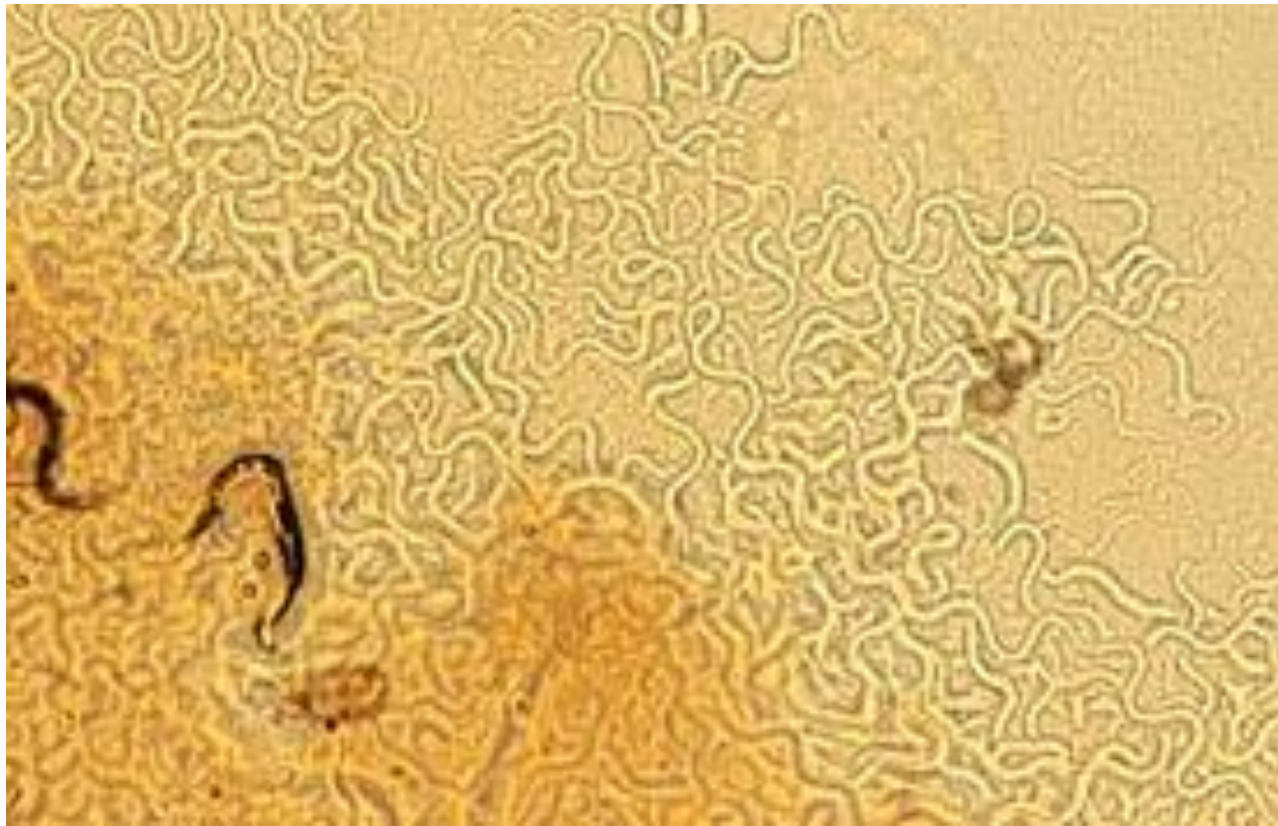
Ancylostoma



Parazitološke pretrage stolice

Koprokultura

- Diferencijacija infekcija parazitom *Ancylostoma*, *Strongyloides*, *Trichostrongylus*
- Kultura na agaru
- Baermanova metoda
- Harada Mori filter papir metoda



BAERMANN TECHNIQUE

Glass Funnel

Seive

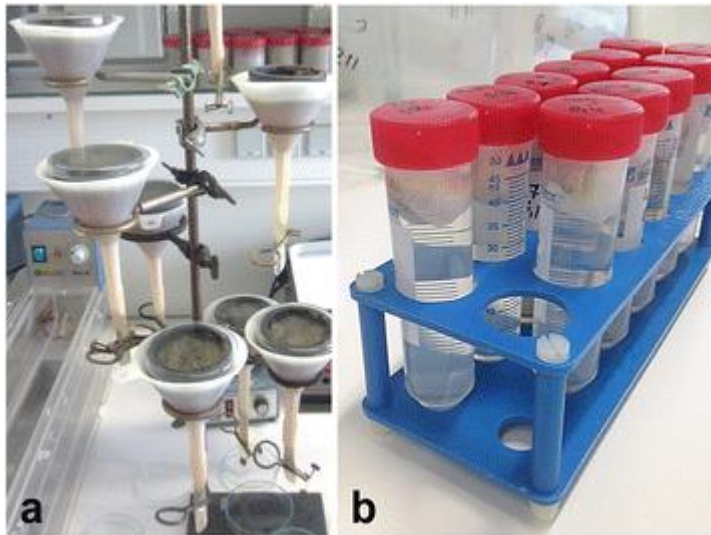
Rubber
Tube

Clip

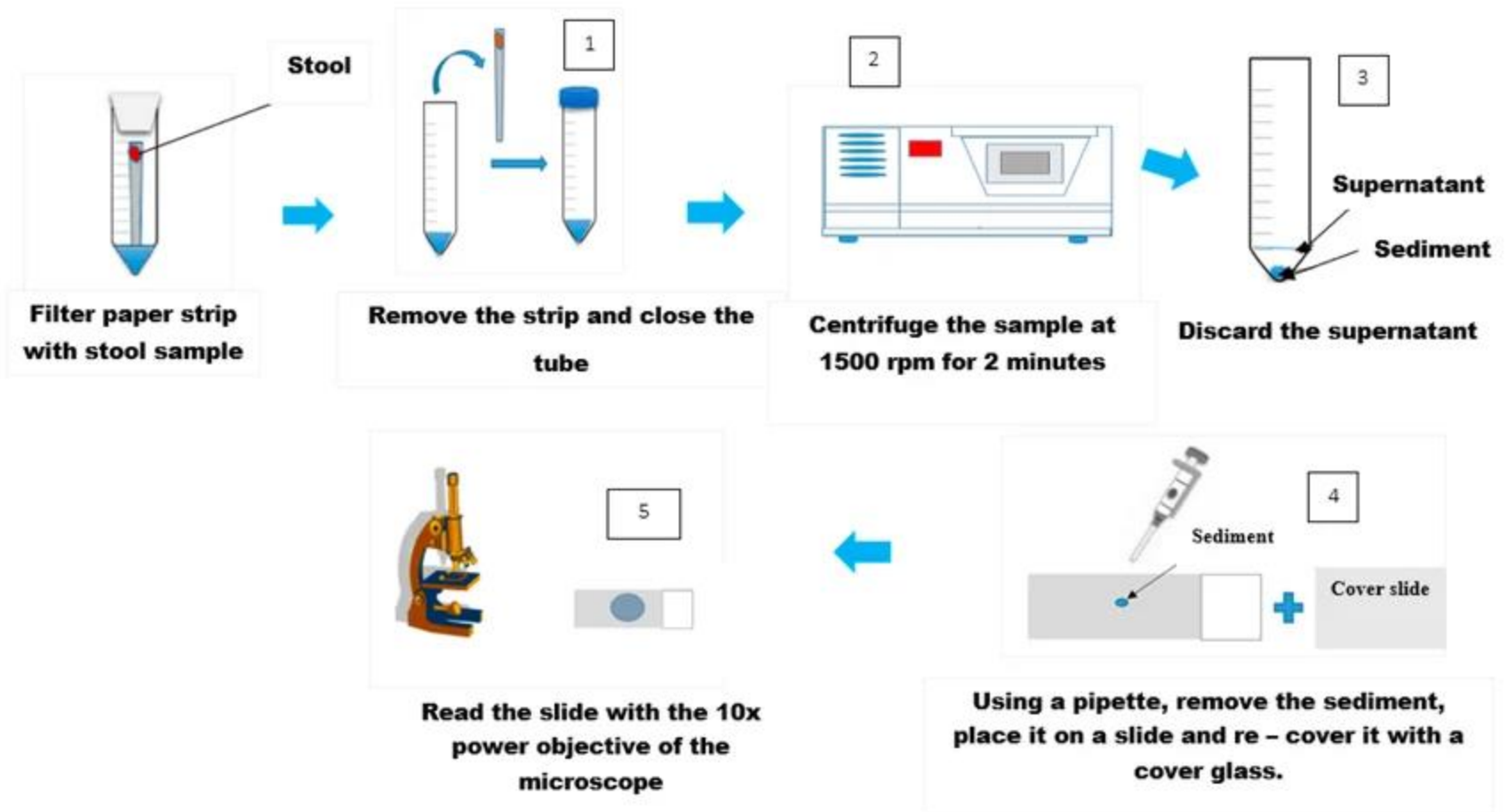
Fresh Feces



CLEVERORCA
VETERINARY TECHNICIAN



- Baermannova metoda radi na principu aktivne migracije živih larva iz uzorka stolice u mlaku vodu, u kojoj one zbog nemogućnosti plivanja tonu na dno. Ova je metoda zlatni standard u parazitologiji za detekciju parazita *Strongyloides stercoralis* jer omogućuje prikupljanje čistih i neoštećenih larva bez upotrebe agresivnih kemikalija koje bi ih mogle deformirati
- koristi kombinaciju topline, vlage i gravitacije kako bi potaknuo žive parazite da sami izađu iz stolice. Larve prolaze kroz gazu u mlaku vodu, gube sposobnost održavanja na vodi i talože se na dnu aparata, odakle se jednostavno uzimaju za mikroskopsku dijagnostiku



- Harada-Mori metoda je jednostavna i jeftina laboratorijska tehnika uzgoja (kulture) koja se koristi za otkrivanje i koncentraciju ličinki parazita u stolici, prvenstveno za *Strongyloides stercoralis*

1. **Nanošenje uzorka:** Oko 0,5 do 1 gram svježje stolice razmaže se po sredini uske trake filter papira. Krajevi papira ostavljaju se čistima
2. **Postavljanje u epruvetu:** Filter papir se umeće u epruvetu u kojoj se nalazi nekoliko mililitara destilirane ili obične vode
3. **Kontakt s vodom:** Papir se postavlja tako da njegov donji, suženi dio dodiruje dno i uronjen je u vodu. Vrlo je važno da **sami razmaz stolice bude iznad razine vode** (oko 1-2 cm) kako se stolica ne bi odmah isprala i zamutila tekućinu
4. **Kapilarni učinak:** Filter papir upija vodu prema gore. Na taj način razmaz stolice stalno ostaje vlažan, što stvara idealne uvjete za preživljavanje i razvoj parazita
5. **Inkubacija:** Epruveta se zatvara (uz minimalan dotok zraka) i ostavlja na sobnoj temperaturi (oko 25–28 °C) kroz **7 do 10 dana**
6. **Migracija ličinki:** Budući da ličinke *Strongyloides stercoralis* u stolici traže vodu i vlagu, one **migriraju s filter papira prema dolje u vodu** na dnu epruvete
7. **Pregled pod mikroskopom:** Nakon završetka inkubacije, pipetom se uzima sediment (tekućina s dna epruvete) i promatra se pod mikroskopom. Ako je infekcija prisutna, u čistoj vodi jasno se vide **žive, pokretne ličinke**

Pretrage na Enterobius

- Perianalni otisak ljepljivom trakom
- Prije kupanja, najbolje rano ujutro
- Preporuča se minimum 3, optimum 6 otisaka
- Analni bris



Parazitološke pretrage urogenitalnih uzoraka

- *Trichomonas vaginalis*
- *Schistosoma haematobium*
- Mikrosporidije
- Mikrofilarije

Parazitološke pretrage urogenitalnih uzoraka

Trichomonas vaginalis

- Uzorak: vaginalni, cervikalni sekret, uretralni iscjedak, sekret prostate, ejakulat, urin (prvi mlaz prvog jutarnjeg urina)
- Nativni preparat
- Pregledati preparat što prije
- Problem: leukociti
- Detekcija antigena – IC test
- Kultivacija – Diamonds, In Pouch (3-7 dana)
- PCR

Schistosoma haematobium

- Uzorak terminalni urin- tijekom mokrenja, kontrakcije mjehura istiskuju jajašca koja su se nakupila u sluznici i stijenci. Zato se najveća koncentracija jajašaca izbacuje u samom završetku mikcije

Parazitološke pretrage krvi

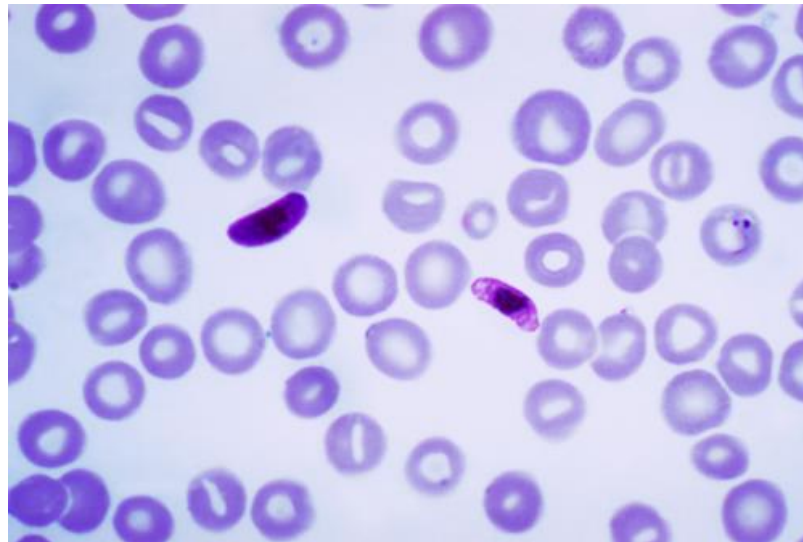
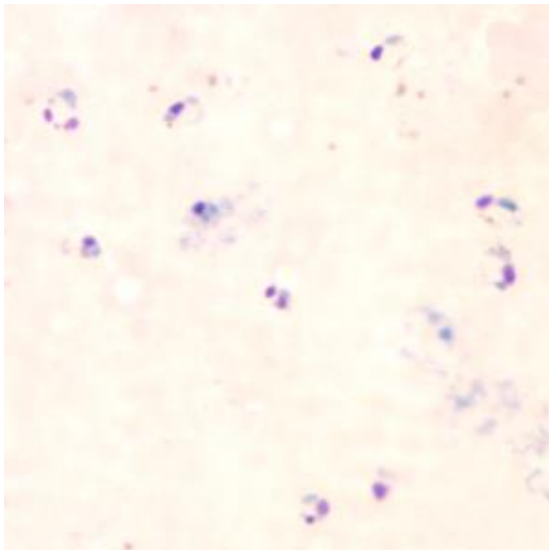
Direktna dijagnostika

- Plasmodium
- Babesia
- Trypanosoma
- Leishmania
- Mikrofilarije
- Svježa krv bez antikoagulansa
- Krv sa antikoagulanom (EDTA)
- Preferirana metoda bojenja Giemsa – nedostatak bojenje ovojnice kod mikrofilarija
- Oprez kod rada s potencijalno visoko infektivnim uzorkom!!!

Parazitološke pretrage krvi

Krvni razmaz i gusta kap

- Gusta kap – veća količina krvi, veća osjetljivost
- Krvni razmaz – konačna determinacija krvnog parazita
- Malarija- sumnja – ako je prvi set negativan – uzimanje uzoraka svakih 6-8 sati
- Preparati – idealno priprema unutar 1 sata od uzimanja krvi



Uzorak

- Uzimanje kapilarne krvi iz jagodice prsta, uške, pete – trenutna izrada guste kapi ili razmaza, antigenski test
- Uzimanje venske krvi u epruvetu s EDTA (preparati, serologija, molekularna)
- Heparin – može uzrokovati promjenu morfologije parazita
- Hitni transport u laboratorij –priprema razmaza i guste kapi unutar 1 sat od uzimanja- razvoj seksualnih oblika parazita, gubitak granulacija

Uzorak

- Jedan set –krvni razmaz + gusta kap – dovoljan u većini slučajeva
- Preporuka međunarodnih tijela za dijagnostiku malarije – optimalno 2 razmaza i 2 guste kapi
- Kada uzimati?

Clinical and laboratory standards institute -Svakih 6-8 sati unutar 3 dana dok se ne isključi malarija

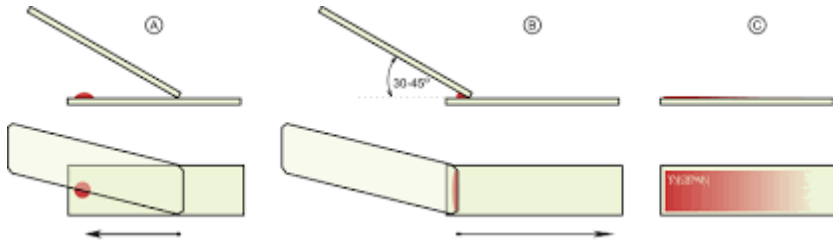
CDC – svakih 12 do 24 sata unutar 3 dana

Krv se može držati u hladnjaku duže vrijeme za molekularnu dijagnostiku

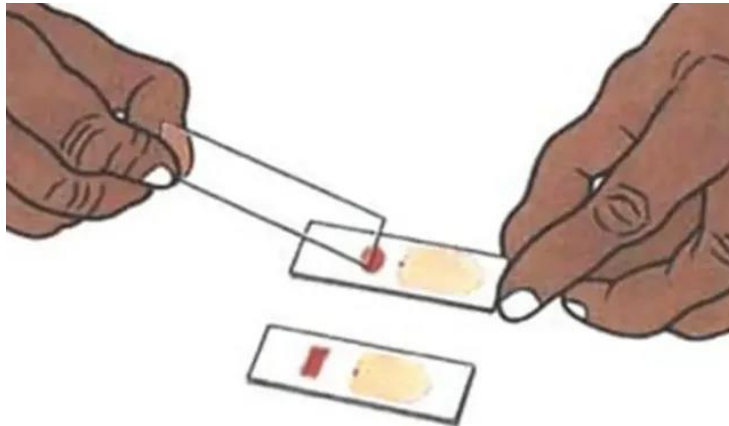
Krvni razmaz i gusta kap



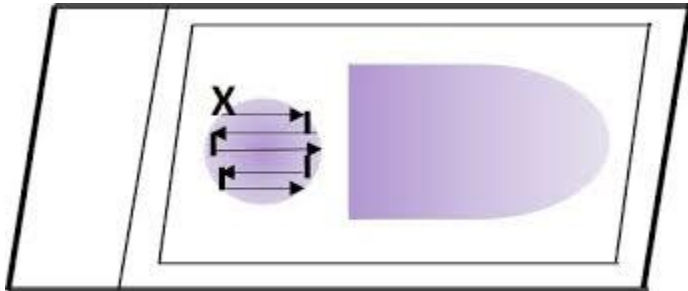
Izrada



- Količine krvi
Krvni razmaz 1- 3 mikrolitra
Gusta kap 3-5 mikrolitra
- Pipeta
- Kap iz prsta 50 mikrolitra



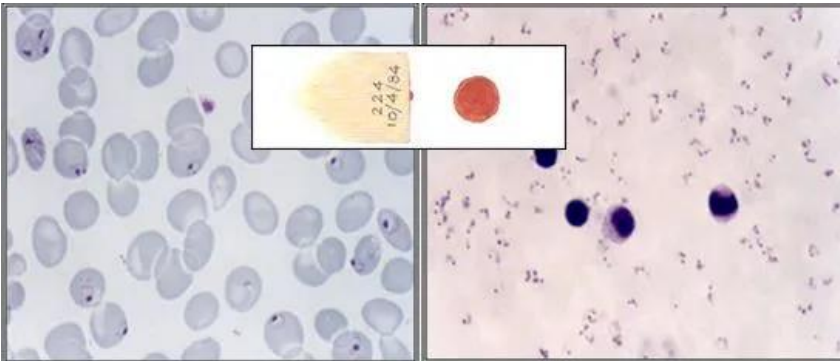
Izrada



- Krvni razmaz fiksacija alkoholom- 1 minuta
- Gusta kap – NE fiksira se
- Gusta kap 10-20 puta osjetljivija od krvnog razmaza -10-50 parazita po mikrolitru krvi (0.0002-0,001 % parazitemija)
- Gusta kap -primarna screening metoda

Izrada

- Najčešća greška korištenje previše krvi
- 1,5- cm promjer – gustoća da se kroz gustu kap mogu čitati novine
- Sušenje- stavljanje u digestor s protokom zraka
- Bojenje preparata– s otopinom Giemsa pH 7,2-7,4



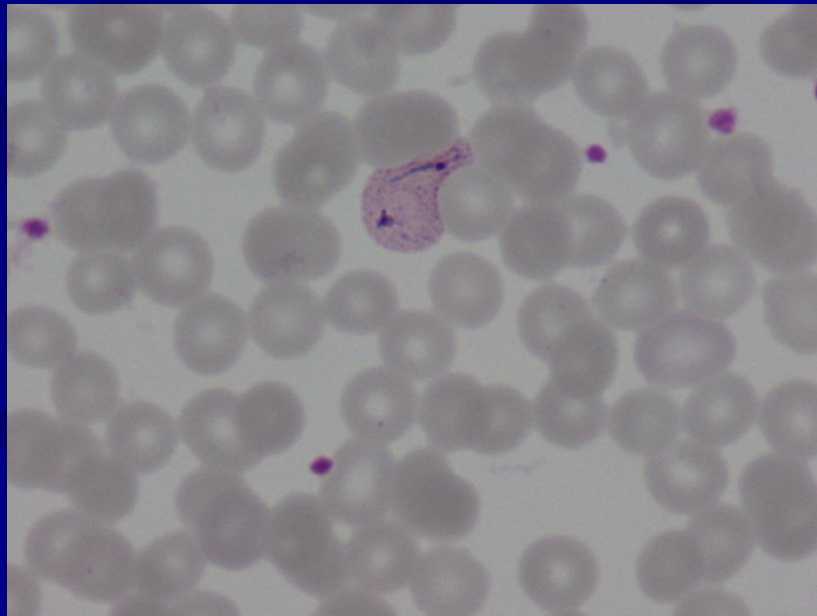
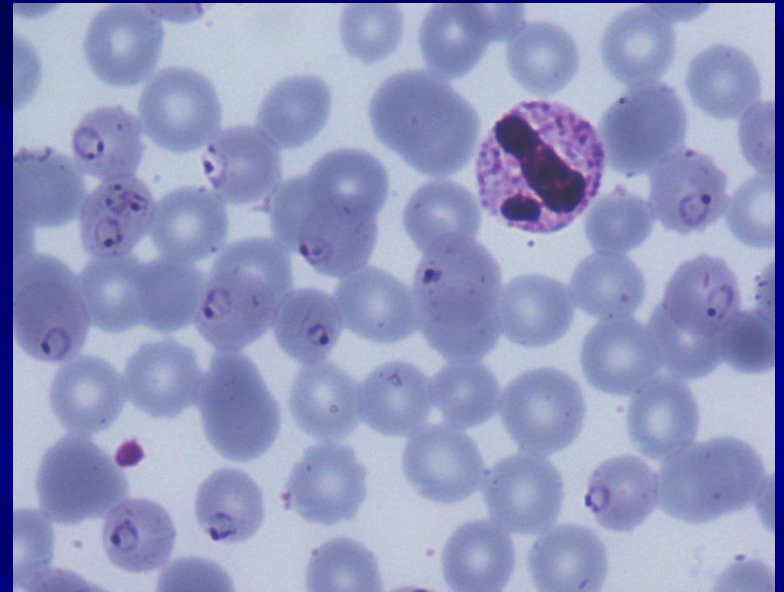
Na_2HPO_4 1gr

KH_2PO_4 0.7 gr

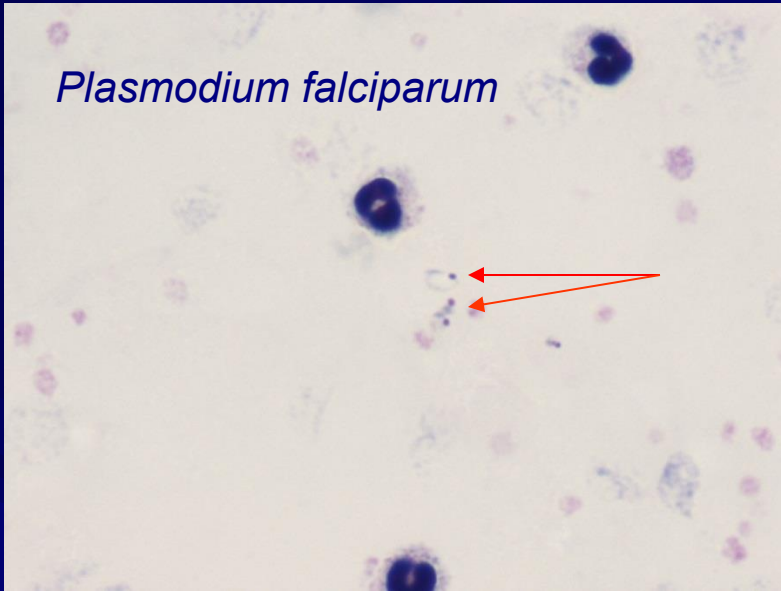
Destilirana voda 1000 ml

Razrjeđenje 1:10/20 – 1-2 kapi Giemse na 1 ml vode

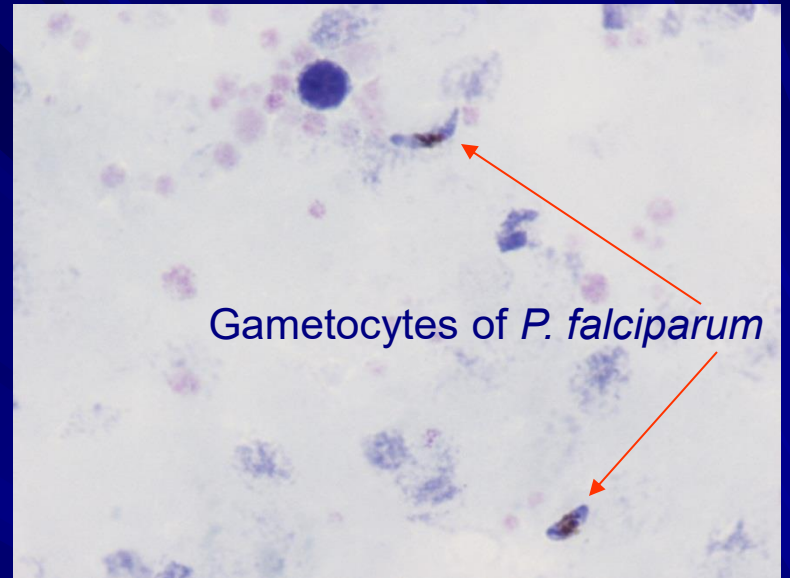
Trajanje 20 do 45 minuta



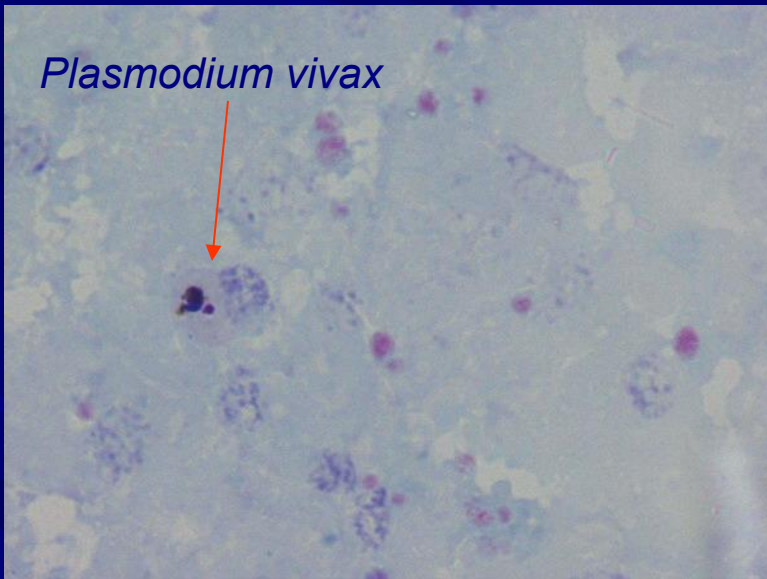
Plasmodium falciparum



Gametocytes of *P. falciparum*



Plasmodium vivax



Babesia sp.

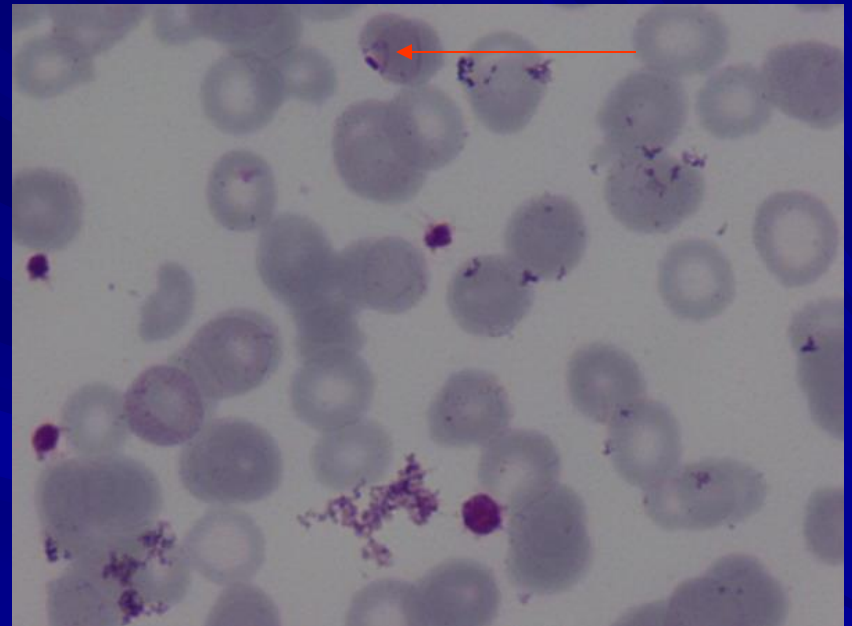


Incorrect pH



Plasmodium vivax –
no dots

Incorrect time (over staining)



Plasmodium falciparum
with stain deposit

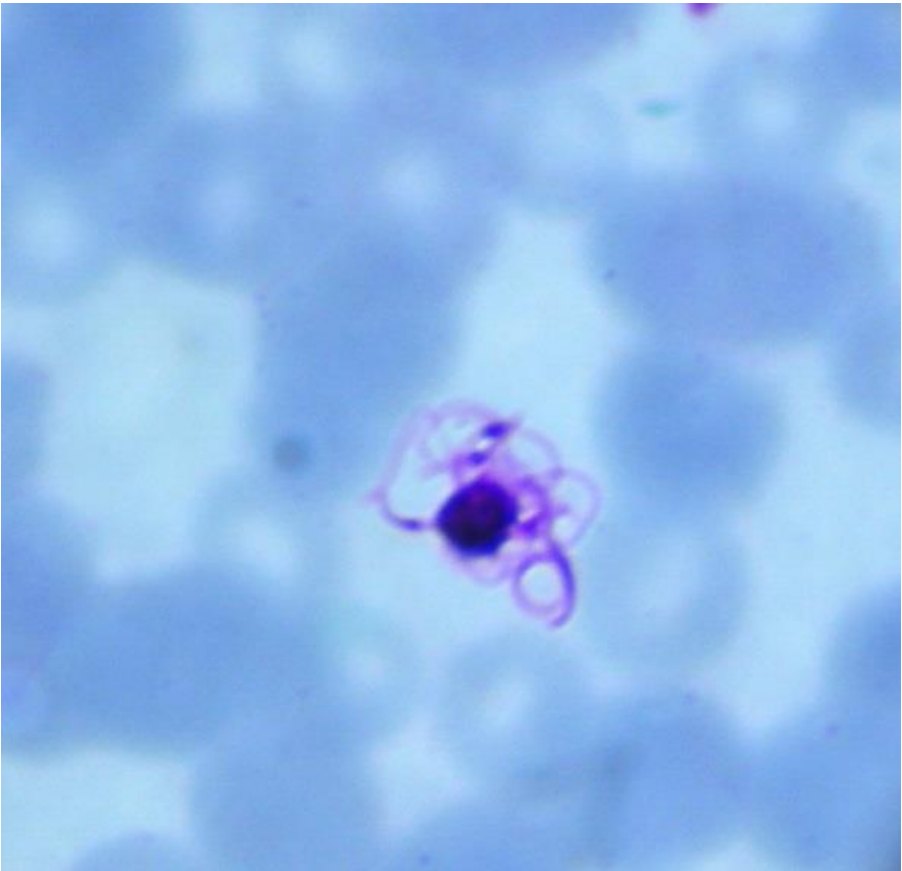
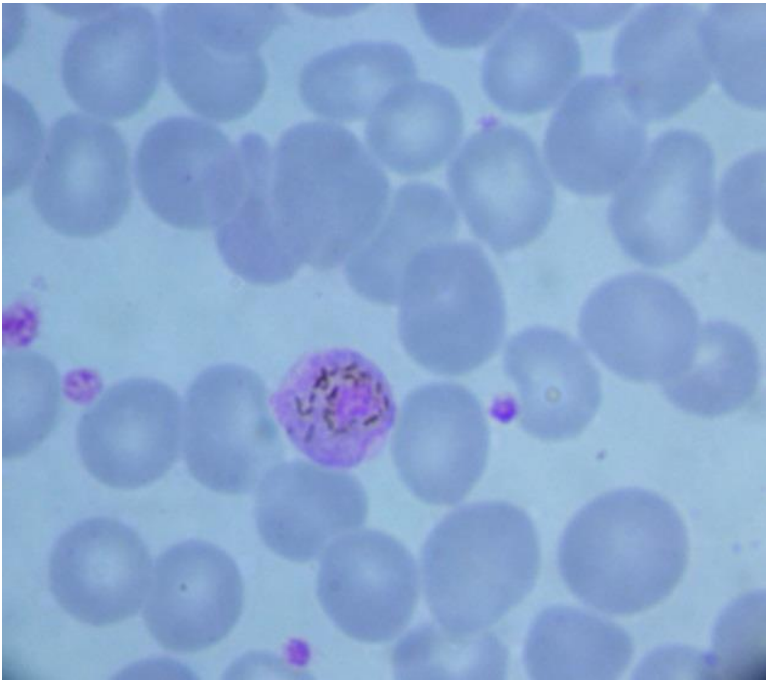




Fig. 1: Too little blood.
Tropo poco sangue.



Fig. 2: Too much blood.
Eccessiva quantità di sangue.



Fig. 3: The correct thickness of the thick film can be judged by the legibility of the printed text seen through the slide.
Corretta quantità di sangue, è possibile leggere attraverso la goccia i comuni caratteri di stampa.

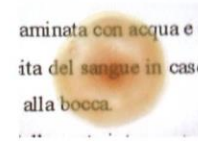


Fig. 4: Enlargement of the previous figure.
Ingrandimento della figura precedente.



Fig. 5: Too little blood.
Tropo poco sangue.



Fig. 6: Too much blood, the end of the film is lost.
Tropo sangue, manca la zona corretta per la lettura dello striscio.



Fig. 7: Edge of the spreader ragged.
Striscio sottile. Vetrino utilizzato per lo striscio con bordi scheggiati.

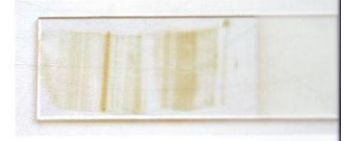


Fig. 8: Discontinuous movement of the spreader.
Movimento discontinuo durante l'esecuzione dello striscio.



Fig. 9: Greasy slide.
Superficie del vetrino con residui di grasso.



Fig. 10: Good thin film.
Striscio sottile di buona qualità.



Fig. 11/A: Thin and thick film on the same slide: make the thin film first.
Goccia spessa e striscio sottile sullo stesso vetrino: prima eseguire lo striscio sottile.

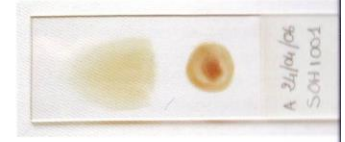
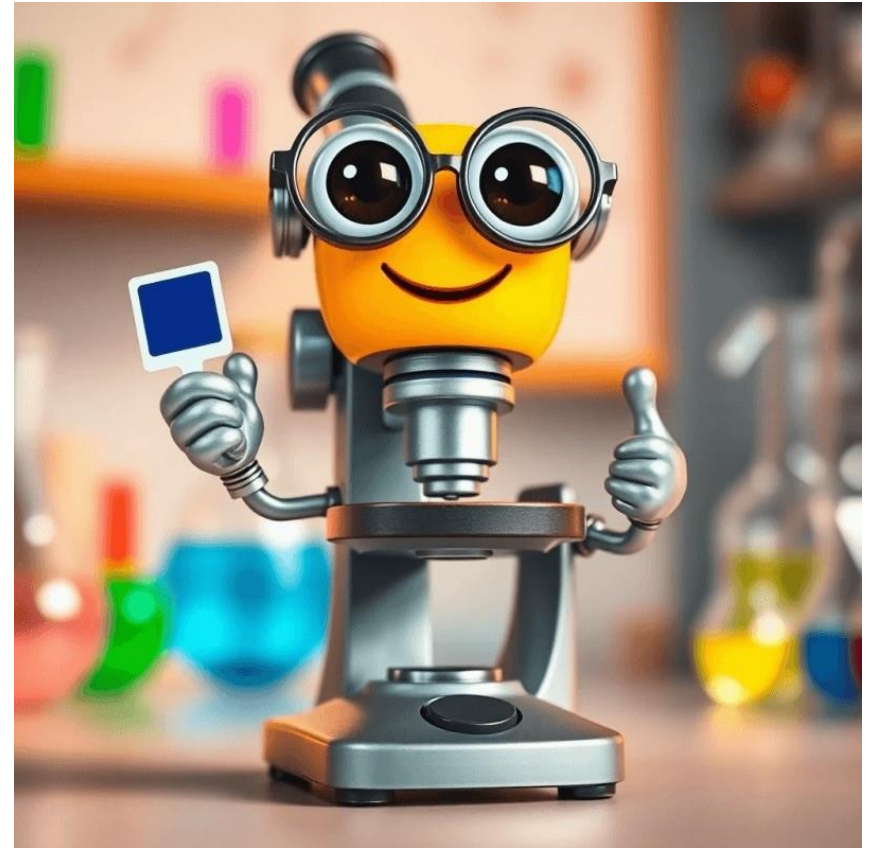


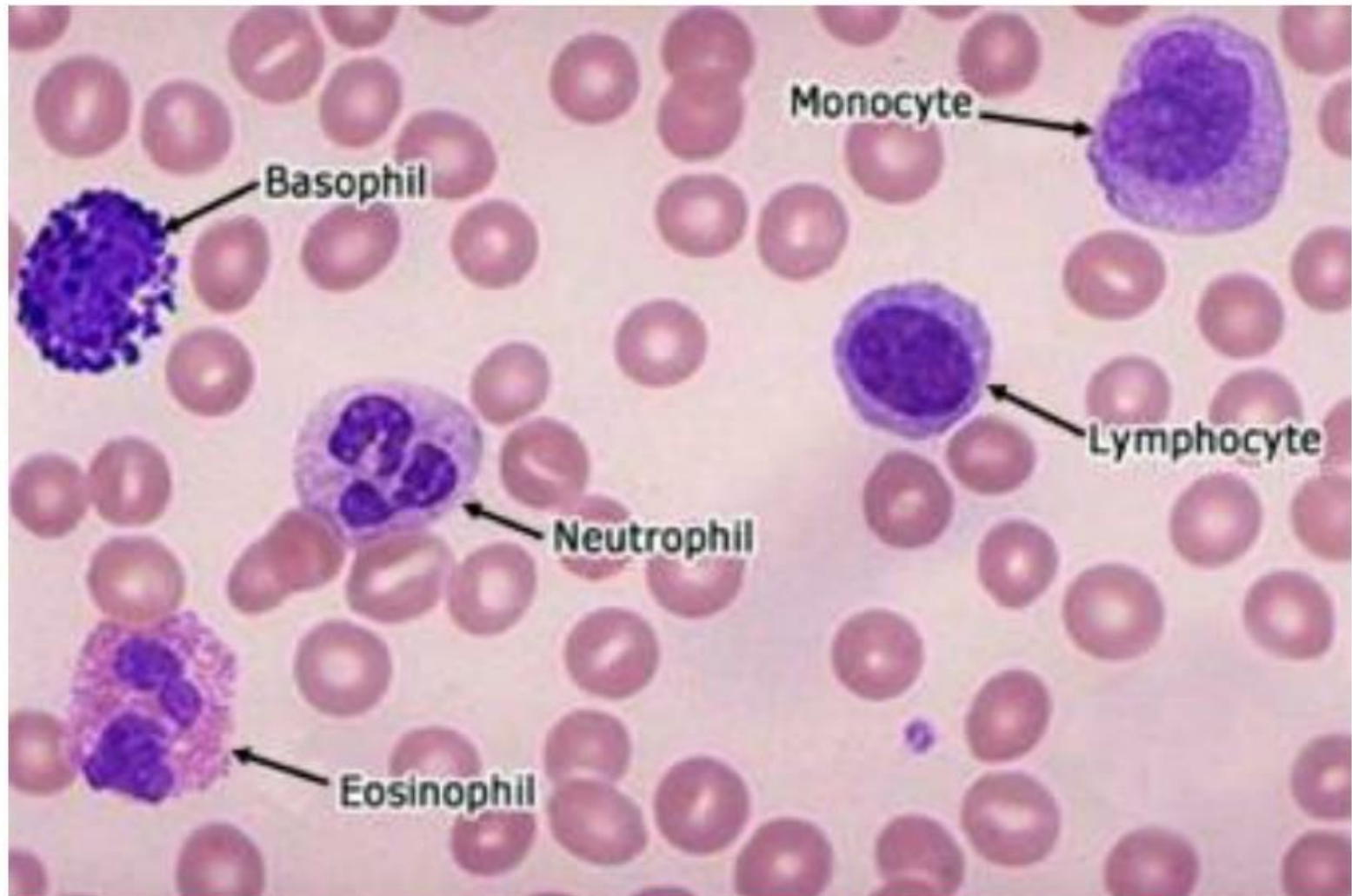
Fig. 11/B: Make the thick film subsequently.
Successivamente eseguire la goccia spessa.

Mikroskopiranje

- Skrining na povećanju od 100X
- Imerzija – 1000 X
- Imerzijsko ulje
- Okrenuti na pravu stranu!
- Pregledati 200 do 300 imerzijskih vidnih polja do izdavanja negativnog razmaza



Blood smear under microscope





Lymphocyte



Monocyte



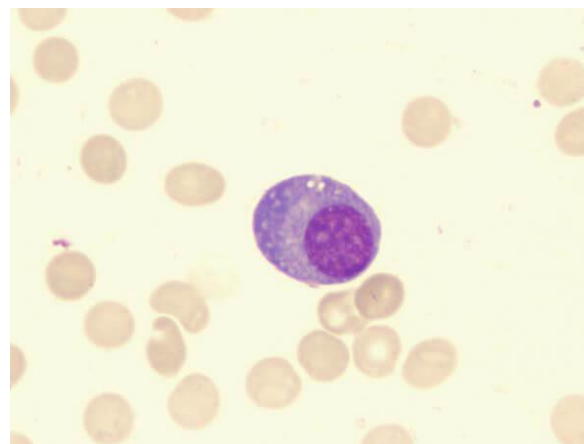
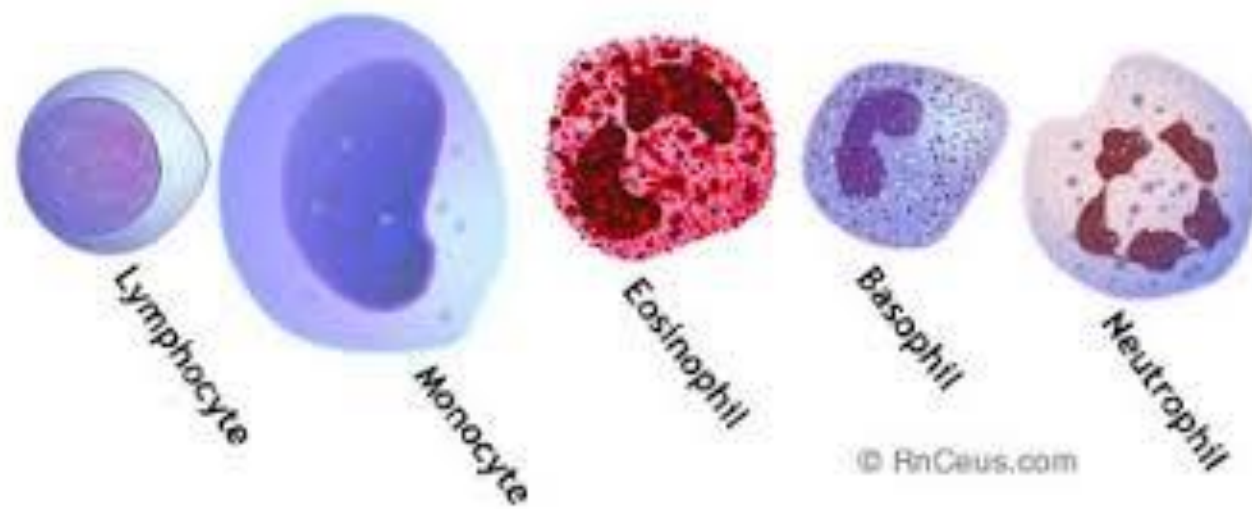
Eosinophil



Basophil



Neutrophil



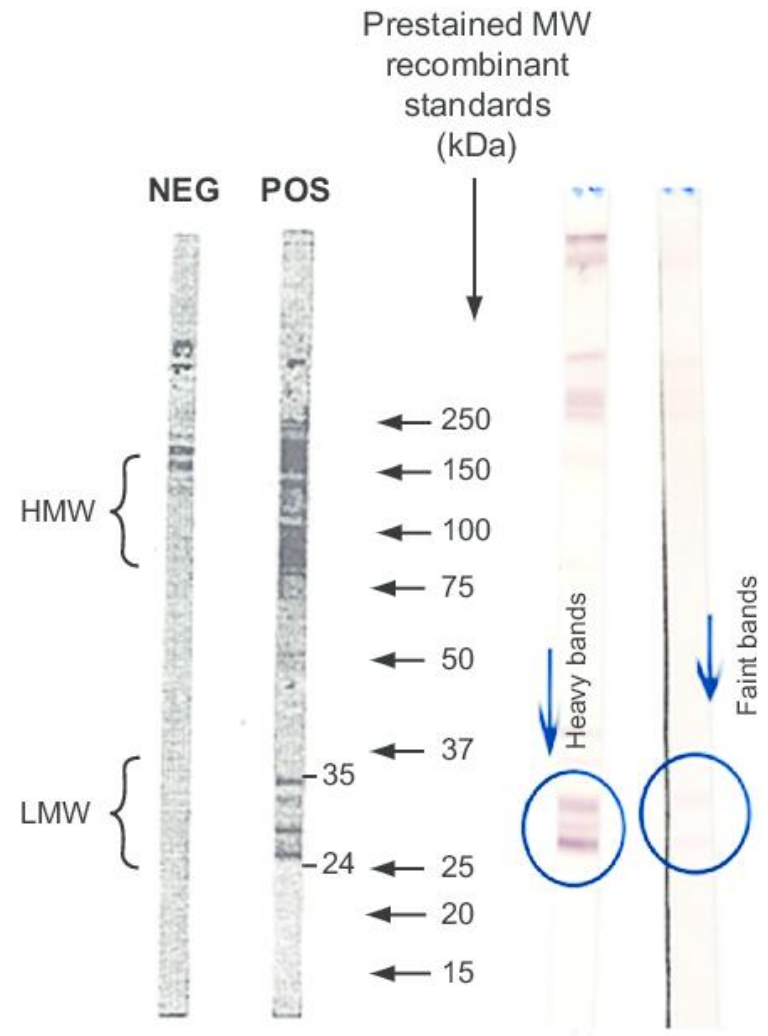
Molekularna dijagnostika

- Sve više ulazi u rutinu
- Osjetljivost i specifičnost uglavnom visoka
- Tipizacija
- Skuplja
- Klasični i real time PCR
- Single ili multiplex
- In house metodologija – nužna standardizacija
- Sindromski testovi

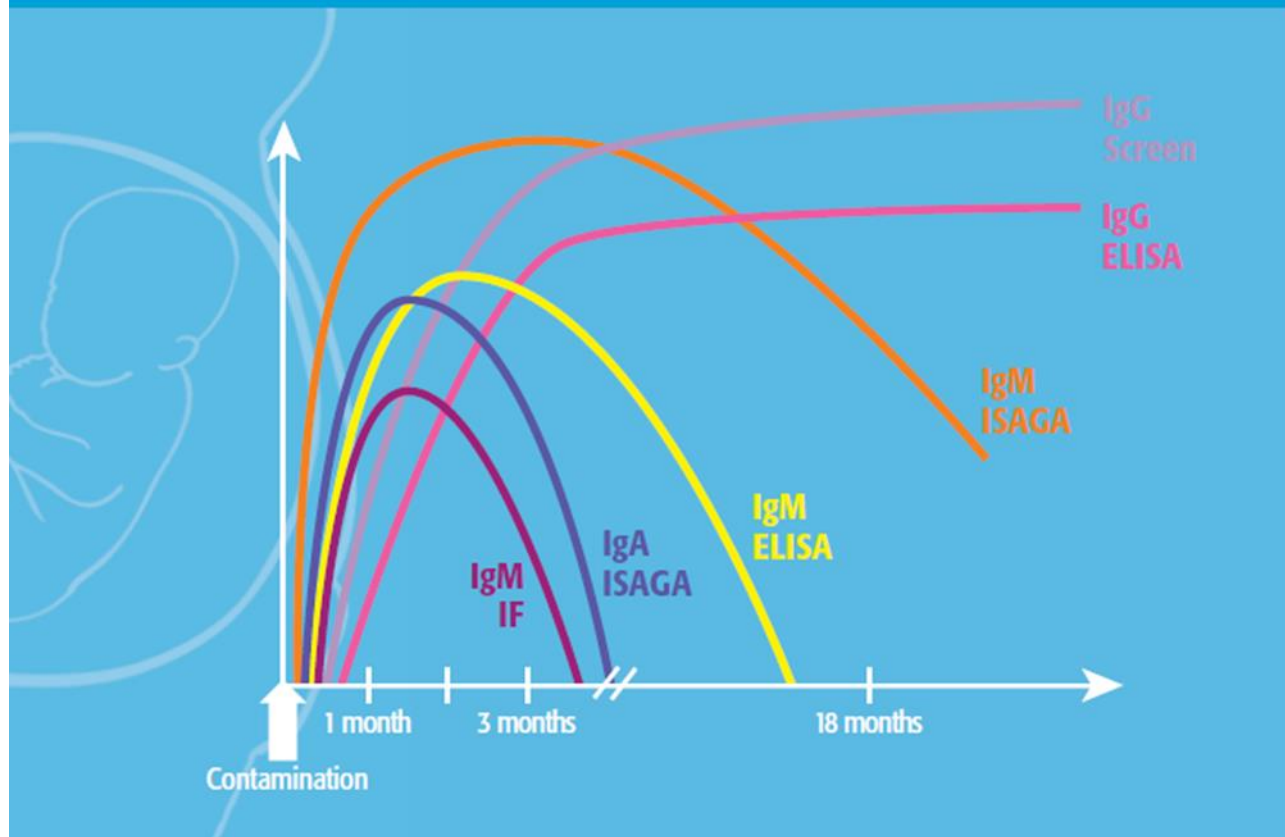
Diagnostic Test	Advantages	Disadvantages
Microscopy	• Relatively low cost • Widely available	• Poor sensitivity • Time consuming • Skilled microscopist essential
Immunoassay based methods	• Good sensitivity • Wide variety of kits available • Convenient adjunct to microscopic analysis	• Not widely available in developing countries due to cost constraints and limited detection spectrum of kits • False positives
Molecular/Nucleic acid amplification methods	• Exceptional sensitivity • Capable of species and subspecies	• Expensive reagents and instrumentation required • Requires skilled technician

Serološka dijagnostika

- Indirektna
- Brojne metode
- Epidemiološka potreba, dokaz akutne infekcije
- Problemi: križne reakcije, razlikovanje akutne od prošle infekcije, imunodeficientni pacijent, novorođenče
- Western blot testovi



Antibody kinetics during toxoplasmosis infection



Ostale metode – intradermalni test

- U nedostatku adekvatnog serološkog testa – intradermalni test
- Casoni test za hidatidnu bolest
- Otkriva staničnu imunost





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DOI: 10.1111/jmi.13433

INVITED REVIEW

Journal of
Microscopy



Artificial intelligence-powered microscopy: Transforming the landscape of parasitology

Mariana De Niz^{1,2} | Sara Silva Pereira³ | David Kirchenbuechler^{1,2} |
Leandro Lemgruber⁴ | Constadina Arvanitis^{1,2}

Key Advantages:

- **High Sensitivity and Accuracy:** often exceeds human performance in identifying parasites, especially in light-intensity infections
- **Reduced Lab Workload & Fatigue:** AI acts as an untiring screening tool, filtering out negative samples and reducing the manual burden on laboratory technicians.
- **Fast Diagnostics:** Automated systems can rapidly process large numbers of images
Standardization: AI provides consistent, objective evaluations, reducing the variability associated with the skill level of human operators
- **Improved Access in Remote Settings:** Portable, smartphone-based, or low-power AI microscopy tools allow for diagnosis in resource-limited, remote areas

Key Disadvantages:

- **Data Dependence & Generalization:** AI models require large, diverse datasets for training; they may struggle with, or fail to recognize, species for which they have insufficient training data
- **High Initial Implementation Costs:** Setting up can be costly
- **Distinguishing Non-Pathogenic Organisms:** AI can sometimes mistake non-pathogenic protozoa or artifacts for parasites
- **Requirement for Expert Verification:** Despite high sensitivity, expert-verified AI often still out-performs fully autonomous AI, meaning human experts are still necessary, particularly for tricky cases or to avoid out-of-focus errors

DIAGNOSIS OF FECAL PARASITIC STAGES

Principle of diagnosis of Fecal parasitic stages

FECAL PROTOZOA:- Diagnostic Stages & Pictorial Key
- Challenges in the identification of fecal parasites

Ayman A. El-Badry, MBBS, M.Sc, M.D.

Prof. and Clinical consultant of Medical Parasitology (Clinical Microbiology)

Ex-Head of Lab of Molecular Medical Parasitology (LMMP)

Ex-Head of Diagnostic and Research Unit of Parasitic Diseases (DRUP)

- Medical Parasitology Dept., Kasr Al-Ainy School of Medicine, Cairo University, Cairo, Egypt.

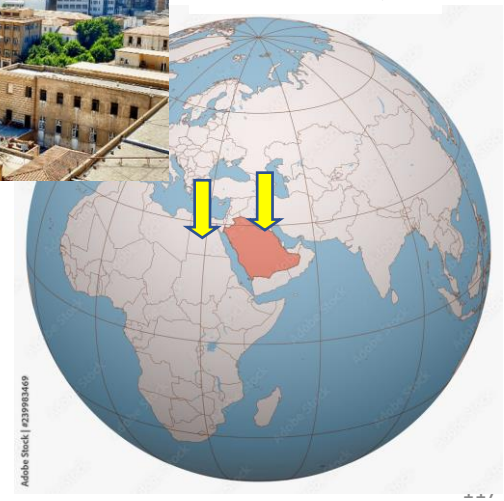
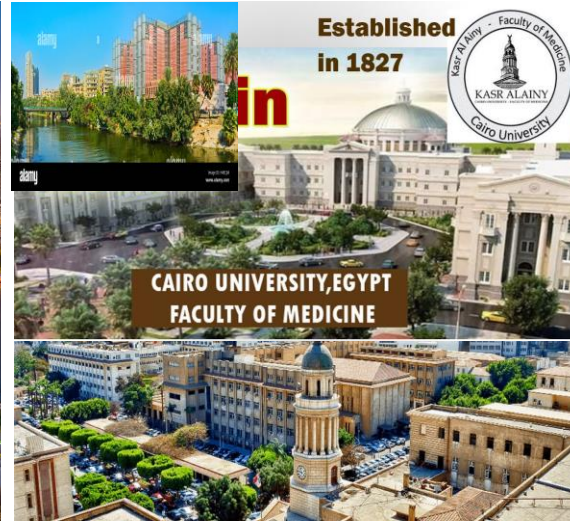
- Department of Microbiology, College of Medicine & King Fahad Hospital of the University,

Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia.

aelbadry@kasralainy.edu.eg aelbadry@iau.edu.sa - Website: www.profelbadry.com



Disclosure: No Conflict of Interest.



Outlines

- I. Case base studies of common fecal protozoa
- II. Pictorial key for identification of fecal protozoa
- III. Spot diagnosis of fecal protozoa
- IV. Successful identification of fecal protozoa
- V. Fecal testing options & workflow chart
- VI. AI in the diagnosis of fecal protozoa

No hand-out
Take notes – Photo
It is personal - Please Do not distribute

Diagnosis of Human Parasitic Infections (PI) = Diagnostic Parasitology

Diagnosis: (Latin)

Dia = through & **Gnosis**
knowledge

Reach **THROUGH KNOWLEDGE**

= “YOU SHOULD HAVE THE **KNOWLEDGE** TO **REACH**”

Without **diagnostics**, medicine is **blind** 

We (para/lab/microbiologists) are **the eyes that can see**,
But NOT SEEN

Diagnosis of parasitic infections in humans

is - **Challenging**,

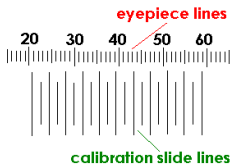
- **Interesting**, and

- **Fun**.

includes

 hat is(are) the diagnostic approach for diagnosing **PI**?

Principles for Successful Microscopic Identification



I. Measurement

→ **Size** (microscope calibration by ocular and stage **micrometry**)

II. Movement

→ **Protozoa trophozoites** (fresh, unpreserved specimens)

III.

→ **Shape,**
Special features,
Color,
Contents (Internal structure(s) & **Nuclear pattern**)

IV. Stains

→ **Internal structure(s) & Nuclear pattern**

Diagnostic key-points

Successful Microscopic Identification depends upon:

- Collecting satisfactory specimens.
- Proper preparation and maintenance of reagents.
- Correct performance of techniques.
- Thorough examination of preparations.

Size is one of the most important and a major diagnostic feature (A must)

Exact size of a microorganism ONLY determined by calibrated microscope (micrometer) – it is an essential tool in diagnostic parasitology

**The Eyes Don't See
What the Mind Doesn't Know**

**A high Index of Suspicion
is the Key to identify **rare** parasite**

Ayman El-Badry

Diagnosis of Human PIs

Diagnostic Approach

- I. Clinical diagnosis “Provisional diagnosis !”
- History** (Age, gender, residency, occupation, previous infection, family...)/Travel-moved
 - Complain** (Symptoms)/**Clinical examinations** (signs)

II. Investigations “Confirm diagnosis = Evidence”



- Imaging** (Diagnostic imaging)



- Laboratory investigations** (Lab diagnosis)

Conventional



- Visualize parasite** (Direct from specimen or after $\pm$ Culture/animal inoculation)

Microscopy = Etiological/Morphological diagnosis



- Detect parasite-Ag OR Host-Ab/sT-lymphocytes** $\pm$ Eosinophilia (CBC = complete blood count)

Immuno-diagnosis



- Detect parasite-nucleic acid (NA)** NA Hybridization/NAAT

Molecular diagnosis



Mol.



+ **Special tests** e.g. Biochemical tests + /**Proteomics**

Diagnostic parasitology (Lab): Detection of parasite stage(s)/product(s) in clinical specimens using laboratory procedures.

Case I

A 24-years-old man had diarrhea for six weeks. He had traveled to South Asia a few weeks prior.

Is it normal to have mucus in stool in healthy individuals?

At the microbiology lab the stool specimen was processed.

The figure shows stool smear under the microscope by **Direct Wet mount**

What is your diagnosis?

Based on which criteria?



At the microbiology lab the stool specimen was processed.

The figure shows stool smear under the microscope by **Direct Wet mount**

What is your diagnosis? Based on which

Concentrated faecal smear under the microscope by **Wet mount**

What is your diagnosis? Based on which

Spherical

S 16 μ in diameter

C Brown (iodine)

C 2 nuclei (vesicular) [= small Nucleolus](Small + Central)

+Peripheral chromatin (Fine + evenly distributed)

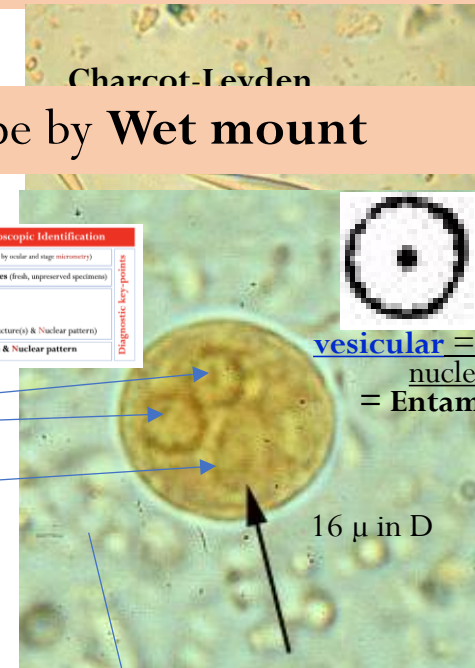
-Diffuse glycogen vacuole (?)

Diagnostic key-points

I. Measurement
II. Movement
III. Morphology
IV. Stains

Principles for Successful Microscopic Identification	
I. Measurement	Size (microscope calibration by ocular and stage micrometry)
II. Movement	Protozoa trophozoites (fresh, unspun specimens)
III. Morphology	Shape, special features, Color, Contents (internal structure(s) & Nuclear pattern)
IV. Stains	Internal structure(s) & Nuclear pattern

Diagnostic key-points



vesicular = bull's eye
nucleus
= Entamoeba

16 μ in D

Dx Tip

focusing up and down on the focal plane of a cyst (also an egg surface)

Principles for Successful Microscopic Identification

16 μ in D

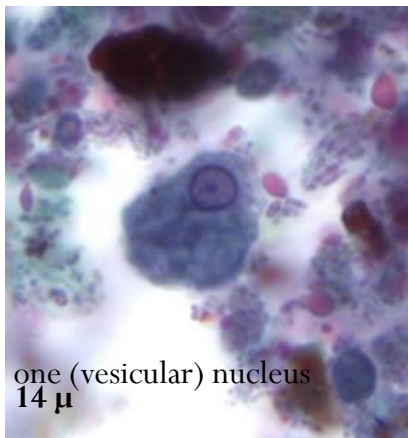
What is the next standard microscopic procedure?

When examining the **permanently stained faecal smear**, the following **images** are seen under the microscope

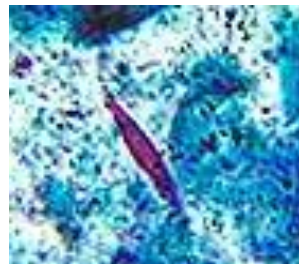
What is your diagnosis?
Based on which criteria?

Why do we have both
trophozoite & cysts

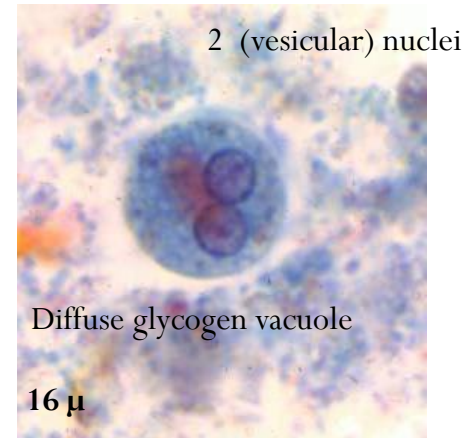
What is the
value of
Trichrome
(permanent)
staining?



Entamoeba
trophozoite



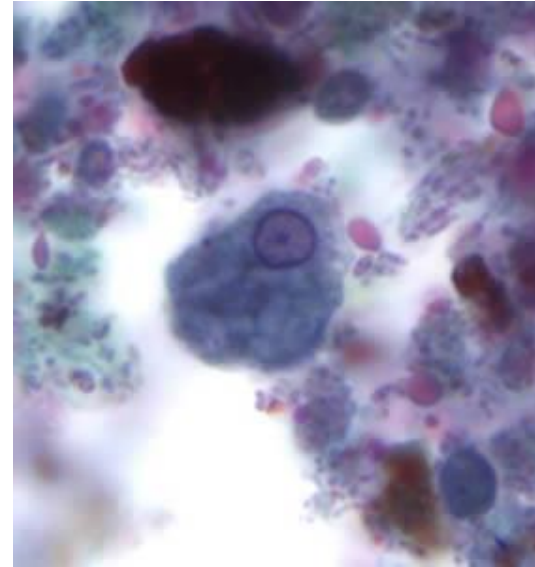
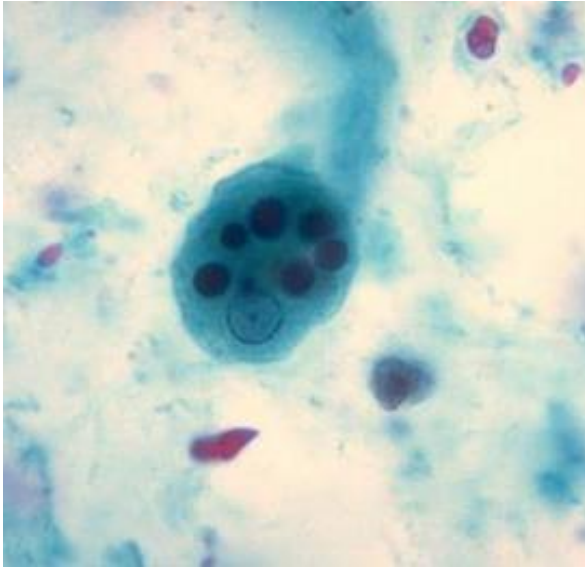
Charcot-Leyden crystal



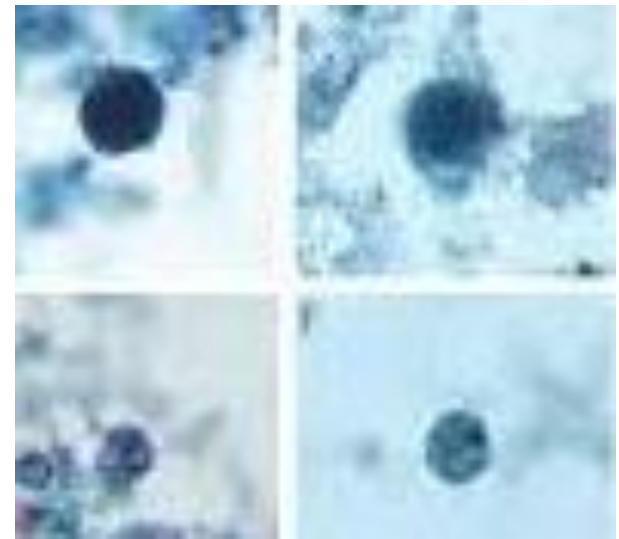
Entamoeba Cyst

reported. "***Entamoeba histolytica* trophozoites and cysts.**"

Identify the following 2 organisms?



What is the difference?



Temporarily *Buffered methylene blue (BMB)* stained wet mount Stains only amoeba Trophozoite:

- Cytoplasm stains **light blue**
- Nucleus & Inclusions (e.g. ingested RBCs or yeast cells) stain **Dark Blue**

Does not stain amoeba cysts or flagellate trophozoites or cysts.

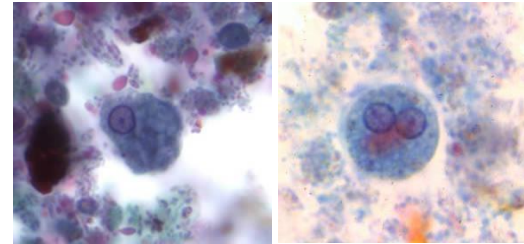


How many *Entamoebae* can inhabit human intestine?

Entamoeba contains many species,

Six of which reside in the lumen of human large intestine

- *E. histolytica*
- *E. dispar* (twin)
- *E. moshkovskii* (twin) (*Entamoeba bangladeshi*)
- *E. hartmanni* (little brother)
- *E. coli* (big sister)
- *E. polecki*



E. histolytica complex (*E. histolytica*/*E. dispar*) ?

E. histolytica complex has THREE indistinguishable forms; two nonpathogenic forms, *E. dispar* & *E. moshkovskii* and pathogenic form, *E. histolytica* which are morphologically indistinguishable from pathogenic *E. histolytica* species.

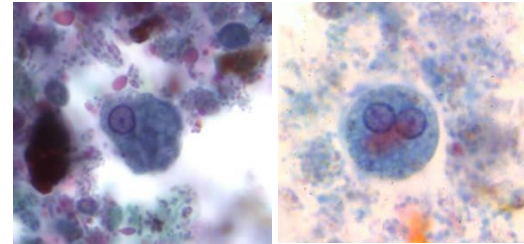
E. dispar & *E. moshkovskii* are responsible for approximately **10** times as many infections as *E. histolytica* & do not require treatment.

How many *Entamoebae* can inhabit human intestine?

Entamoeba contains many species,

Six of which reside in the lumen of human large intestine

- *E. histolytica*
- *E. dispar* (twin)
- *E. moshkovskii* (twin) (*Entamoeba bangladeshi*)
- *E. hartmanni* (little brother)
- *E. coli* (big sister)
- *E. polecki*



Why the identified organism is not *E. hartmanni*?

E.H complex: trophozoites $>12\mu\text{m}$ & the cysts $>10\mu\text{m}$.

Organisms that measure below these measurements should be identified as *E. hartmanni* [5–10 μm (usual range, 6–8 μm)].

Practice tip.

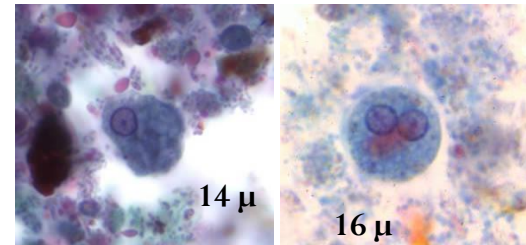
Cysts tend to shrink - "halo"

How many *Entamoebae* can inhabit human intestine?

Entamoeba contains many species,

Six of which reside in the lumen of human large intestine

- *E. histolytica*
- *E. dispar* (twin)
- *E. moshkovskii* (twin) (*Entamoeba bangladeshi*)
- *E. hartmanni* (little brother)
- *E. coli* (big sister)
- *E. polecki*



Why the identified organism is not *E. coli*?

In vesicular nucleus: chromatin irregularly stained; karyosome eccentric

Trophozoites are often mistaken for *E. histolytica* due to their overlap in size [10–35 μm (usual range, 15–25 μm)].

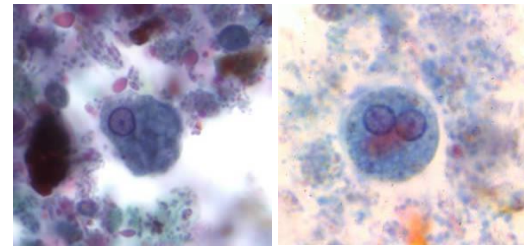
The cysts are distinguished by noticing the 5-8 nuclei found **in the** mature form.

How many *Entamoebae* can inhabit human intestine?

Entamoeba contains many species,

Six of which reside in the human intestinal lumen

- *E. histolytica*
- *E. dispar*
- *E. moshkovskii* (*Entamoeba bangladeshi*)
- *E. hartmanni*
- *E. coli*
- *E. polecki*



Others amoebae & intestinal protozoa in addition to pseudoparasites (artifacts) are morphologically different but sometimes there is morphological overlap with

E. histolytica

- *Endolimax nana*
- *Iodamoeba butschlii*
- *Blastocystis* spp.
- *Dientamoeba fragilis*

AMEBAE							
	<i>Entamoeba histolytica</i>	<i>Entamoeba hartmanni</i>	<i>Entamoeba coli</i>	<i>Entamoeba polecki</i> ¹	<i>Endolimax nana</i>	<i>Iodamoeba butschlii</i>	<i>Dientamoeba fragilis</i> ²
Trophozoite							
Cyst							No cyst

¹Rare, probably of animal origin
²Flagellate

Scale: 0 5 10 μm

Adapted from Brooks and Metcalf, 1968

¹Rare, probably of animal origin

²Flagellate

Scale: 0 5 10 μ m

Adapted from Brooke and Melvin, 1964

E. histolytica is **Over-Diagnosed & Over-treated**

What are other diagnostic option for giardiasis?

Detect – *Entamoeba histolytica* complex antigen (**IAs**), **or**
- *Entamoeba histolytica* DNA (**PCR**)

Entamoeba Faecal Immunoassays (Faecal IAs)

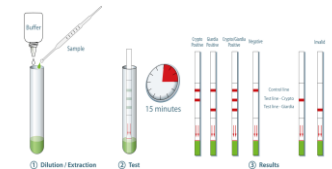
- Enzyme/florescent immunoassay (Screening).
- Rapid immunochromatographic (ICT) assay [cartilage/casset]

Detects *Entamoeba* antigen.

Also available to detect antigens of *Giardia* & *Cryptosporidium* **OR**
Entamoeba, *Giardia* & *Cryptosporidium*.

Quick & easy to perform and **no special** equipment is needed.

- **Borderline positives & Questionable negative** results,
require further confirmed by additional testing.



Entameoba Faecal DNA (Faecal PCR)

Confirm diagnosis & differentiate *Entamoeba histolytica* complex species

E.histolytica, What is the problem

PCR assays, a practical non-microscopic method, can differentiate between amoebae infections.

So **PCR assays** → Determine the true prevalence of *E. histolytica* → avoid unnecessary treatment (Overmedication).

Over-Diagnosis + Over-Medication
A Need for Molecular Diagnosis

CASE II

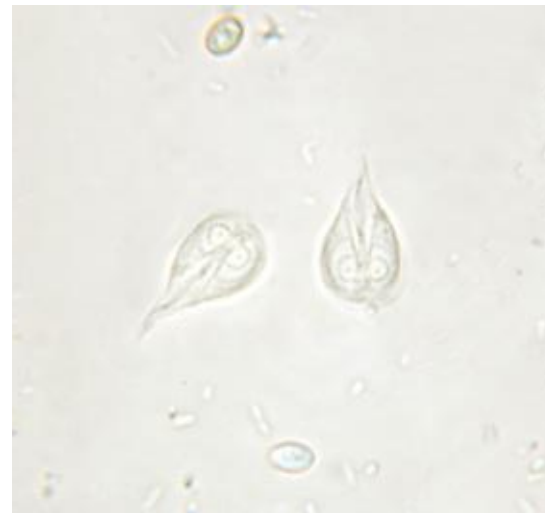
A 7-year-old girl was brought by her mother to GIT clinic. She had diarrhea for 4 days.

Her symptoms included epigastric pain, abdominal cramps, watery foul-smelling fatty stool, and excess gas.

At the microbiology lab the stool specimen was processed direct wet mount.

Figure show what was detected in stool smear under the microscope (**unstained faecal smear**)

What is your diagnosis?
Based on which criteria?



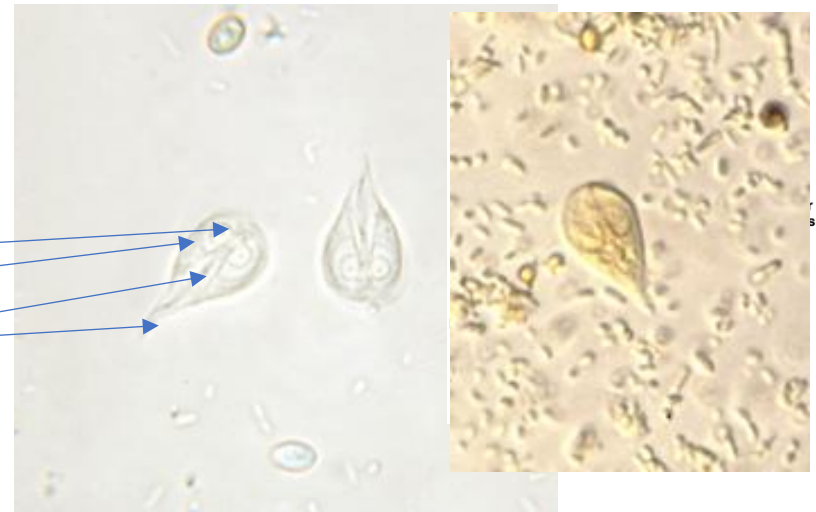
http://www.atlas-protozoa.com/gallery.php?SOT_CAP=ARTE#0

At the microbiology lab the stool specimen was processed direct wet mount.

Figure show what was detected in stool smear under the microscope (**unstained faecal smear**)

What is your diagnosis?
Based on which criteria?

- S pyriform
- S 5-10x10-20 (7-14) um
- C - 2 nuclei
- C -2 sucking discs
- C - Flagella
- Axostyle, &
- comma-shaped **parabasal bodies** (Not seen in unstained trophozoite)



http://www.atlas-protozoa.com/gallery.php?SOT_CAP=ARTE#0

At the microbiology lab the stool specimen was processed direct wet mount.

Figure show what was detected in stool smear under the microscope (**Iodine stained faecal smear**)

What is your diagnosis?
Based on which criteria?

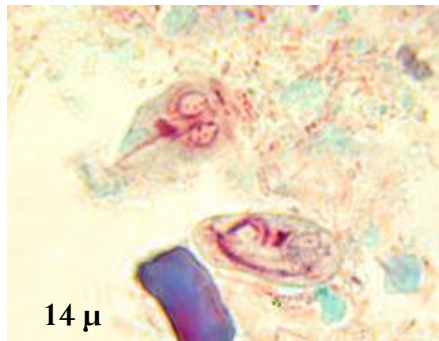
- S** Oval (ellipsoid)
- S** 7-10x11-14 (8-12) um
- C** - 4(mature)/2(immature) **nuclei** at one end.
- C** - **Prominent longitudinal fibers** = **filaments: S-shaped** (longitudinally located) remnants of **retracted flagella** & **comma shaped parabasal bodies** (maybe seen)
Axonemes = **Axostyle** (maybe seen)



What is the next standard microscopic procedure? (work flow chart for processing of stool for microscopic examination)

When examining the **permanently stained faecal smear**,
the following **images** are seen under the microscope

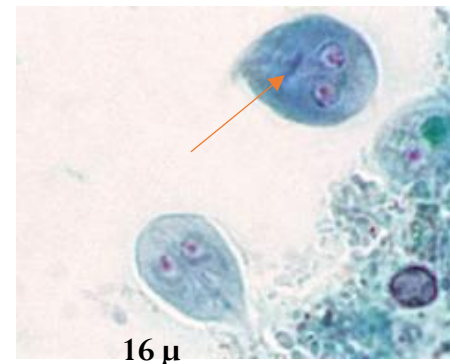
What is your diagnosis?
Based on which criteria?



Giardia trophozoite

Axostyle

No. of **Nuclei**



Giardia 2 trophozoites

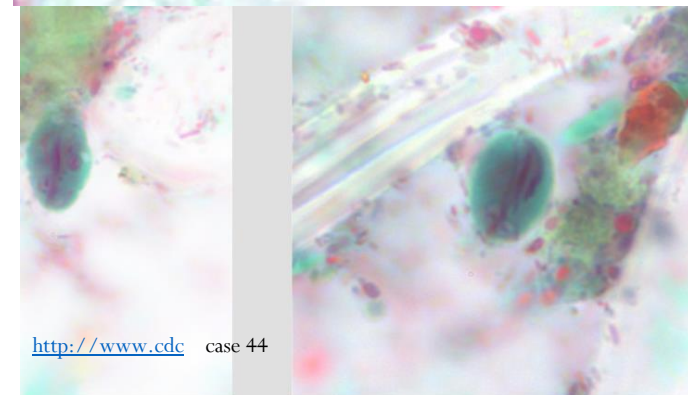
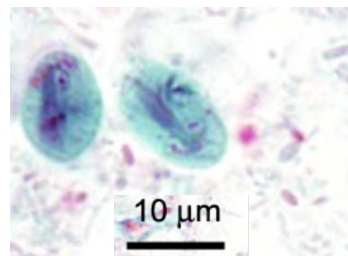
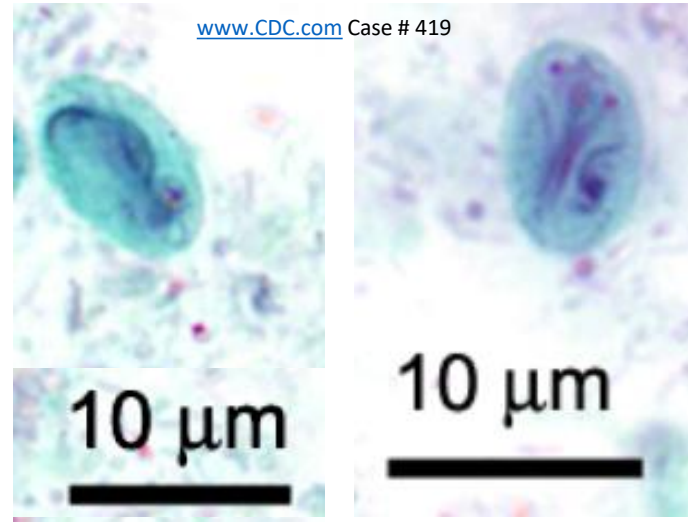
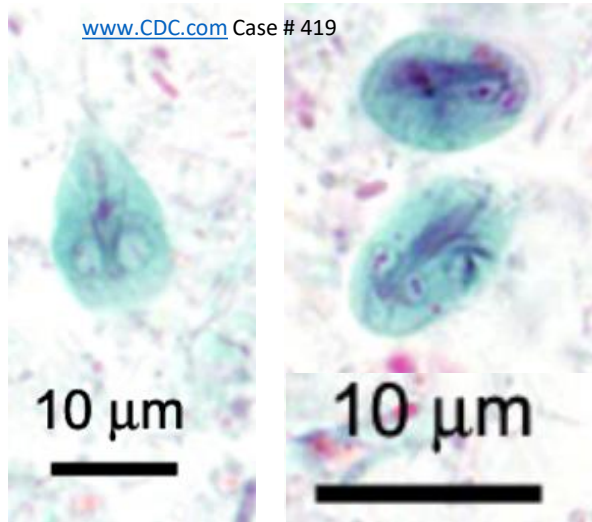


Figure C

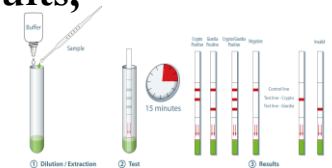
Giardia Faecal Immunoassays (Faecal IAs)

- Enzyme/florescent immunoassay (Screening).
 - **Rapid immunochromatographic** (ICT) assay [cartilage/casset]
- Detects *Giardia* antigen.

Also available to detect antigens of *Giardia* & *Cryptosporidium* **OR**
Entamoeba, *Giardia* & *Cryptosporidium*.

Quick & easy to perform and **no special** equipment is needed.

- **Borderline positives & Questionable negative** results,
require further confirmed by additional testing.



Giardia Faecal DNA (Faecal PCR)

Confirm diagnosis & also some detect *Giardia* assemblage.

Pictorial key of protozoa (oo)cyst & trophozoite

See Next **(oo)Cyst** YES **Rigid Wall** NO **Trophozoite**

Contents
• Flagella
• Cilia
• N.

Permanent Stain

Nuclei

- Chromatin pattern

3) Pseudopodia = Amoeba

Changeable shape

NO 2) Flagella NO 1) Cilia

Oval

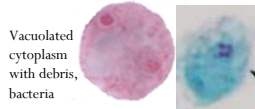
2(80%) [1 (20%)]
Peripheral chromatin: Absent
- Endosome: Large, fragmented
(4-8 granules (fragments))

No. of Nuclei 2 1 One

Nuclear Chromatin

Peripheral Chromatin (PC)
Karyosomal Chromatin (Endosome)

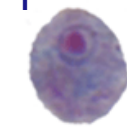
Present
Relatively small
Vesicular nucleus
(Kart Wheel) =



5-12 μ
Dientamoeba fragilis

Large Irregular endosome
(blot-like/ bird eye)

Large Regular endosome
(basket nucleus) Surrounded by refractile, indistinct achromatic granules

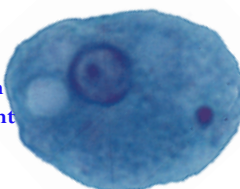


6-12 μ
Endolimax nana
thin layer of pigment around the nucleus



8-20 μ
Iodamoeba butschilii

Sluggish movement

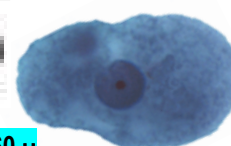


20-50 μ
Entamoeba coli

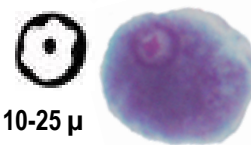


15-60 μ
Entamoeba histolytica
/dispar/moshkovskii (+RBCs)

Directional movement



<15 μ
Entamoeba hartmanni
(often eccentric small endosome)



10-25 μ
Entamoeba polecki

Resemble E.coli, but occasionally with central regular peripheral Chromatin
Naturally in Pigs & Monkeys



Balantidium coli

Color

Shape
Size (~5-100 μ)
Color

Prof. Ayman El-Badry
Adapted and redrawn, WHO, 1991

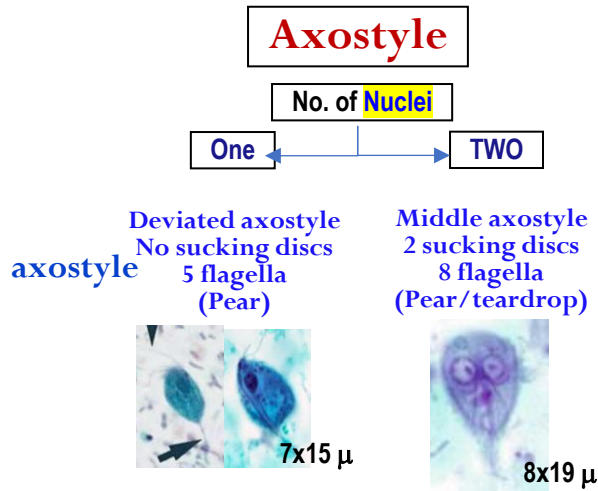
Round/oval/Irregular or Y-shaped
Central (bird eye) / eccentric

basket nucleus
= If chromatin on one side
(more common in cyst)

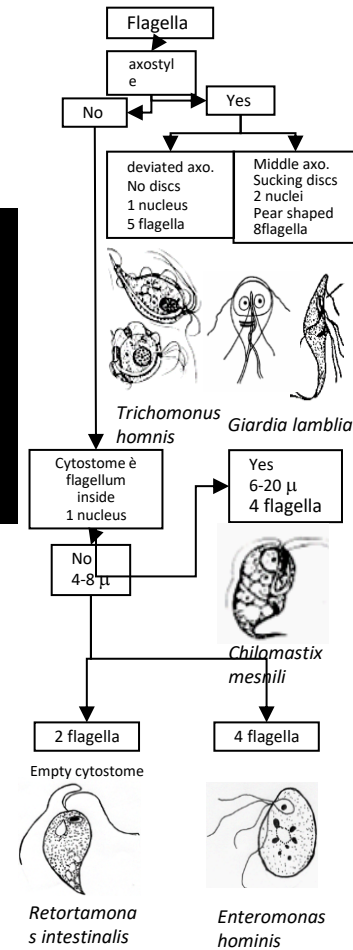
I. butschilii / *E. nana*: Difficult to distinguish
between trophozoites of *I. butschilii* (larger size)
and *E. nana*

The key feature in Giardia is **Axostyle**

Other fecal parasites has axostyle:



Pentatrichomonas hominis



Pictorial Key for Protozoa (Oo) Cysts (Temporarily stain)

Rigid (Oo) Cyst Wall

No filaments

Filaments inside

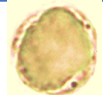
Vacuole

Yes

No

Large up to 90%
+ 1-4 Nuclei

Small
+ 1 Nucleus



Blastocystis sp.



Iodamoeba butschilii

Spindle



Cystoisospora belli

Mass of protoplasm
12x30μ

Oval/
Spherical

Vesicular Nucleus

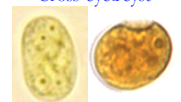
No

Yes

Nuclei

Large Endosome
Absent Peri. Chromatin

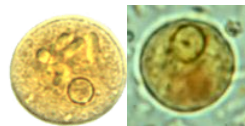
Nuclei paired at 2 poles
"Cross-eyed cyst"



Endolimax nana
gibbous appearance

1 Nucleus

Large Nucleus 1/3
Full of chromatoid bodies



E. polecki

R.H: Pig

+ cysts e 2-4 nuclei



E. histolytica precyst

≥ 2 nuclei

Nuclei

eccentric
uneven

5-8

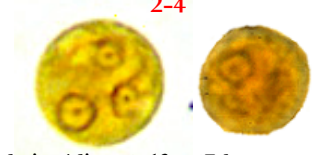


E. coli

Small Endosome
Peri. Chromatin

central
even

2-4



E. histolytica / dispar >12μ <*E. hartmanni*

1 Nucleus

Lemon Shape
5-9 μ



Chilomastix mesnili

Pear shaped
4-7 μ



Retortamonas intestinalis

≥ 2 nuclei

4 nuclei at 2
pole



Entromonas hominis
Resembles *E. nana* cyst. Fibrils
or flagella are usually not seen

2-4 nuclei at
1 pole
+ parabasal



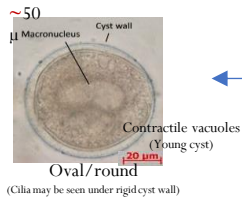
Giardia intestinalis

By Ayman El-Badry

Adapted and redrawn, WHO, 1991

<https://www.cdc.gov>

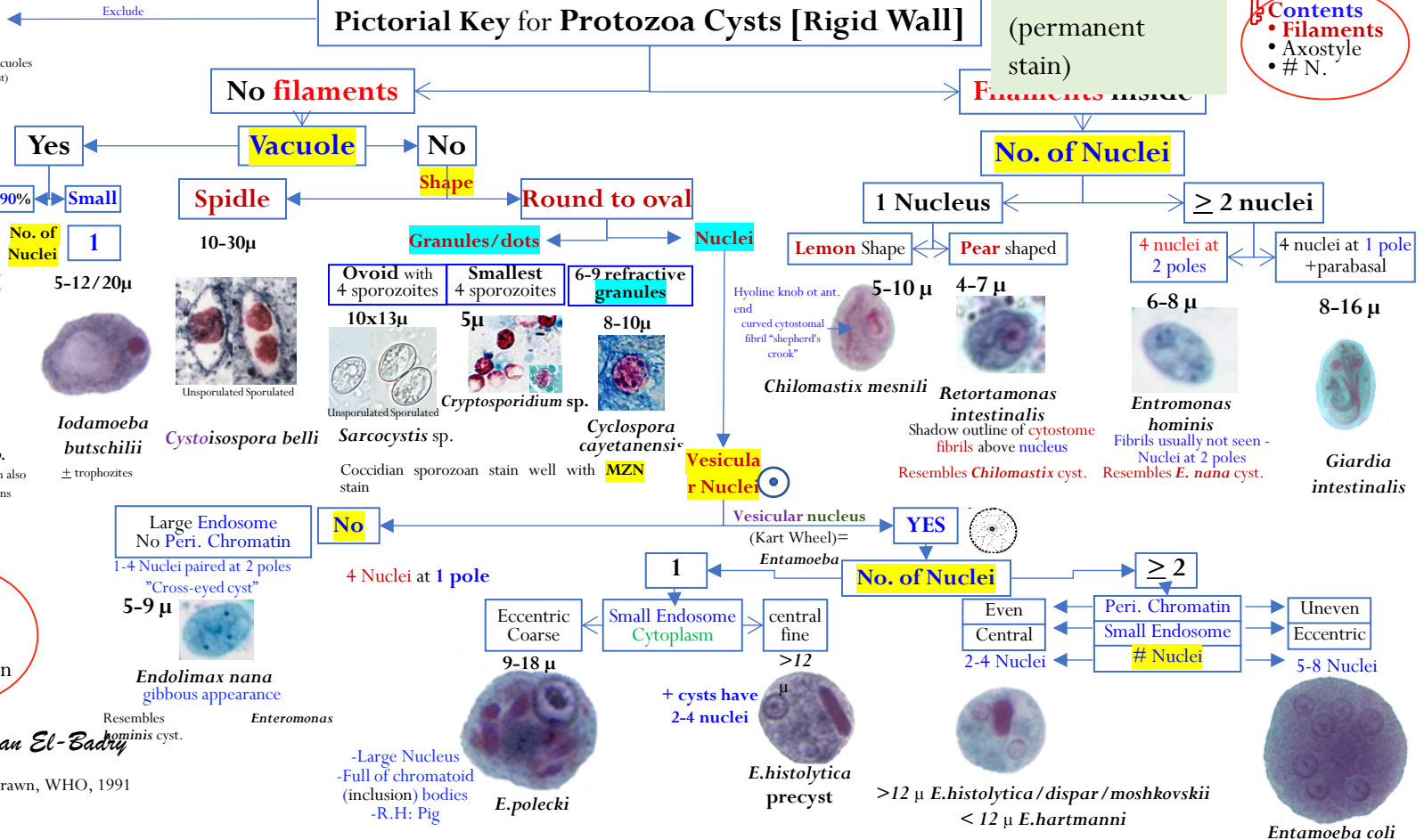
<http://www.atlas-protozoa.com>



Pictorial Key for Protozoa Cysts [Rigid Wall]

(permanent stain)

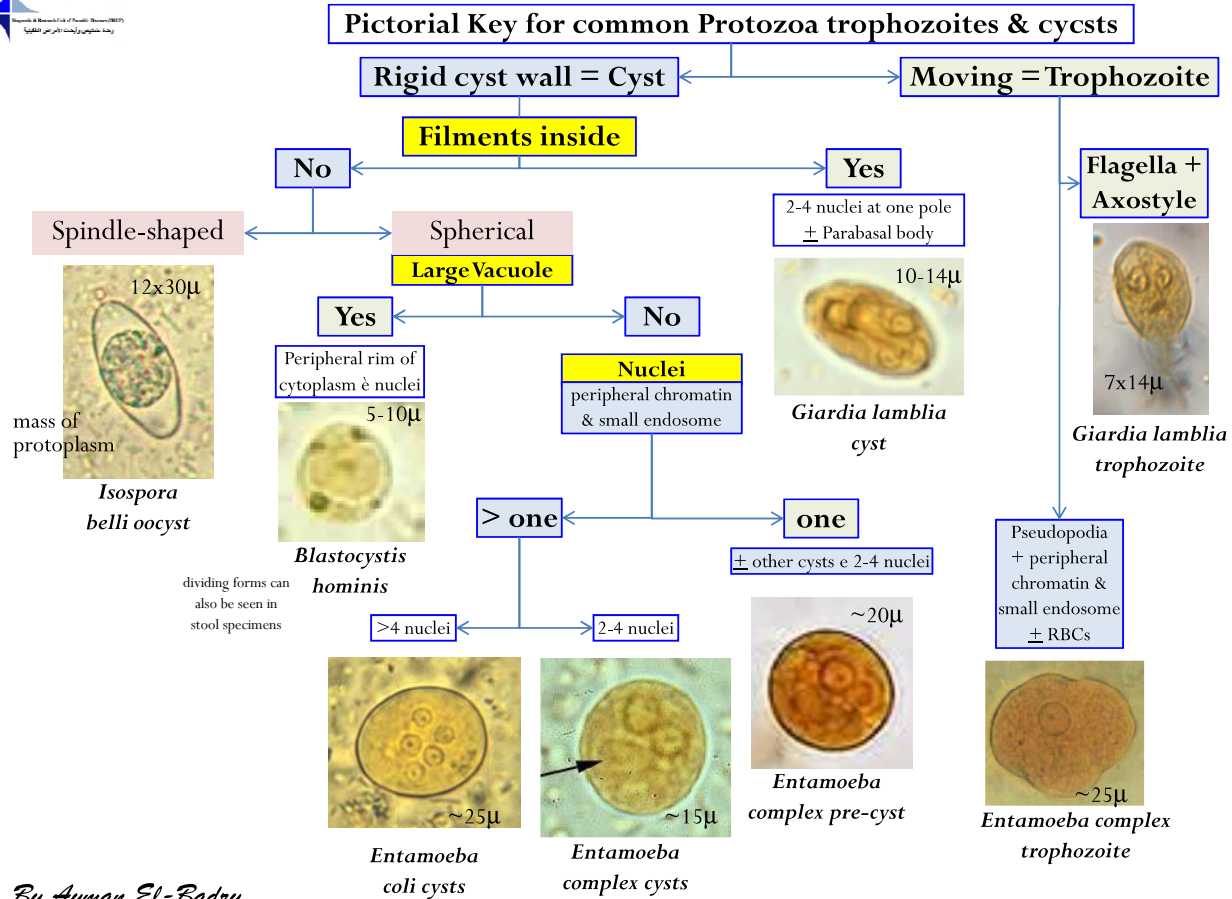
Contents
• Filaments
• Axostyle
• # N.



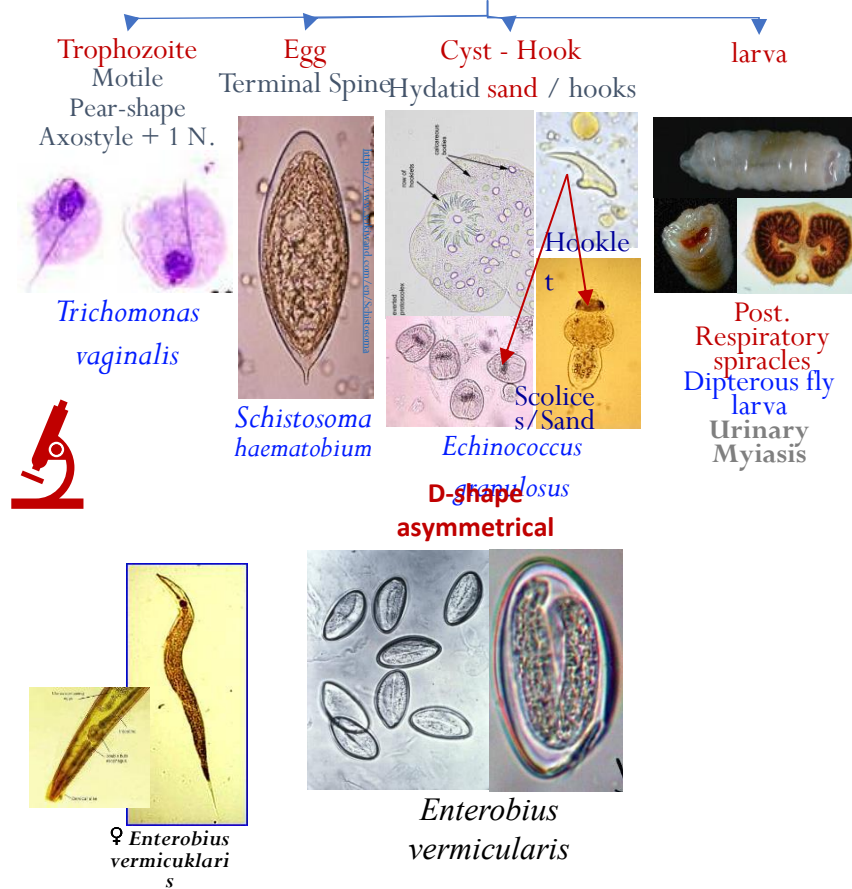
Shape Contents
• Vacuole
• N.: # - chromatin

Prof. Ayman El-Badry

Adapted and redrawn, WHO, 1991



Urine (U. discharge) Microscopy



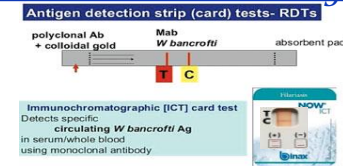
Immunodiagnosis of UG parasites

1. Antigen detection

(RDT Rapid diagnostic test)

I. Tichomonas vaginalis RDT

II. *Wuchereria bancrofti* RDT



III. Malaria **RDT**



2-band malaria
RDTs (P.v or P.f)

3-band malaria
RDTs (Pan, P.v,
P.f)

4-band malaria
RDTs (Pan, P.v,
P.f, P.m)

IV. Schistosoma **RDT**

Mention the principle & value of RDT

2. Serology (Antibodies detection)

Mention the role of serology in diagnosing urogenital parasites.

Protozoi krvi i tkiva

Medicinsko značajni krvno - tkivni protozoi

- Plasmodium
- Babesia
- Toxoplasma
- Acanthamoeba
- Balamuthia
- Naegleria
- Leishmania
- Trypanosoma

Medicinsko značajni krvno - tkivni protozoi

Protisti tjelesnih šupljina

- Filogenetski stariji
- Bolje prilagođeni
- Rijetko izazivaju ozbiljne smetnje

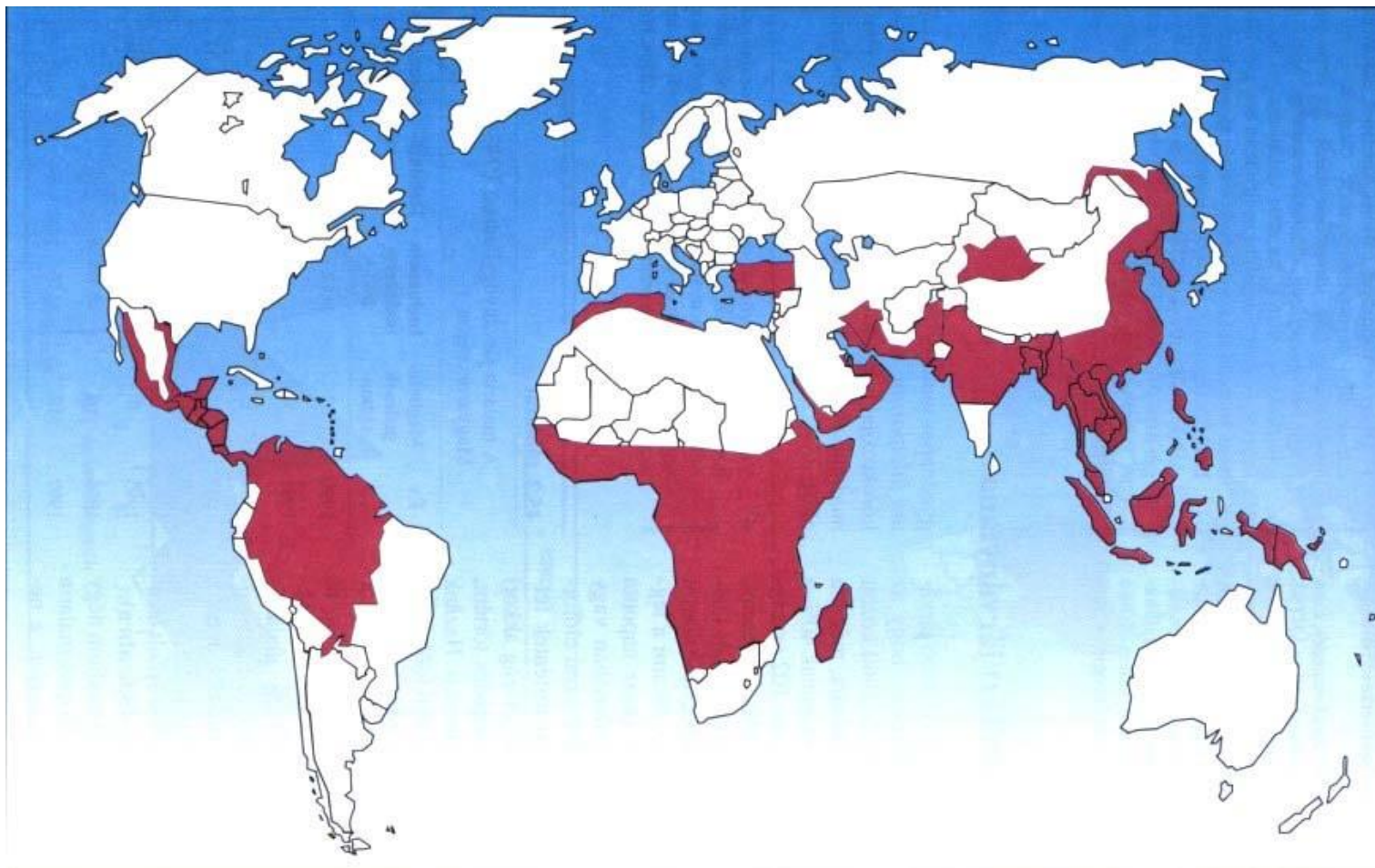
Krvno-tkivni protisti

- Većina se prenosi hematofagnim člankonošcima
- Bolesti dramatične, mogu imati smrtni ishod

Malaria – Plasmodium

- Uzročnik malarije
- Jedna od najznačajnijih infektivnih bolesti današnjice
- WHO (2009) - endemska u 100 zemalja svijeta (40% ljudi, 2,5 milijarde ljudi izloženo)
- Godišnja incidencija- 300-400 mil.kliničkih slučajeva
- Godišnji mortalitet oko 0,5 mil.ljudi

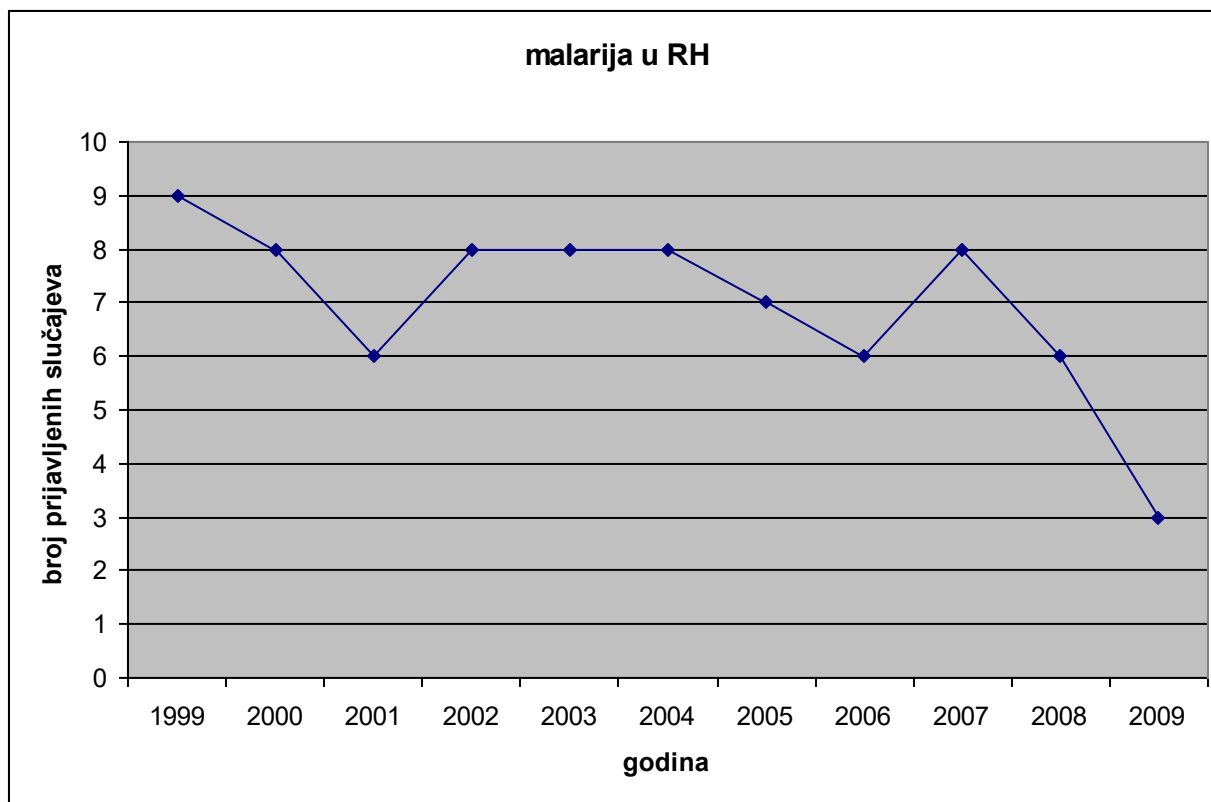
Malarija – Plasmodium



Malaria – Plasmodium

- Malaria putnika (turisti, radnici)
- Malaria zračnih luka
- Transfuzijska malaria
- Malaria u i.v. korisnika droga

Malaria – Plasmodium



Malaria – Plasmodium

- Poznato oko 172 vrste
- Za čovjeka specifični

Plasmodium vivax

Plasmodium ovale

Plasmodium malariae

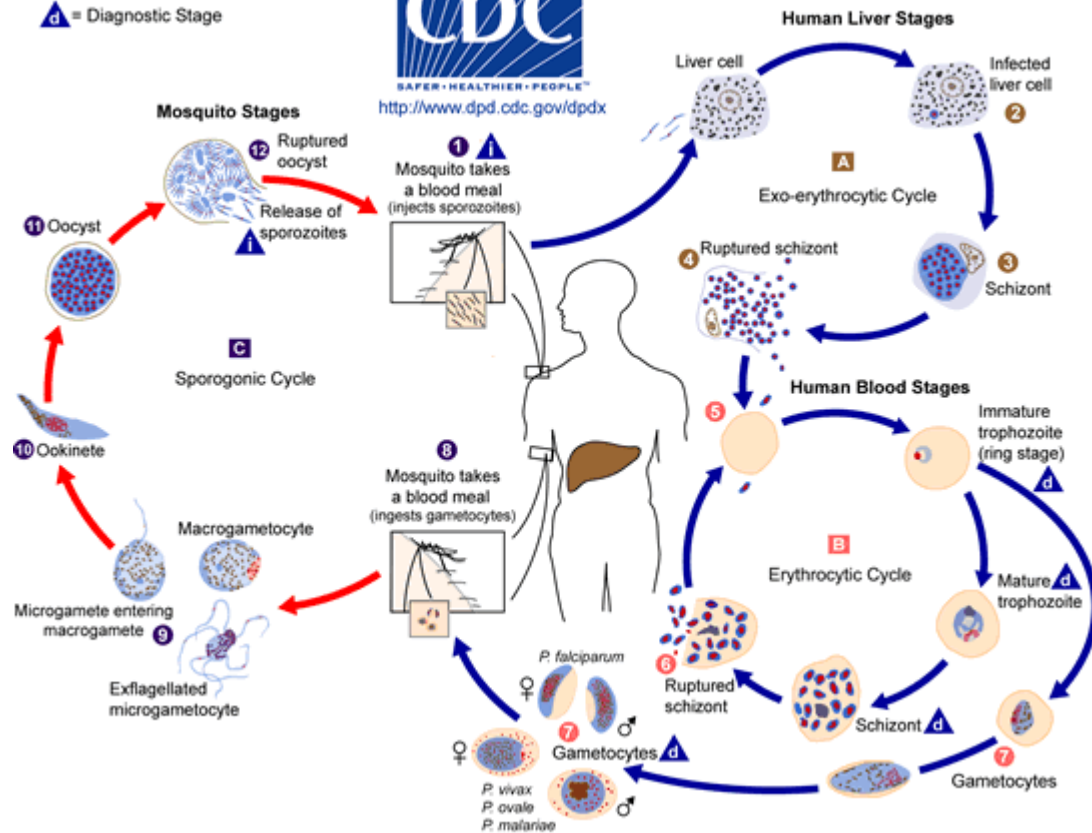
Plasmodium falciparum

Plasmodium knowlesi

i = Infective Stage
 d = Diagnostic Stage



<http://www.dpd.cdc.gov/dpdx>



Malaria – Plasmodium

- Inkubacija 7-35 dana
- Klinička slika - ovisna o vrsti plazmodija, imunom statusu bolesnika, dobi
- Simptomi: nespecifični inicijalni simptomi - glavobolja, bolovi u mišićima, opća slabost, mučnina, tresavica, visoka temperatura (kontinua, intermitentna), znojenje, proljev
- Anemija, cerebralna malaria, konvulzije, respiratorni distres, cirkulatorni kolaps, zatajenje bubrega

Malaria tertiana

- P.vivax i P.ovale
- Inkubacija: 9-20 dana (rjeđe nekoliko tjedana do mjeseci)
- Parazitemija: niska (max. 1-2 %)
- Tijek: obično benignan
- Česti relapsi (do 5 godina)- hipnozoiti

Malaria quartana

- *P.malariae*
- Inkubacija: 15-40 dana
- Parazitemija: niska (max. 1%)
- Tijek: obično benignan
- Česte rekrudescence (do 30 godina)

Malaria tropica

- P.falciparum
- Inkubacija: 7-15 dana ili duže
- Parazitemija: visoka (20% i više)
- Tijek:
 - inicijalni simptomi jače naglašeni
 - težak tijek u neimunih osoba
 - visok letalitet u neliječenih
(50-60% u neimunih Europljana)

Malaria tropica

- Rekrudescenca rijetka, obično unutar godine dana
- Moguće teške komplikacije:
cerebralna malarija, teška anemija, edem pluća i respiratorna insuficijencija, bubrežna insuficijencija, cirkulatorni kolaps, hemoragije, DIK, hiperpireksija

Malaria quotidiana – *P. knowlesi*

- Uzročnik malarije u dugorepih majmuna makaka (*Macaca fascicularis*)
- Prije par godina prihvaćen kao “novi” uzročnik malarije u čovjeka
(broj slučajeva, prijenos čovjek-komarac-čovjek)



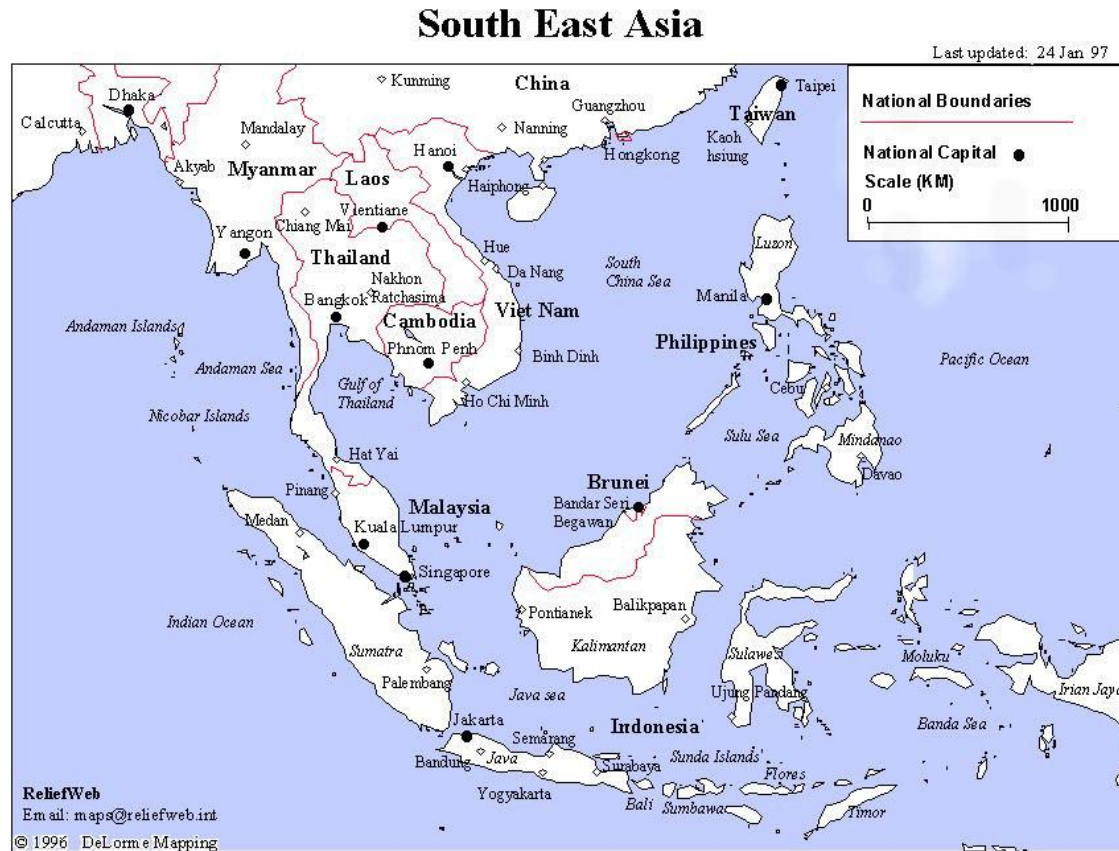
Malaria quotidiana – P. knowlesi

- 1931. godina-Knowles-opis-majmun Macaca
- 1932. godina – eksperimentalno dokazano da može uzrokovati klinički manifestnu bolest kod ljudi
- Do 1955. godine – korišten kao piretički agens za liječenje sifilisa kod pacijenata s neurosifilisom

Malaria quotidiana – *P. knowlesi*

- 1965 – prvi slučaj prirodno stečene *P. knowlesi* malarije Malezija
- 1971– drugi opisan slučaj prirodno stečene bolesti (Malezija)
- 2004 – povećani broj izvješća o ovoj infekciji u jugo istočnoj Aziji
- Molekularna djagnostika
- Spremljeni serum, preparati (1990 – *P. knowlesi* prisutan)
- Do 70% slučajeva malarije u jugoistočnoj Aziji uzrokovano ovim parazitom (Singh et al. Lancet, 2004. 369: 1017-1024)

Malaria quotidiana – P. knowlesi



The boundaries and names shown on this map do not imply official endorsement or acceptance by the United Nations or ReliefWeb. These maps may be freely distributed. If more current information is available, please update the maps and return them to ReliefWeb for posting.

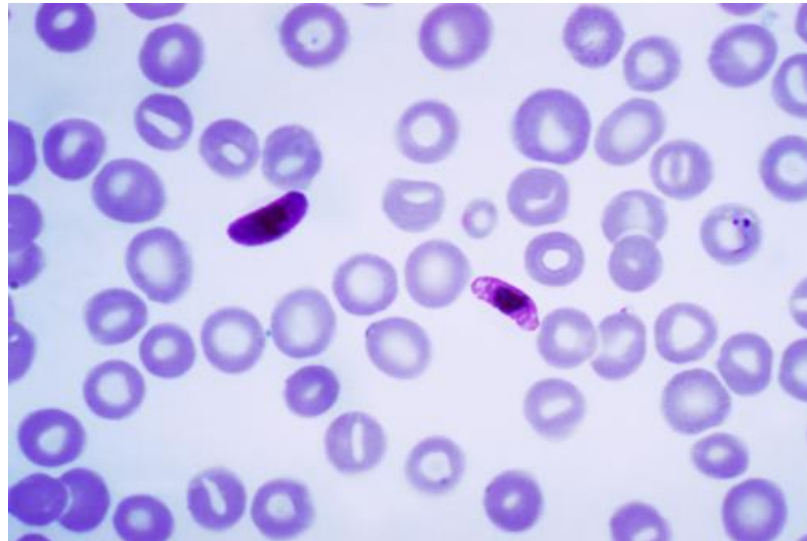
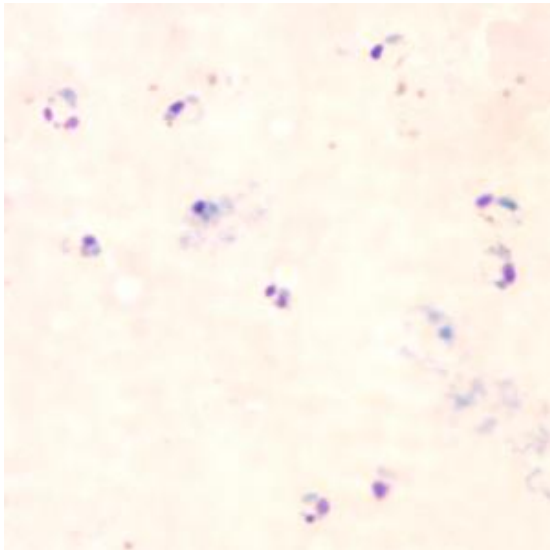
Malaria quotidiana – *P. knowlesi*

- Život u eritrocitu – 24 sata
- Visoka parazitemija, klinička slika
- Hipnozoiti nisu nađeni

Malaria - dijagnostika

Krvni razmaz i gusta kap

- Gusta kap – veća količina krvi, veća osjetljivost
- Krvni razmaz – konačna determinacija krvnog parazita



Malaria - dijagnostika

- Mikroskopija
- Veličina
- Oblik eritrocita
- Oblici - svi ili samo neki, nazubljeni eritrocit, plastičan, forme uz membranu, slušalice
- Granulacije
- Trfozoiti, ameboid
- Merozoiti – broj, forma
- Gametociti
- Parazitemija

P. vivax



ring form



mature ring form



trophozoite



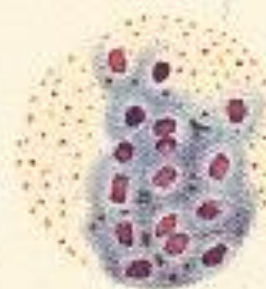
trophozoite



early schizont



schizont



mature schizont



developing gametocyte

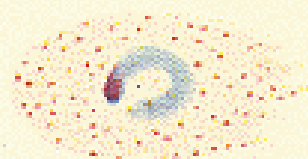
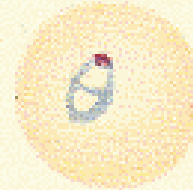


female gametocyte



male gametocyte

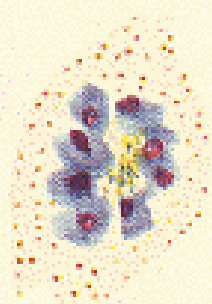
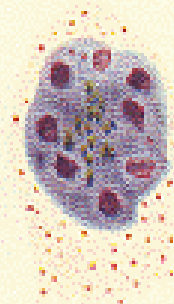
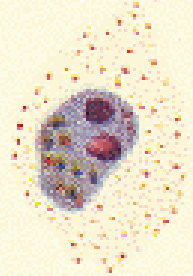
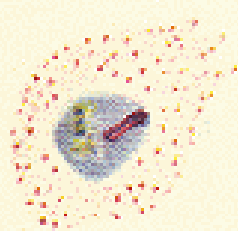
P. ovale



young ring

older ring

comet form



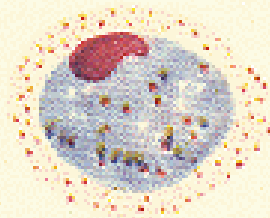
trophozoite

trophozoite

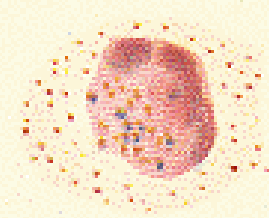
young schizont

schizont

mature schizont



female gametocyte



male gametocyte

P. falciparum



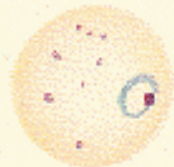
marginal form



ring form



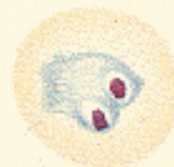
double dotted rings



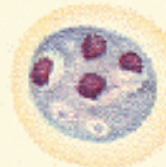
ring form



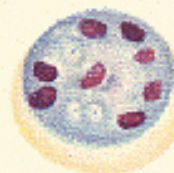
young trophozoite



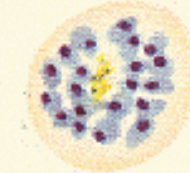
trophozoite



early schizont



schizont



mature schizont



female gametocyte

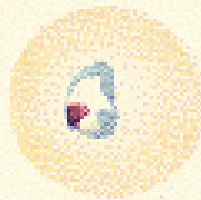


male gametocyte

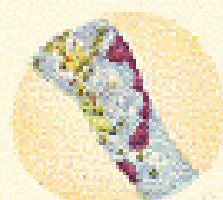
P. malariae



ring form



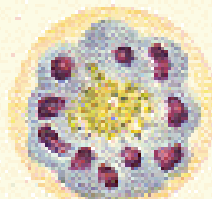
early band form



band form



early schizont



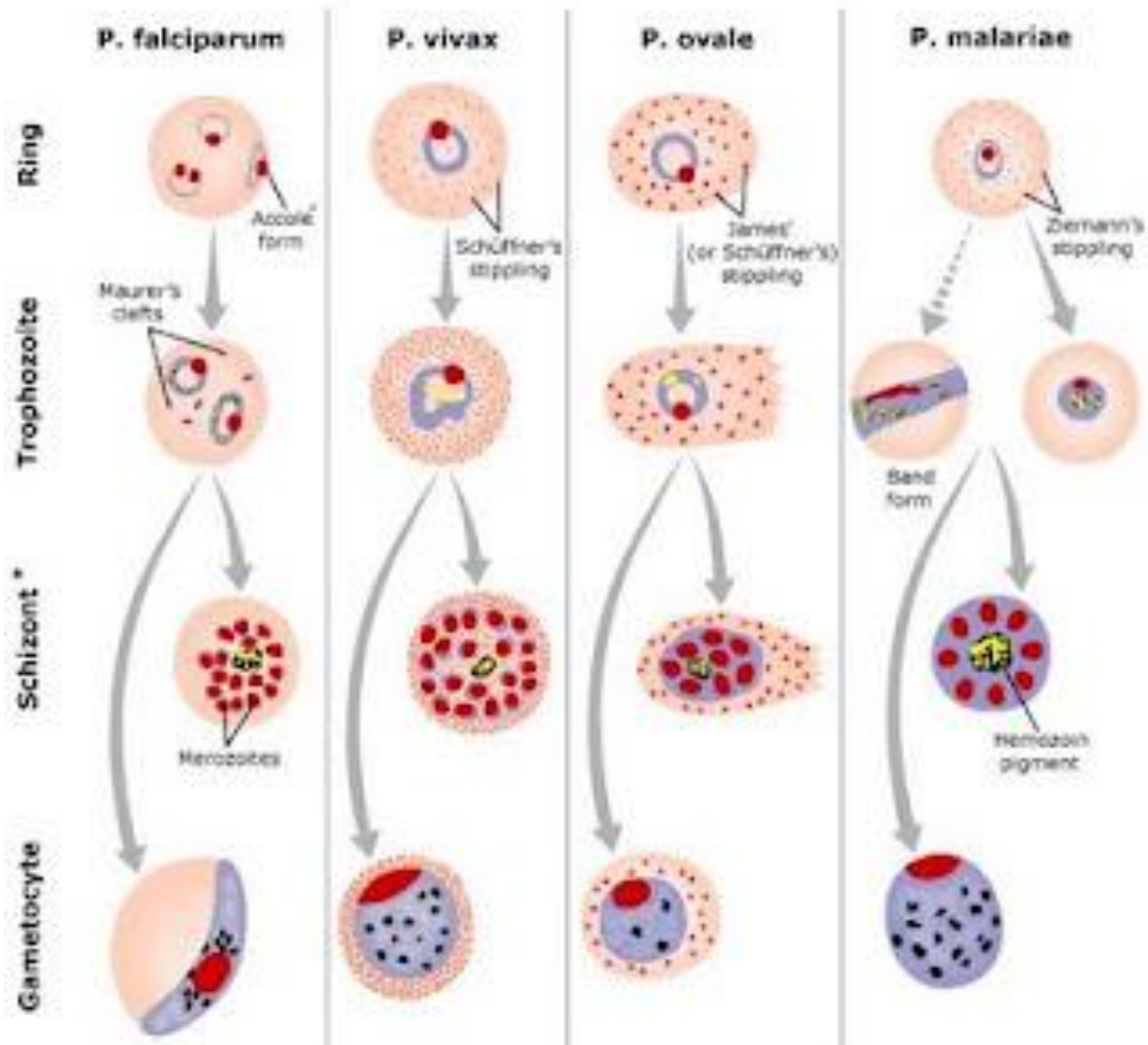
mature schizont

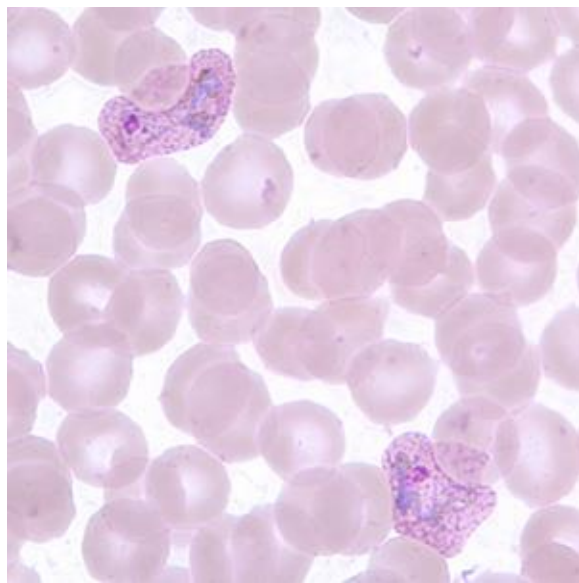


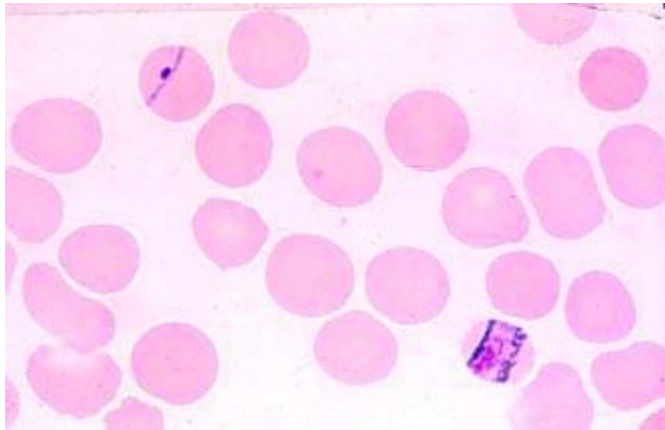
female gametocyte

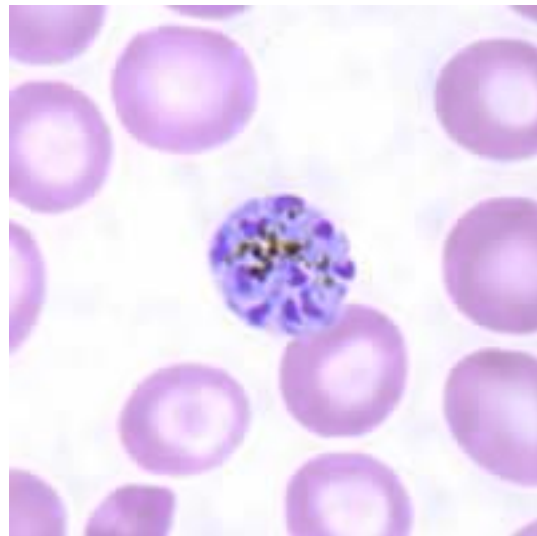


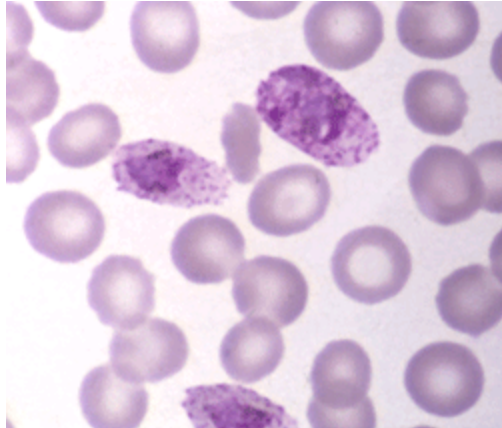
male gametocyte

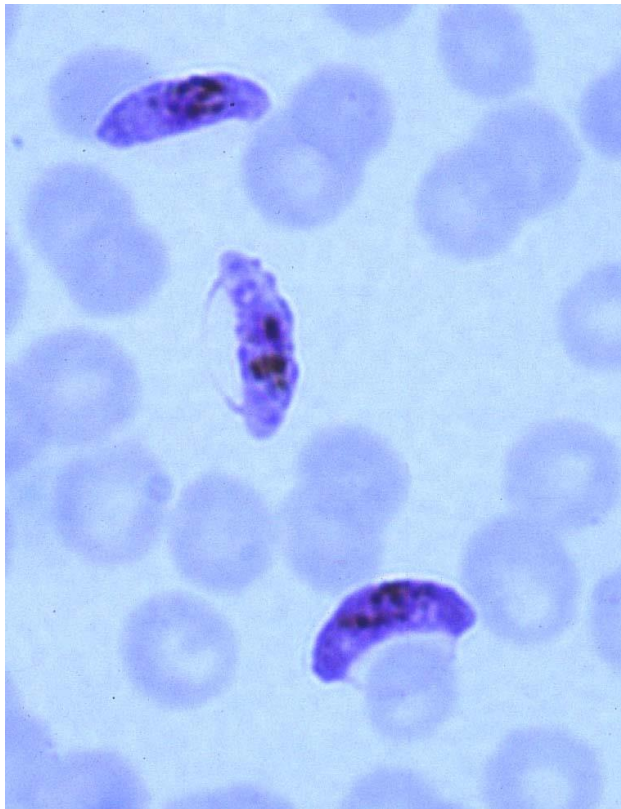


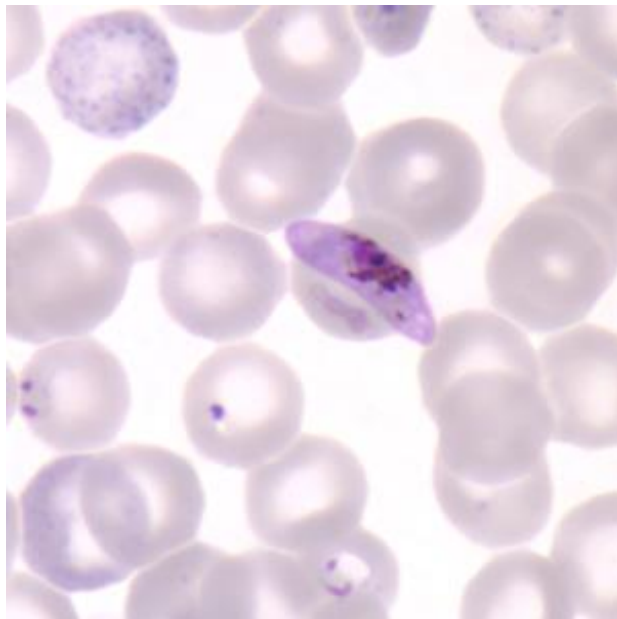


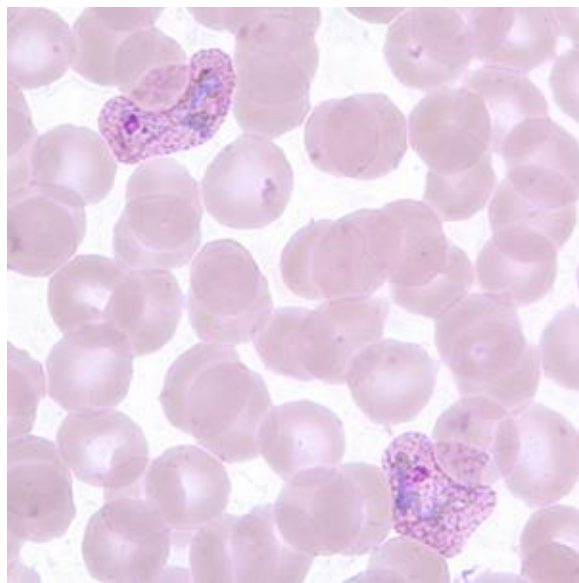


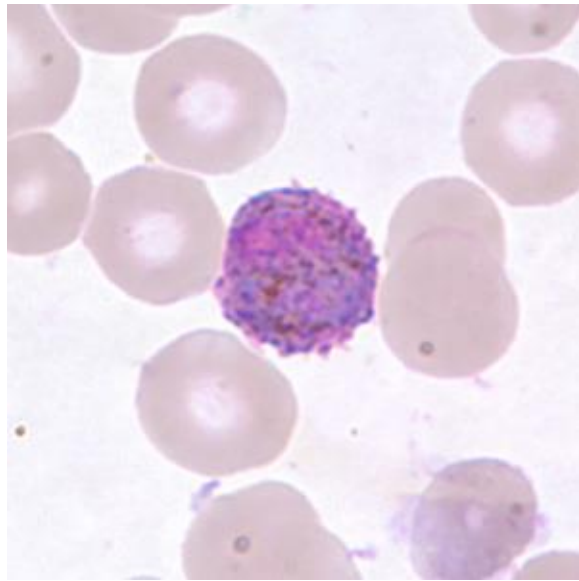


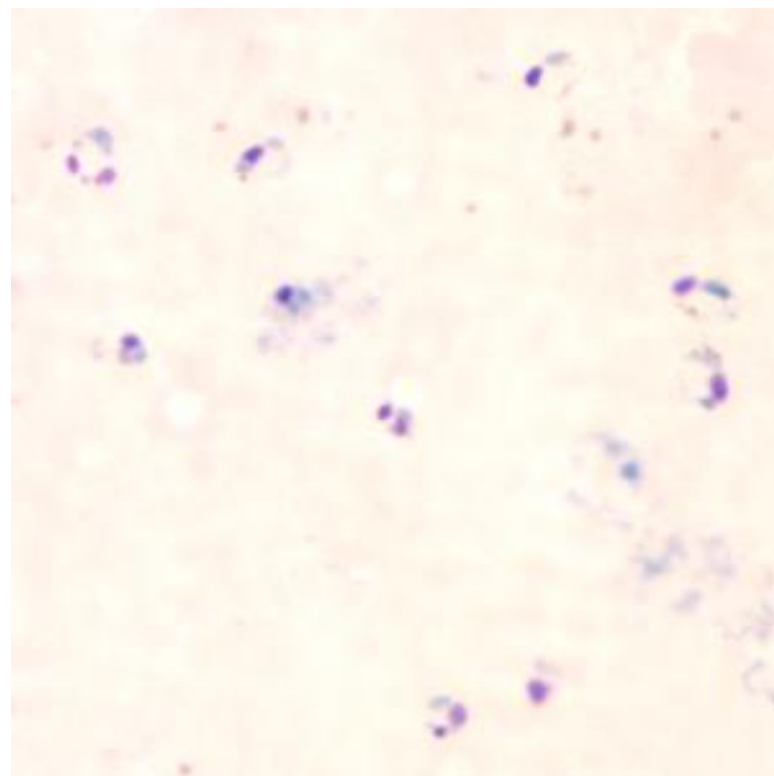


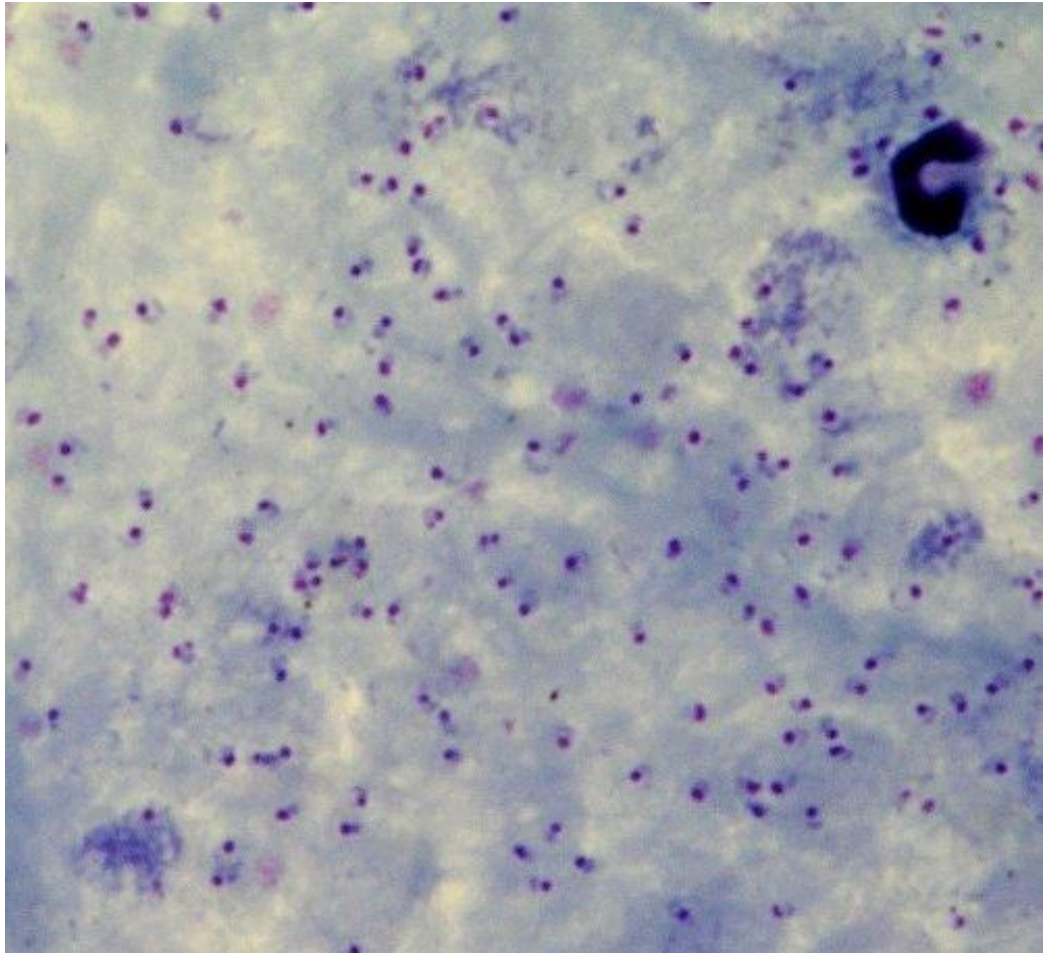


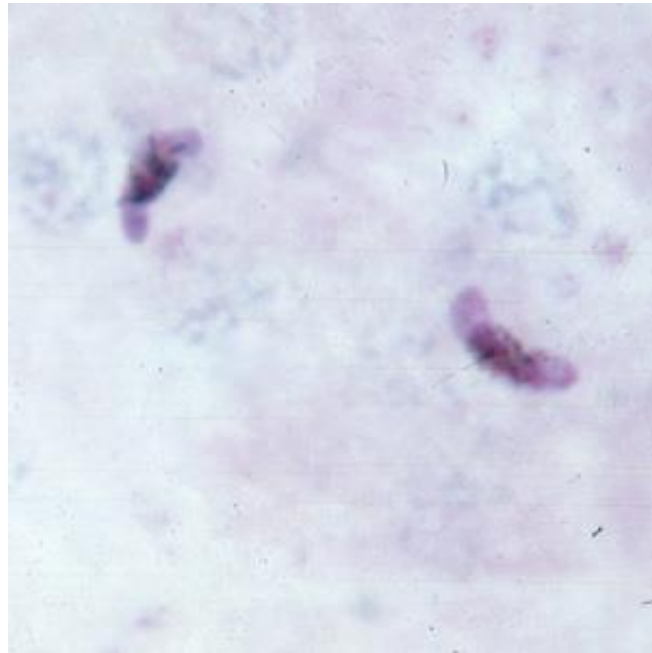










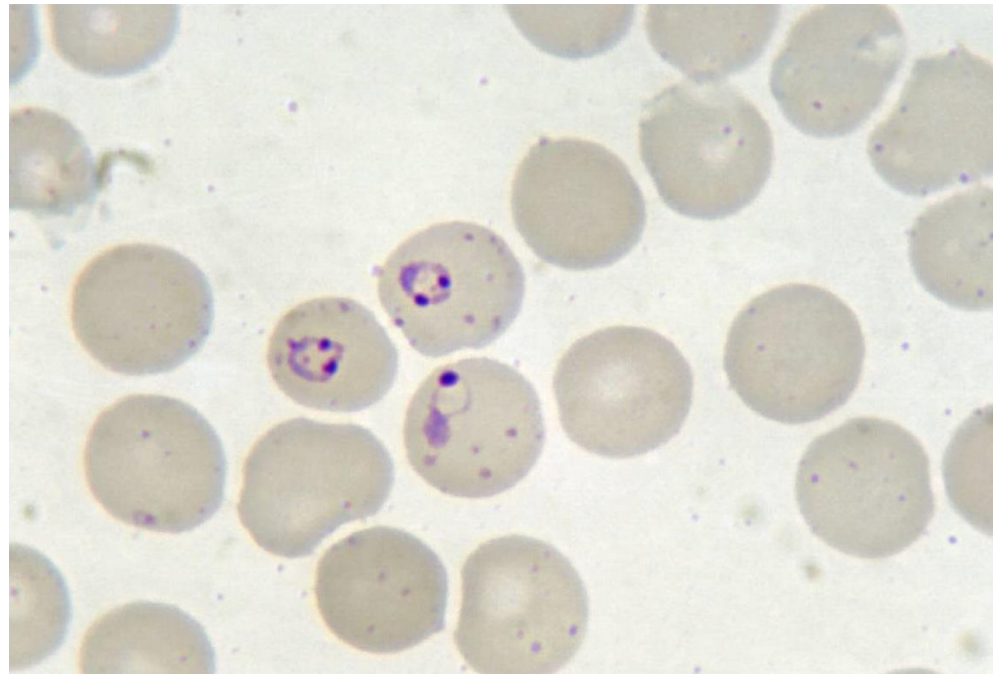


P. knowlesi

Krvni razmaz i gusta kap

Rani trofozoit

- Prsten ispunjava do 1/3 eritrocita
- Ponekad više prstenova u jednom eritrocitu
- Dvostruki kromatin

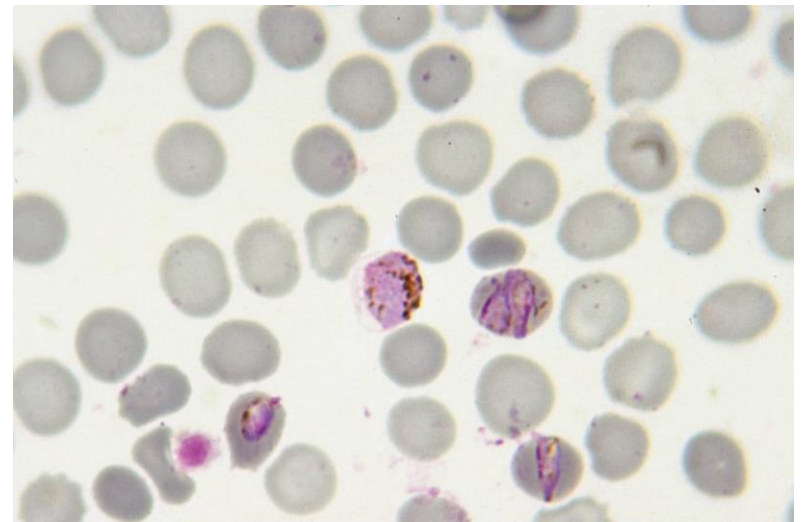


P. knowlesi

Krvni razmaz i gusta kap

Kasni trofozoit

- Zauzima ne više od 2/3 eritrocita
- Citoplazma kompaktna, ne ameboidna, “poput vrpce”

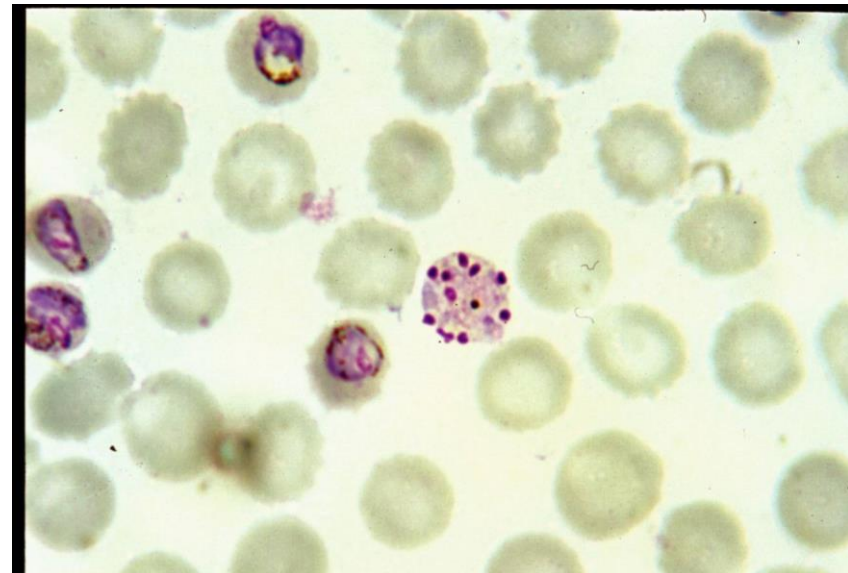


P. knowlesi

Krvni razmaz i gusta kap

Šizonti

- 8-16 merozoita
- Crni malarični pigment
- Rozeta česta



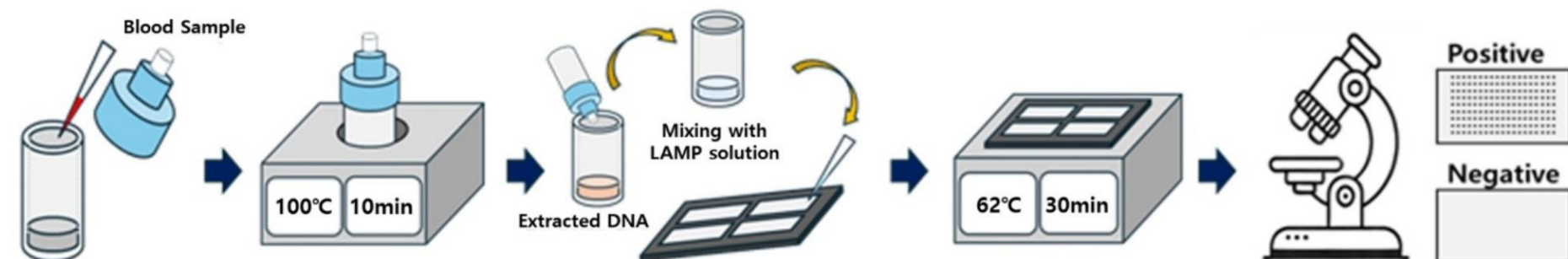
Malaria - dijagnostika





DNA extraction

LAMP amplification/LAMP-MS detection



① Mixing chelex-100 solution with sample



② Boiling 100°C, 10min



③ Filtering and adding to LAMP solution



④ LAMP reaction at 62°C, 30min

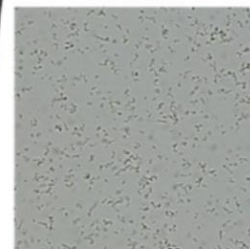


⑤ LAMP reaction at 62°C, 30min

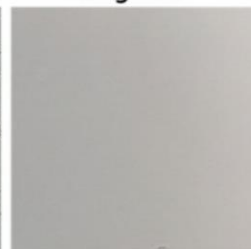


⑥ LAMP-MS detection

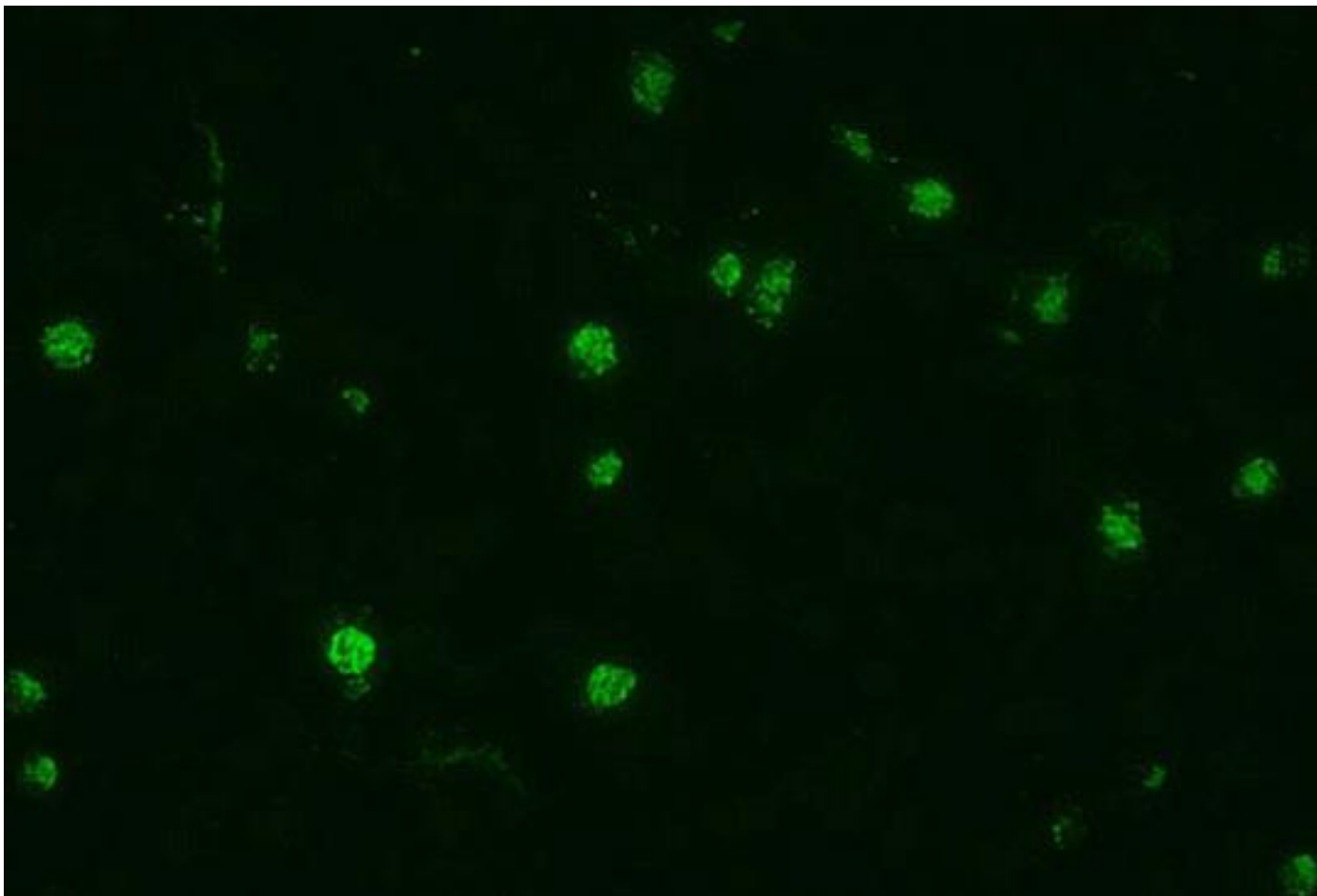
Positive



Negative



Malaria - dijagnostika



Malarija terapija



How **Malaria Vaccines** Are Changing the Game



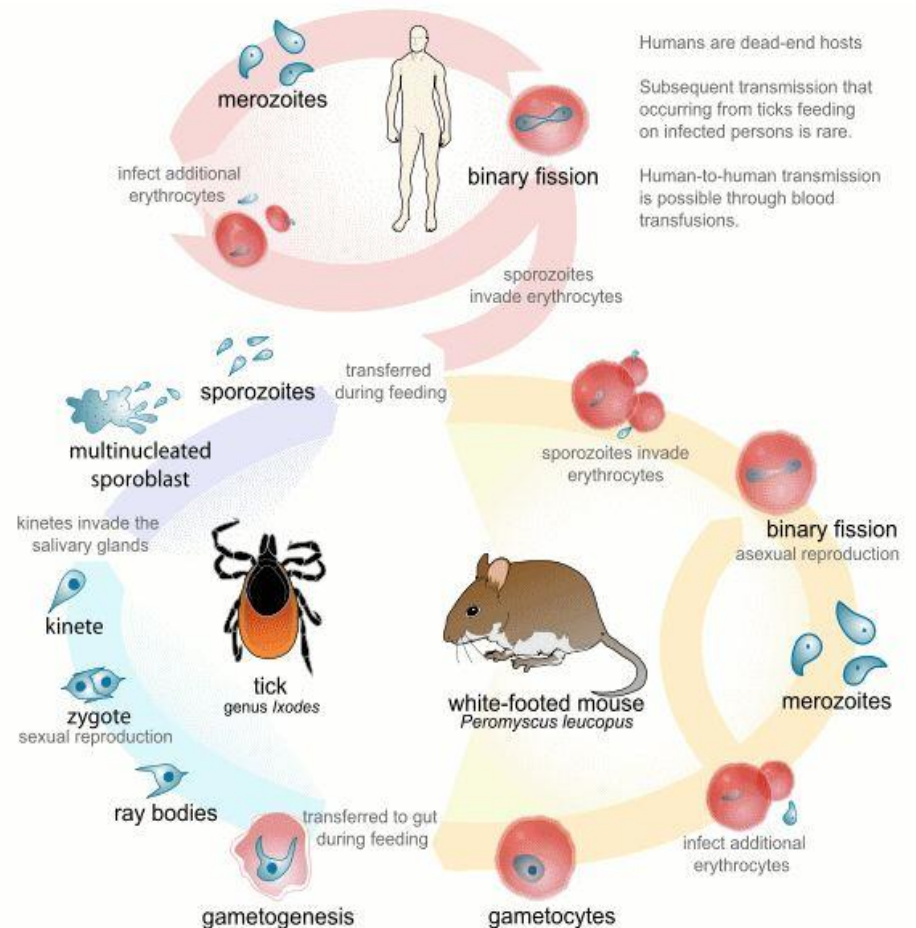
Babesia

- Inficira brojne divlje i domaće životinje
- Krpelji
- Mali (trofozoiti 1-2,5 μm) – *B. microti* i *B. gibsoni*
- Veliki (trofozoiti 2,5-5,0 μm) – *B. bovis* i *B. canis*

Babesia

Životni ciklus

- Nema egzoeritrocitnog oblika
- Unutar eritrocita dioba dvojnog diobom ne shizogonijom
- Gametociti postoje no morfološki se ne mogu razlikovati od trofozoita



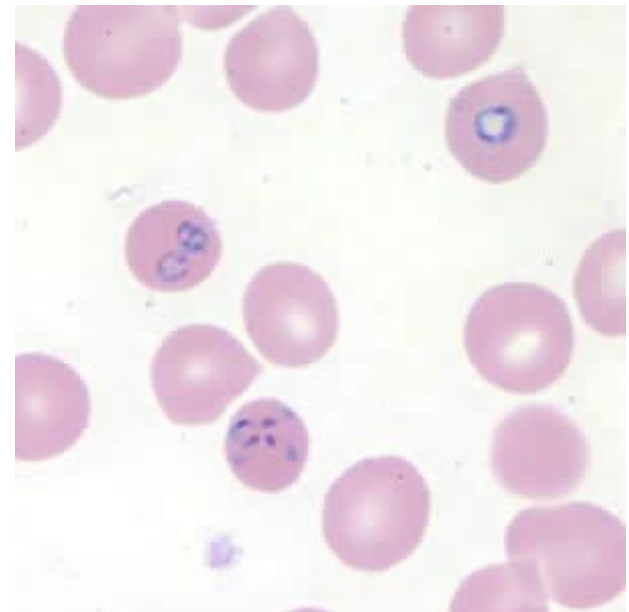
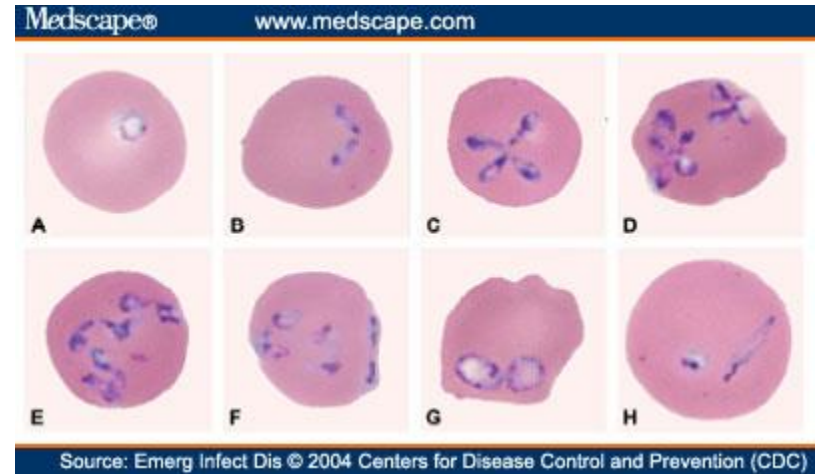
Babesia

Klinička slika

- Asimptomatska – fulminantna bolest
- Predisponirajući čimbenici – imunosupresija, splenektomija, starija životna dob

Babesia

- Dijagnostika
- Krvni razmaz
- Mali trofozoiti
- Ekstracelularni trofozoiti
- Više trofozoita u jednom eritrocitu
- Vakuole u trofozoitima
- Tetrade
- Serologija
- PCR



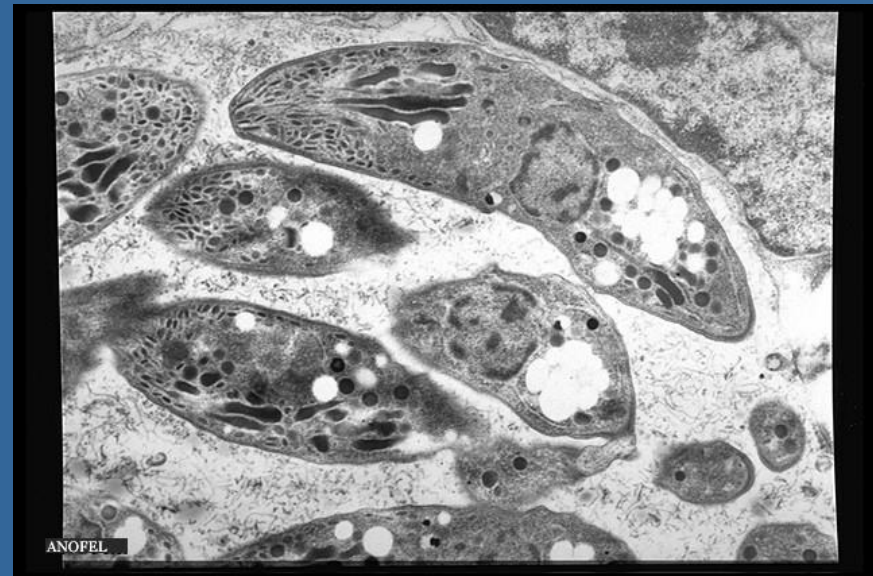
Medicinsko značajni krvno - tkivni protozoi

- Plasmodium
- Babesia
- Toxoplasma
- Acanthamoeba
- Balamuthia
- Naegleria
- Leishmania
- Trypanosoma

Toxoplasma gondii

Toxoplasma gondii

- Jednostanični parazit iz koljena kokcidija
- Tri morfološka oblika:
 - Oocista
 - Bradizoit
 - Tahizoit
- Tri klonalna genotipa: I, II, III
Za čovjeka najznačajniji genotip II



Povijest

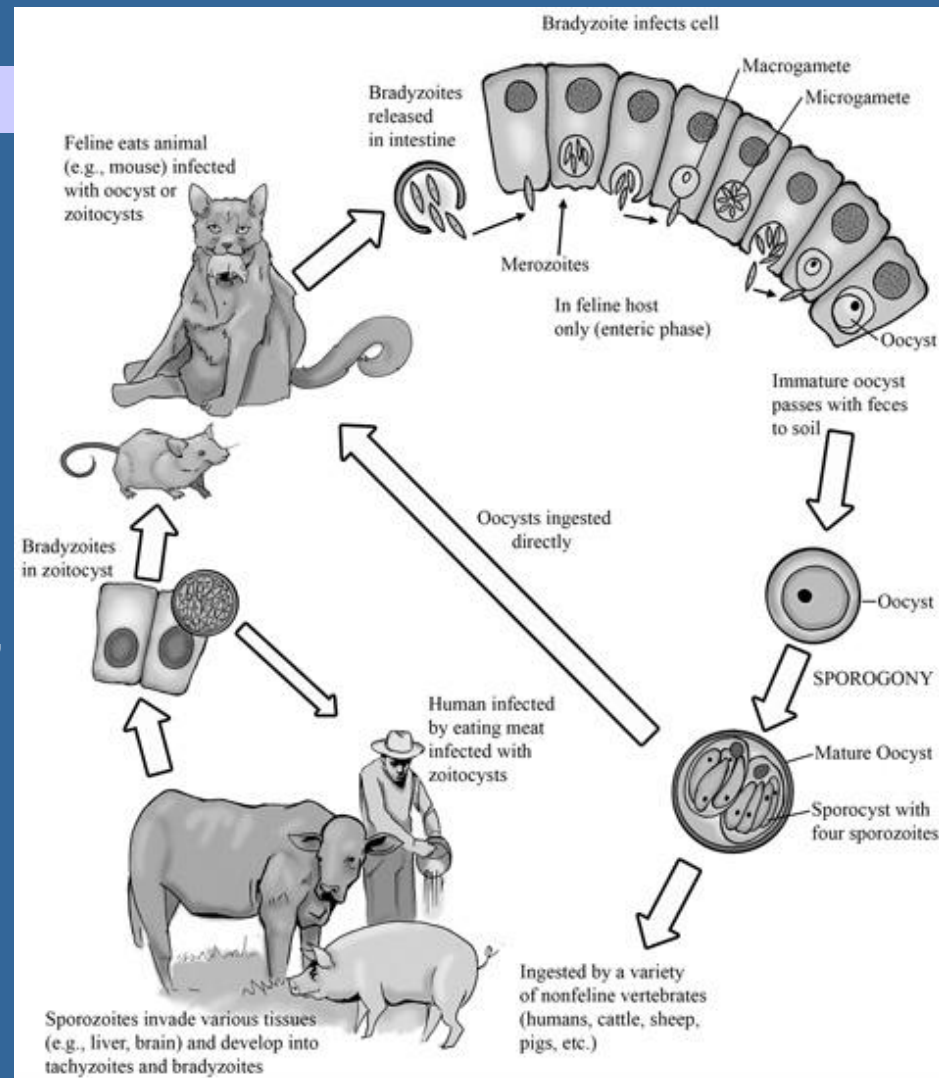
- 1908. Nicolle i Manceaux – *T. gondii* merozoit - afrički glodavac *Ctenodactylus gondii*
- 1923. Janku – toksoplazmoza u 11 mjesecnog dojenčeta s hidrocefalusom i mikroftalmijom
- 1937. Pinkerton – diseminirana toksoplazmoza sa smrtnim ishodom u 22 godišnjeg mladića
- 1942. Paige – opisan vertikalni prijenos bolesti
- 1948. Sabin i Feldman – Dye test
- 1970. Dubey – u potpunosti razjašnjen životni ciklus parazita
- 1981. opisan *T. gondii* encefalitis u AIDS pacijenta

Epidemiologija

- Antropozoonoza – bolest toplokrvnih životinja (ptice, sisavci) i čovjeka, biljojeda, svejeda, mesojeda

Putevi prijenosa

- Konzumacija termički nedovoljno obrađenog ili sirovog mesa, jaja, mlijeka (kuhinjski pribor – noževi, daska za rezanje)
- Ingestija oocista iz okoliša (geofagija, zagađena voda, hrana, muhe, žohari)
- Transplacentarni put prijenosa
- Transfuzija krvi, transplantacija organa
- Laboratorijski put prijenosa



Epidemiologija

Svijet

- 1/3 svjetske populacije inficirana
- Seroprevalenca - ovisi o geografskom području i prehrambenim navikama

Seroprevalenca u žena generativne dobi:

- Skandinavija 11-28%
- Središnja Europa 37-58%
- Afrika 54-77%

Epidemiologija

Hrvatska

- Seroprevalenca - žene s područja grada Zagreba (1) -51%
-stanovnici grada Kutine (2) – 72,4%
 - Serumi 1464 žitelja Splitsko-dalmatinske županije u dobi od 2-84 godine testirani su EIA metodom na prisustvo IgG protutijela na *T. gondii* (3)
U 533 seruma (36,4%) nađena su protutijela na toksoplazmu
 - Serumi 502 žene generativne dobi (16-45 godina) testirani su na prisustvo protutijela na *T. gondii* (4)
U 146 (29,1%) žena nađena su protutijela na toksoplazmu
-
- 1.Derkos-Mikulić V. *Prevalence of antibodies to T. gondii in parurients and nonpregnant women in a larger area of the city of Zagreb*. Acta Parasitol Iugoslav 1974;5:67-72.
 - 2.Konjević P. *Izloženost kongenitalnoj toksoplazmozi na području općine Kutina* (magistarski rad). Zagreb: Medicinski fakultet Sveučilišta u Zagrebu; 1978.
 - 3. Tonkić M, Punda Polić V, Sardelić S, Čapkun V. *Učestalost protutijela za T. gondii u populaciji Splitsko-dalmatinske županije*. Liječnički vjesnik 2002;124:19-22.
 - 4. Vilibić Čavlek T, Ljubin Sternak S, Ban M, Kolarić B, Sviben M, Mlinarić Galinović G. *Seroprevalence of TORCH infections in women of childbearing age in Croatia*. The Journal of Maternal-Fetal & Neonatal Medicine 2011;24:280-283.

Klinička slika

Toksoplazmoza u:

- Imunokompetentne osobe
- Imunodeficijentne osobe
- Okularna
- U trudnoći
- Kongenitalna



Klinička slika

Toksoplazmoza u imunokompetentne osobe

- U 10-20% pacijenata simptomatska bolest

Toksoplazmoza u imunodeficijentne osobe

- Mortalitet 100%

Očna toksoplazmoza

- Infekcija *T. gondii* jedan je od najčešćih uzroka uveitisa i najčešći uzrok retinitisa u imunokompetentne osobe
- Posljedice: potpuni ili parcijalni gubitak vida, glaukom

Klinička slika

Toksoplazmoza u trudnoći

- Najčešće asimptomatska
- Moguć prijenos infekcije na plod (neovisan o prisutnosti simptomatske infekcije)
- Rizik gotovo u potpunosti ograničen na trudnice koje su infekciju stekle tijekom trajanja trudnoće
- Iznimke
 - infekcija novim agresivnijim sojem parazita kod kronično inficiranih trudnica
 - infekcija imunodeficientne trudnice - reaktivacija parazita

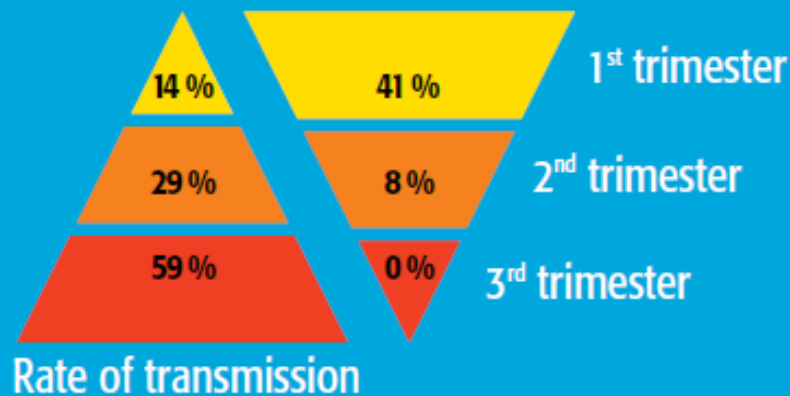


Klinička slika

Toksoplazmoza u trudnoći

**Frequency
of transplacental
transmission
of toxoplasma
and severity
of congenital
infection**

**Percentage of severe infections and
stillborn infants among congenital infections**



Slika preuzeta: www.biomerieux.com/toxoplasmosis

Klinička slika

Kongenitalna toksoplazmoza

Posljedice:

- Smrt, korioretinitis, strabizam, sljepoća, epilepsija, psihomotorna i mentalna retardacija, anemija, žutica, osip, petehije, encefalitis, pneumonitis, mikrocefalija, intrakranijske kalcifikacije, hidrocefalus
- Infekcija tijekom trudnoće – spontani pobačaj, mrtvorođenje, prijevremeni porod

Dijagnostika

Direktna

- Mikroskopija
- Detekcija antigena
- Metode detekcije nukleinske kiseline
- Kultivacija

Indirektna

- Serološka dijagnostika specifičnih protutijela

Dijagnostika

Uzorci

- Serum/krv
 - Likvor
 - Očna vodica
 - Amnionska tekućina
 - Bioptati organa
-
- Serum – spremanje do tjedan dana na $+4^{\circ}\text{C}$ ili kod dužeg spremanja na -20°C
 - Transport seruma na temperaturi okoline (put < 1 tjedan; temperatura okoline < 30°C)
 - Ako je nužna usporedba dva seruma – istovremeno testiranje oba seruma
 - Testiranje likvora/očne vodice + seruma – uzorci uzeti isti dan i testirani isti dan
 - PCR – krv se uzima s antikoagulansom (transport $+4^{\circ}\text{C}$)

Dijagnostika

Direktna dijagnostika – mikroskopija

- Tjelesne tekućine (BAL, AT), bioptati tkiva i organa, heparinizirana krv, likvor
- Tekući uzorci → centrifugiranje → sediment → metanol (fiksacija) → bojenje po Giemsi
- Uzorci tkiva/organa → imprint preparat → metanol (fiksacija) → bojenje po Giemsi
- Elektronska mikroskopija



Dijagnostika

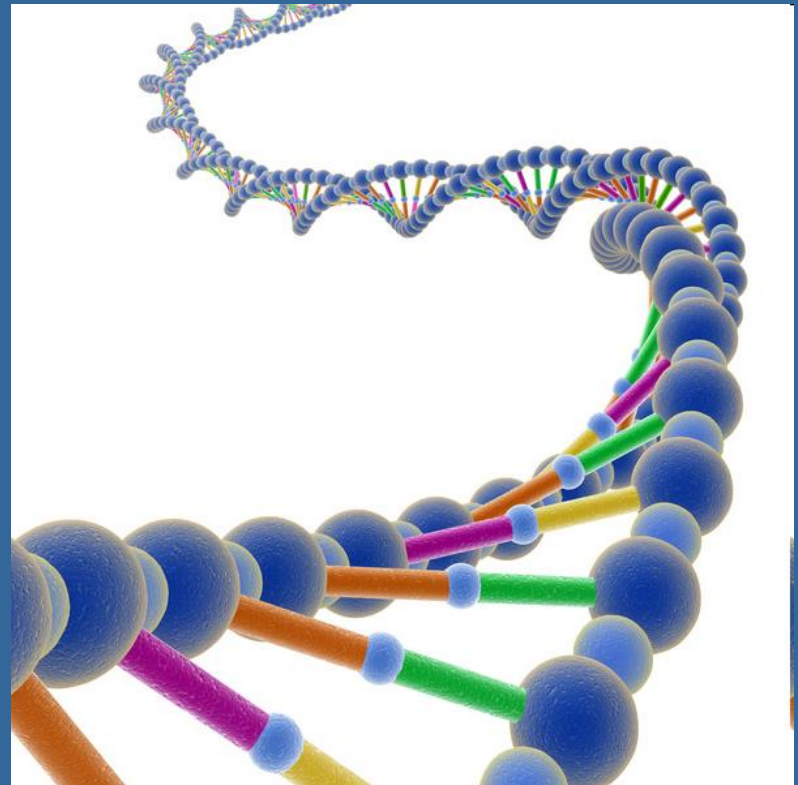
Direktna dijagnostika – detekcija antigena

- Uzorci – bioptati, kultura stanica
- DFA – koristi monoklonalna anti *T. gondii* protutijela obilježena fluorescein-isotio-cijanatom
- Imunoperoksidazna tehnika

Dijagnostika

Direktna dijagnostika – metoda detekcije nukleinske kiseline

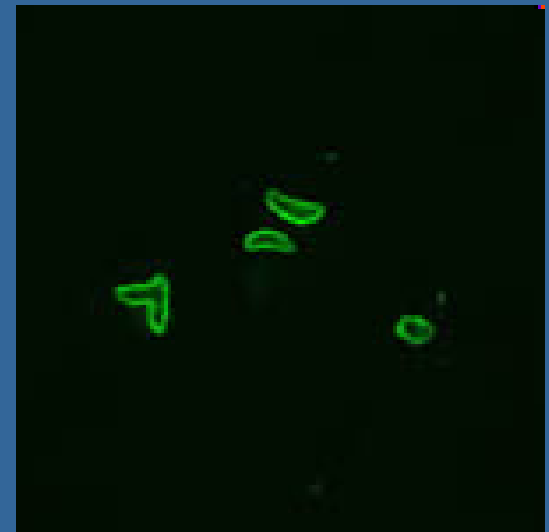
- PCR
- Dijagnostika kongenitalne infekcije, encefalitisa, očne toksoplazmoze
- Komercijalni kitovi za sada nisu dostupni
- Primer - B1 gen *T. gondii*
- PCR iz amnionske tekućine -dijagnostika intrauterine infekcije – izbjegavanje invazivnih procedura po fetus
- PCR iz likvora, krvi, urina, placente, fetalnog tkiva
- Osjetljivost -85 %, specifičnost 100 %



Dijagnostika

Direktna dijagnostika – izolacija

- Na pokusnoj životinji (miš) – 4 - 6 tjedana – serologija, pregled mozga miša
- Na kulturi stanica (24 – 96 sati) – bojenje po Giemsi, DFA



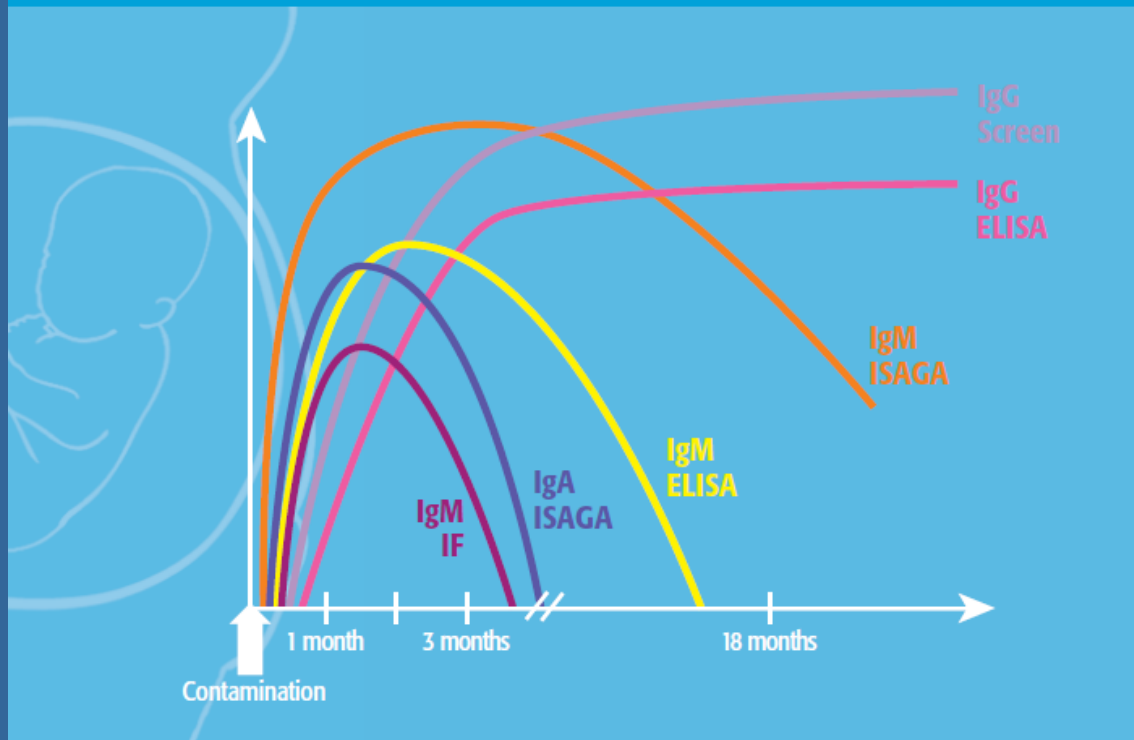
Dijagnostika

Serološka dijagnostika

- Najčešće se koristi u dijagnostici toksoplazmoze
- Sabin Feldman dye test – zlatni standard
- Na tržištu brojni komercijalni testovi
(aglutinacija, IFA, EIA, ELFA, ISAGA, WB)

Dijagnostika – serološka dijagnostika

Antibody kinetics during toxoplasmosis infection



Slika preuzeta: www.biomerieux.com/toxoplasmosis

Dijagnostika

Serološka dijagnostika – IgG protutijela

- 1-2 tjedna nakon infekcije
- Najveća vrijednost 1-2 mjeseca
- Prisutna u niskom titru čitavog života
- Titar IgG protutijela izražava se u IU/ml

Dijagnostika

Serološka dijagnostika – IgG protutijela

- EIA (Enzyme linked immunosorbent assay)
- ELFA (Enzyme linked fluorescent assay)
- EIA / ELFA test aviditeta IgG protutijela
- Niski, granični, visoki aviditet
- Visoki aviditet – infekcija “starija” od 4-6 mjeseci
- Niski aviditet – infekcija “mlađa” od 4-6 mjeseci
- Biološka varijabilnost !!! – visoki aviditet garancija “stare” infekcije, niski aviditet upućuje na “mlađu” infekciju

Dijagnostika

Serološka dijagnostika – IgM protutijela

- Par dana nakon infekcije
- Prisutni u serumu > godine dana
- Samo nalazom IgM protutijela NE MOŽE se postaviti dijagnoza akutne toksoplazmoze

Dijagnostika

Serološka dijagnostika – IgM protutijela

- IgM Immunosorbent Agglutination Assay (IgM ISAGA)
- Komercijalno dostupan
- Najosjetljiviji test za određivanje IgM protutijela, visoko specifičan
- Vremenski i tehnički zahtjevan

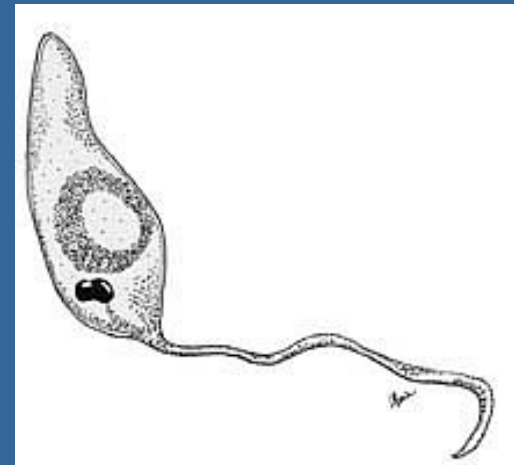
Dijagnostika

Serološka dijagnostika – IgA protutijela

- Dinamika kao i IgM protutijela
- Komercijalno dostupan kit (EIA, ISAGA)
- Prednost – veća osjetljivost
- Dijagnostika akutne i kongenitalne toksoplazmoze

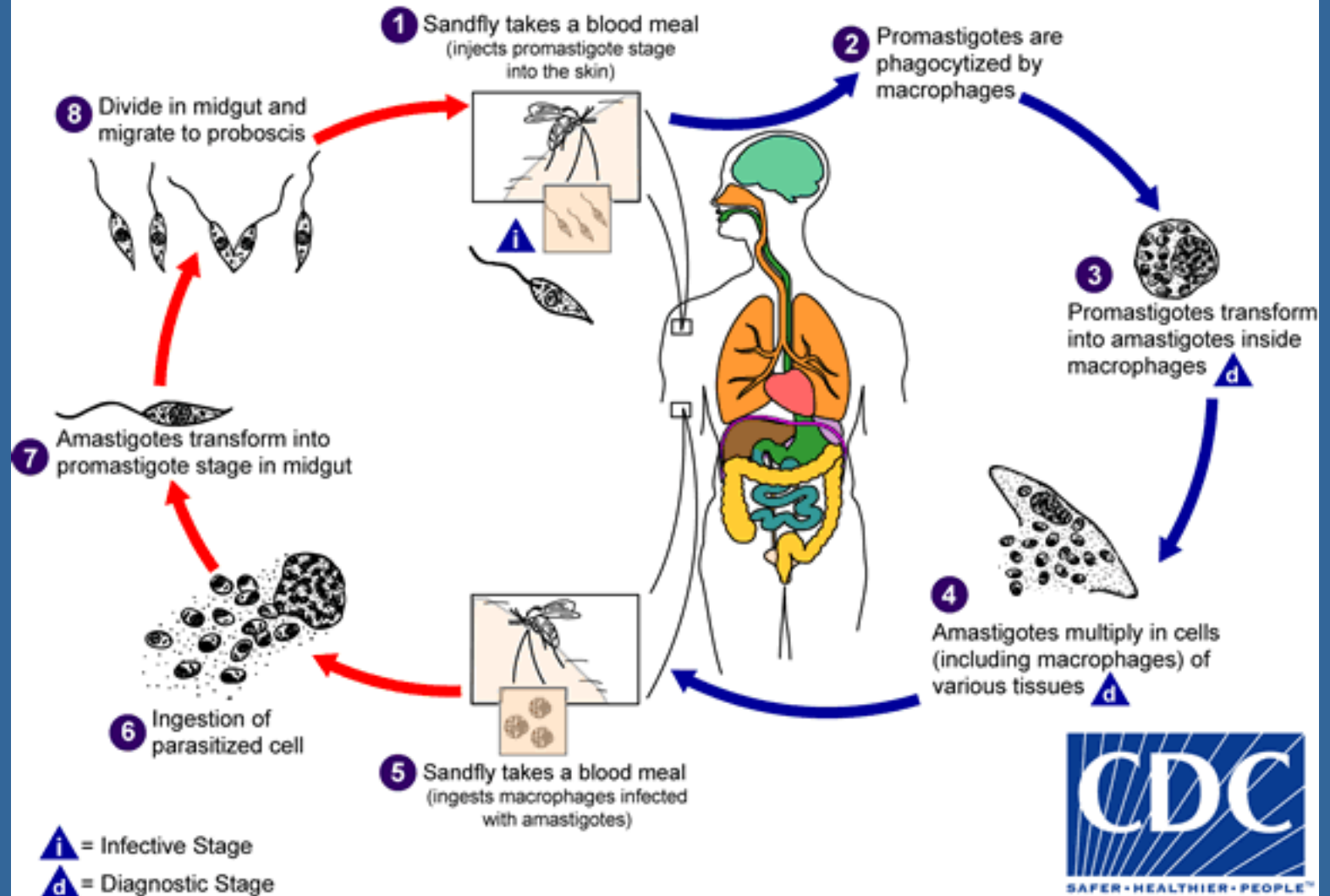
Leishmania

- Hemoflagelat
- Vektor-nevid iz roda *Phlebotomus* (Stari svijet), *Lutzomyia* (Novi svijet)
- Antropozoonoza



Sandfly Stages

Human Stages



Leishmania

Klinička slika

- Visceralna lišmanioza (kala azar, dum dum groznica)
L.donovani, L.infantum, L.chagasi
- Kutana lišmanioza (orientalni ulcus)
L.maior, L.tropica, L.aethiopica
- Američka mukokutana lišmanioza (espundia, chiclero ulcus)
L.mexicana complex, L.brasiliensis complex, L.peruviana

Leishmania

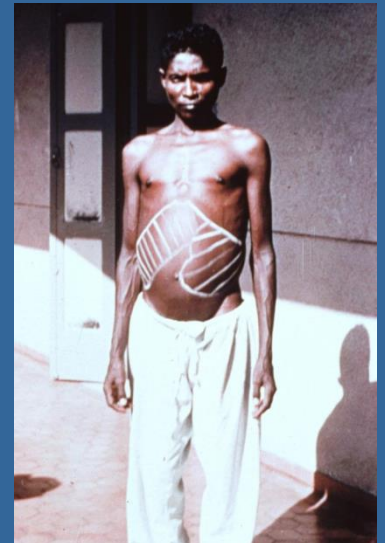
Visceralna lišmanijaza

Inkubacija-nekoliko tjedana do godinu dana

Postepeni nastup povišene temperature, proljev, pancitopenija, pojavljuju se tresavice i znojenja, inapetencija (nespecifični simptomi)

Hepatosplenomegalija, oštećenje bubrega

Tamna pigmentacija kože (crna smrt)



Leishmania

Kožna lišmanioza

Inkubacija-2 tjedna do
2 mjeseca

Crvena papula na
mjestu uboda
nevida (svrbež,rast)→
ulcus (krusta,serozni
iscjedak)→sek.bakteri-
jska infekcija→ožiljak



Leishmania

Mukokutana lišmanioza

Inkubacija-2 tjedna
do 2 mjeseca

Destrukcija mukoznih
membrana i priležećih
struktura

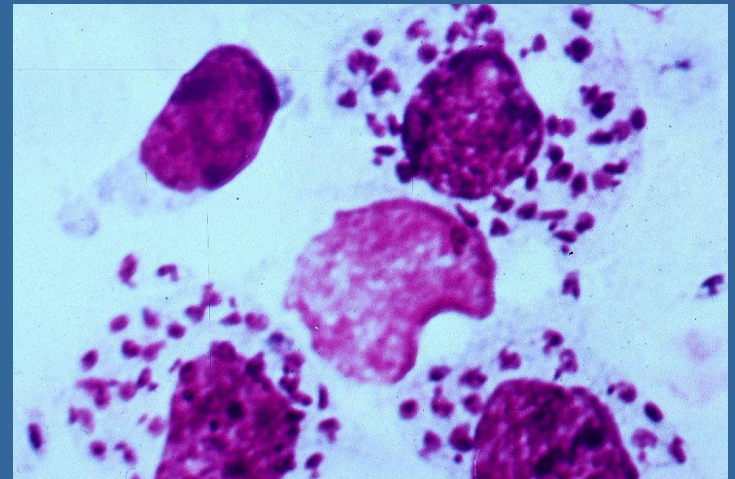
Teške i mutilirajuće
promjene, smrt



Leishmania

Dijagnostika

- Nalaz uzročnika u aspiratu koštane srži (mikroskopija, kultura-NNN podloga)
- PCR
- Serologija (ITFA, ELISA)
- Western blot



Trypanosoma

- Hemoflagelat
- **Afrička tripanosomoza**
(bolest spavanja)
T.brucei gambiense
T.bruci rhodensiense-
enzootija
- **Američka tripanosomoza**
(Chagasova bolest)
T.cruzi



Trypanosoma brucei

- Endemska u subsaharskoj Africi
- Vektor ce ce muha (Glossina)
- WHO-50.000 inficiranih godišnje



Trypanosoma brucei

- **1.stadij-febrilno glandularni**

ulcus

povišena temperatura, tresavica, bolovi u zglobovima i mišićima, edemi, astenija, generalizirana

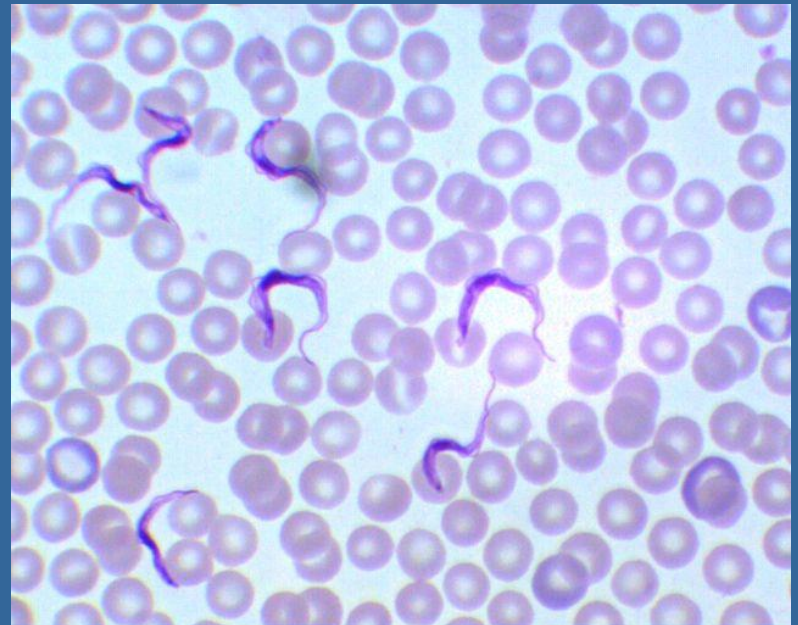
limfadenopatija (Winterbottomov znak), miokarditis, anemija, trombocitopenija

- **2.stadij-meningoencefalitični**

konvulzije, somnolencija, apatija, koma, smrt

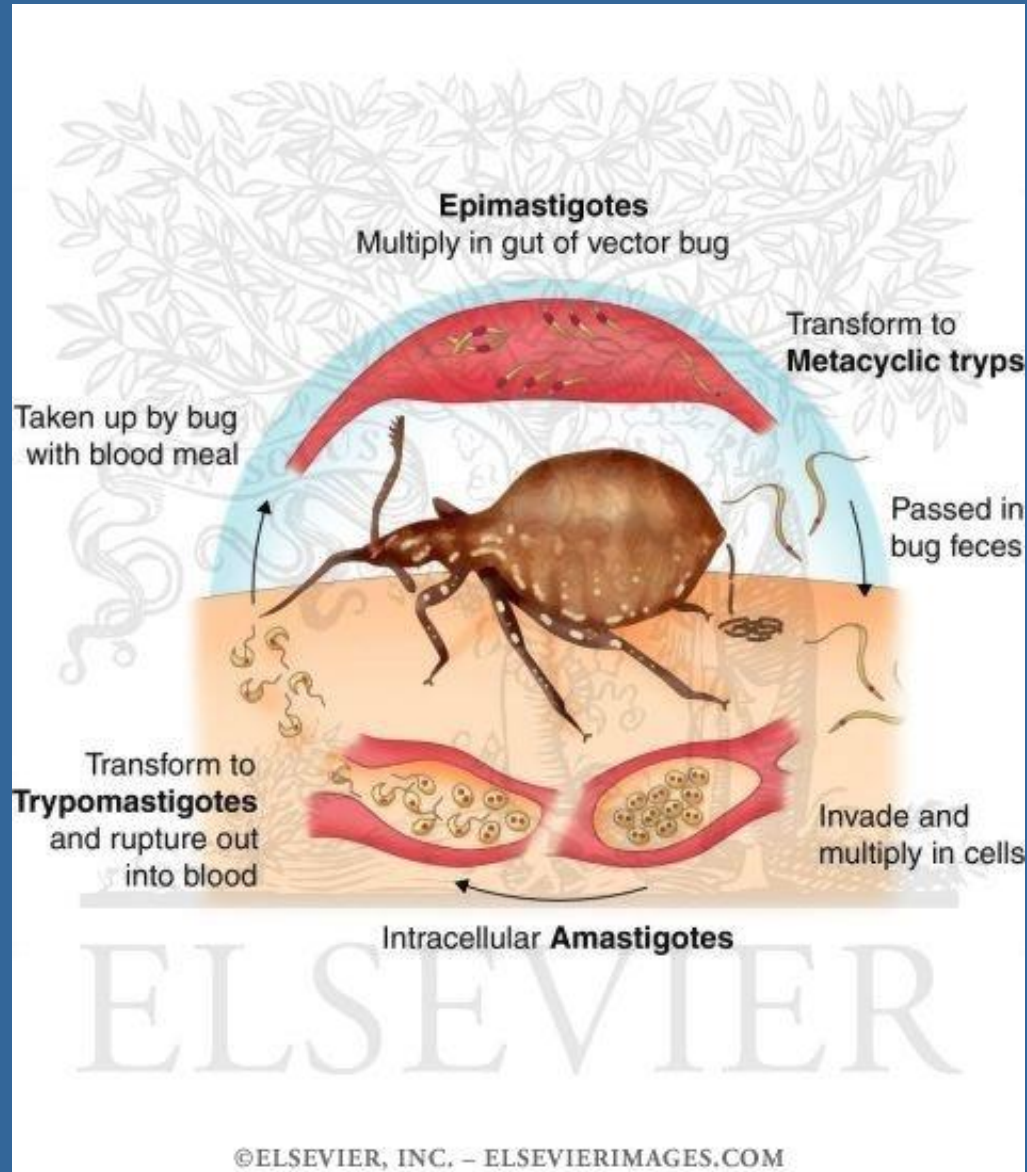
Trypanosoma brucei

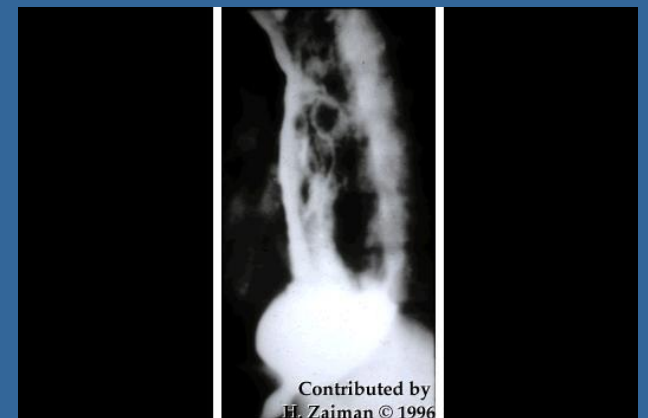
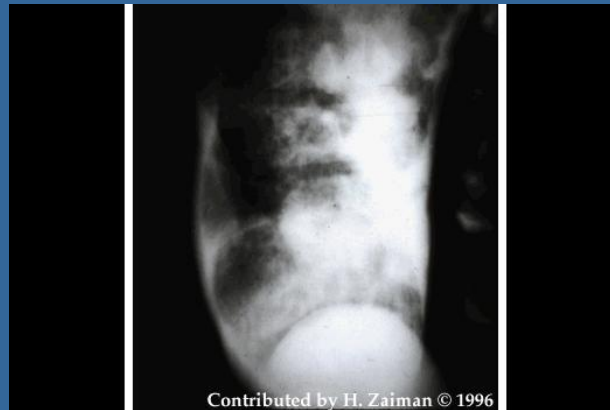
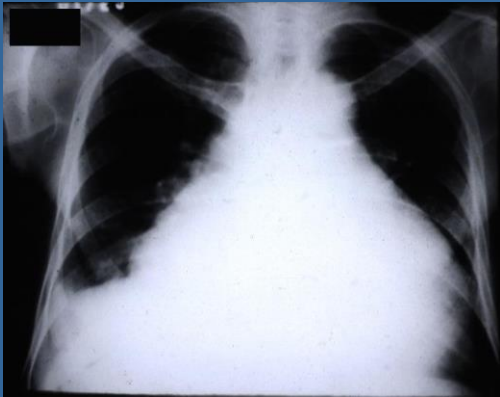
- Direktna
Nalaz tripanosoma u krvi,
aspiratu limfonoda, likvoru
(krvni razmaz, gusta kap)
Poželjna koncentracija
uzorka, više uzoraka
Kultivacija (miš)
- Indirektna - serologija



Trypanosoma cruzi







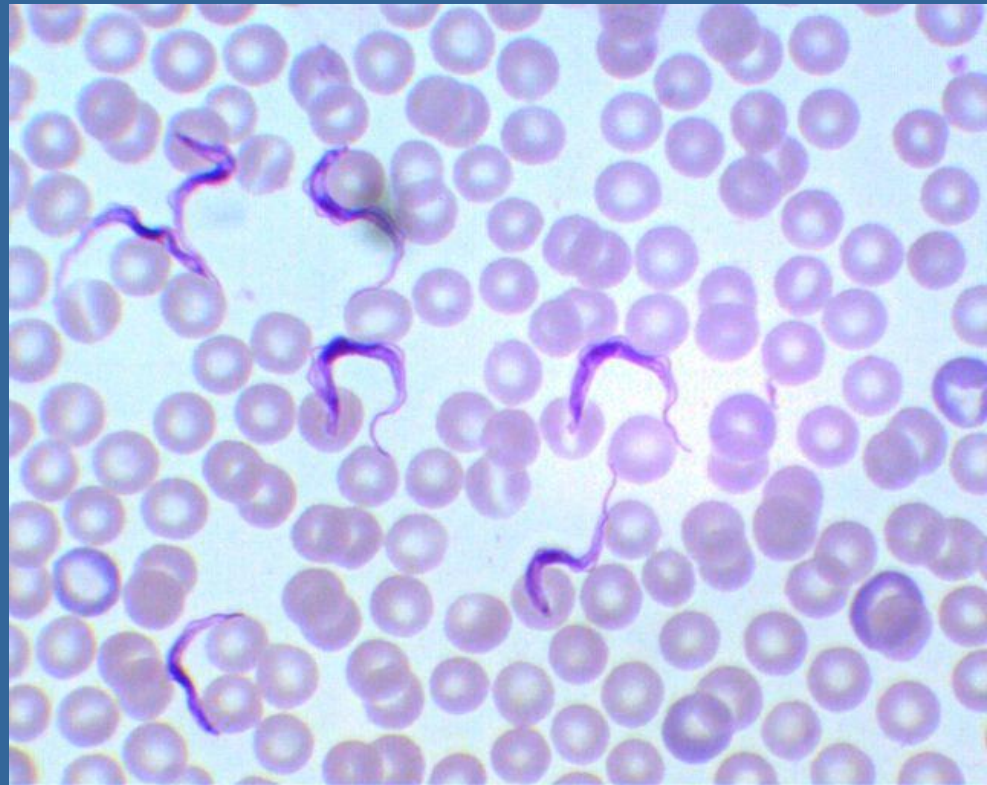
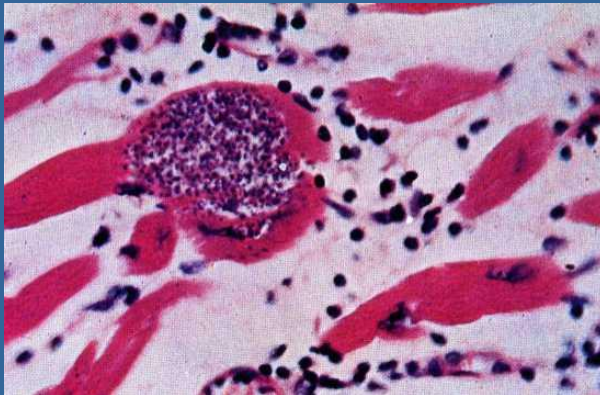
Trypanosoma cruzi

- Direktna
- Krvni razmaz, gusta kap (koncentriranje uzorka)
- Biopsija limfonoda, jetre, slezene, koštane srži
-amastigoti
- Ksenodijagnostika
- PCR
- Indirektna - Serologija (ITFA, ELISA, WB)

Trypanosoma cruzi



Trypanosoma cruzi



Slobodno živeće amebe

- Infekcije u ljudi i životinja
- Nisu česte
- Životno ugrožavajuće ili s teškim posljedicama
- Obolijevaju imunodeficijentne osobe, ali i zdrave osobe

Slobodno živeće amebe

Organizmi

- Naegleria
- Balamuthia
- Sappinia
- Acanthamoeba

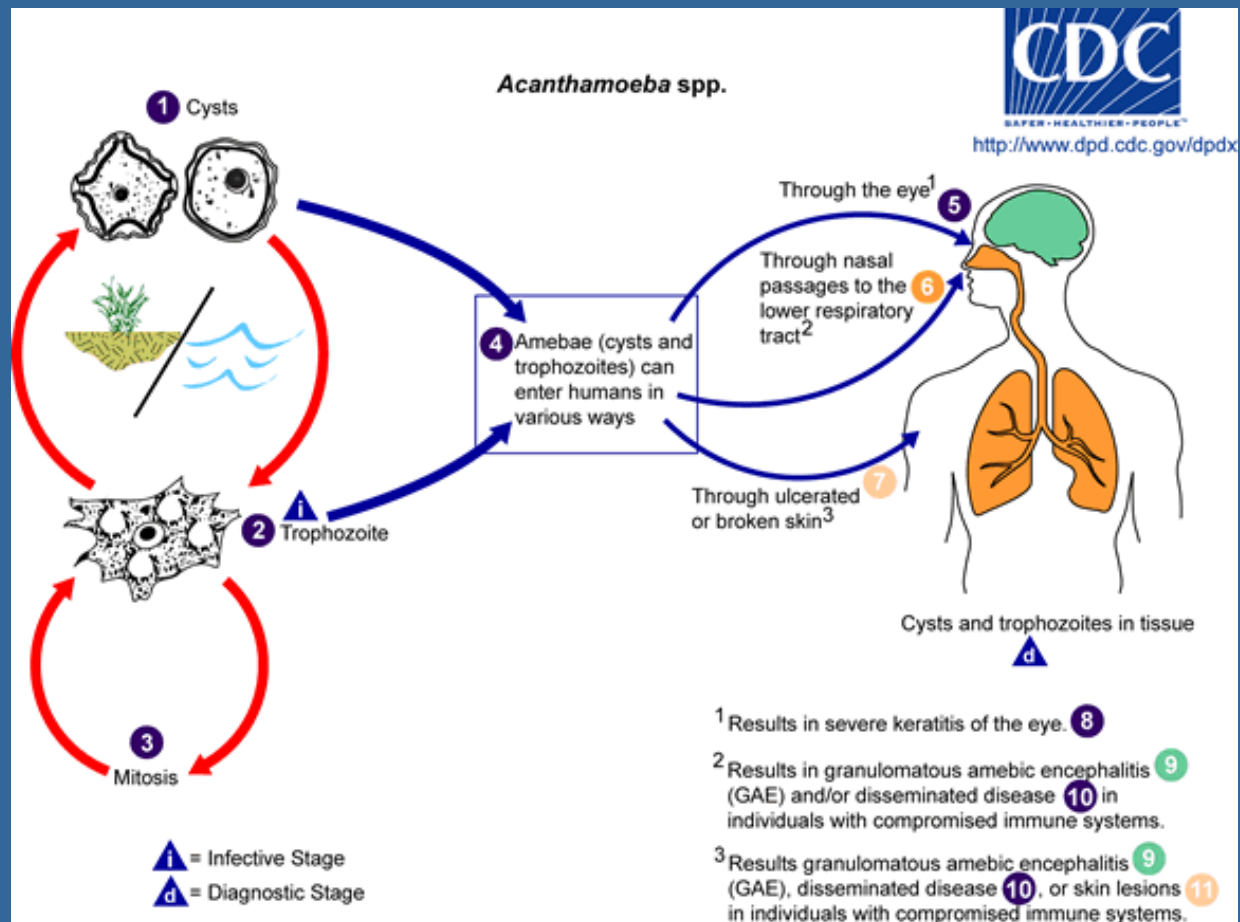
Slobodno živeće amebe

- Razlikuju se od drugih patogenih protozoa
- Slobodni život u prirodi
- Primarno nisu parazitarni organizmi – bolest – “pravo vrijeme na pravom mjestu”
- Nemaju vektora
- Nema kliconoštva
- Ne postoji povezanost između loše sanitacije i širenja infekcije

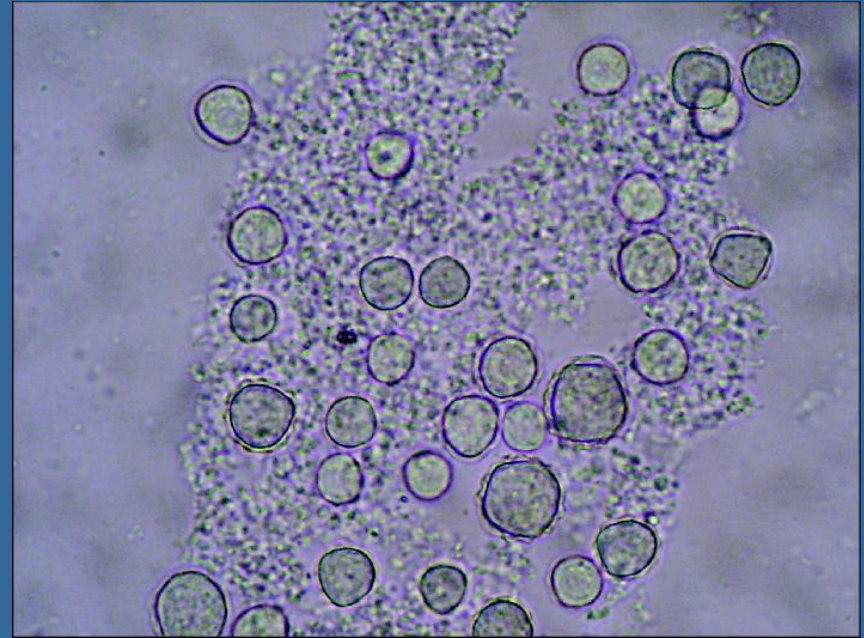
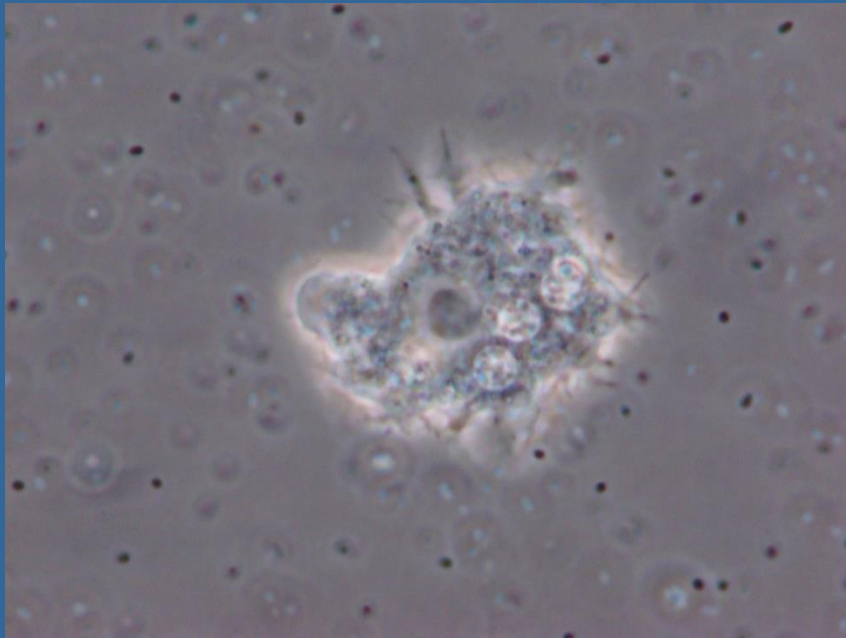
Slobodno živeče amebe

Sindrom, bolest	Uzročnik
Primarni amebni meningoencefalitis (PAM)	<i>Naegleria sp.</i>
Granulomatozni amebni encefalitis (GAE)	<i>Acanthamoeba sp.</i> <i>Balamuthia mandrilaris</i> <i>Sappinia diploidea</i>
Diseminirana granulomatozna amebna bolest (DGAB)	<i>Acanthamoeba sp.</i> <i>Balamuthia mandrilaris</i>
Amebni keratitis	<i>Acanthamoeba sp.</i>

Acanthamoeba



Acanthamoeba



Acanthamoeba

Laboratorijska dijagnostika-uzorci

- Strugotina, bioptat rožnice, suze
- Kontaktna leća, posudica i otopina za leće-indirektna dijagnostika!
- Bris oka nije adekvatan uzorak
- Laboratorijska obrada uzoraka unutar 24 sata (optimum 2-3 sata)
- Uzorak se može par sati ostaviti stajati na sobnoj temperaturi, ne stavljati u frižider ili zamrzivač

Acanthamoeba

Laboratorijska dijagnostika

- Direktna (mikroskopija, kultivacija, molekularna dijagnostika)
- Indirektna – serološka dijagnostika

CONTACT LENSES AND WATER DON'T MIX

MICROSCOPIC ORGANISMS FOUND IN TAP WATER MAY CAUSE EYE INFECTIONS IN CONTACT LENS WEARERS IF PROPER CARE IS NOT FOLLOWED.

TIPS FOR SOFT CONTACT LENS WEARERS:

- > Carefully wash and dry hands before handling lenses.
- > Don't use tap water to rinse contact lenses or storage cases.
- > Take lenses out before doing any activity involving water like showering, using a hot tub or swimming.
- > Talk to your eye care professional about proper contact lens cleaning and storage methods.




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www.midwesteyebanks.org

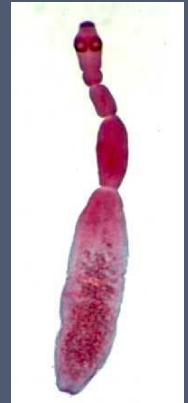
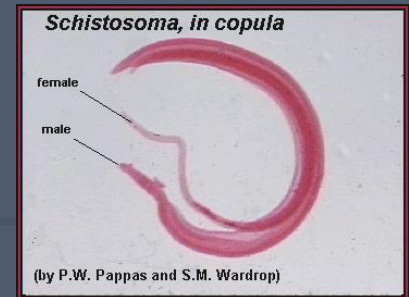
 **Prevent
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100 Years. Our Vision is Vision®
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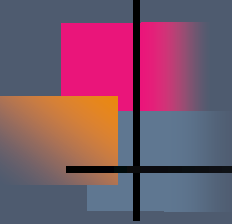


Laboratorijska dijagnostika plosnatih crva

Koljeno Platyhelminthes

- Plosnati crvi –
višestanične životinje
nalik na list ili vrpca,
rjeđe valjkasta oblika,
bilateralno simetrični,
građeni od triju zametnih
listića
- Nemaju tjelesne šupljine,
mnogi nemaju probavni
sustav

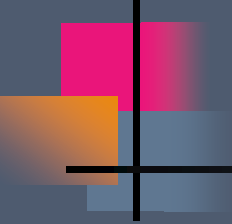




Koljeno Platyhelminthes

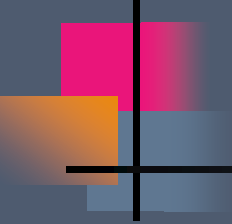
Tri razreda

- Turbellaria – slobodne životinje
- Trematoda – metilji
- Cestoidea - trakavice



Trematoda (metilji)

- Plosnati, oblik poput lista vrbe
- Oralna i ventralna prijanjalka
- Većina dvospolci
- U životnom ciklusu svima potreban prijelazni domaćin (prvi bez iznimke puževi) – aseksualni životni ciklus
- Drugi prijelazni domaćini različiti
- Jaja metilja - operkulum (Schistosoma iznimka)



Trematoda (metilji)

Medicinski značajni predstavnici

- Fasciolopsis buski – veliki crijevni metilj
- Fasciola hepatica - ovčji jetreni metilj
- Opisthorchis (clonorchis) sinensis – kineski jetreni metilj
- Paragonimus westermani – plućni metilj
- Schistosoma spp. - krvni metilji

Fasciola hepatica

- Fasciola hepatica – ovčji jetreni metilj
- Uzročnik fascioloze
- Infekcija u oko 50 zemalja na svim kontinentima
- Prevaljencija WHO 1995. 2 milijuna ljudi

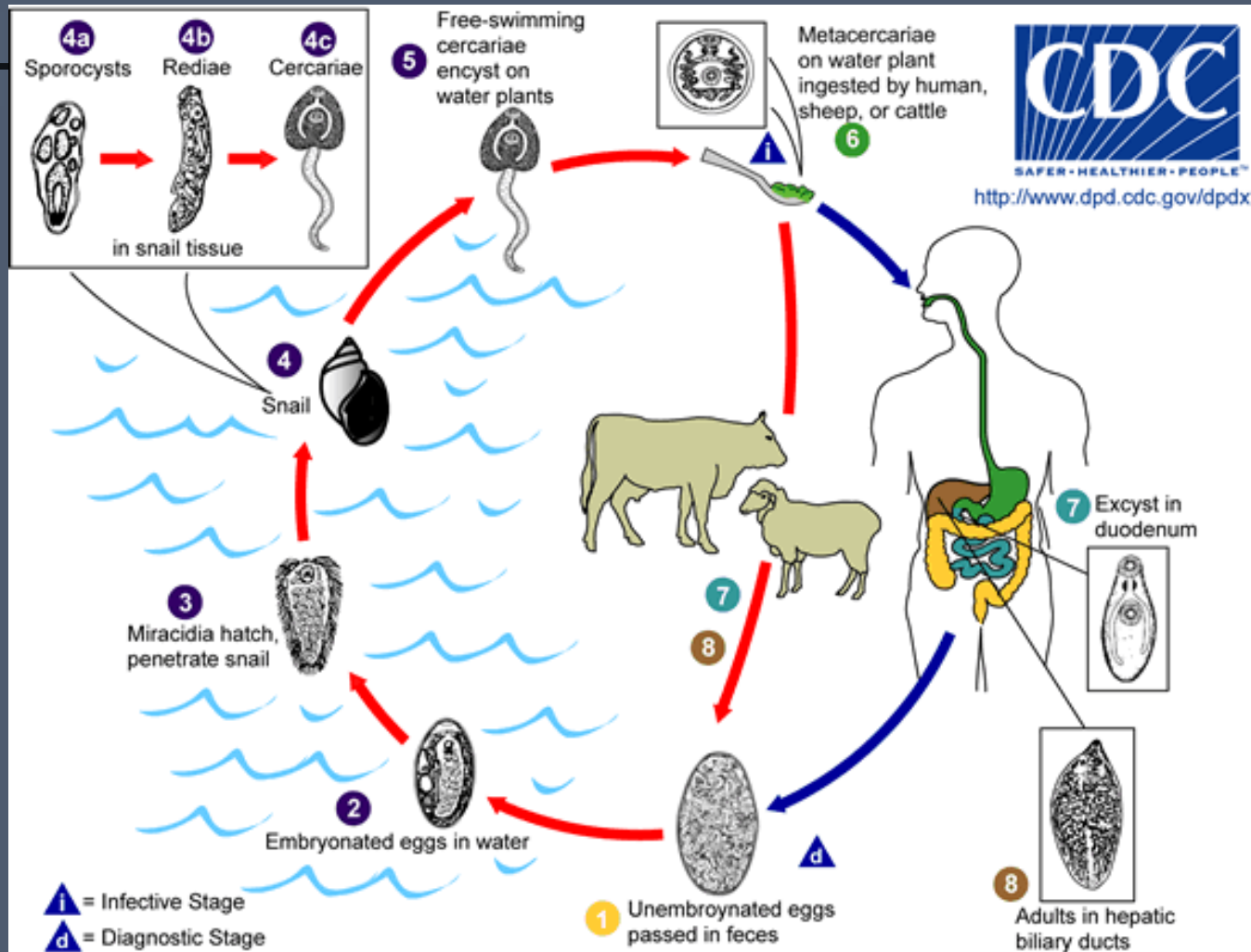


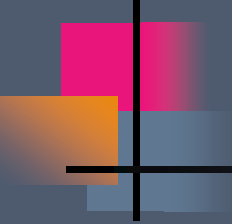
Fasciola hepatica

- Plosnat, poput lista breze, dužina 2-5 cm, širina 1 cm
- Adulti parazitiraju u žučnim vodovima
- Jajašca – 130x85 μm , zlatno žute boje, s operkulumom



Fasciola hepatica





Fasciola hepatica

Klinička slika

- Asimptomatska
- Simptomatska –
 - akutna faza: bolovi u abdomenu, hepatomegalija, febrilitet, leukocitoza, eozinofilija
 - kronična faza: hepatokolangitički simptomi (žutica), anemija
- Migracija parazita u druge organe

Fasciola hepatica

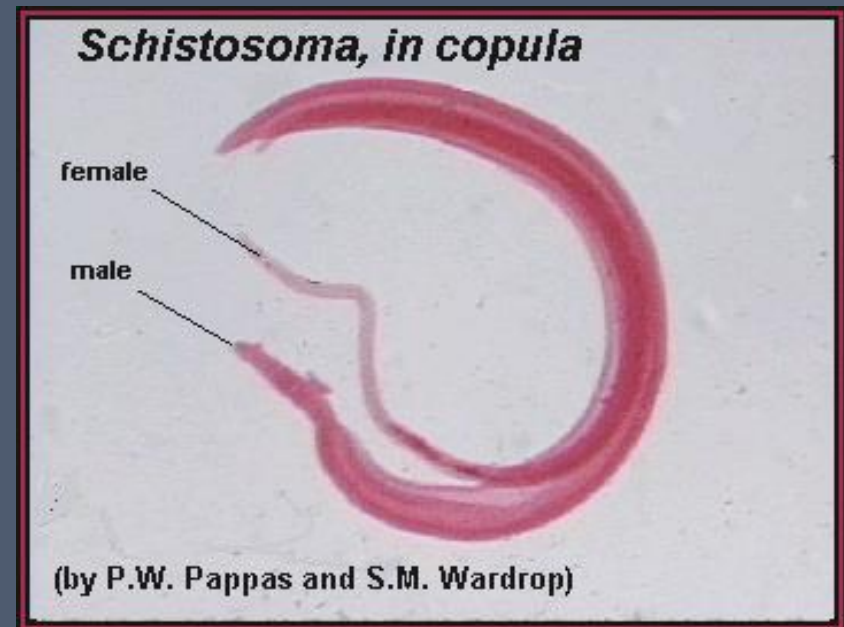
Laboratorijska dijagnostika

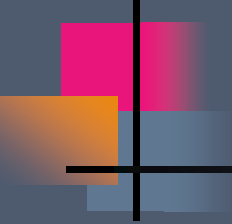
- Uzorci: stolica, duodenalni sok, krv
- Detekcija jaja (2-3 mjeseca nakon infekcije)
- Serologija (EIA, WB)



Schistosoma

- Krvni metilji
- Uzročnik šistosomoze, bilharcioze
- Endemska u tropskim i subtropskim zemljama Afrike, Južne Amerike i Azije (80 ak zemalja)
- Prevalencija WHO 2008 god. 200 milijuna ljudi





Schistosoma

- *S. hematobium*
- *S. mansoni*
- *S. japonicum*
- *S. intercalatum*
- *S. mekongi*

Distribution of Schistosomes

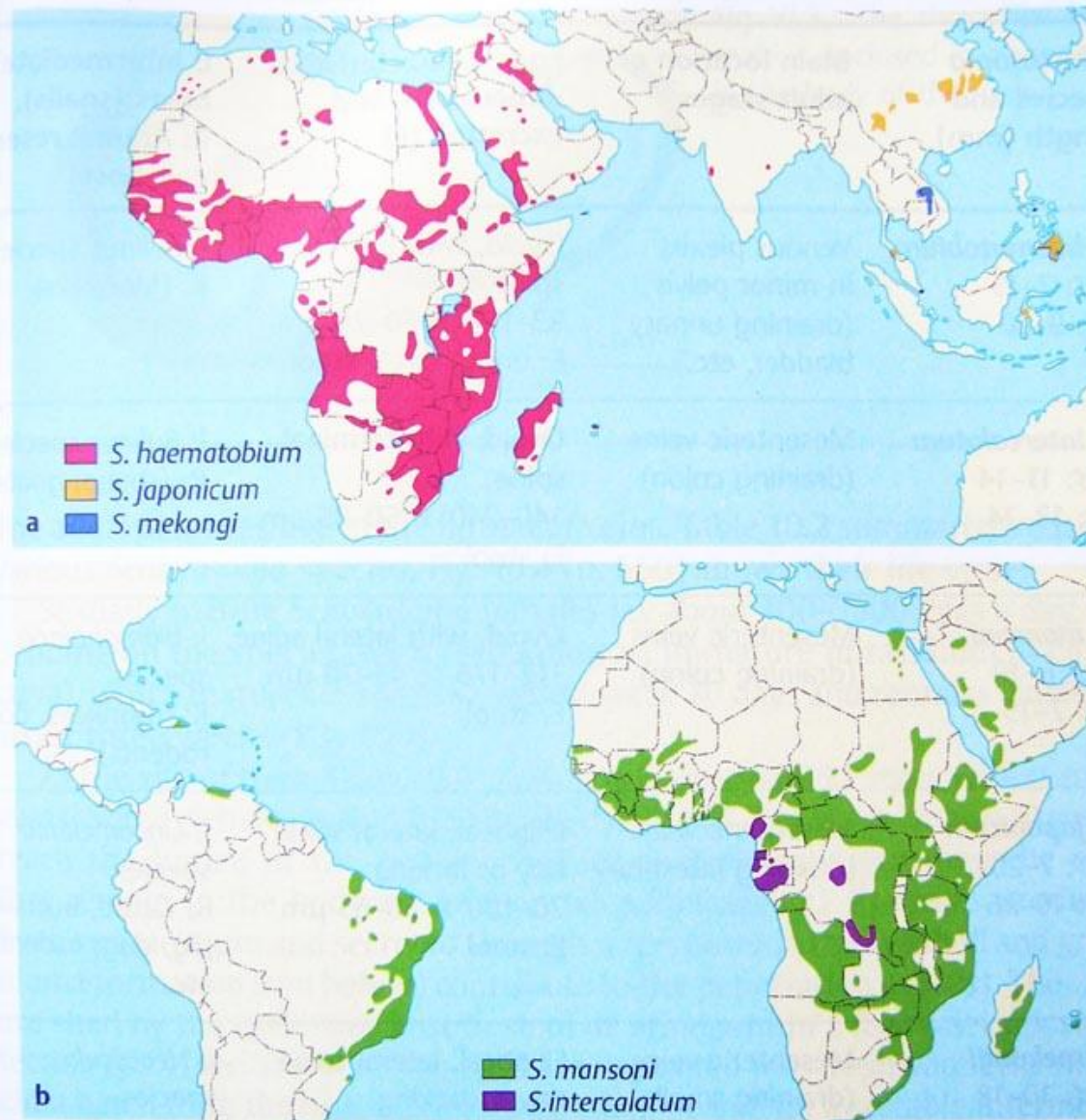
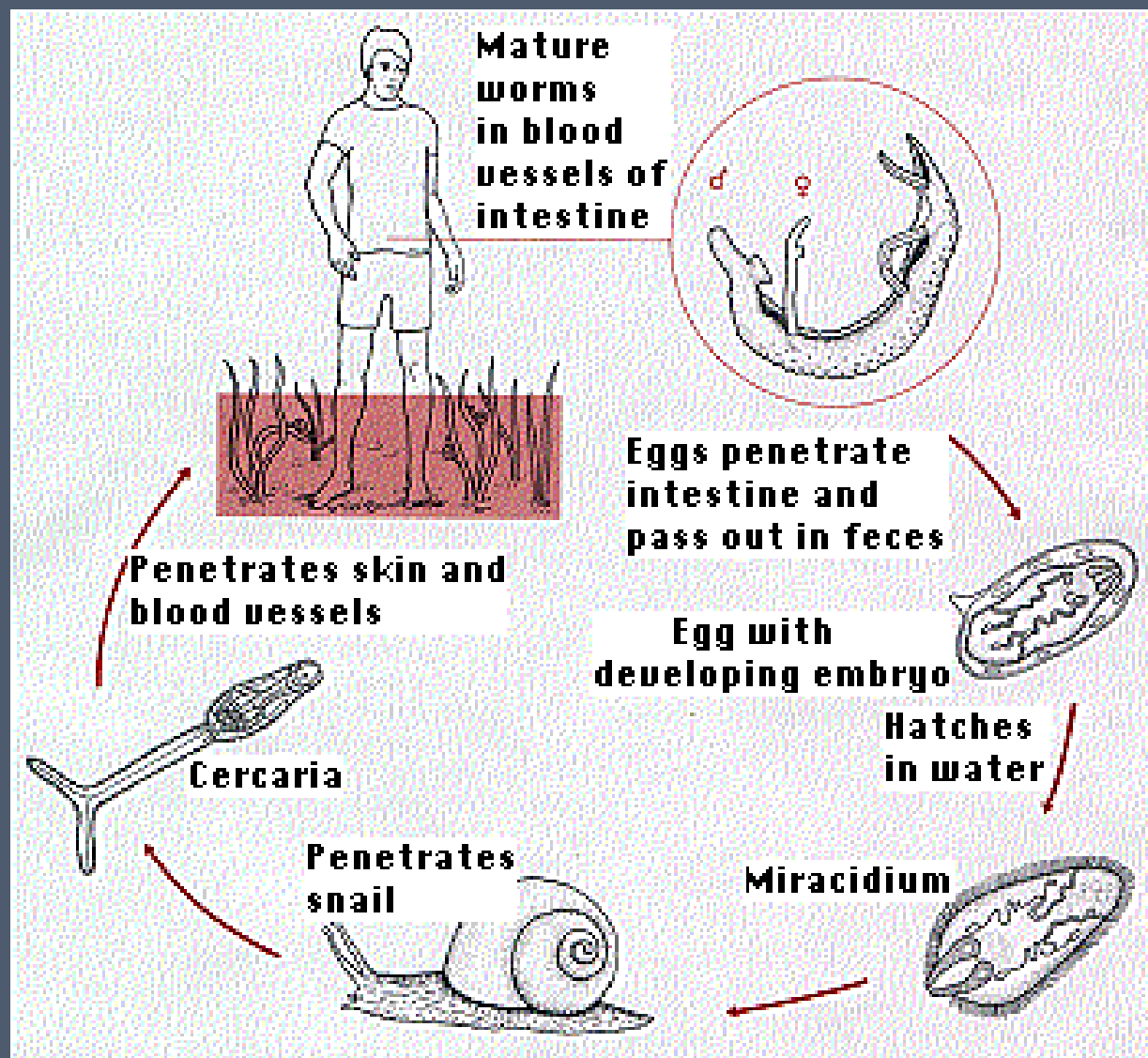


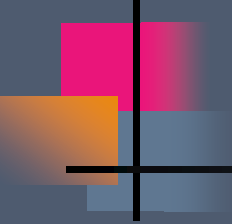
Fig.10.2 a *Schistosoma haematobium*, *S. japonicum*, and *S. mekongi*;
b *S. mansoni* and *S. intercalatum* (according to WHO Technical Report Series No. 830.
Geneva: World Health Organization; 1993).

Schistosoma

- Adulti žive u lumenu vena
- Zrele ženke legu 100 – 3500 jaja na dan
- Jaje sadrži nezreli miracidijum (cilijarna larva)
- Izlučuju se mokraćom i/ili stolicom

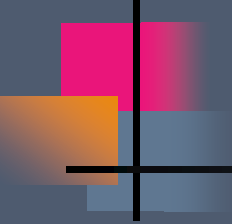






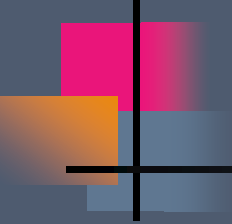
Schistosoma

- Kliničke manifestacije
- Infekcija se može podijeliti u nekoliko faza
 1. Faza penetracije (asimptomatska, simptomatska – svrbež, crvenilo, papule)
 2. Akutna faza (asimptomatska, simptomatska – Katayama groznica)
2-10 tjedana nakon infekcije
febrilitet, glavobolja, bolovi u mišićima, urtikarija, bolovi u abdomenu, hepatosplenomegalija, limfadenopatija, eozinofilija



Schistosoma

3. Kronična faza
2 mjeseca –
Započinje ovipozicijom
50 % jaja ostane u tkivima
Hematogena diseminacija – CNS, koža
Formiranje granuloma - vezivo



Schistosoma

Lokalizacija lezija

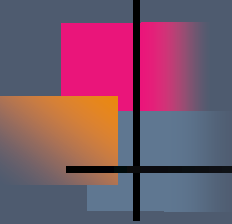
1. Urinarna - *S. hematobium*
2. Intestinalna – *S. mansoni*, *S. japonicum*, *S. mekongi*
3. Hepatosplenična – *S. japonicum*, *S. mansoni*
4. Pulmonarna – *S. mansoni*
5. Cerebralna – *S. japonicum*

Schistosoma

Laboratorijska dijagnostika

- Nalaz jaja u stolici, urinu
- Biopsija crijeva, mokraćnog mjehura
- Serologija (EIA, WB)
- Miracidijski test





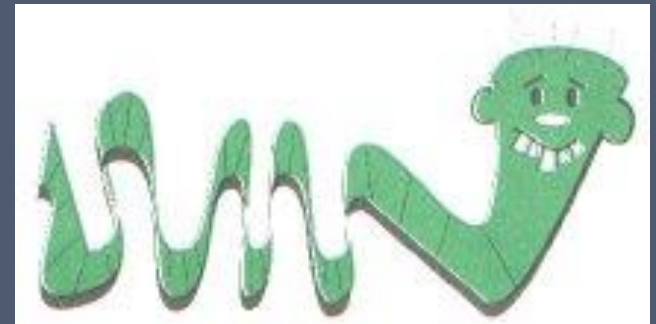
Cestoidea (trakavice)

- Poput trake izduženi, člankovito građeni plosnati crvi
- Adulti: nametnici crijeva
- Građa: scolex, proglotide
- Dvospolci
- Jaja – nemaju operkulum, sadrže embrio
- Nemaju probavni sustav
- Kompleksni životni ciklusi

Cestoidea (trakavice)

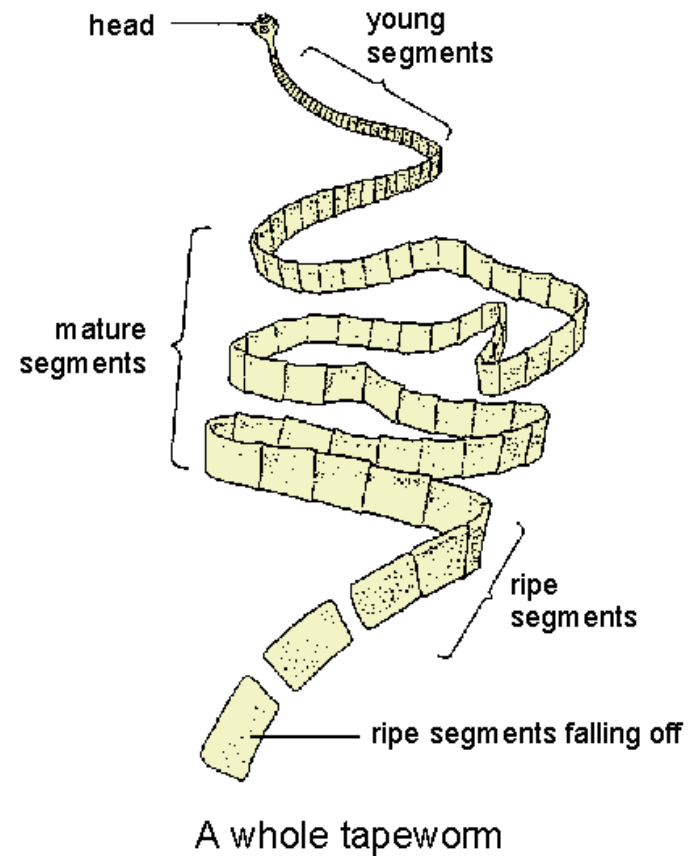
Medicinski značajne trakavice:

- *Taenia solium*
- *Taenia saginata*
- *Diphyllobothrium latum*
- *Echinococcus granulosus*
- *Echinococcus multilocularis*
- *Hymenolepis nana*
- *Hymenolepis diminuta*



Taenia

- Grč. traka
- Scolex – četiri acetabula
- Gravidni članci – raščlanjeni uterus
- Adulti – žive u tankom crijevu mesoždera i sveždera
- Vrste:
 - Taenia saginata
 - Taenia solium



Taenia saginata

- Goveda trakavica
- Rasprostranjena širom svijeta
- Prevalencija: 40-60 milijuna ljudi
- Prevalencija cisticerkoze u goveda
Europa 0,3 %
Mexico – više od 20%



Taenia saginata

- Nametnik tankog crijeva, češća od *T. solium*
- Veličina do 10 metara
- Četvrtasti zaobljen skolex, 4 prisisne zdjelice (nema rosteluma, niti vijenca kukica)
- Proglotide duguljaste
- Razgranat uterus s 15-20 kolateralnih ogranaka

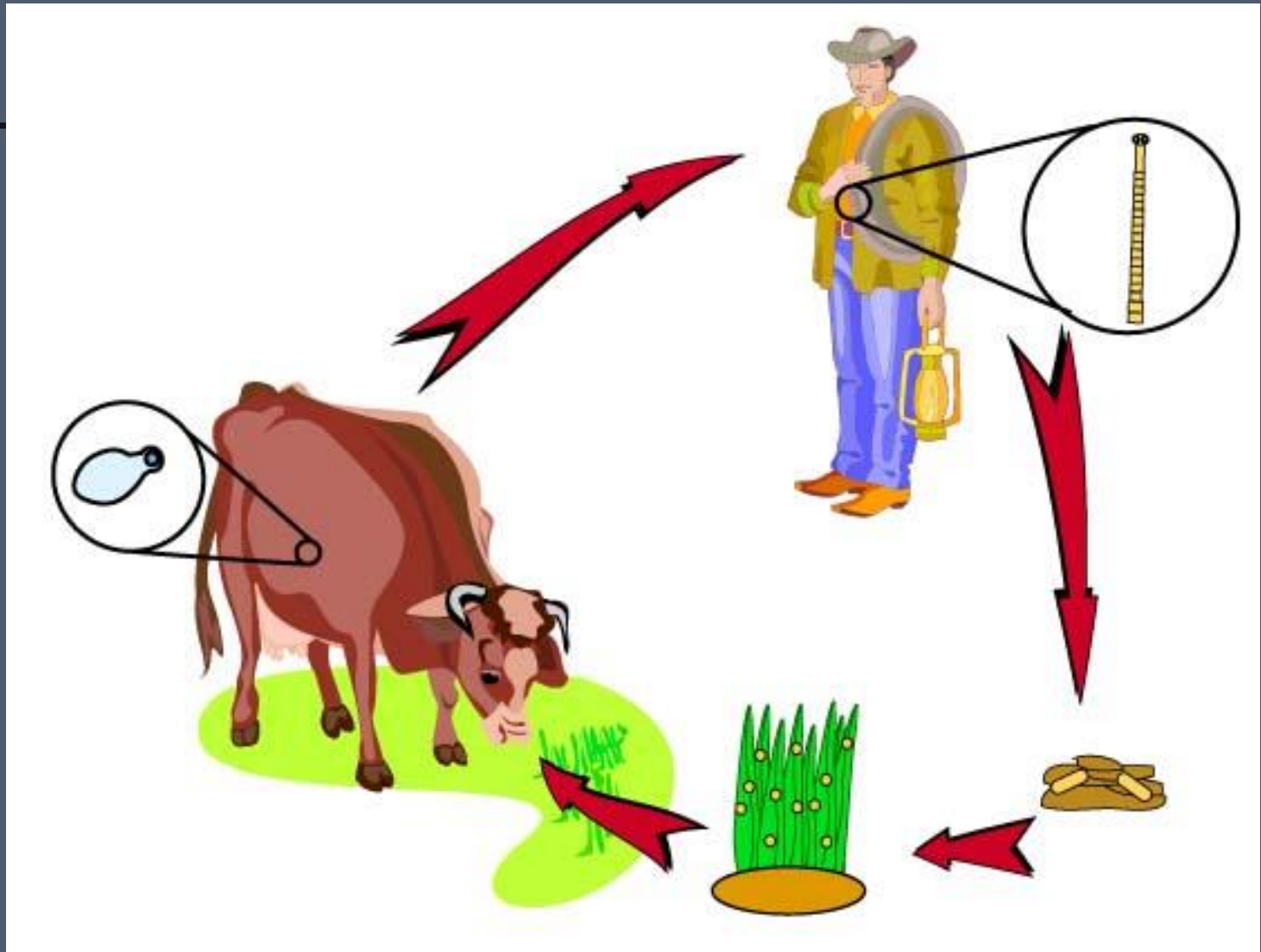


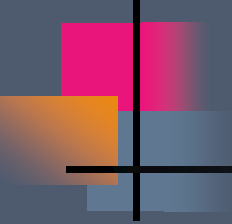
Taenia saginata

- Jaja – 30-40 μm , okrugla, debela, radijarno isprugana ljuska smeđe boje
- Sadrže onkosferu
- Otporna u vlažnoj sredini (nekoliko mjeseci)



Taenia saginata

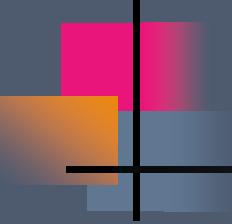




Taenia saginata

Klinička slika

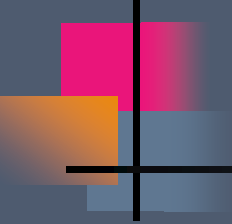
- Asimptomatska
- Simptomatska infekcija:
mučnina, povraćanje,
mršavljenje, bolovi u
abdomenu, proljev,
zatvor, smanjen ili
povećan apetit



Taenia saginata

Laboratorijska dijagnostika:

- Nalaz proglotida i jaja u stolici
- Diferencijacija vrste ponekad nemoguća – PCR
- Detekcija antigena u stolici – EIA
- Serologija na cisticerkozu – za isključenje infekcije trakavicom *T. solium*

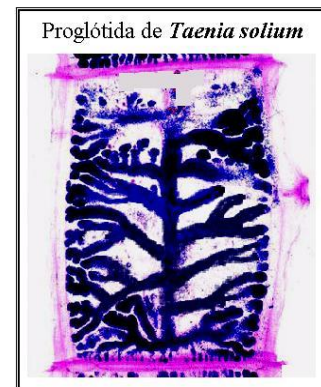


Taenia solium

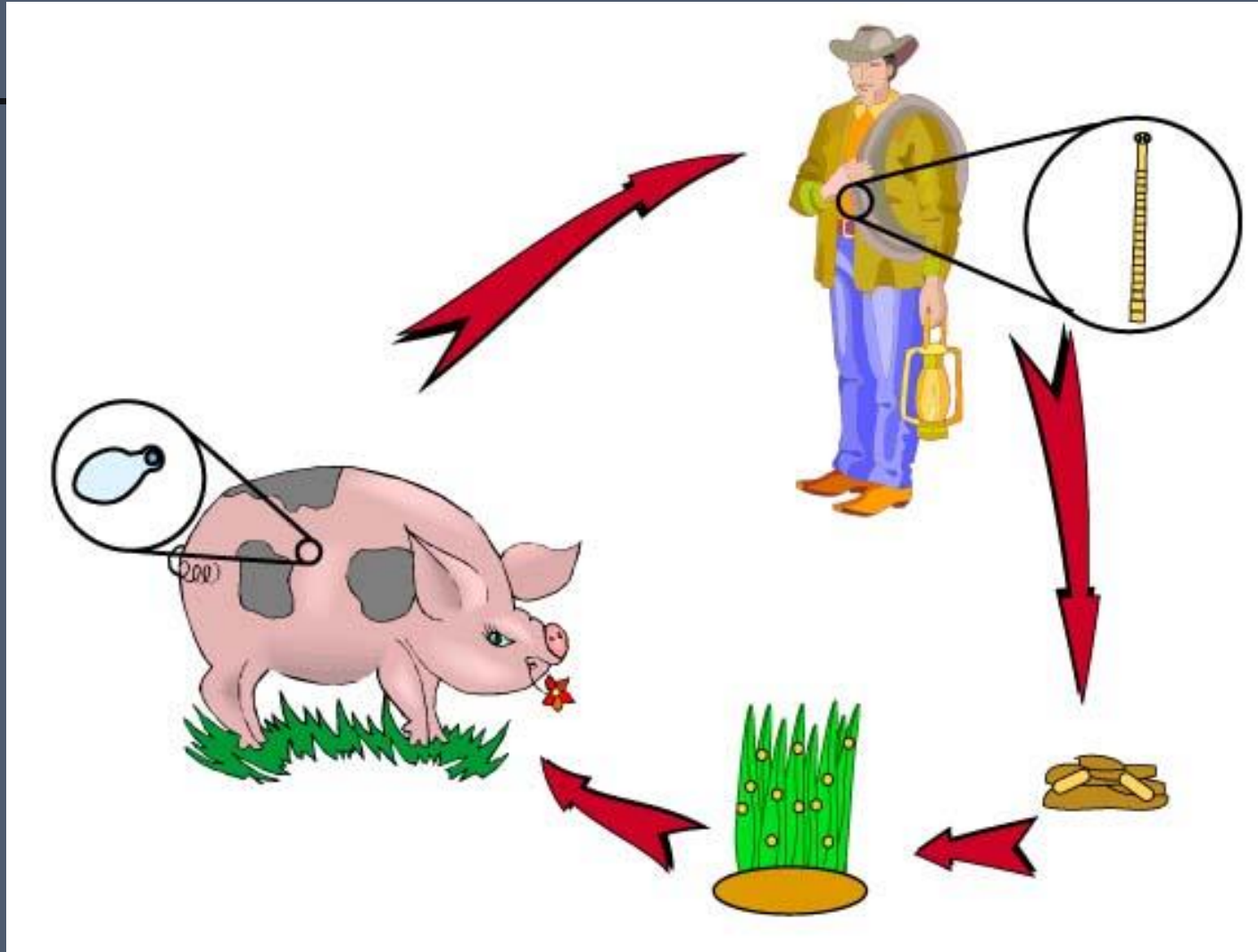
- Svinjska trakavica
- Uzročnike T. solium tenioze (cisticerkoze)
- Endemska u Centralnoj i Južnoj Americi, Africi i Aziji, sporadična pojava u SAD-u i Europi
- (Mexico 0,1-7 % ruralne populacije nosioci adultnog parazita, 25 % svinja nosioci ličinki)

Taenia solium

- Nametnik tankog crijeva čovjeka
- Dužina 2-7 m, 1 000 članaka
- Skolex – ima rostellum s kukicama
- Proglotide – uterus se grana u 7-12 bilateralnih ogranaka
- Jaja morfološki jednaka onima *T. saginata*

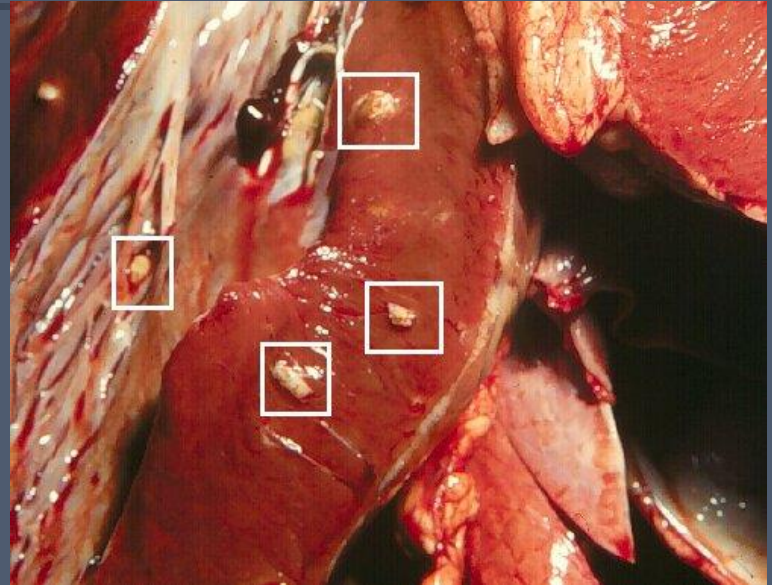


Taenia solium



Taenia solium

- Klinička slika
- Asimptomatska
- Simptomatska:
- Adulti – osjećaj težine i boli u trbuhu, proljev, zatvor, povraćanje, slab apetit, mršavljenje
- Ličinke (cisticerkoza) – ovisno o broju i smještaju ličinki
Mozak, oko, potkožno tkivo, srce, skeletna muskulatura



Taenia solium

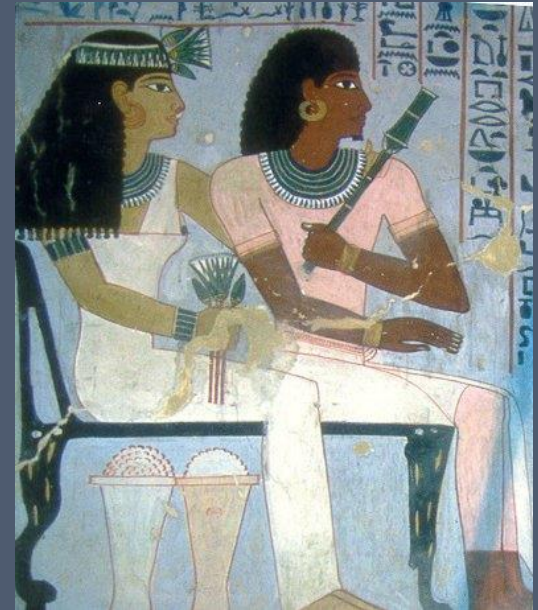
Laboratorijska dijagnostika

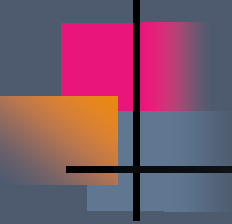
- Nalaz jaja, proglotida u stolici (nativni preparat, MIFC, grahamova metoda)
- Detekcija antigena (ELISA)
- Serologija: EIA, Western blot



Echinococcus granulosus

- Uzročnik ehinokokoze
- Mala trakavica (4-7 mm, skolex i 2-6 proglotide)
- Rasprostranjen širom svijeta



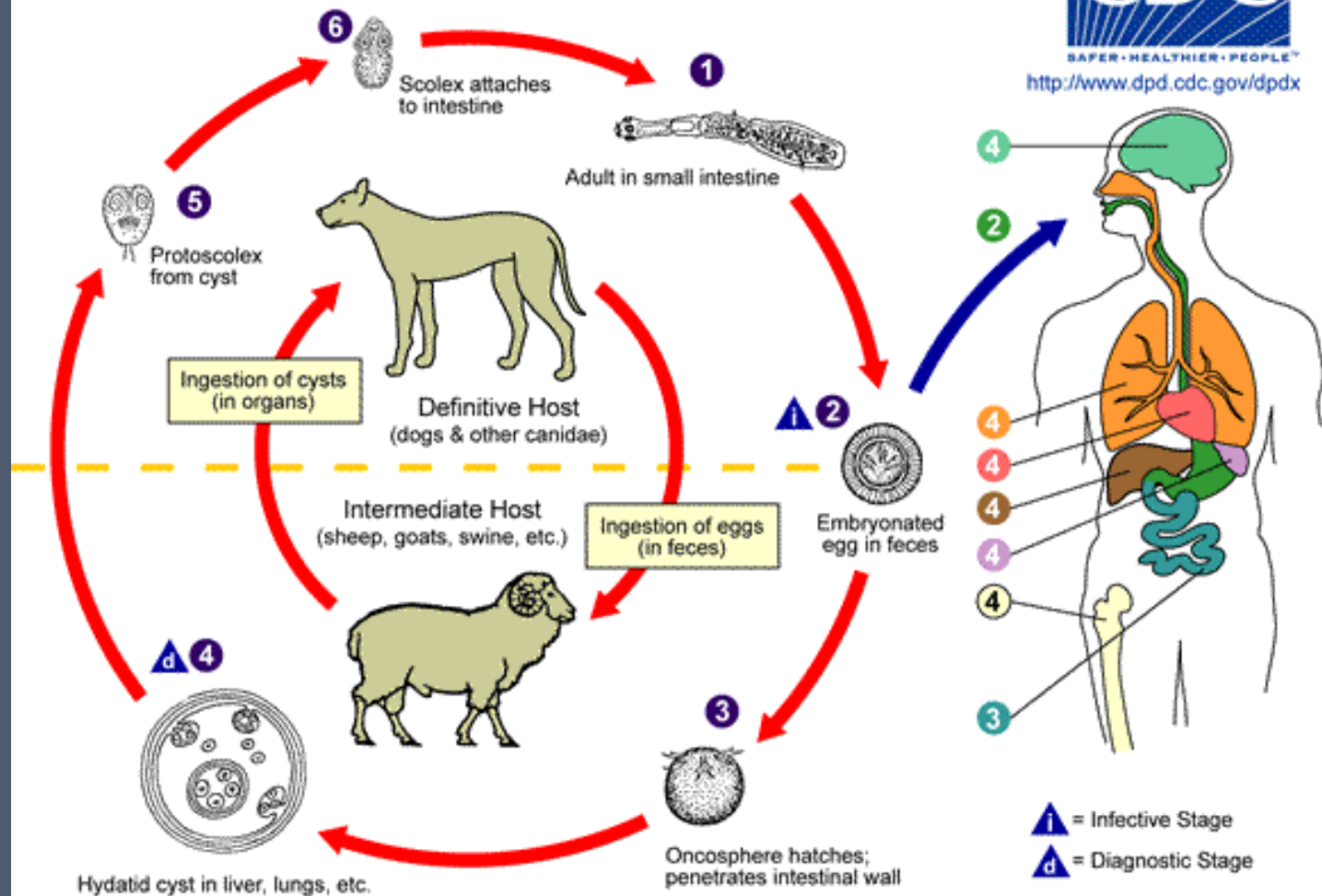


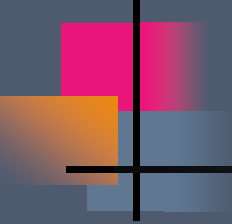
Echinococcus granulosus

- Najznačajniji konačni
domaćin – pas
- Herbivorni i omnivorni
vertebrati – prijelazni
nosioci



<http://www.dpd.cdc.gov/dpdx>

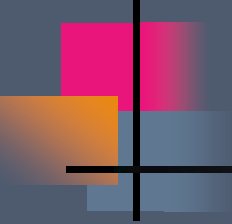




Echinococcus granulosus

Klinička slika

- Asimptomatska infekcija
- Simptomatska infekcija – cista svojim rastom oštećuje strukture i funkcije organa u kojem se nalazi (jetra, pluća, slezena, bubrezi, mišići, CNS)
 - solitarna, multiple
- Ruptura ciste – moguć anafilaktički šok
 - sekundarna ehinokokoza
- Spontano izlječenje



Echinococcus granulosus

- Laboratorijska dijagnostika
- Serologija – EIA, IFA, WB
- PCR (uzorak – sadržaj ciste) – oprez – sekundarna ehinokokoza
- Jaja parazita NE TRAŽE se u stolici

Human Alveolar Echinococcosis, Croatia

Davorka Dušek, Adriana Vince, Ivan Kurelac, Neven Papić, Klaudija Višković, Peter Deplazes, Relja Beck

Author affiliations: University Hospital for Infectious Diseases, Zagreb, Croatia (D. Dušek, A. Vince, I. Kurelac, N. Papić, K. Višković); University of Zagreb School of Medicine, Zagreb (D. Dušek, A. Vince, N. Papić); University of Zurich, Zurich, Switzerland (P. Deplazes); Croatian Veterinary Institute, Zagreb (R. Beck)

DOI: <https://doi.org/10.3201/eid2602.181626>

Alveolar echinococcosis is a parasitic disease caused by the tapeworm larval stage of *Echinococcus multilocularis*. This zoonotic disease has not been known to occur in Croatia. We report a confirmed case of human alveolar echinococcosis in a patient in Croatia who had never visited a known *E. multilocularis*-endemic area.

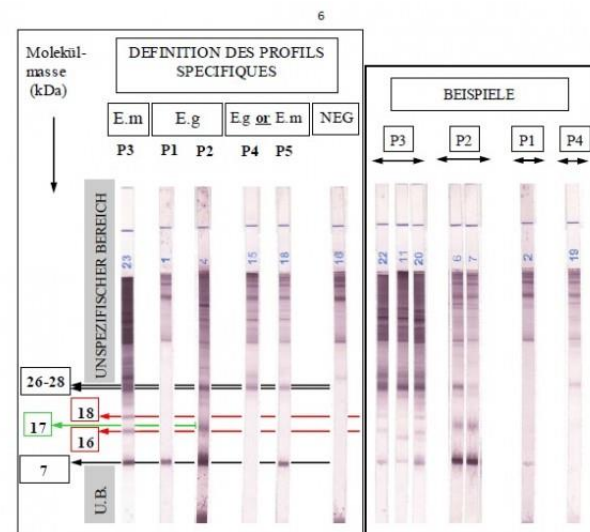


Abb. 1: Beispiele positiver und negativer Ergebnisse

Patient 1 (2017): male, 63 y.

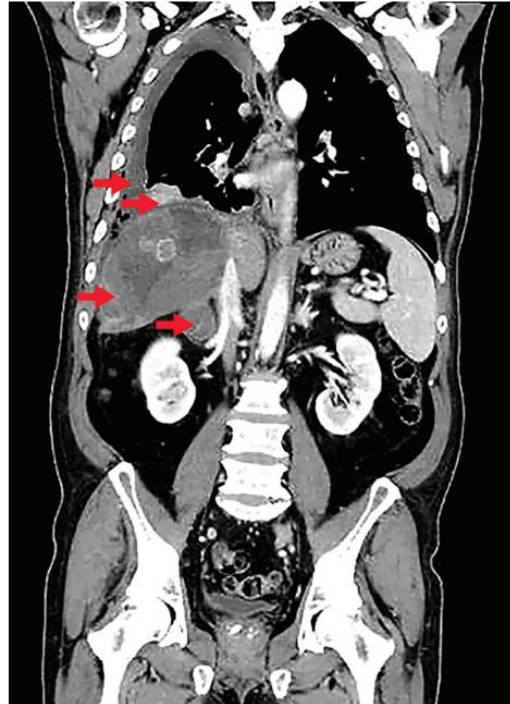


Figure. Computed tomography scan of a patient diagnosed with alveolar echinococcosis, Croatia. Arrows indicate right pleural effusion, lung lesions, an enlarged right adrenal gland, and a 13 × 12 × 12 cm lesion in the liver caused by *Echinococcus multilocaris*.



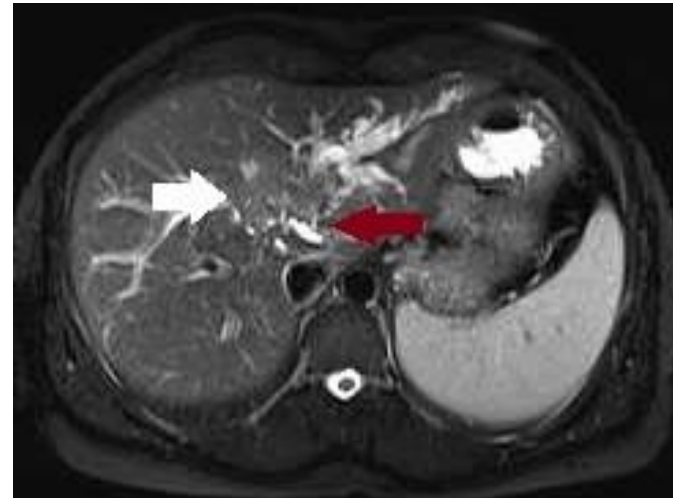
Patient 2 (2019): male, 64 y.

Patient 2: a sagittal postcontrast multidetector computed tomography (MDCT) scan in the late arterial phase demonstrated an infiltrating tumorlike liver mass in the left liver lobe with irregular margins and heterogeneous enhancement, infiltrating a gall bladder (arrow). A moderate dilatation of the intrahepatic bile ducts was noted (star).

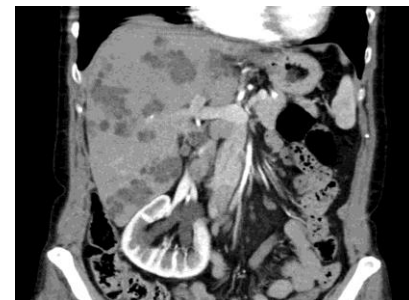
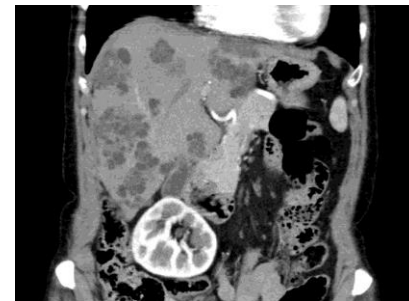
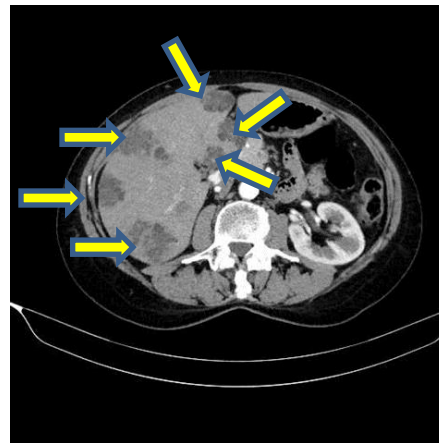
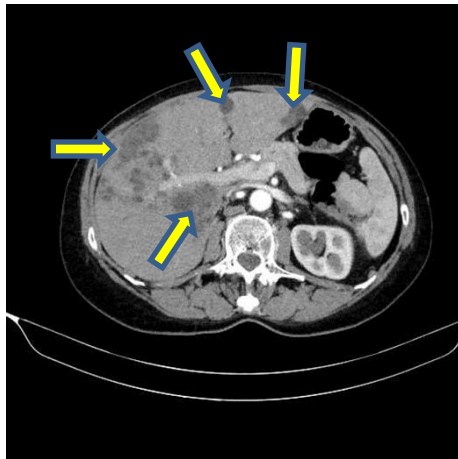


Patient 3 (2021): female, 37 y.

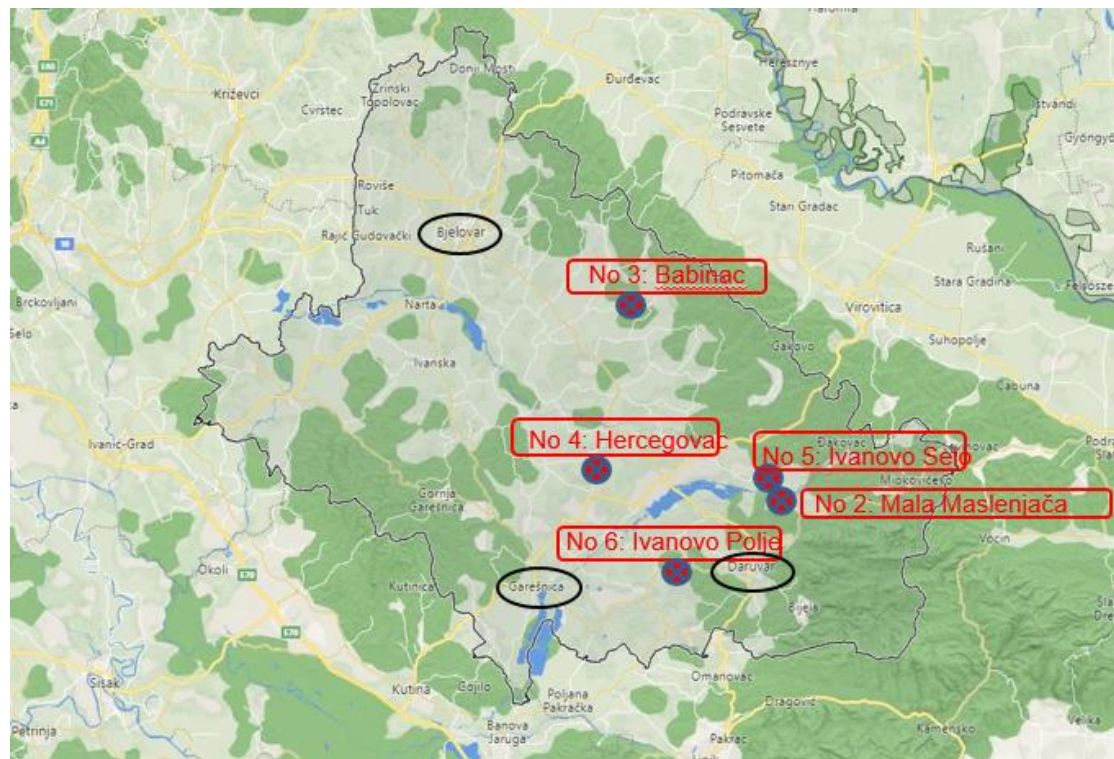
Patient 3: magnetic resonance imaging (MRI) revealed obstruction of the left and right hepatic duct with a hypovascular focal lesion measuring 4 cm in the IV.A and VIII. liver segment (white arrow). The lesion infiltrates a patent median hepatic vein (red arrow).

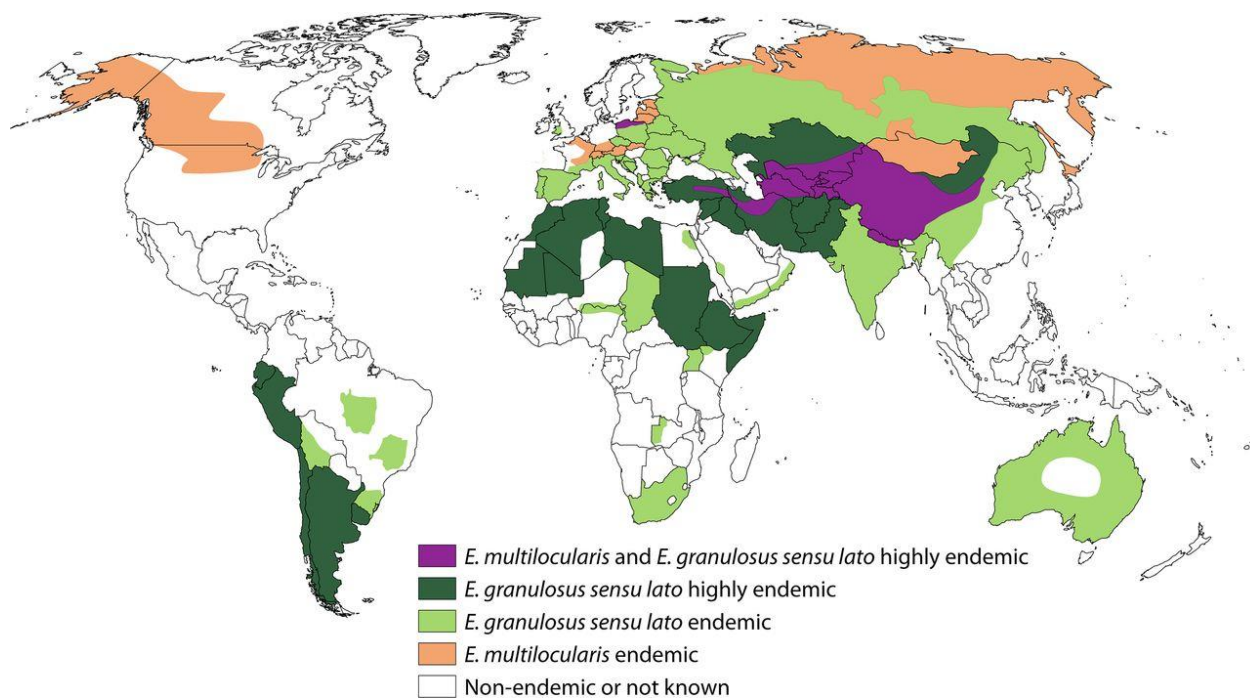


Patient 4 (2022): female, 58 y.



Distributon of human cases in Bjelovarsko – Bilogorska County







RESEARCH

Open Access

First report of *Echinococcus multilocularis* in cats in Poland: a monitoring study in cats and dogs from a rural area and animal shelter in a highly endemic region



Jacek Karamon^{1*}, Jacek Sroka, Joanna Dąbrowska, Ewa Biłska-Zajac, Jolanta Zdybel, Maciej Kochanowski, Mirosław Różycki and Tomasz Cencek

Abstract

Background: Alveolar echinococcosis is a dangerous zoonotic disease caused by larval forms of *Echinococcus multilocularis*. In its life-cycle, the principal definitive host is the red fox; however, domesticated carnivorous animals (dogs and cats) can also act as definitive hosts. Until now, there were no data concerning this infection in cats in Poland. The aim of this study was to estimate the prevalence of *E. multilocularis* in cats and dogs originating from rural areas and animal shelters in a region characterised by a high prevalence of *E. multilocularis* in red foxes.

Methods: Samples of faeces were collected from 67 cats and 268 dogs from a rural area (villages and animal shelters) of a highly endemic region in southeastern Poland. Samples were examined using nested PCR (*E. multilocularis*), multiplex PCR (*E. multilocularis*, *Taenia* spp.) and PCR [*E. granulosus* (s.l.)]. Additionally, faeces were examined microscopically (flotation). Moreover, intestines from 110 red foxes shot in the investigated area were examined (sedimentation and counting technique).

Results: Positive PCR results for *E. multilocularis* were obtained in 4 cats (6.0%) and 4 dogs (1.5%). There were no significant differences between groups of animals (from a shelter and with an owner) concerning the prevalence of *E. multilocularis* in both cats and dogs. *Taenia* spp. were found in 10 cats (14.9%) (*Taenia taeniiformis* and *T. hydatigena*) and 26 dogs (9.7%) (*T. hydatigena*, *T. serialis*, *T. taeniiformis*, *T. crassiceps*, *T. pisiformis* and *T. ovis*) and *Mesocostoides litoreus* was found in 4 cats (6.0%) and 3 dogs (1.1%). All samples were negative for *E. granulosus* by PCR. Taking into consideration PCR and flotation results, 29 cats (43.3%) and 73 dogs (27.2%) were infected with helminths (26.9 and 11.9%, respectively, were infected with tapeworms). The highly endemic status of the investigated area was confirmed by examination of red foxes: 48.2% of examined red foxes were infected with *E. multilocularis*.

Conclusions: To the best of our knowledge, this study reports the presence of *E. multilocularis* in cats for the first time in Poland and confirms the role of dogs in this infection in highly endemic areas.

Keywords: Cat, Dog, *Echinococcus multilocularis*, Helminths, Hookworms, *Mesocostoides*, *Taenia*, *Toxocara*, Poland

RESEARCH ARTICLE

Potential risk factors associated with human alveolar echinococcosis: Systematic review and meta-analysis

Franz J. Conraths^{1*}, Carolina Probst¹, Alessia Possenti^{2,3}, Belgees Boufana^{2,4}, Rosella Saulle⁵, Giuseppe La Torre⁵, Luca Busani⁶, Adriano Casulli^{2,4}

1 Friedrich-Loeffler-Institut, Institute of Epidemiology, Greifswald-Insel Riems, Germany, **2** Department of Infectious Diseases, Istituto Superiore di Sanità (ISS), Rome, Italy, **3** European Union Reference Laboratory for Parasites (EURLP), ISS, Rome, Italy, **4** World Health Organization Collaborating Centre for the epidemiology, detection and control of cystic and alveolar echinococcosis (in humans and animals), ISS, Rome, Italy, **5** Sapienza University of Rome, Department of Public Health and Infectious Diseases, Rome, Italy, **6** Department of Food Safety and Veterinary Public Health, Istituto Superiore di Sanità (ISS), Rome, Italy

* franz.conraths@flf.de



Table 1. Meta-analysis of potential and protective risk factors identified in case-control studies on human alveolar echinococcosis.

POTENTIAL RISK FACTOR	Effect model	Odds ratio (95% CI)	Overall effect	Number of sub- studies	Number of participants in studies	References
Dog ownership	M-H, Fixed	2.50 [1.73–3.62]	p<0.00001	5	1,068	19, 21, 22, 23
* Allowed dog into the house	M-H, Fixed	1.80 [0.90–3.62]	p = 0.10	2	216	19, 23
* Play with dogs	M-H, Random	1.24 [0.39–3.91]	p = 0.71	2	216	19, 23
Cat ownership	M-H, Fixed	2.63 [1.42–4.85]	p = 0.002	2	265	19, 22
* Living in a rural area	M-H, Random	3.07 [0.83–11.37]	p = 0.09	3	803	21, 23
Having a kitchen garden	M-H, Fixed	5.21 [2.65–10.22]	p<0.00001	2	746	21
Occupation: farmer	M-H, Fixed	4.50 [2.74–7.39]	p<0.00001	4	1,011	19, 21, 22
Haymaking in meadows not adjacent to water	M-H, Fixed	3.50 [1.63–7.55]	p = 0.001	2	238	19, 22
Went to forests for vocational reasons	M-H, Fixed	2.61 [1.13–6.05]	p = 0.03	2	266	19, 22
* Ate unwashed strawberries	M-H, Fixed	1.39 [0.87–2.23]	p = 0.17	4	1,006	19, 21, 22
Chewed grass	M-H, Fixed	3.20 [1.65–6.20]	p = 0.00006	2	252	19, 22
* Hunting	M-H, Fixed	1.13 [0.69–1.83]	p = 0.63	5	1,064	19, 21, 22, 23
Handling foxes	M-H, Fixed	2.27 [1.35–3.81]	p = 0.002	4	959	19, 21, 23
§ Ate mushrooms	M-H, Fixed	0.72 [0.38–1.39]	p = 0.33	2	255	19, 22
* Ate wild vegetables and fruit	M-H, Fixed	1.38 [0.90–2.10]	p = 0.14	5	1,046	19, 21, 22, 23
HLA (protective factor)	M-H, Fixed	0.50 [0.32–0.80]	p = 0.003	2	743	20, 24

Bold: significantly increase of odds ratio.

*: increased odds ratio, but not statistically significant.

§: influence on infection risk uncertain.

RESEARCH ARTICLE

Potential risk factors associated with human alveolar echinococcosis: Systematic review and meta-analysis

Franz J. Conraths^{1*}, Carolina Probst¹, Alessia Possenti^{2,3}, Belgees Boufana^{2,4}, Rosella Saulle⁵, Giuseppe La Torre⁶, Luca Busani¹, Adriano Casulli^{2,4}

1 Friedrich-Loeffler-Institut, Institute of Epidemiology, Greifswald-Insel Riems, Germany, **2** Department of Infectious Diseases, Istituto Superiore di Sanità (ISS), Rome, Italy, **3** European Union Reference Laboratory for Parasites (EURLP), ISS, Rome, Italy, **4** World Health Organization Collaborating Centre for the epidemiology, detection and control of cystic and alveolar echinococcosis (in humans and animals), ISS, Rome, Italy, **5** Sapienza University of Rome, Department of Public Health and Infectious Diseases, Rome, Italy, **6** Department of Food Safety and Veterinary Public Health, Istituto Superiore di Sanità (ISS), Rome, Italy

* franz.conraths@flf.de



Table 1. Meta-analysis of potential and protective risk factors identified in case-control studies on human alveolar echinococcosis.

POTENTIAL RISK FACTOR	Effect model	Odds ratio (95% CI)	Overall effect	Number of sub-studies	Number of participants in studies	References
Dog ownership	M-H, Fixed	2.50 [1.73–3.62]	p<0.00001	5	1,068	19, 21, 22, 23
* Allowed dog into the house	M-H, Fixed	1.80 [0.90–3.62]	p = 0.10	2	216	19, 23
* Play with dogs	M-H, Random	1.24 [0.39–3.91]	p = 0.71	2	216	19, 23
Cat ownership	M-H, Fixed	2.63 [1.42–4.85]	p = 0.002	2	265	19, 22
* Living in a rural area	M-H, Random	3.07 [0.83–11.37]	p = 0.09	3	803	21, 23
Having a kitchen garden	M-H, Fixed	5.21 [2.65–10.22]	p<0.00001	2	746	21
Occupation: farmer	M-H, Fixed	4.50 [2.74–7.39]	p<0.00001	4	1,011	19, 21, 22
Haymaking in meadows not adjacent to water	M-H, Fixed	3.50 [1.63–7.55]	p = 0.001	2	238	19, 22
Went to forests for vocational reasons	M-H, Fixed	2.61 [1.13–6.05]	p = 0.03	2	266	19, 22
* Ate unwashed strawberries	M-H, Fixed	1.39 [0.87–2.23]	p = 0.17	4	1,006	19, 21, 22
Chewed grass	M-H, Fixed	3.20 [1.65–6.20]	p = 0.00006	2	252	19, 22
* Hunting	M-H, Fixed	1.13 [0.69–1.83]	p = 0.63	5	1,064	19, 21, 22, 23
Handling foxes	M-H, Fixed	2.27 [1.35–3.81]	p = 0.002	4	959	19, 21, 23
§ Ate mushrooms	M-H, Fixed	0.72 [0.38–1.39]	p = 0.33	2	255	19, 22
* Ate wild vegetables and fruit	M-H, Fixed	1.38 [0.90–2.10]	p = 0.14	5	1,046	19, 21, 22, 23
HLA (protective factor)	M-H, Fixed	0.50 [0.32–0.80]	p = 0.003	2	743	20, 24

Bold: significantly increase of odds ratio.

*: increased odds ratio, but not statistically significant.

§: influence on infection risk uncertain.

Table 2. Meta-analysis of potential and protective risk factors identified in cross-sectional studies on human alveolar echinococcosis.

POTENTIAL RISK FACTOR	Effect model	Odds ratio (95% CI)	Overall effect	Number of sub-studies	Number of participants	References
Dog ownership	M-H, Fixed	1.95 [1.52–2.51]	p<0.00001	4	12,571	26, 29, 33, 34
Play with dogs	M-H, Fixed	3.48 [2.20–5.52]	p<0.00001	3	5,916	10, 33
* Hand wash before eating	M-H, Random	4.90 [0.80–29.87]	p = 0.08	3	5,348	10, 33
Gender: female	M-H, Random	1.66 [1.31–2.10]	p<0.00001	10	42,812	10, 25–27, 29–34
Age over 20 years	M-H, Random	3.65 [1.15–11.62]	p = 0.03	8	24,988	10, 25, 26, 28, 31–33
Ethnic group: Tibetan	M-H, Fixed	2.03 [1.56–2.63]	p<0.00001	4	25,952	10, 27, 29, 34
Low income	M-H, Fixed	3.92 [2.42–6.36]	p<0.00001	2	4,124	10
Source of drinking water other than well or tap	M-H, Fixed	1.81 [1.52–2.17]	p = 0.001	5	23,714	10, 29, 33, 34
* Occupation: farmer	M-H, Random	1.16 [0.31–4.35]	p = 0.82	5	17,878	10, 26, 27, 29, 34
Occupation: herder	M-H, Random	2.20 [1.51–3.19]	p<0.00001	5	21,045	10, 26, 27, 29
Drink un-boiled water	M-H, Fixed	0.63 [0.48–0.84]	p = 0.002	2	7,096	10, 29
* Hunting / handling foxes	M-H, Random	1.25 [0.71–2.20]	p = 0.44	3	9,442	26, 29, 33
Low education	M-H, Fixed	4.81 [2.73–8.48]	p<0.00001	2	5,297	10

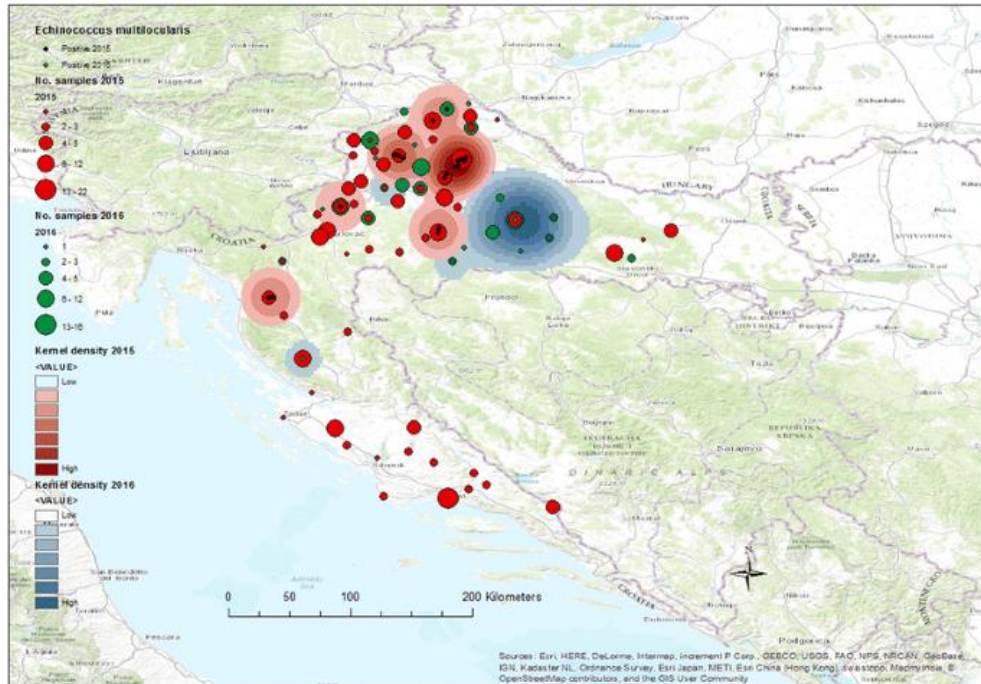
Bold: significantly increase of odds ratio.

* Increased odds ratio, but not statistically significant.

<https://doi.org/10.1371/journal.pntd.0005801.t002>







Beck R, Mihaljević Ž, Brezak R i sur. First detection of *E. multilocularis* in Croatia. *Parasitology Research* 2018;117:617-621.

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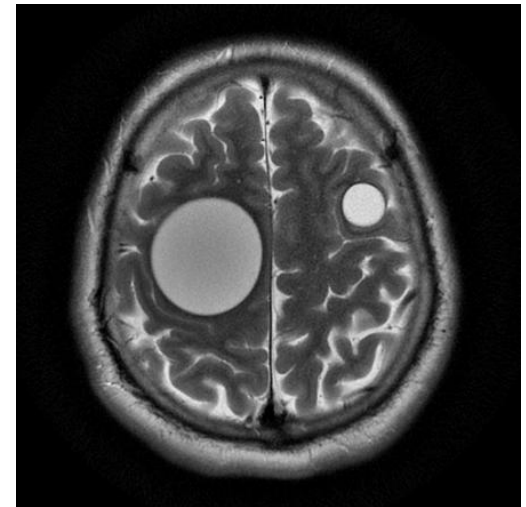
Differences in Cysts

Echinococcus granulosus

- Slow development of cyst
- Cysts have thick-walled chambers
- Separated by connective tissue
- Cyst is fluid filled
- Cyst is free of host material

Echinococcus multilocularis

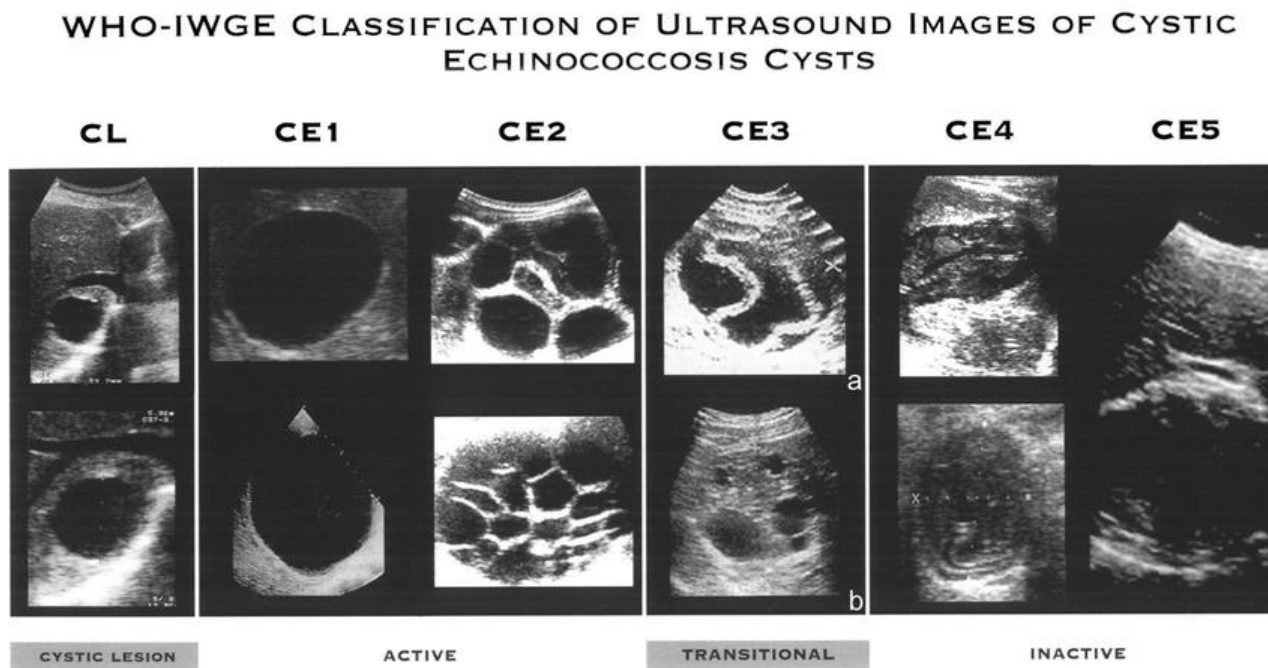
- Rapid development of cyst
- Cyst has thin-walled chambers
- Not separated by connective tissue
- Cyst is gelatinous filled
- Cyst is contaminated by host material



Diagnosis - general

- Imaging techniques essential for diagnosis and follow up
- Relatively inexpensive and portable ultrasound (US) widely used to diagnose CE and AE liver lesions
- X rays, CT, MR for other cysts
- Serology – confirmatory step – various levels of sensitivity/specificity – involved species, lesion location, antigen used
- US-mass population screening – diagnosis of asymptomatic cases
- Systemic follow up of patients with different conditions – increase of asymptomatic cases to

Diagnosis - general

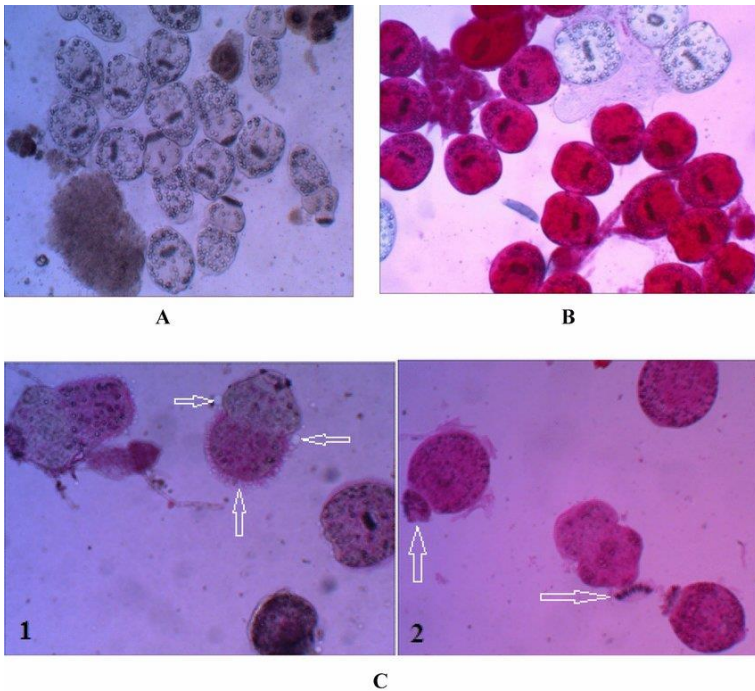


Diagnosis - general

P	Primary lesion localized to the liver
PX	Primary lesion cannot be assessed
P0	No detectable hepatic lesion
P1	Peripheral hepatic lesion with no proximal hepatic vascular or biliary involvement
P2	Central hepatic lesion with proximal involvement of vessels or biliary ducts in 1 lobe
P3	Central hepatic lesion with involvement of hilar vessels or biliary ducts in both lobes or with involvement of 2 hepatic veins
P4	Hepatic lesion with extension along the vessels and biliary tree
N	Extrahepatic involvement of neighboring organs or tissues (diaphragm, lung, pleura, pericardium, heart, gastric or duodenal wall, adrenal gland, peritoneum, retroperitoneum, parietal wall [muscles, skin, bone], pancreas, regional lymph nodes, hepatic ligaments, kidney)
NX	Cannot be evaluated
NO	No regional involvement
N1	Regional involvement of contiguous organs or tissues
M	Absence or presence of distant metastasis (in lung, distant lymph nodes, spleen, central nervous system, orbits, bone, skin, muscle, kidney, distant peritoneum, and retroperitoneum)
MX	Not completely evaluated
M0	No metastasis
M1	Metastasis

Diagnosis

- Images of living, dead, and partially dead *E. granulosus* protoscolices following staining with 0.1 percent eosin



Casoni skin test



- The test is positive in about 90% of cases of hydatid disease affecting the liver, but positive in less than 50% of patients with hydatid disease elsewhere in the body; false positive results are also common

Diagnosis- antibody detection

- AE and CE are lacking pathognomonic clinical signs, imaging technologies and serology remain the main pillars for diagnosis
- Methods: IHA, IFA, EIA, CF, La, immunoelectrophoresis, immunoblotting
- Response – location and viability of cysts/lesions
- Calcified, senescent or dead cysts – often negative serologic results
- Liver cysts – greater serological response 90%, lung cysts 50%, brain or spleen less than 50%
- Cross reactions – in low titres in patients with other parasitic diseases, liver cirrhosis and collagen disease

Diagnosis- serology

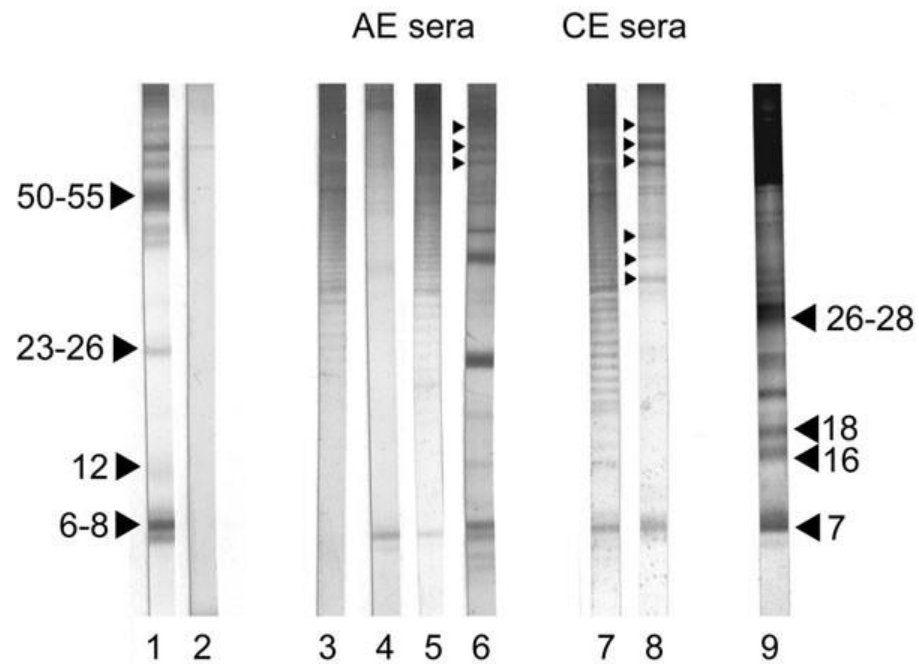
- Hidatid fluid (HF) major antigenic source for immunodiagnosis
- Test with recombinant antigens (arc-5, 8 kDa antigen) increased sensitivity
- Test positivity in screening test – immunoblotting
- Immunoblotting – monitor therapy, EIA and IHA positive results for years
- AE patients usually serologically positive when *E. granulosus* antigens are used

Diagnosis- serology

- Em2, Em2G11, Em18
- Sensitivity more than 95%
- Specificity 99%



Diagnosis- serology



DNA detection

- Recently developed DNA based methods – quantitative and/or nested PCR assays
- Highly sensitive
- Reasonably specific able to distinguish species
- Determine genotypes
- Routinely used on biopsy specimens in unusual imaging aspects and/or in patients with negative serology
- Detection of eggs in contaminated areas

Conclusion

- From 2017 the number of cases of autochtoneous human AE in Croatia is rising

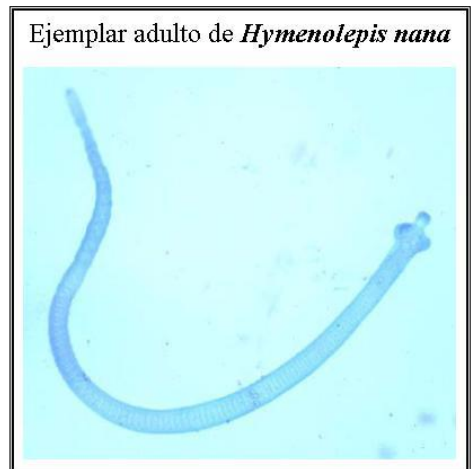


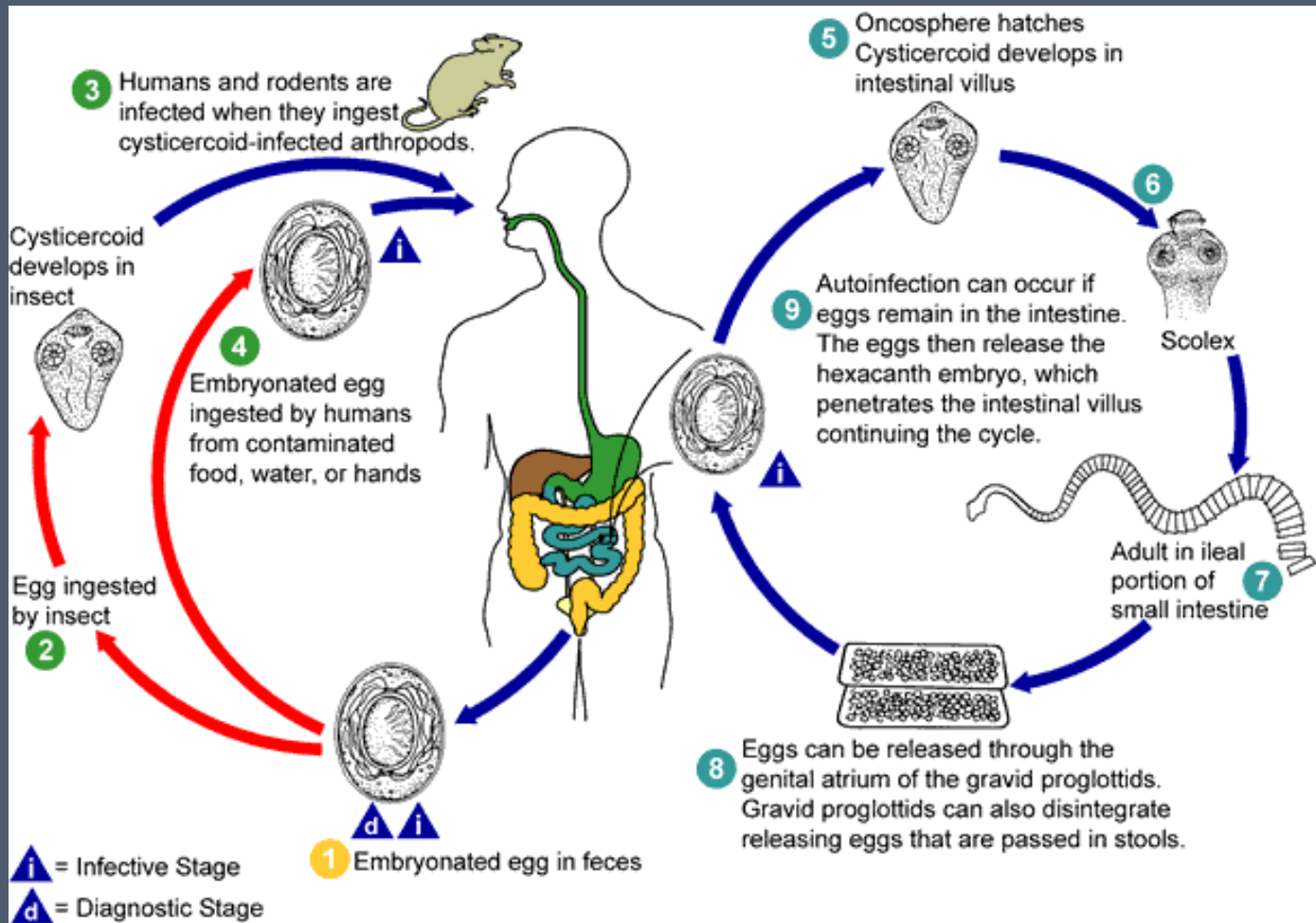
- US screening of persons from endemic regions?
- Serolgy in persons from endemic regions with tumorous hepatal lesions
- Education of physicians (internal medicine, surgeons, oncologist, radiologist)
- Reporting!!!

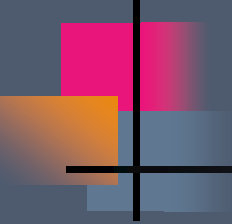


Hymenolepis (Vampirolepis) nana

- Patuljasta trakavica
- Veličina 1 – 4 cm
- Rasprostranjena širom svijeta
- Konačni domaćin glodavci i čovjek
- Prijelazni buhe (alternativno)







Hymenolepis nana

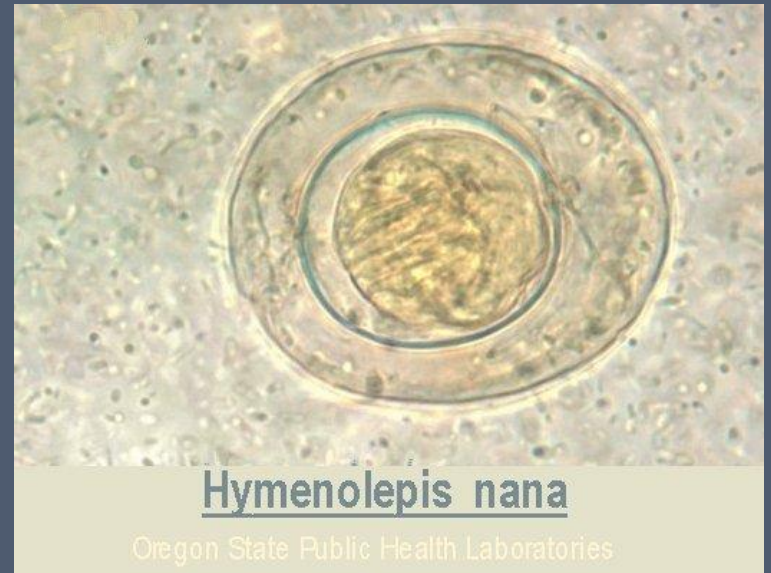
Klinička slika

- Najčešće asimptomatska
- Simptomatska – nespecifični (glavobolje, vrtoglavice, proljevi, inapetenca)

Hymenolepis nana

Laboratorijska dijagnostika

- Uzorak stolica
- Nalaz velikih, prozirnih, karakterističnih jaja (polarni filamenti)





Laboratorijska dijagnostika valjkastih crva

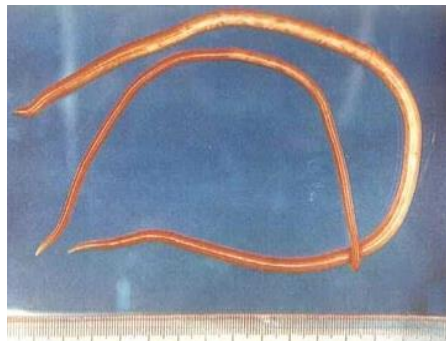
Koljeno Nematoda

- ✿ Neseegmentirani beskralješnjaci valjkaste, crvolike građe
- ✿ Veličina: manje od mm – 1 m
(mužjaci obično manji od ženki)
- ✿ Odvojeni spolovi
- ✿ Kompleksni tegument i probavni sustav

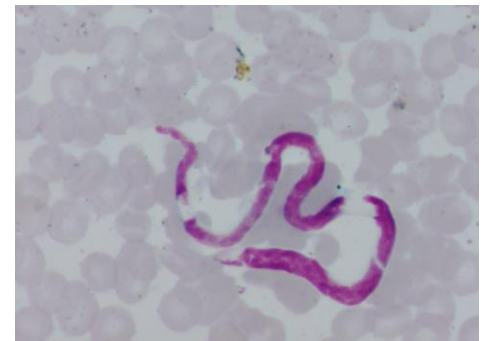
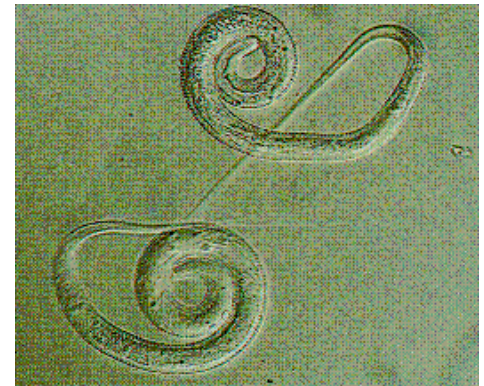


Nematode

crijevne



krvno - tkivne



Medicinski značajne nematode

- ✿ Enterobius vermicularis
- ✿ Ascaris lumbricoides
- ✿ Toxocara canis
- ✿ Toxocara cati
- ✿ Trichuris trichiura
- ✿ Ancylostoma duodenale
- ✿ Necator americanus
- ✿ Ankylostoma braziliense
- ✿ Strongyloides stercoralis
- ✿ Trichinella spiralis
- ✿ Wuchereria bancrofti
- ✿ Brugia malayi
- ✿ Loa loa
- ✿ Mansonella spp.
- ✿ Onchocerca volvulus
- ✿ Dirofilaria immitis
- ✿ Dracunculus medinensis



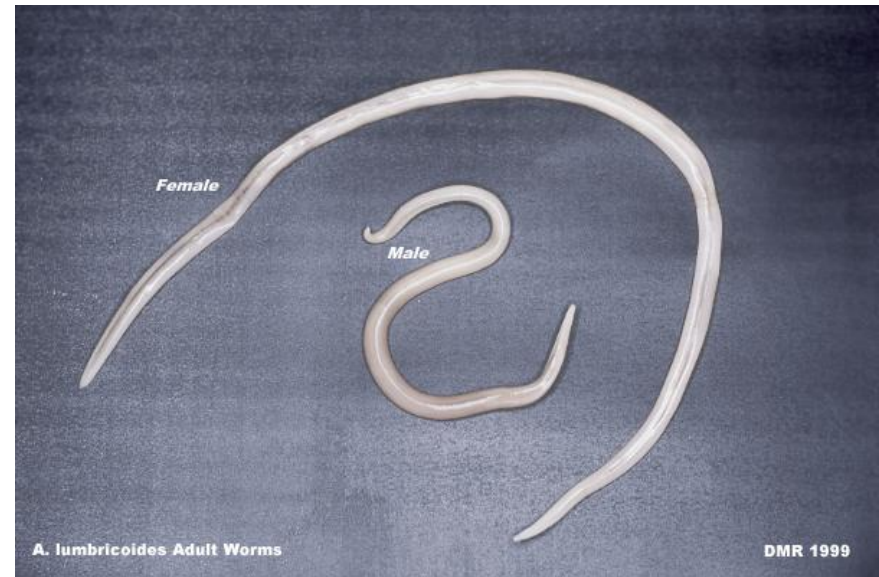
- ✿ *Ascaris lumbricoides*
- ✿ *Trichuris trichiura*
- ✿ *Ancylostoma duodenale*
- ✿ *Strongyloides stercoralis*
- ✿ *Enterobius vermicularis*
- ✿ *Trichinella spiralis*
- ✿ Filarije

Ascaris lumbricoides

- ✚ Velika dječja glista
- ✚ Uzročnik askarioze
- ✚ Rasprostranjen širom svijeta
- ✚ WHO – 2018. god. – 1,4 milijarda inficiranih

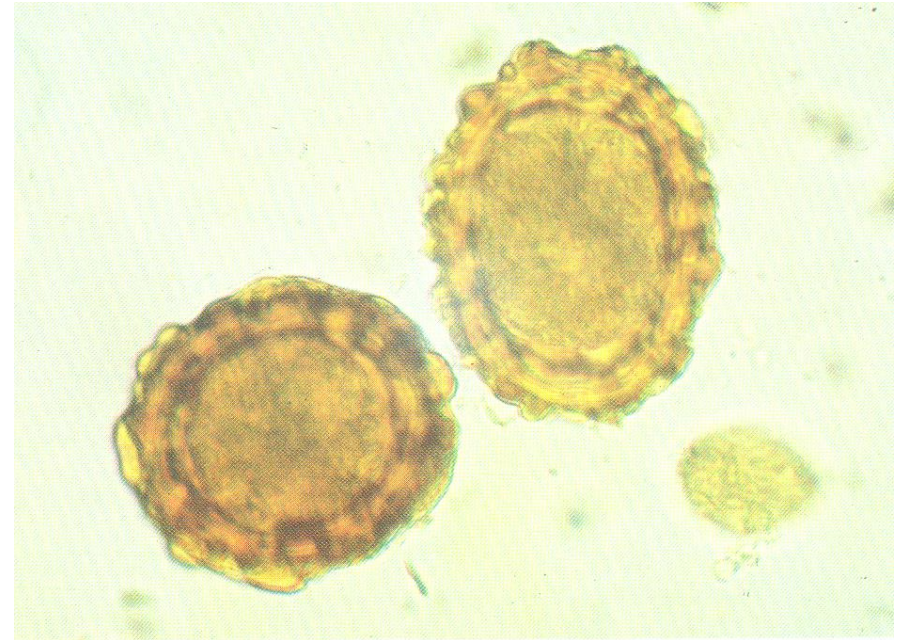
Ascaris lumbricoides

- Adulti žive u tankom crijevu
- Veličina:
mužjaci 15-20 cm x 3-4 mm, ženke 20-30 cm x 5-6 mm

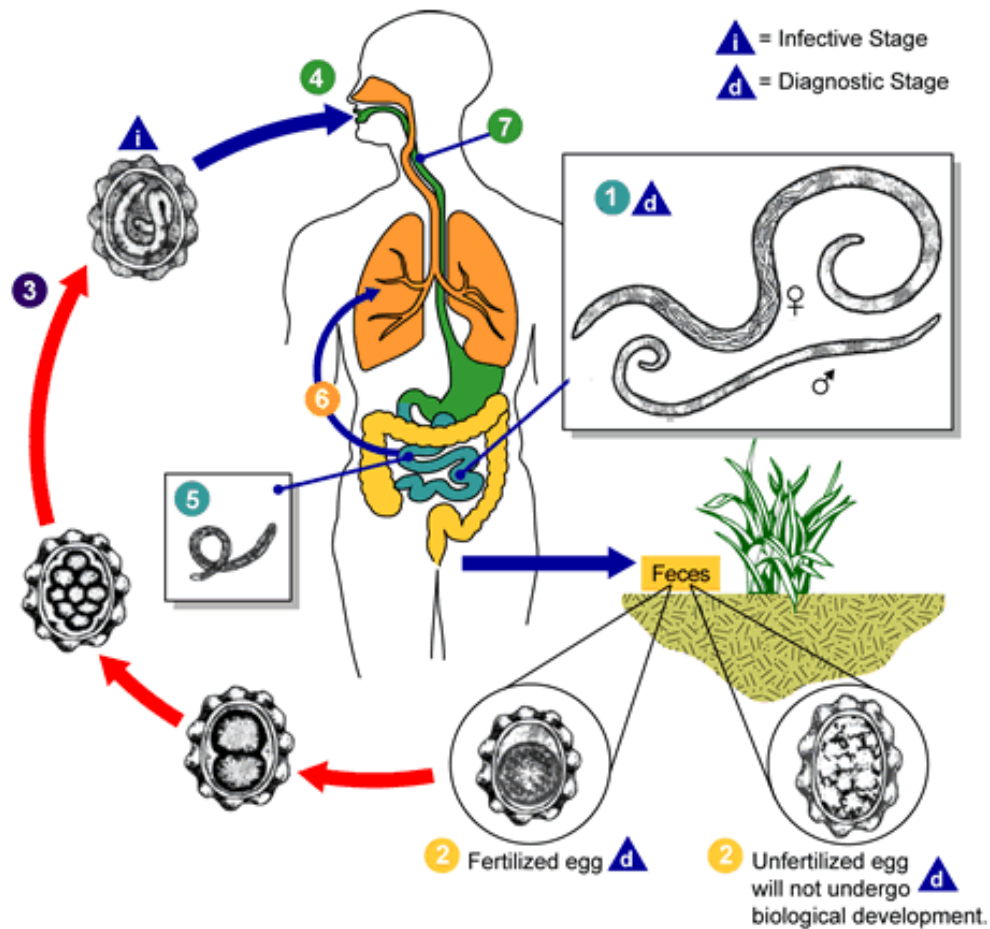


Ascaris lumbricoides

- ✚ Ženka polaže oko 200 000 jaja na dan
- ✚ Jaja neembrionirana
- ✚ Veličine 60x45 μm , debela, smeđa ljuska, najčešće čvoraste površine



Ascaris lumbricoides



Ascaris lumbricoides

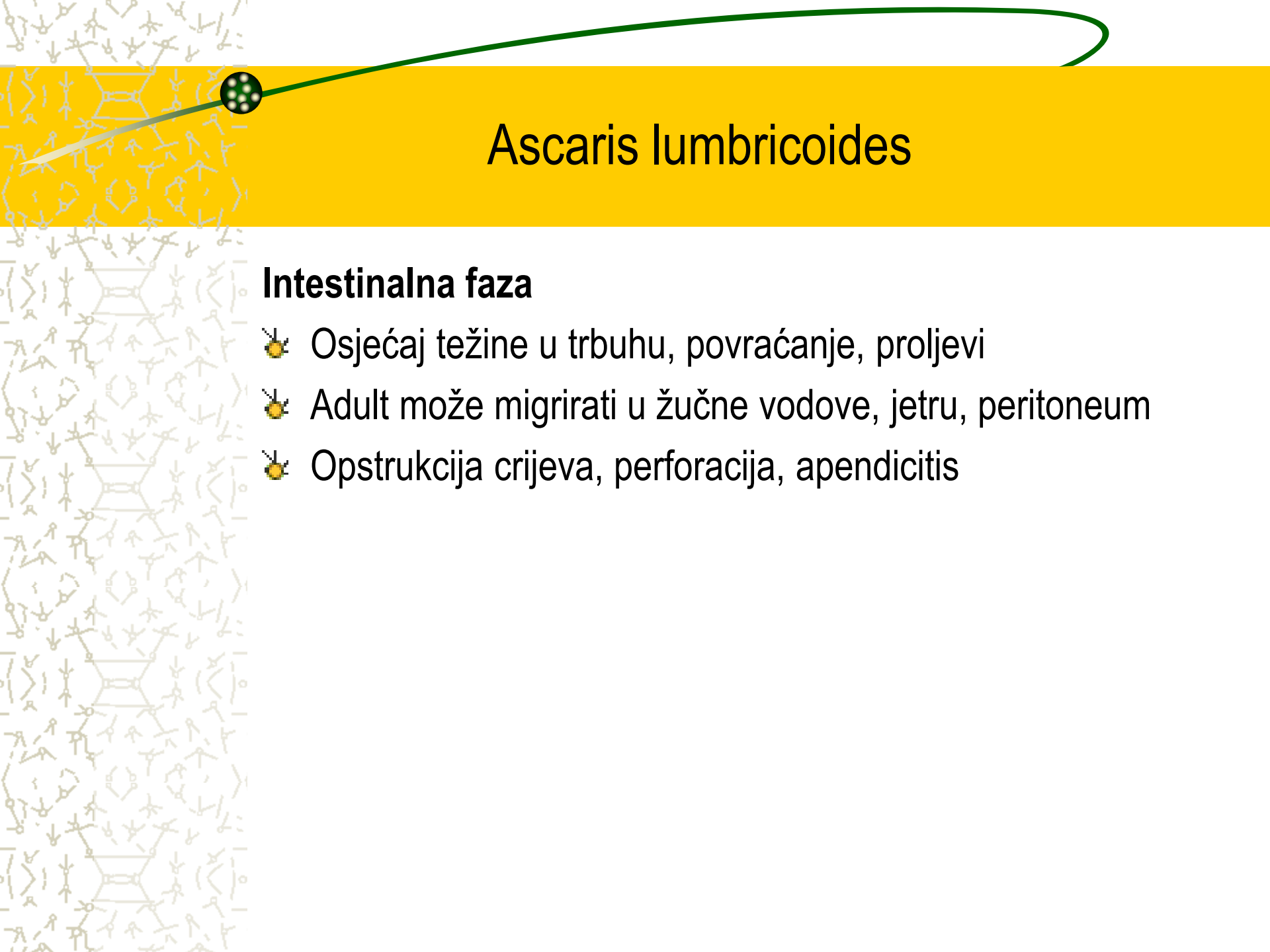
Epidemiologija

- ☼ Humani parazit
- ☼ Jaja vijabilna nekoliko godina u vlažnom tlu
- ☼ Infekcija zagađenom hranom, tlom (geofagija), vodom
- ☼ Prevalencija najveća u djece

Ascaris lumbricoides

Klinička slika

- ☼ Infekcija često asimptomatska
 - ☼ U težim slučajevima bolesti **migracija ličinki** može dovesti do pneumonitisa
- Löfflerov sindrom – kašalj, dispneja, febrilitet, eozinofilija



Ascaris lumbricoides

Intestinalna faza

- ✚ Osjećaj težine u trbuhu, povraćanje, proljevi
- ✚ Adult može migrirati u žučne vodove, jetru, peritoneum
- ✚ Opstrukcija crijeva, perforacija, apendicitis

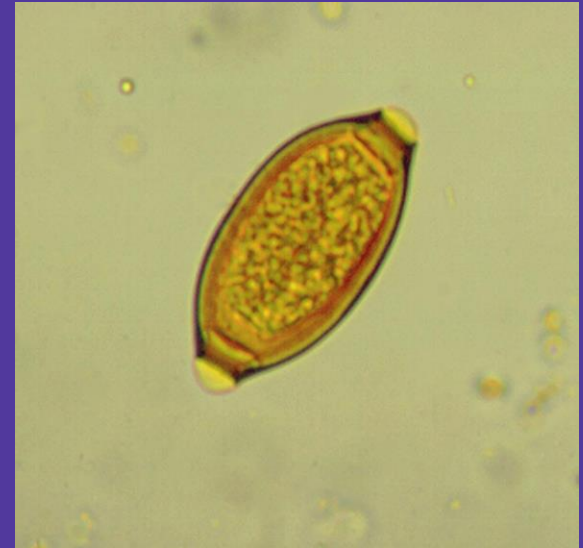
Ascaris lumbricoides

Laboratorijska dijagnostika

- ✚ Uzorak: stolica, sputum, krv
- ✚ Stolica: nativni preparat, MIFC - jajašca
- ✚ Sputum: nalaz ličinki
- ✚ Serologija: EIA

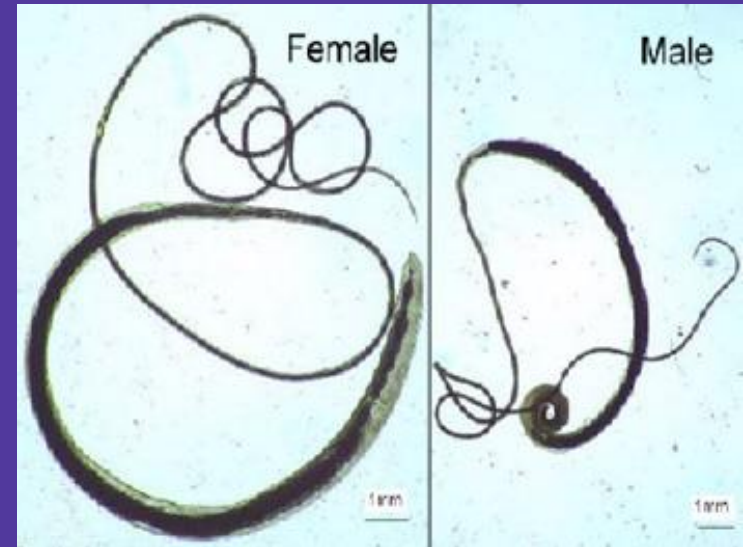
Trichuris trichiura

- 🪲 Crv vlašnjak
- 🪲 Uzročnik trihurioze
- 🪲 Parazit primata (ljudi i majmuni)
- 🪲 Rasprostranjen širom svijeta
- 🪲 WHO 2018.god.- inficirana 1 milijarda ljudi



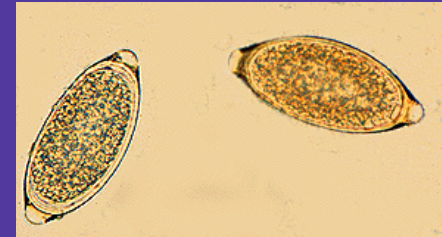
Trichuris trichiura

- Živi u ileocekalnom području i u debelom crijevu
- Ružičasto crvene boje
- Mužjak 3-4 cm, ženka 3,5-5 cm
- Prednji dio tanak, zadak zadebljan, u mužjaka spiralno savijen

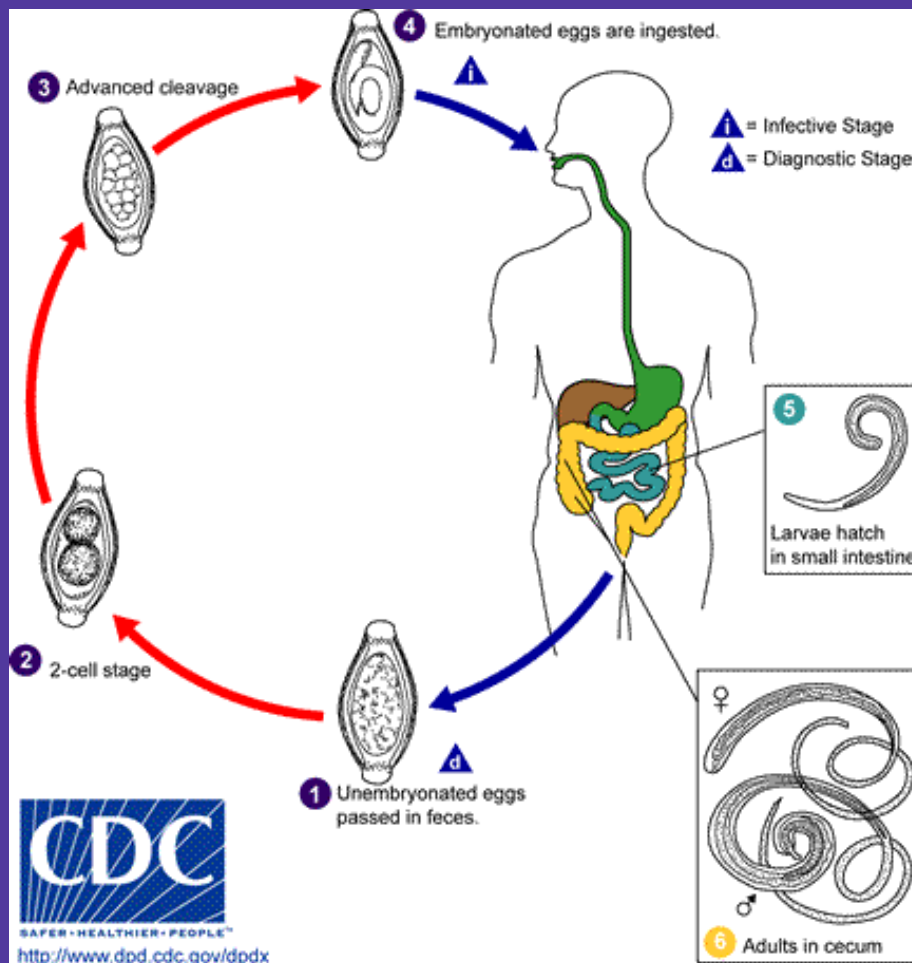


Trichuris trichiura

- Ženka leže 2 000-14 000 jajašca dnevno
- Jajašce u obliku bačvice ili limuna
- Veličine 50-55 μm x 35-50 μm
- Vanjska ljuska smeđe obojena, tri unutarnja sloja bezbojna, hijalini polarni čepovi
- Jajašce nije segmentirano
- Kisik nužan za sazrijevanje
- U našem podneblju –10 tjedana- u jajašcu invazivna ličinka
- Otporno, u vlažnoj sredini vijabilno nekoliko godina



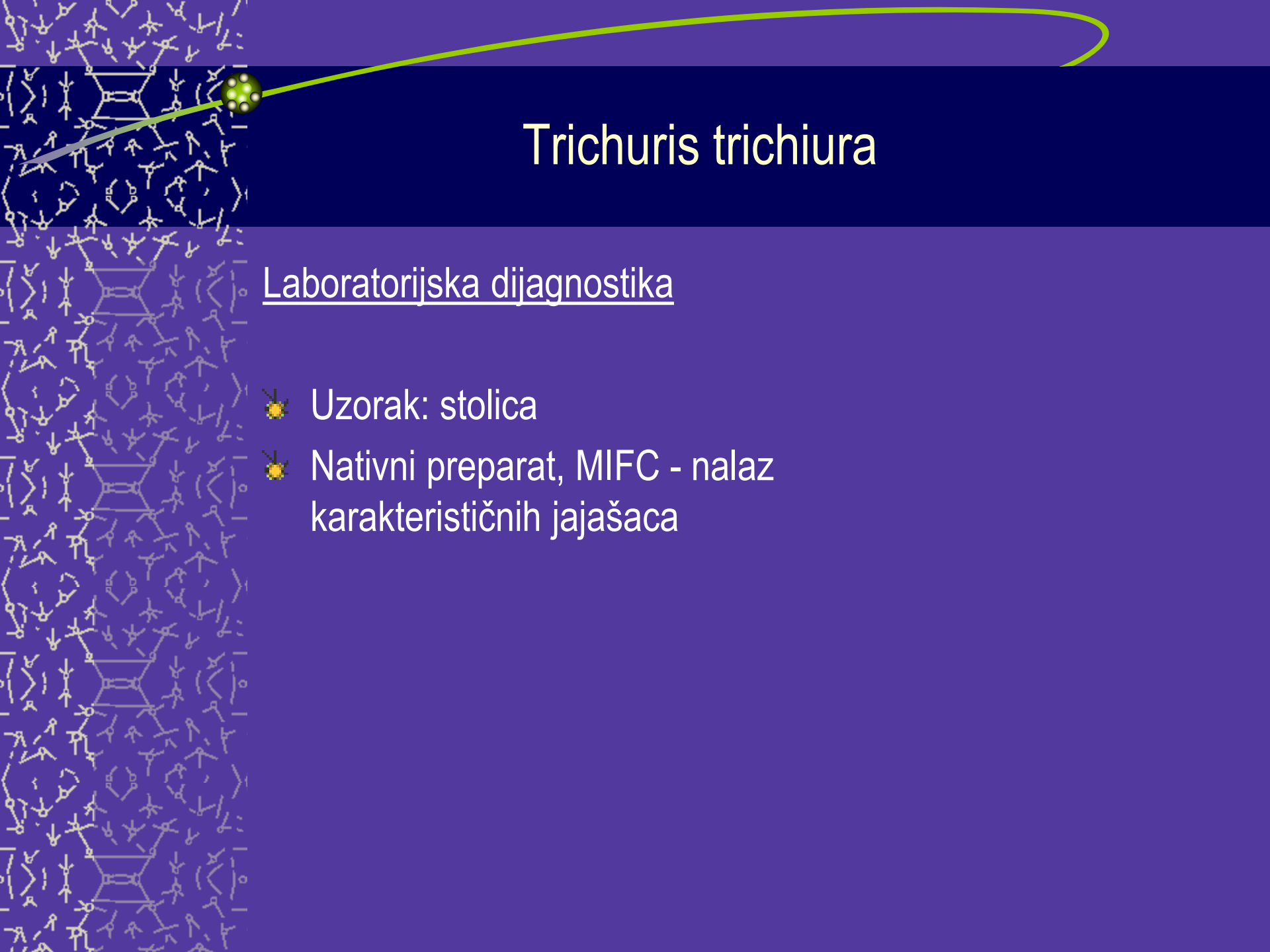
Trichuris trichiura



Trichuris trichiura

Klinička slika

- ☀ Ovisna o broju parazita
- ☀ Mali broj parazita - nema simptoma
- ☀ Veliki broj, mala djeca – bolovi u trbuhu, krvavi proljevi, gubitak apetita, mršavljenje, apendicitis, anemija



Trichuris trichiura

Laboratorijska dijagnostika

- ☀ Uzorak: stolica
- ☀ Nativni preparat, MIFC - nalaz karakterističnih jajašaca



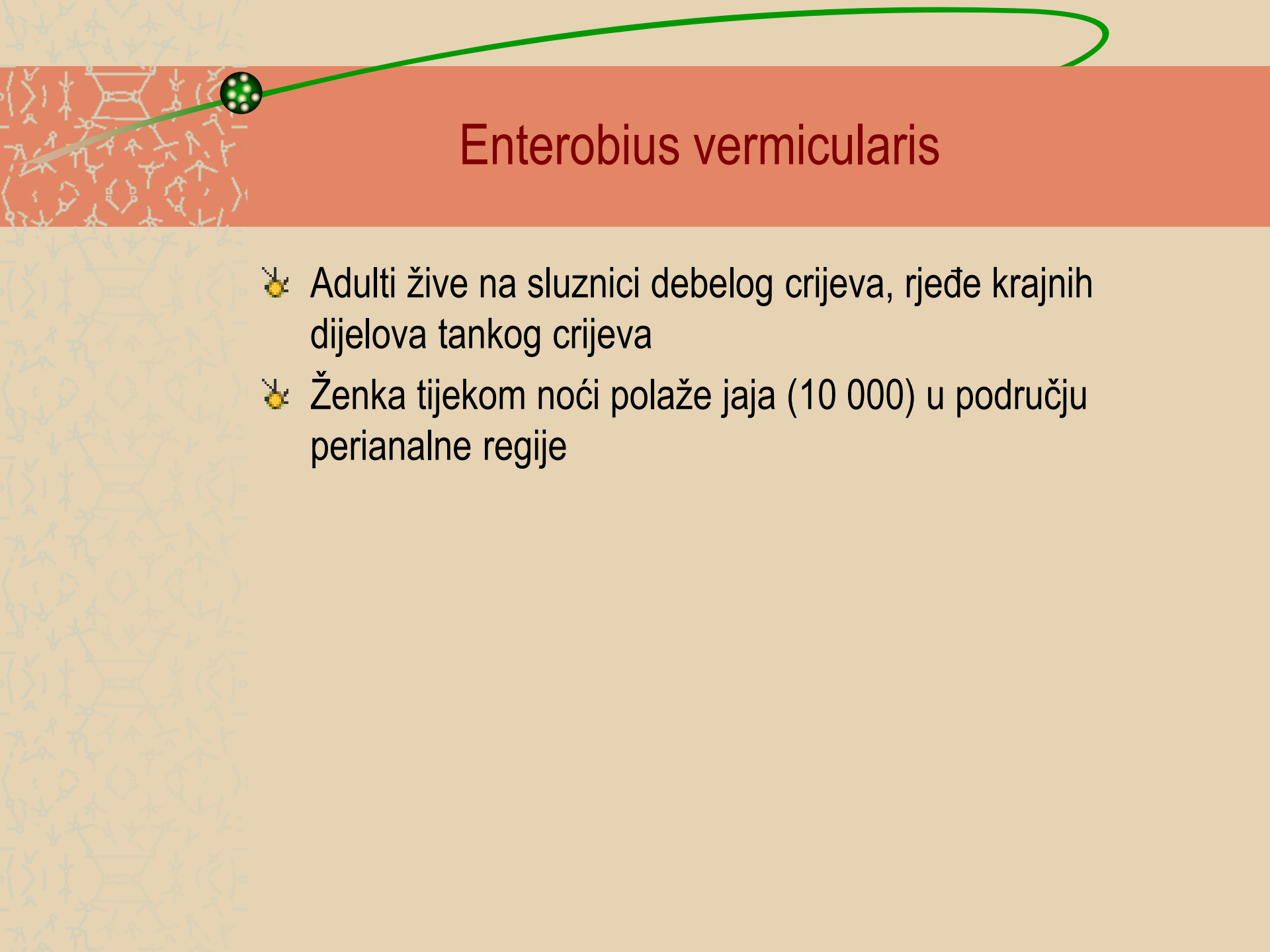
Enterobius vermicularis (Oxyuris vermicularis)

- ✎ Mala dječja glista
- ✎ Uzročnik enterobioze, oksiuroze
- ✎ Rasprostranjen po čitavom svijetu
- ✎ WHO – 2018.god.- 500 mil. ljudi inficirano

Enterobius vermicularis

- ✎ Makroskopski podsjeća na komadiće konca
- ✎ Bijele boje
- ✎ Mužjak 2-5 mm, tanak, vitak, zavijen ventralno
- ✎ Ženka 8-13 mm, vitka, ispruženog tijela
- ✎ Oboljeva samo čovjek

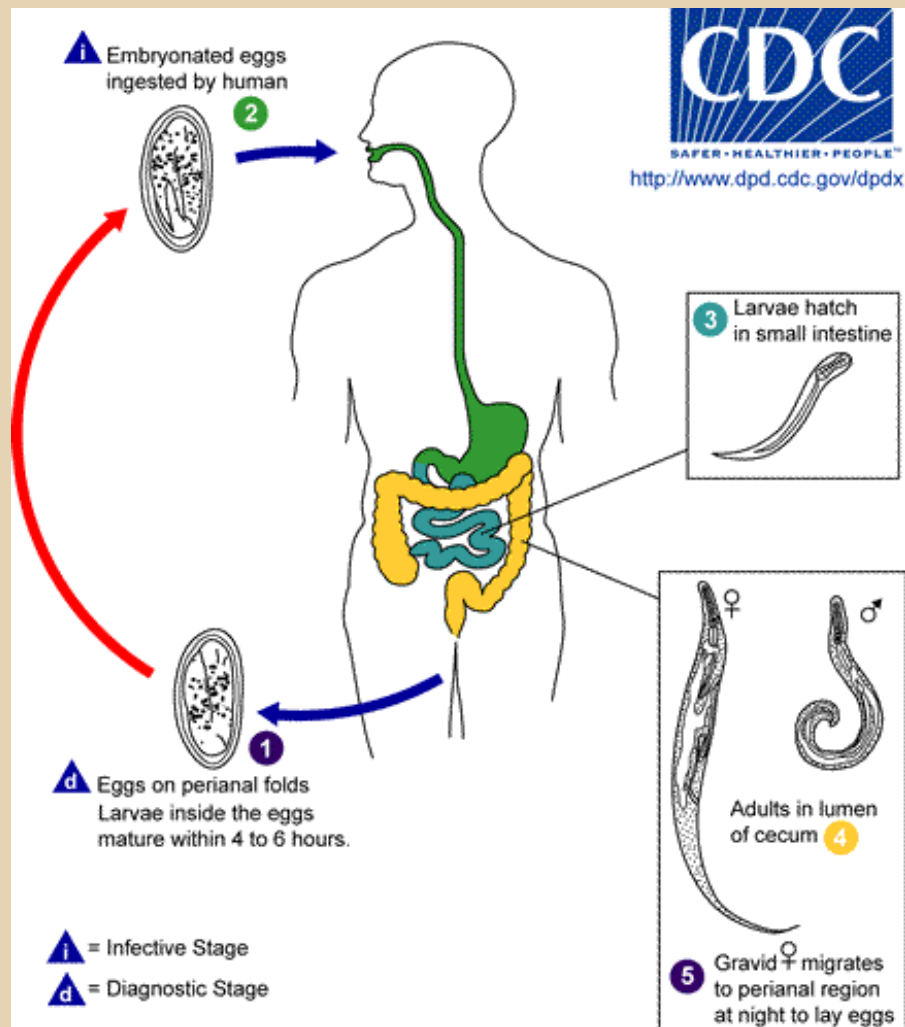




Enterobius vermicularis

- ✎ Adulti žive na sluznici debelog crijeva, rjeđe krajnih dijelova tankog crijeva
- ✎ Ženka tijekom noći polaže jaja (10 000) u području perianalne regije

Enterobius vermicularis

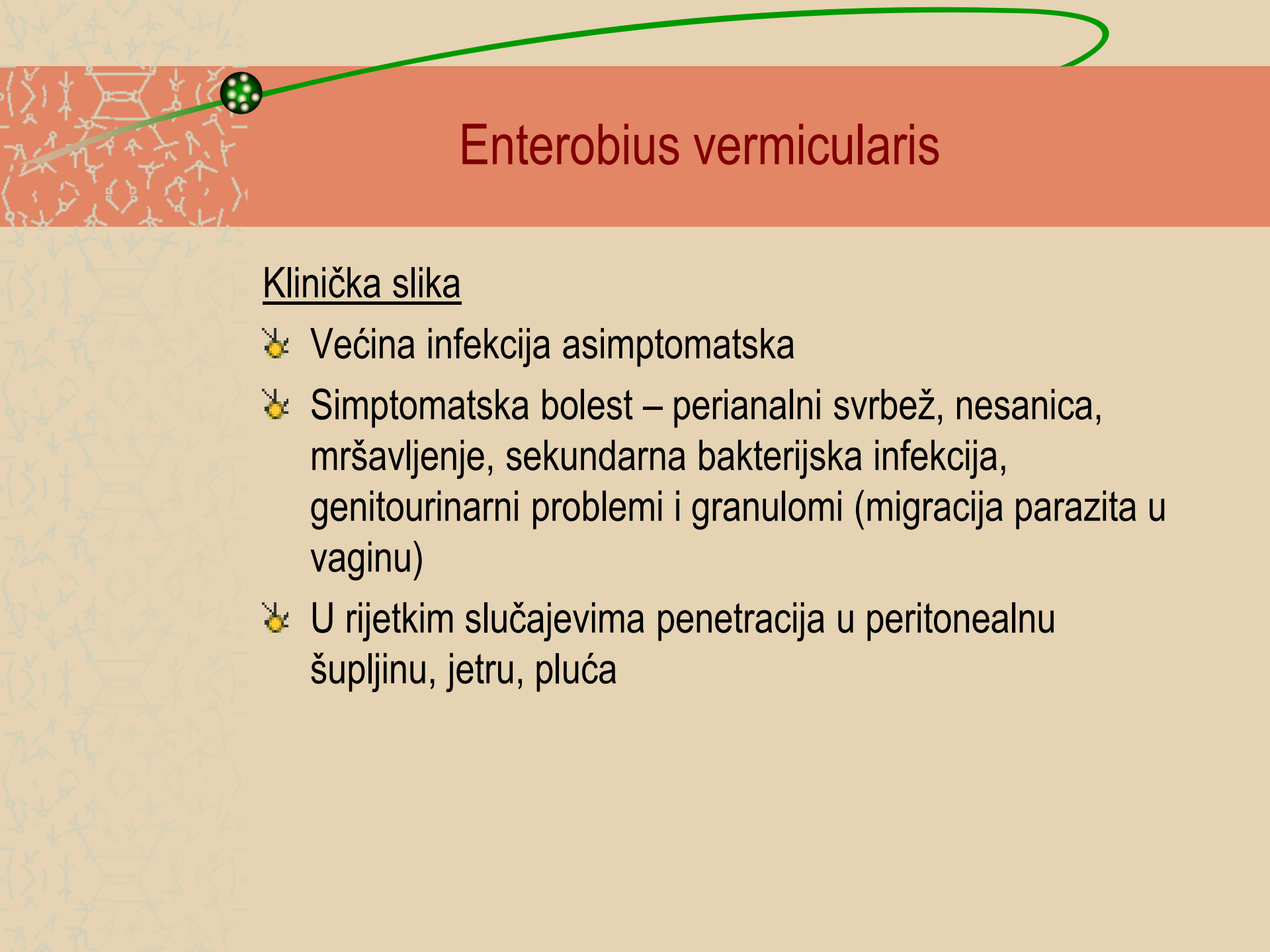


Enterobius vermicularis

Jajašca

- ✿ Nesimetrično ovalna, veličine 50-60 x 20-30 μm
- ✿ Glatke i prozirne ljuske
- ✿ Sadrže embrio koji se za 4-6 sati razvije u infektivnu ličinku
- ✿ Vijabilna 4 tjedna u vlažnoj sredini
- ✿ Autoinfekcija!

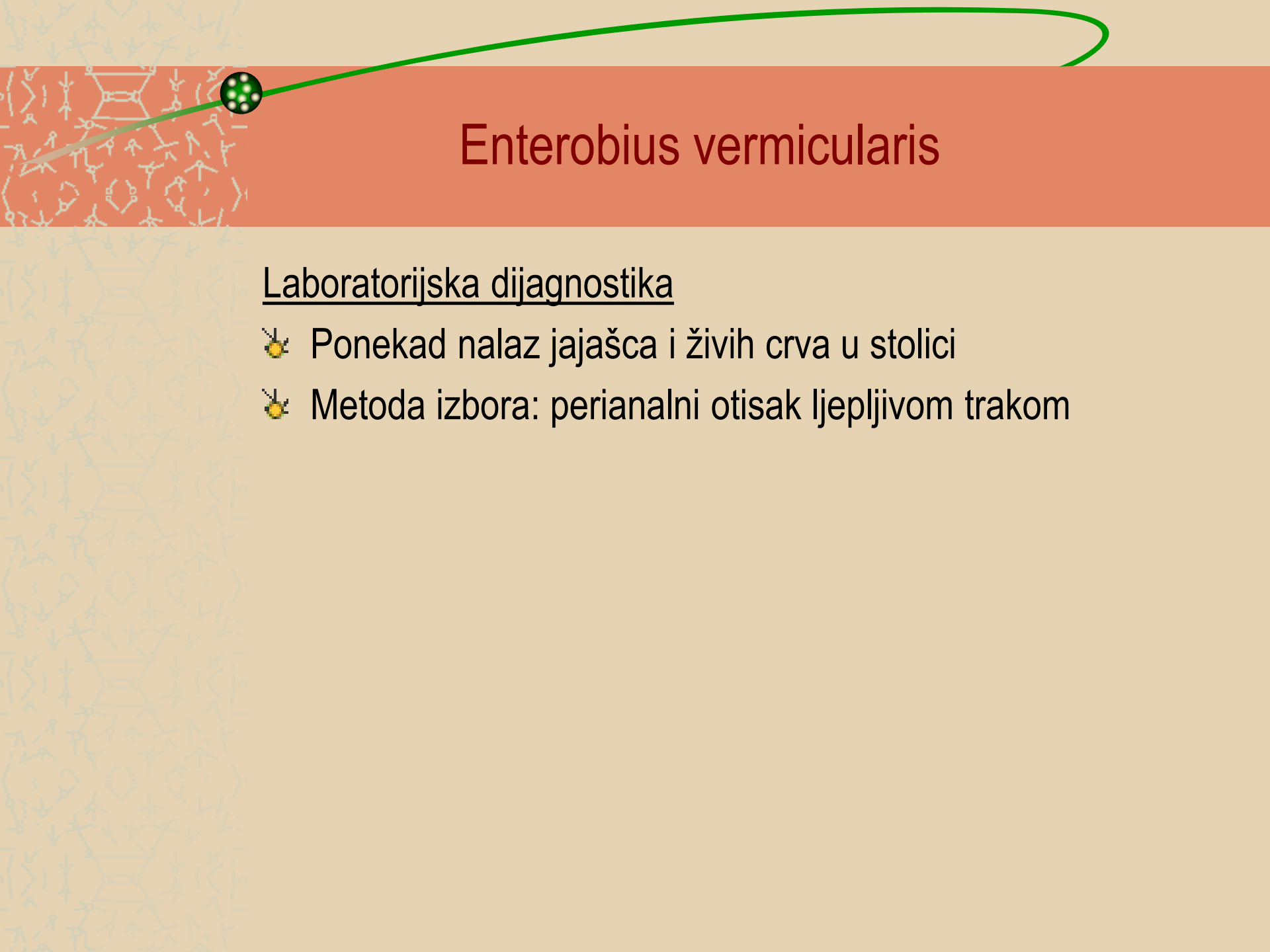




Enterobius vermicularis

Klinička slika

- ✚ Većina infekcija asimptomatska
- ✚ Simptomatska bolest – perianalni svrbež, nesanica, mršavljenje, sekundarna bakterijska infekcija, genitourinarni problemi i granulomi (migracija parazita u vaginu)
- ✚ U rijetkim slučajevima penetracija u peritonealnu šupljinu, jetru, pluća



Enterobius vermicularis

Laboratorijska dijagnostika

- ✎ Ponekad nalaz jajašca i živih crva u stolici
- ✎ Metoda izbora: perianalni otisak ljepljivom trakom

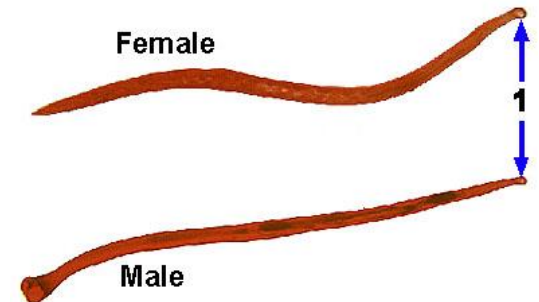
Ancylostoma duodenale

- ✦ Rudarska glista
- ✦ WHO 2018.god. – 900 milijuna ljudi inficirano
- ✦ Uz malariju najozbiljniji medicinsko - socijalni problem tropskih i subtropskih zemalja
- ✦ Ankilostomoza – Ebersov papirus (1600 g.p.n.e)



Ancylostoma duodenale

- ✶ Parazit duodenuma i gornjeg dijela tankog crijeva
- ✶ Adult: mužjaci 8-11 mm, ženke 10-13 mm
- ✶ Sivkaste boje
- ✶ Razjapljen usni otvor (krivousta glista)
- ✶ U usnom otvoru čvrste kukice za prihvaćanje uz sluznicu tankog crijeva
- ✶ Mužjak - karakteristično građen kopulacijski aparat u obliku krilca

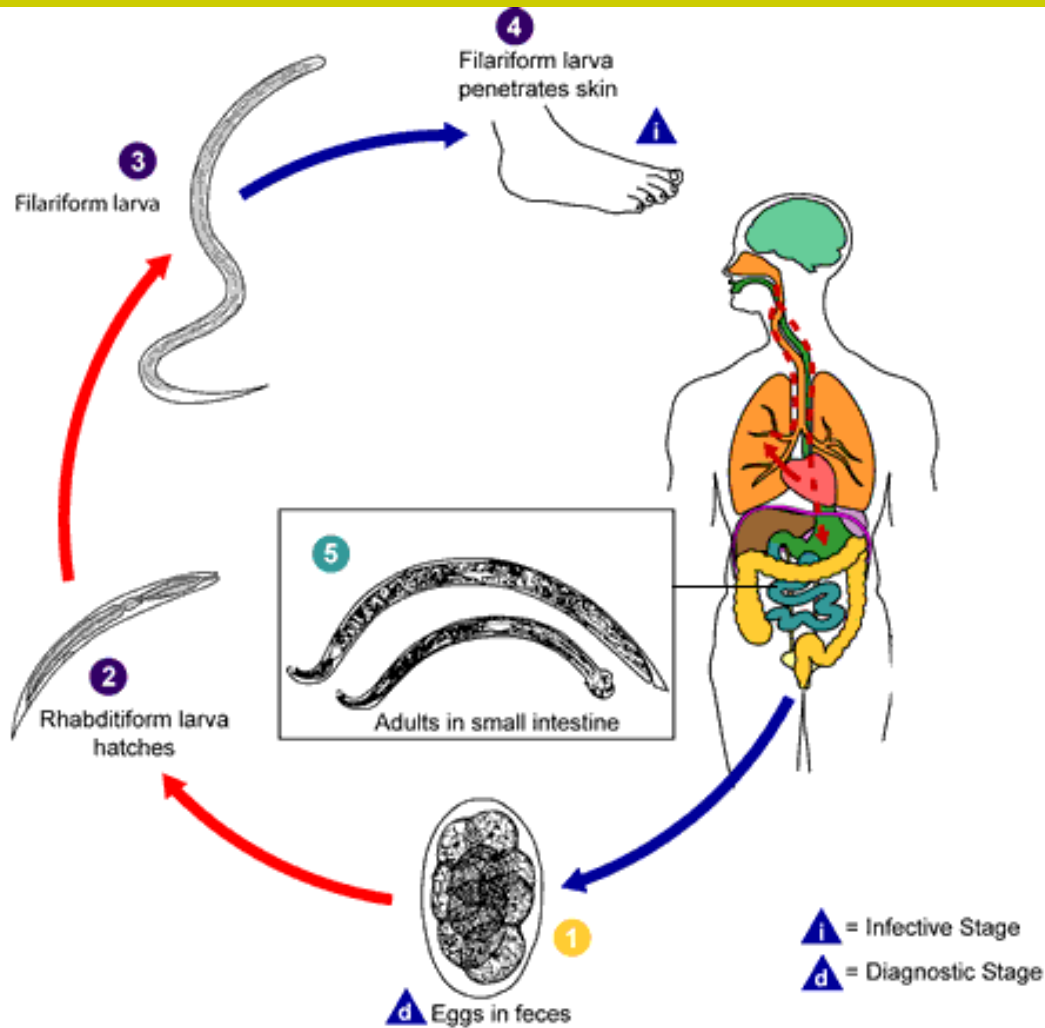


Ancylostoma duodenale

- ✚ Oplođene ženke nesu jajašca (- 20 000 na dan)
- ✚ Jajašca – 60x40 μm , tanka prozirna ljuska, 4 zametne stanice
- ✚ Vlažna i topla zemlja – rabditoidne ličinke – filariformne ličinke (infektivne)



Ancylostoma duodenale



Ancylostoma duodenale

Klinička slika

- ✚ Penetracija ličinki kroz kožu – alergijska reakcija i osip
- ✚ Migracija – pneumonitis
- ✚ Adulti mučnina, povraćanje, proljev, mršavljenje, hipokromna anemija, edemi (hipoproteinski)

Ancylostoma duodenale

Laboratorijska dijagnostika

- ✚ Uzorak: stolica
- ✚ Nalaz jajašca (nativni preparat, MIFC)
- ✚ Koprokultura
- ✚ Serologija – nije rutinska

Strongyloides stercoralis

- Uzročnik strongiloidoze
- Parazit čovjeka, majmuna i pasa
- Infekcija najčešće u vlažnim, toplim krajevima, rjeđe u umjerenj klimatskoj zoni
- WHO – 2018. 100 mil. inficiranih
- Opisan 1876. u stolici i sluznici tankog crijeva od proljeva umrlih francuskih vojnika u Indoneziji

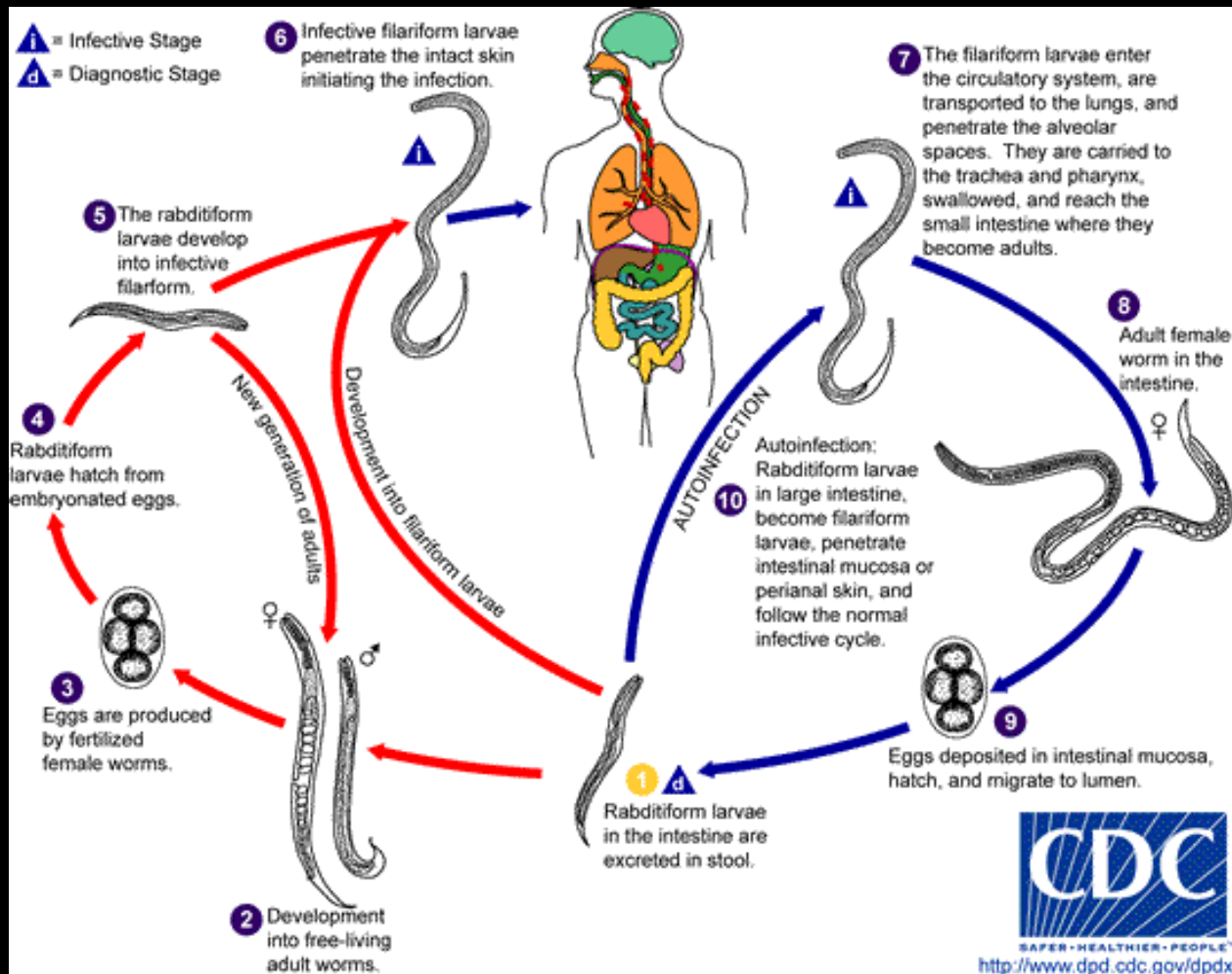
Strongyloides stercoralis

Morfologija i biologija

- U pravilu ima jednu slobodnu i jednu parazitsku generaciju
- Ženke veličine 1 mm, mužjaci 0,7 mm



Strongyloides stercoralis



Strongyloides stercoralis

Klinička slika

- ☀ Kožne lezije – jak svrbež, crvenilo kože
- ☀ Pluća – pneumonitis (eozinofilija)
- ☀ Tanko crijevo – bez simptoma
- ☀ Simptomi : povraćanje, proljev, bolovi, malapsorpcija

Strongyloides stercoralis

- ☼ Sistemska infekcija – kod imunosuprimiranih pacijenata (AIDS, bolesnici s malignim bolestima, na kortikosteroidnoj terapiji, transplantirani)
- ☼ Diseminacija ličinki u virtualno svaki organ (pluća, jetra, slezena, bubreg, pankreas, srce, mozak)
- ☼ Mortalitet 90 %

Strongyloides stercoralis

Laboratorijska dijagnostika

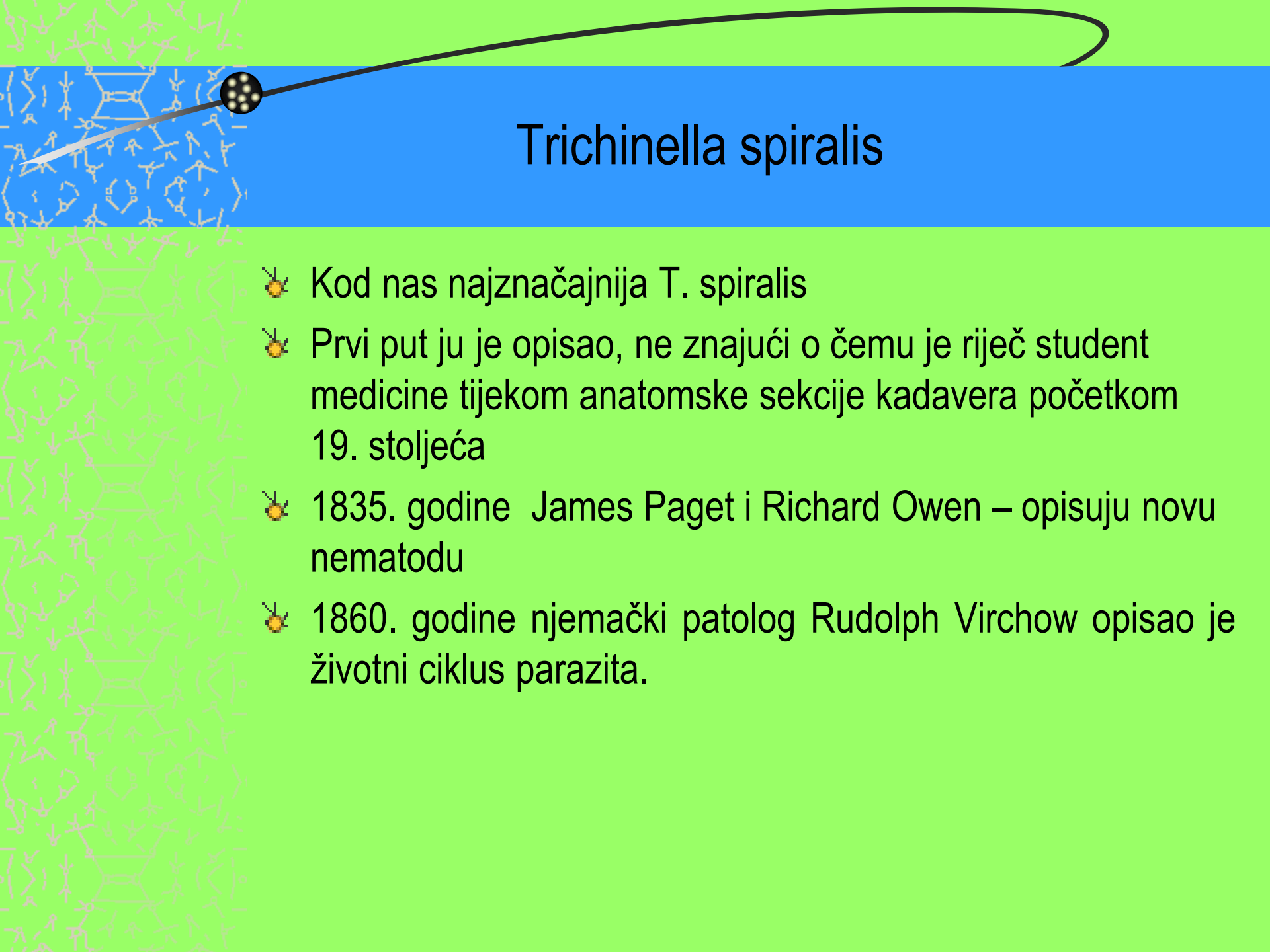
- Uzorak: stolica, duodenalni aspirat, sputum
- Stolica: nativni preparat, MIFC, koprokultura
- Nalaz ličinki rabditoidnog tipa
- Jajašca se ne traže
- Obraditi najmanje 3 uzorka stolice
- Serologija: EIA



Trichinella



- ✎ Uzročnik trihineloze
- ✎ Poznato više vrsta
- ✎ **T. spiralis**
- ✎ T. britovi
- ✎ T. murelli
- ✎ T. nativa
- ✎ T. nelsoni
- ✎ T. pseudospiralis
- ✎ T. papuae
- ✎ T. zimbabwensis

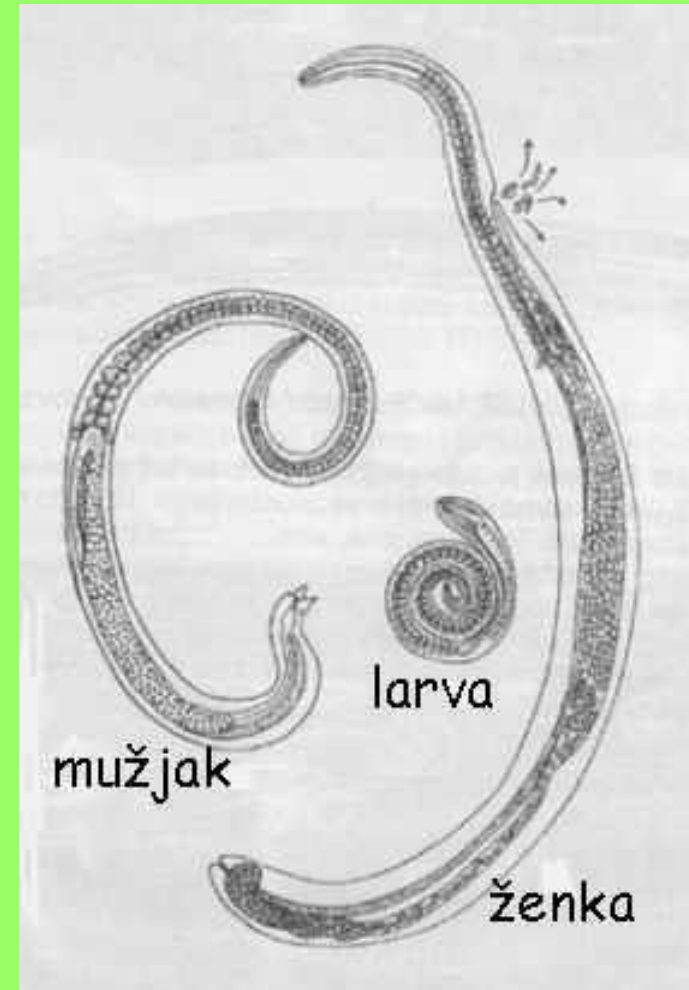


Trichinella spiralis

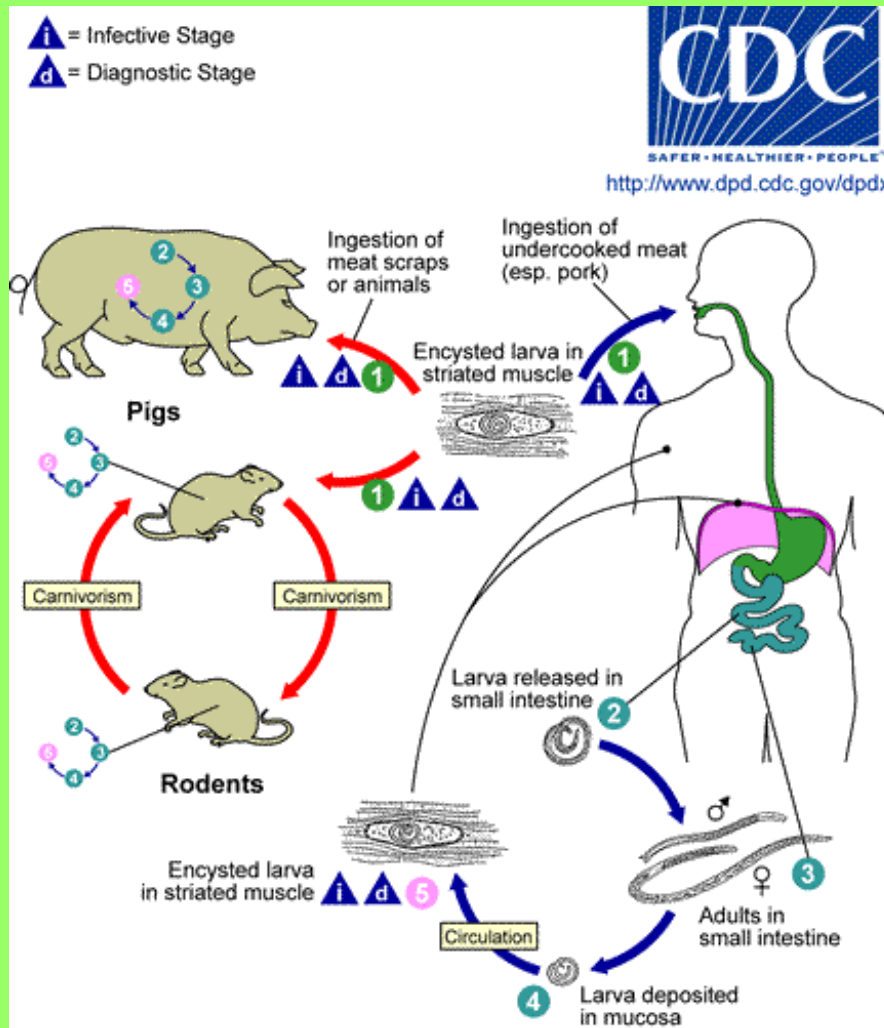
- ✚ Kod nas najznačajnija *T. spiralis*
- ✚ Prvi put ju je opisao, ne znajući o čemu je riječ student medicine tijekom anatomske sekcije kadavera početkom 19. stoljeća
- ✚ 1835. godine James Paget i Richard Owen – opisuju novu nematodu
- ✚ 1860. godine njemački patolog Rudolph Virchow opisao je životni ciklus parazita.

Trichinella spiralis

- ✎ Parazit mesoždera i sveždera (divljih i domaćih životinja, čovjeka)
- ✎ Odrasli paraziti kratkotrajni su stanovnici tankog crijeva
- ✎ Tanki poput konca, mužjaci veličine 1-2, ženke 2-4 mm



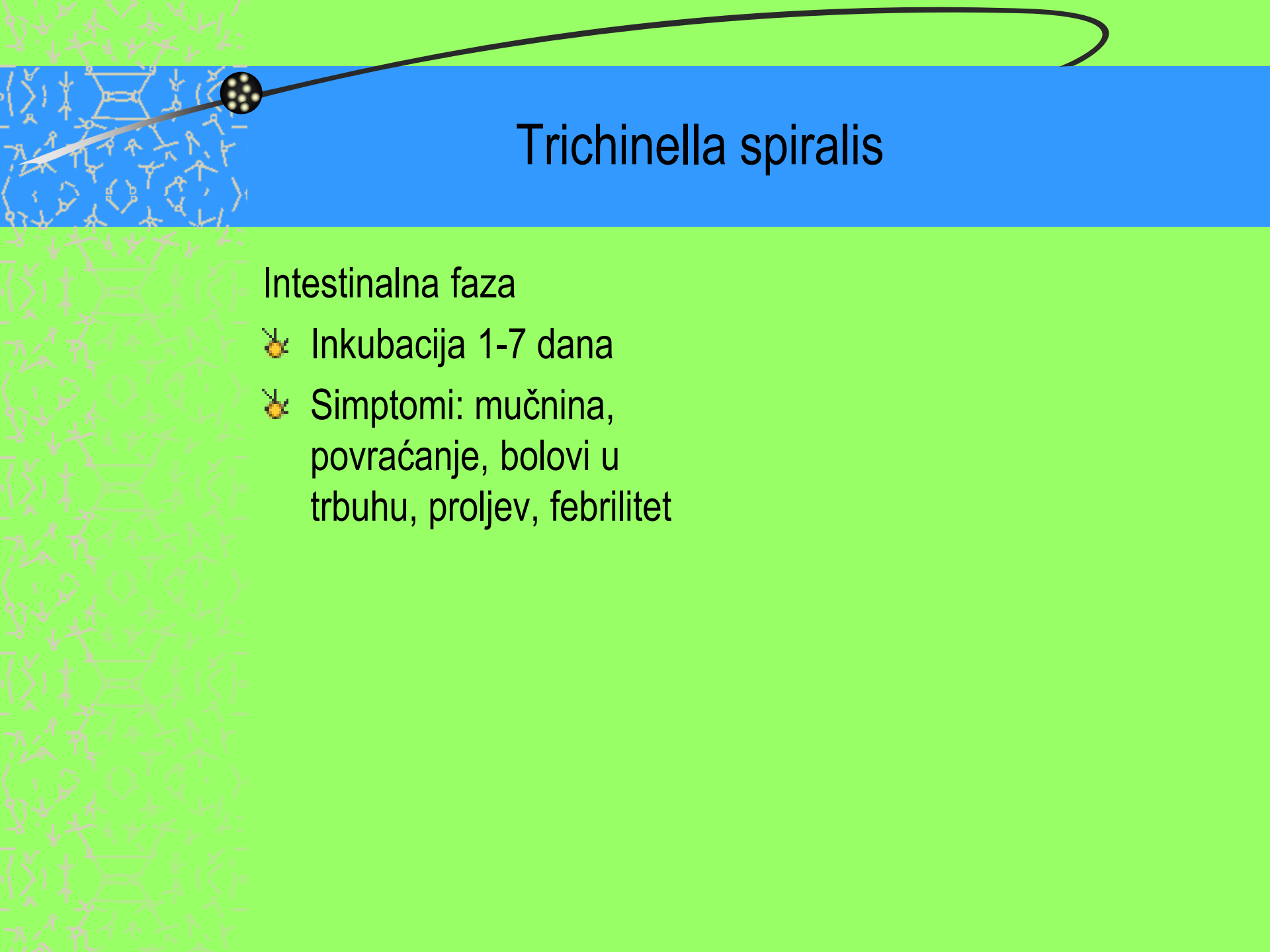
Trichinella spiralis



Trichinella spiralis

Klinička slika

- 🦋 Kliničke manifestacije ovise o infektivnoj dozi (50-70 ličinki *T. spiralis* može uzrokovati klinički manifestnu bolest u ljudi)
- 🦋 *T. spiralis* najpatogenija
- 🦋 Infekcija bifazični tijek:
 - Intestinalna faza
 - Ekstraintestinalna faza



Trichinella spiralis

Intestinalna faza

- ✎ Inkubacija 1-7 dana
- ✎ Simptomi: mučnina, povraćanje, bolovi u trbuhu, proljev, febrilitet



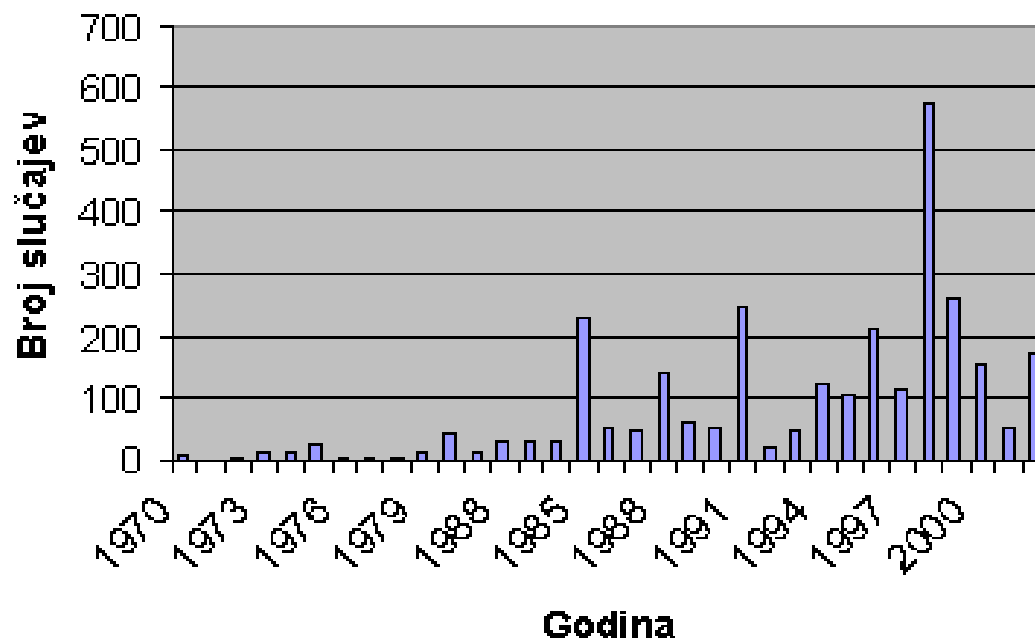
Trichinella spiralis

Ekstraintestinalna faza

- ✂ Inkubacija 7- dana
- ✂ Simptomi od invazije ličinki u tkivo oboljelog
- ✂ Miozitis (bolovi, ukočenost, otežano disanje i gutanje), febrilitet, edemi kapaka i lica, kožni osip
- ✂ Opasnost: miokarditis, meningoencefalitis
- ✂ Eozinofilija, povišeni LDH, CPK, kreatinurija
- ✂ Traje 1-6 tjedana

Trichinella spiralis

**Trihineloz a u Hrvatskoj od 1970-2002. godine
(podaci HZJZ)**





Trichinella spiralis

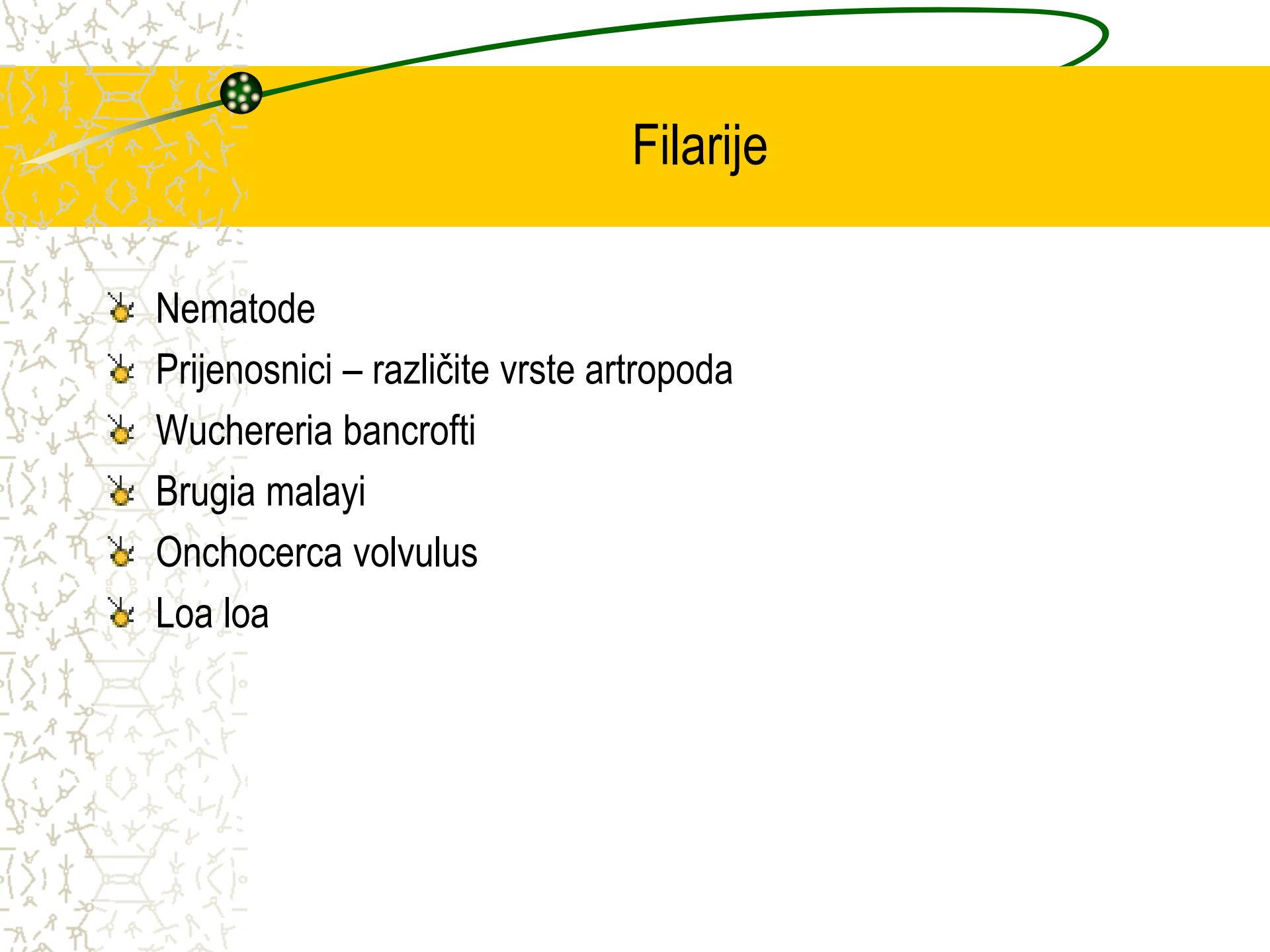
Laboratorijska dijagnostika

Direktna dijagnostika:

- ✎ Trihinela se rijetko može naći u stolici i duodenalnom soku
- ✎ Nalaz ličinki u bioptatu mišića (mikroskopski, PCR), umjetna digestija inkriminiranog mesa

Indirektna dijagnostika:

- ✎ Serologija (ELISA, ITFA, WB)



Filarije

- ✿ Nematode
- ✿ Prijenosnici – različite vrste artropoda
- ✿ *Wuchereria bancrofti*
- ✿ *Brugia malayi*
- ✿ *Onchocerca volvulus*
- ✿ *Loa loa*

Filarije

- ☛ Kompleksni životni ciklusi
- ☛ Adultne ženke legu mikrofilarije
- ☛ Artropod – razvoj do infektivnog oblika
- ☛ Paraziti limfnog sustava, potkožnog tkiva, elefantijaza, sljepoća
- ☛ Periodicitet – nokturnalni (W. Bancrofti, Brugia), diurnalni (Loa loa)
- ☛ Vermiformne, neke vrste imaju ovojnicu
- ☛ Krvni razmaz, gusta kap, serologija

Filarije

- ✱ *Wuchereria bancrofti*
- ✱ Adulti – limfno tkivo
- ✱ Mikrofilarija – veličina 230-320x10 mikrona, repić zašiljen nema jezgara, ima ovojnicu
- ✱ Limfangitis, limfadenopatija, elefantijaza
- ✱ Dijagnostika –periodicitet 10 pm-2am
- ✱ Krvni razmaz, gusta kap, filtracija, metode koncentracije, ELISA



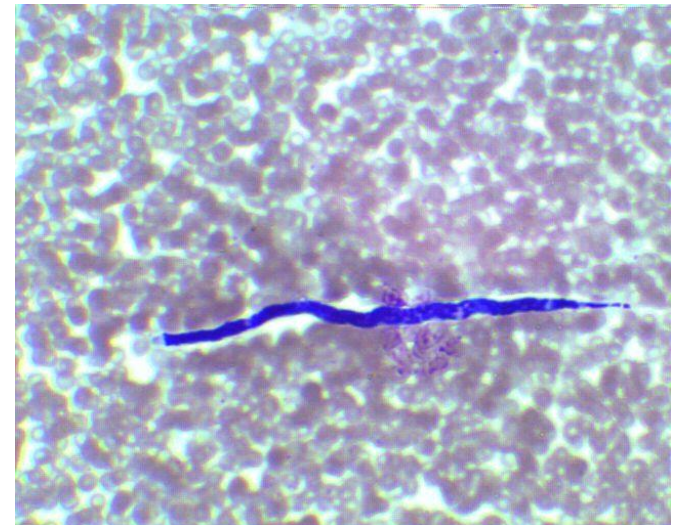
Filarije

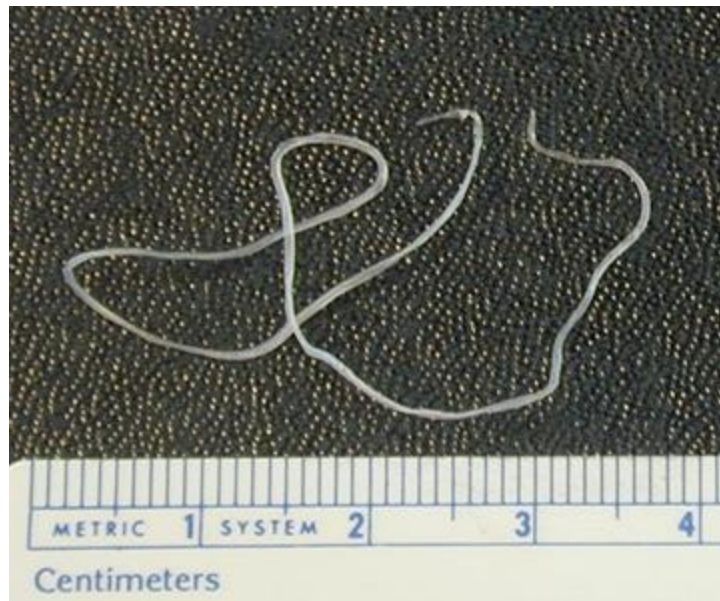
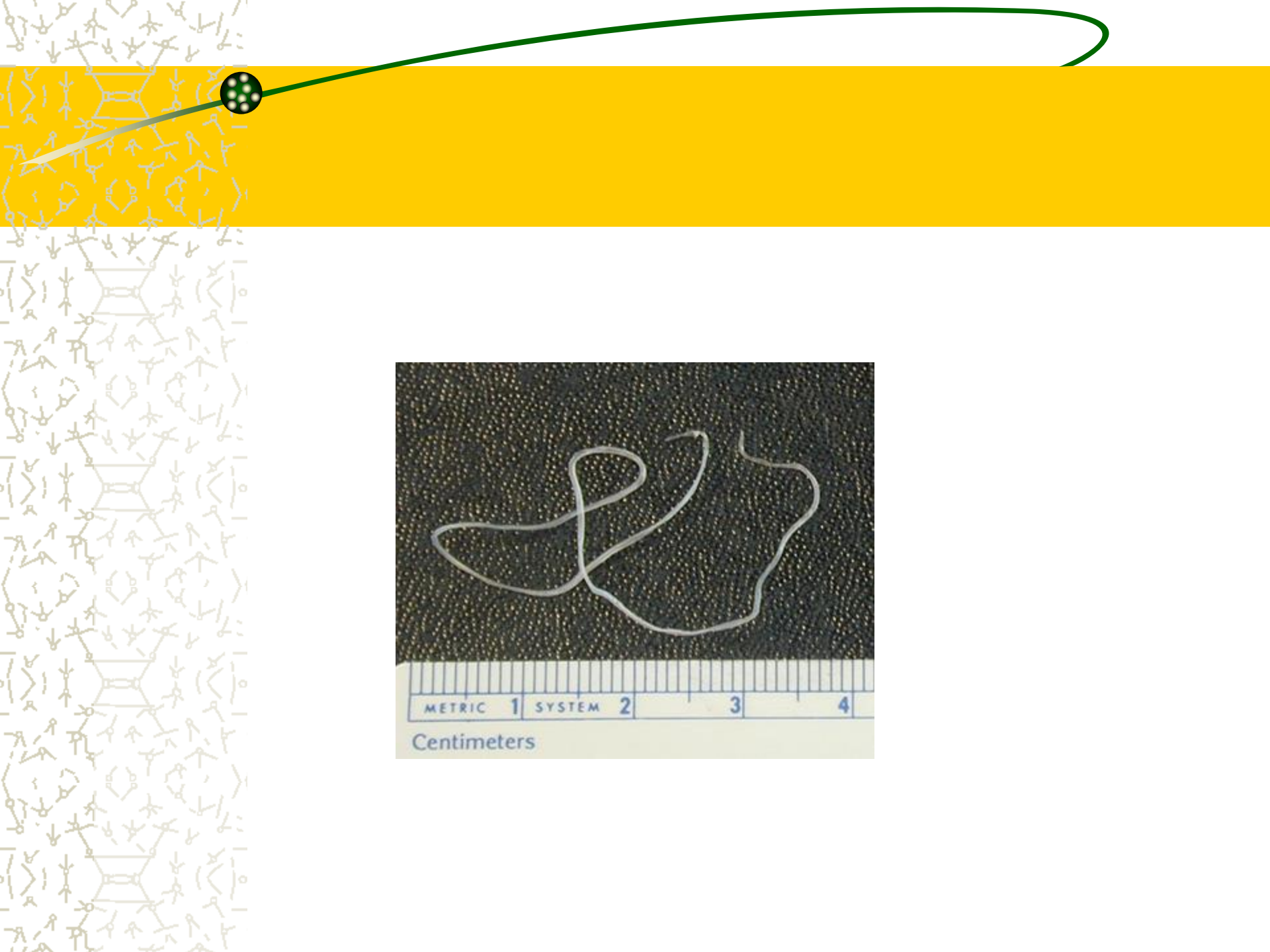
- ☛ *Brugia malayi*
- ☛ Adulti limfno tkivo
- ☛ Mikrofilarija – veličina 170-260 x 5-6 mikrona, u repiću dvije jezgre, ovojnica
- ☛ Dijagnostika –periodicitet 10 pm-2am
- ☛ Krvni razmaz, gusta kap, filtracija, metode koncentracije, ELISA



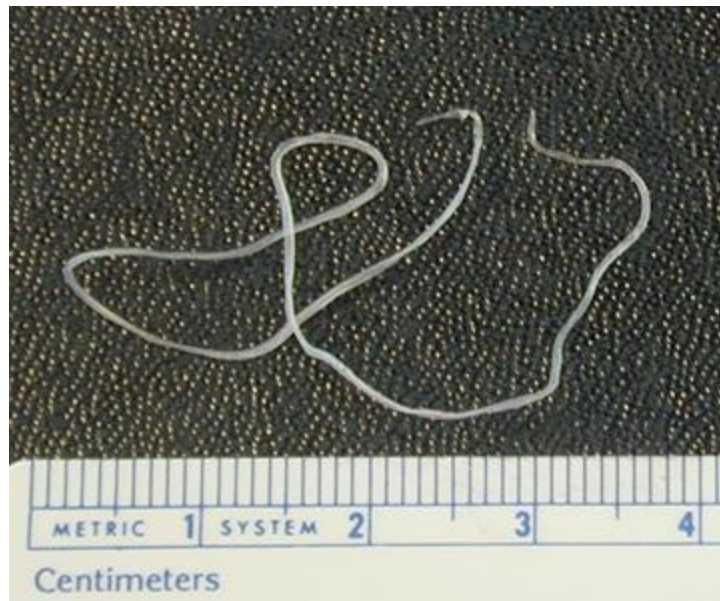
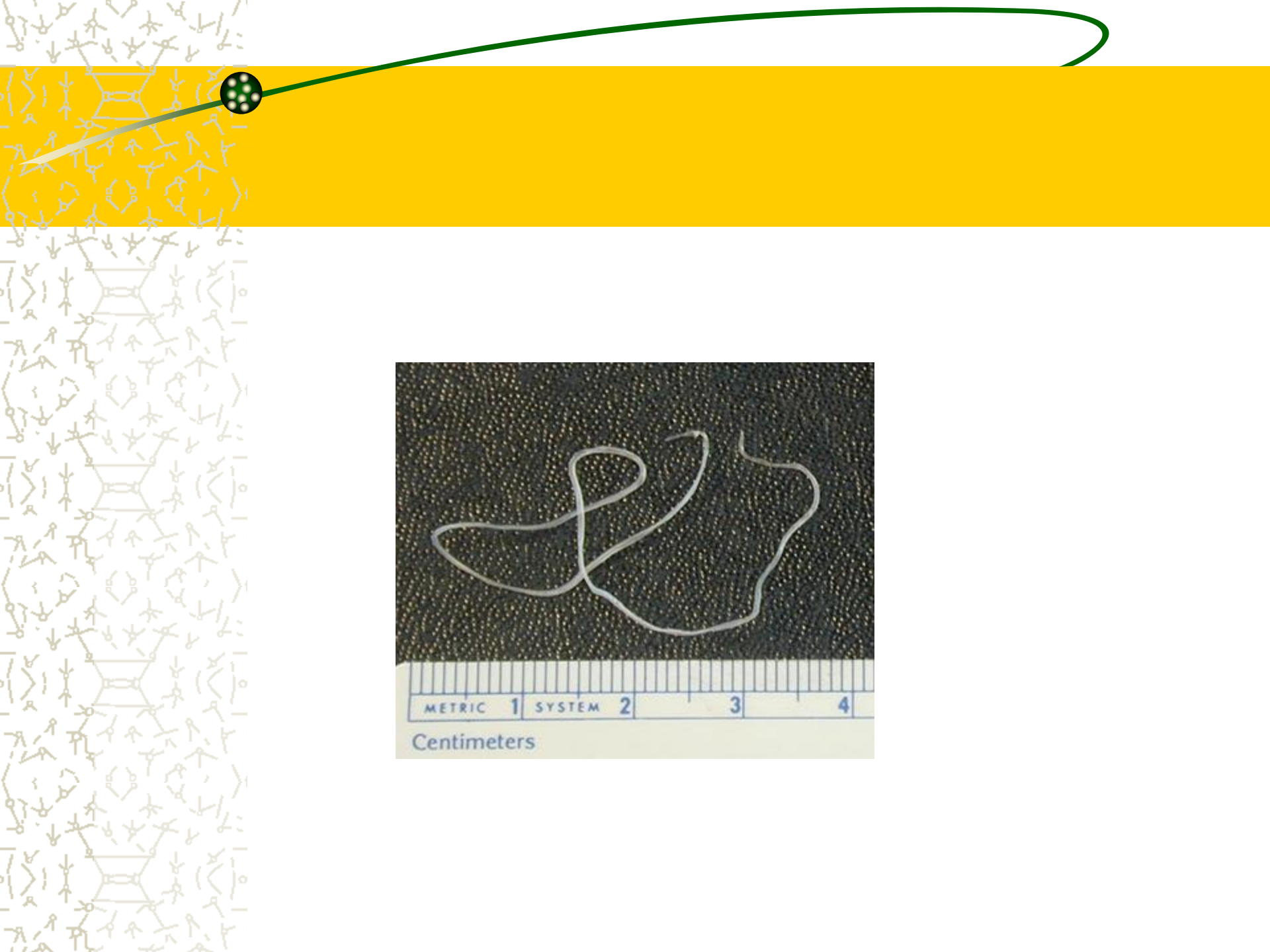
Filarije

- ☼ Loa loa
- ☼ Adulti – pokožno tkivo
- ☼ Subkutani nodusi, ispod konjunktive
- ☼ Diurnalni periodicitet
- ☼ Mikrofilarija – veličina 250-300 x 6-8,5 mikrona, u repiću jezgre, ima ovojnicu
- ☼ Krvni razmaz, gusta kap, membranska filtracija, metode koncentracije, serologija

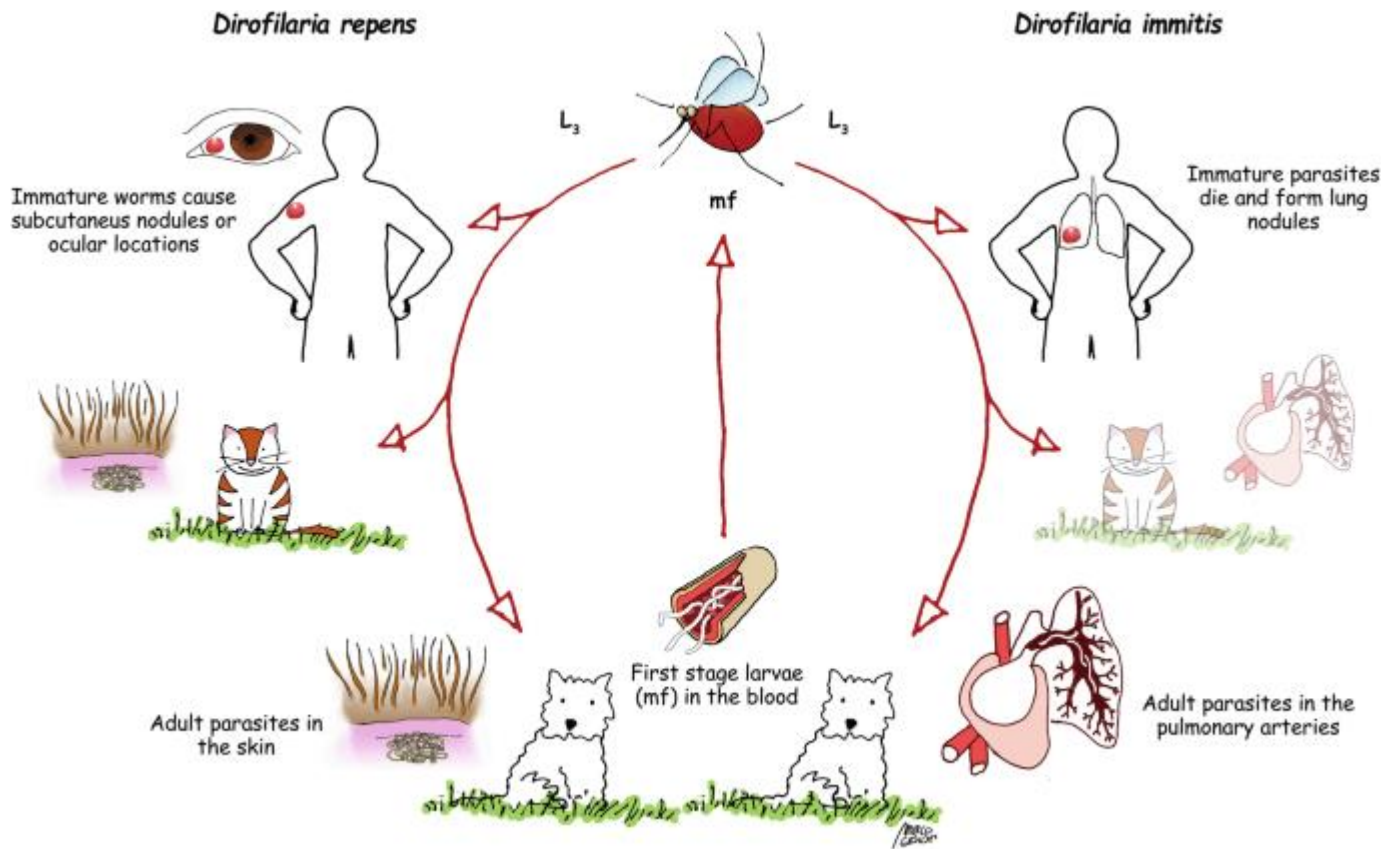












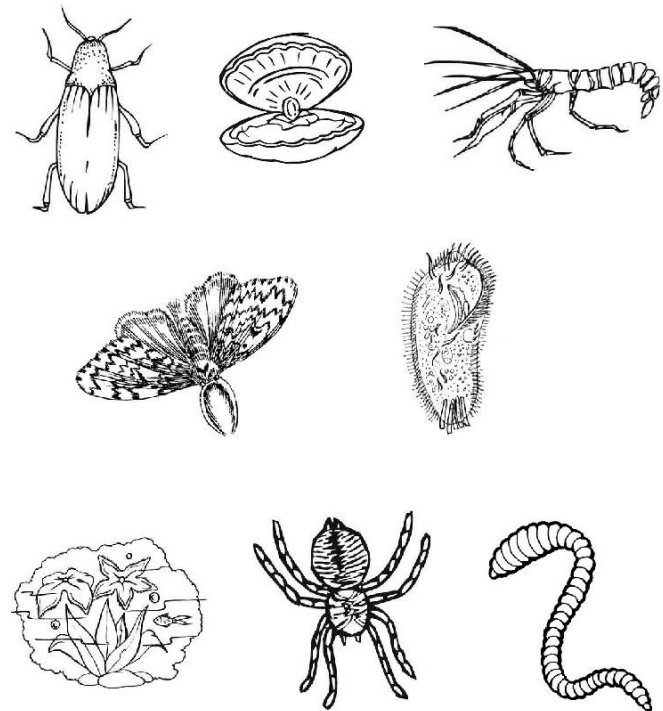


Artropodi

Artropodi

- Najbogatije koljeno u životinjskom carstvu
- Izvanredno prilagođeni uvjetima okoline, napučili vode, kopno i zrak
- Mogu nanjeti veliku štetu gospodarstvu i čovjekovom zdravlju
- Medicinski značaj – često su prenosioci različitih uzročnika bolesti

Arthropods



Artropodi

- Višestanični organizmi
- Građa :metamerija – ponavljanje jednakih segmenata (članaka)
- Pojedini segmenti – diferencijacija i stapanje u anatomske cjeline – nastali glava, prsiše i zadak
- Na tim segmentima se nalaze lokomotorni organi – noge, krila



Artropodi

- Odvojeni spolovi
- Jaje – metamorfoza
 - razvojni oblici (ličinke ili larve) - adulti



Artropodi

Značaj za medicinu

- Direktan – upalne promjene, alergija, toksično, mijaze
- Indirektno – biološki vektori uzročnika zaraznih bolesti



Artropodi

- Suzbijanje artropoda
- Insekticidi
- Larvicidi, adulticidi



Artropodi

Koljeno – 13 razreda

- Za čovjeka značajna 4:
- Eucrustacea – rakovi
- Diplopoda i Chilopoda – stonoge
- Arachnida – paučnjaci
- Insecta - kukci



Ušljivost

- Koljeno člankonošci, razred kukci ili insekti
- Životinjske vrste – 70%
- Uši – sitni kukci prilagođeni parazitizmu na krznu sisavaca
- Odvojeni spolovi
- Krila potpuno zakržljala
- Snažne nožice s jakim pandžama za veranje među dlakama
- Hrane se krvlju
- Trajno ostaju na nosiocu za koga su strogo specifični
- Adulti ne mogu preživjeti duže od 48 sati bez svog nosioca

Ušljivost

- Za čovjeka specifične dvije vrste
- *Pediculus humanus* – dvije odlike *P. humanus* var. *capitis* i *P. humanus* var. *corporis*
- *Pediculus* (*Phthirius*) *pubis*



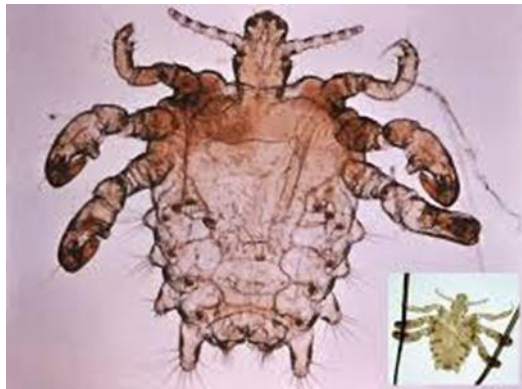
Table 1. Bacterial infections transmitted by body lice

Disease name	Epidemic typhus, "jail fever"	Relapsing fever ("yellow fever")	Trench fever, quintan fever
Species of pathogen	<i>Rickettsia prowazekii</i>	<i>Borrelia recurrentis</i>	<i>Bartonella quintana</i>
Infection in lice	Gut, intestinal, generalized	Hemolymph	Gut, intestinal
Human contamination	Louse feces	Louse hemolymph	Louse feces
Disease in human relapse	Yes, one single late (Brill-Zinsser)	Yes, usually one, for ~2 days	Yes, usually several (quintan fever)
Chronic carriage	Yes	No	Yes
Reservoir	Human (+ sylvatic)	Human	Human
Fatality rate (without treatment)	30%	10%—40%	<1%
Treatment	Doxycycline, 200 mg once	Tetracycline	Doxycycline and gentamicin

Modified from Raoult and Roux (CID 29: 888-911, 1999)

Pediculus (Phthirius) pubis

- Adulti 1-2 mm, smeđa
- Prijenos uglavnom spolnim putem
- Primarno živi na dlakama pubične regije



Pediculus (Phthirius) pubis

- Adulti 1-2 mm, smeđa
- Prijenos uglavnom spolnim putem
- Primarno živi na dlakama pubične regije
- Rjeđe – pazuha, obrve i trepavice, brkovi, brada, abdomen i drugi dlakom pokriveni dijelovi tijela
- Životni ciklus jaje- adult 17-25 dana



Pediculus (Phthirius) pubis



- Papillon d'amour
- Svrbež zahvaćene regije, najintenzivniji u pubičnom dijelu tijela
- Ekskorijacije i male (0.5-1cm) plavkaste makule, ingvinalna limfadenopatija
- Smeđe crveni izmet – uz bazu dlake, ekzorijacije ili na donjem rublju



Pediculus (Phthirius) pubis

Dijagnostika

- Nalaz uši ili gnjida na dlakama
- Korištenje povećala
- Woodova svjetiljka – fluorescenca vijabilnih gnjida



Pediculus (Phthirius) pubis

- Malathion 0.5%
- Permethrin 1%
- Phenothrin 0.2%
- Carbaryl 0.5-1%
- Lindan 1%
- Druga aplikacija nakon 7-10 dana
- Ivermectin tablete -1 tableta, druga nakon 10 dana
- Obrve,trepavice-vazelinska mast
- Liječenje spolnih partnera
- Mrtve grinje ostaju pričvršćene za dlaku – uklanjanje češljanjem
- Rezistencija, trovanje organofosfornim insektidicidima

Svrab

- Infestacija grinjom *Sarcoptes scabiei* var. *hominis*
- Životinjski svrab
 - svrbež, upala, gubitak dlaka
 - mačke, psi, svinje, konji
 - fakultativni ektoparaziti kod ljudi
 - samo ograničavajuća infekcija
 - nema kompletnog životnog ciklusa
 - čovjek „dead end” domaćin
 - terapija se preporuča



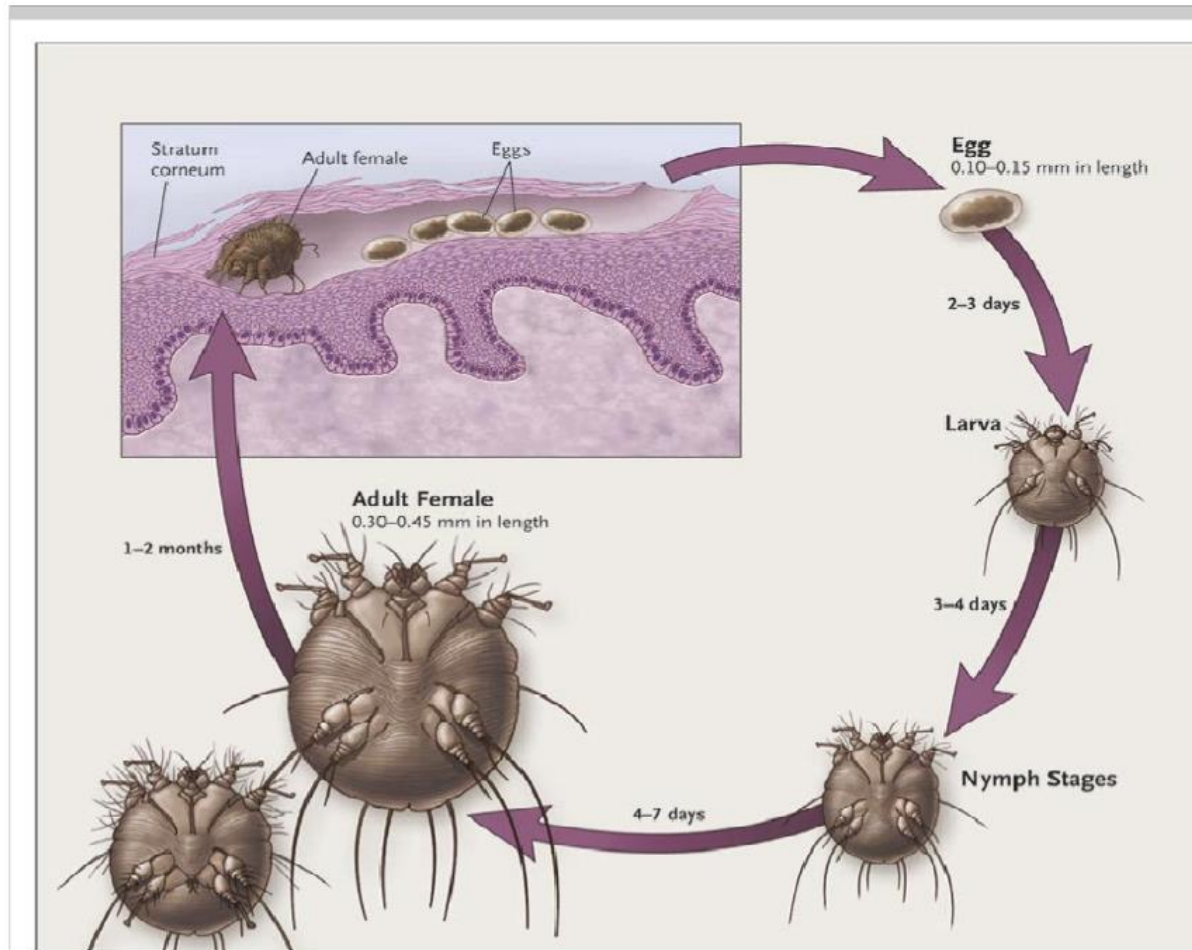
Svrab

- Razred paučnjaci, red Acarina ili grinje
- Parazit površnih slojeva kože sisavaca
- Sitni (300-350 microm., okruglasta oblika, 4 para nogu)



Svrab

- Čeljustima kopa rov u epidermisu i hrani se keratinom
- Ženka živi 1-2 mjeseca dana i za to vrijeme polaže dnevno 3-4 jaja
- Jaje – ličinka – adult -11-22 dana
- Jače aktivan tijekom noći



Life Cycle of Scabies (SARCOPTES SCABIEI)

Svrab

- Epidemiologija
- Učestalost svijet – 300 mil. ljudi
- Češće -beskućnici, pacijenti u institucijama za ljude sa mentalnim poteškoćama, zatvorenici
- Prijenos – blizak dodir kože
- Moguć posredno – grinja do 7 dana na životu
- Širom svijeta, sve dobi, sve socioekonomske grupe
- Hiperendemski proširen u Subsaharskoj Africi, Indiji, kod Aboriđina



VJESTI ► HRVATSKA SVIJET CRNA KRONIKA KULTURA NOVAC ZNANOST

HRVATSKA

SPRIJEČITE EPIDEMIJU

SVE VIŠE SLUČAJEVA SVRABA U HRVATSKOJ: Ako uočite ove simptome, odmah se javite liječniku



11:49 29.11.2015

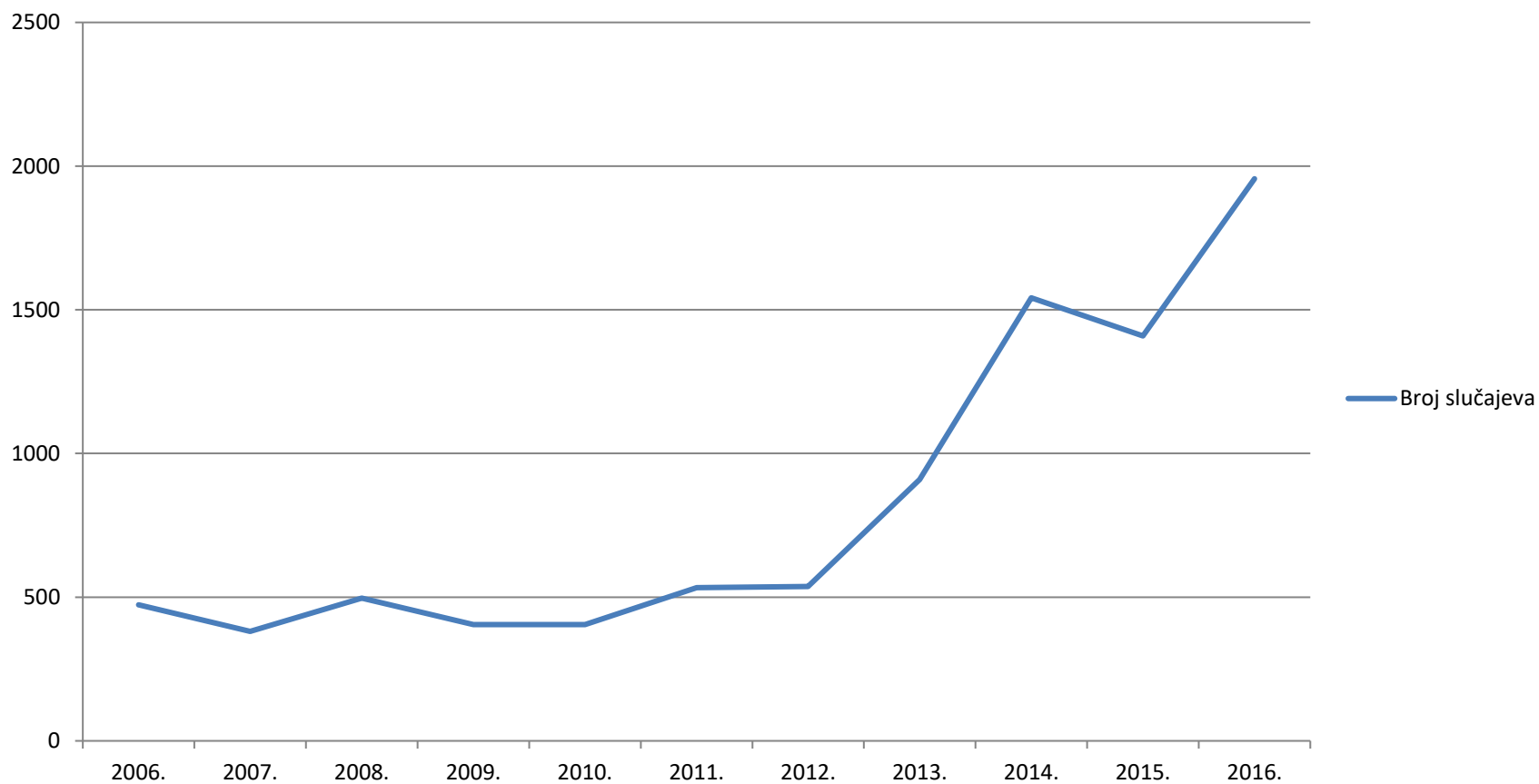
Autori: Danas.hr

Foto: Tharakorn



Poveći broj slučajeva svraba zabilježen je ove godine u odnosu na dvije godine prije. Osobe koje uoče simptome odmah bi se trebale javiti liječniku kako bi se spriječilo širenje bolesti i pojava epidemije.

Svrab u Hrvatskoj



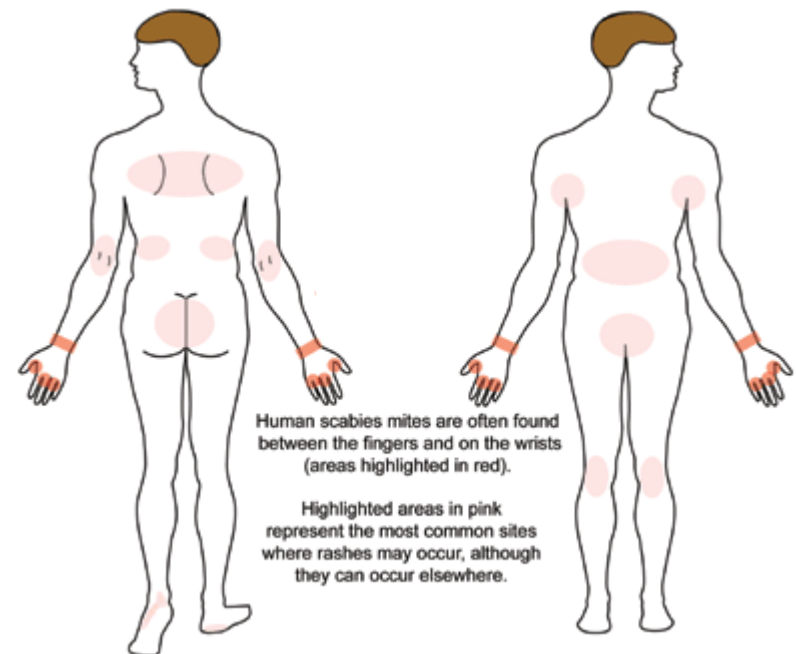
Svrab

- Kliničke manifestacije
- Simptomi – primarna infekcija – 2-6 tjedana nakon infekcije
- Ponovna infekcija – nakon 1-4 dana



Svrab

- Kliničke manifestacije
- Tipični simptomi
- Generalizirani, intenzivni noćni svrbež – interdigitalni prostor, poplitealna fosa, fleksorne strane zapešća, umbilikalno područje, genitalije, unutarnja strana bedara, dojke kod žena



Svrab



- U umjerenoj klimatskoj zoni – lice i vlasište pošteđeno osim kod djece i starijih ljudi
- Sekundarna infekcija
- Koža – polimorfne promjene -2-3 mm papule i vezikule, ekzorijacije, kruste, kanalići, ponekad bule i urtike, indurirani upalni čvorovi – plastični infiltrati

Svrab

- Kliničke manifestacije
- Norveški svrab –
krustozni – iznimno
kontagiozan oblik
- Na koži tisuće grinja
- Svrbež odsutan ili
minimalan
- Obično zahvaća i
vlasište
- Imunodeficijencija,
malnutricija, visoka
životna dob,
neurološka bolest



Svrab



- Dijagnostika
- Dermatoskopija
- Mikroskopija
- Biopsija kože



Svrab

- Terapija

Aktivna komponenta	Doziranje	Sigurnosni profil	Kontraindikacije
5% permethrin (krema)	Aplikacija od vrata na niže; ispiranje 8-14 sati nakon; rezidualna aktivnost, druga aplikacija nakon tjedan dana	Odličan; bockanje i svrbež kod aplikacije	Poznata alergija na lijek; novorođenčad mlađa od 2 mjeseca, dojenje
1% lindan (losion ili krema)	Aplikacija od vrata na niže; ispiranje 8-14 sati nakon; nema rezidualne aktivnosti, ponoviti nakon tjedan dana	Organofosforni insekticid, CNS toksičnost	Epilepsija, novorođenčad mlađa od 6 mjeseci, trudnoća, dojenje
10 krotamiton (krema ili losion)	Aplikacija od vrata na niže tijekom dva dana, ispiranje 24 sata nakon druge aplikacije	Odličan, ne pretjerano efikasan	Nema
2-10% sumpor u vazelinu premaz	Aplikacija 2-3 dana , ispiranje	Odličan, ne pretjerano efikasan	Alergija na sumpor
10-25% benzoil benzoat (losion)	Dvije aplikacije, sa jedno dnevnim i jedno tjednim razmakom	Iritans	Ekzem
0.5 % malathion (losion)	95% ovidan, brzo ubijanje – 5 min., otporost	Zapaljiv, organofosforni insekticid	Novorođenčad mlađa od 6 mjeseci, trudnoća, dojenje
Ivermektin	200 microgr/kg jedna PO doza, ponavljanje za 14 dana	Odličan, mučnina, povraćanje	Sigurnost u trudnoći nepoznata, ne preporuča se djeci lakšoj od 15 kg

Format: Abstract ▾

Send to ▾

Indian J Dermatol Venereol Leprol. 2012 Sep-Oct;78(5):605-10. doi: 10.4103/0378-6323.100571.

Comparative efficacy and safety of topical permethrin, topical ivermectin, and oral ivermectin in patients of uncomplicated scabies.

Chhaiva SB¹, Patel VJ, Dave JN, Mehta DS, Shah HA.

Author information

Abstract

BACKGROUND: Ivermectin has opened a new era in the management of scabies as orally effective drug. However, topical route has been little explored for ivermectin.

AIMS: To compare the efficacy and safety of topical permethrin, oral ivermectin, and topical ivermectin in the treatment of uncomplicated scabies.

METHODS: This was an open-label, randomized, comparative, parallel clinical trial conducted in 315 patients, randomly allocated to 3 groups. First group received permethrin 5% cream as single application, second group received tablet ivermectin 200 mcg/kg as single dose, and third group received ivermectin 1% lotion as single application. All the patients received anti-histaminic for pruritus. The patients were followed up at intervals of 1, 2, 3, and 4 weeks. If there were no signs of cure, the same intervention was repeated at each follow up. Primary efficacy variable was clinical cure of lesions. Statistical analysis was done by chi square test and one way ANOVA test using SPSS version 12.

RESULTS: At the end of first week, cure rate was 74.8% in permethrin group, 30% in oral ivermectin group, and 69.3% in topical ivermectin group ($P < 0.05$). At the end of second week, cure rate was 99% in permethrin group, 63% in oral ivermectin group, and 100% in topical ivermectin group ($P < 0.05$). At the end of third week, 100% cure rate was observed in permethrin and topical ivermectin group while 99% in oral ivermectin group ($P = 0.367$). No serious adverse events were observed.

CONCLUSIONS: Permethrin and topical ivermectin were equally effective against scabies while oral ivermectin was significantly less effective up to 2 weeks. Topical ivermectin can be used as an alternative to permethrin.

PMID: 22960817 DOI: 10.4103/0378-6323.100571

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Future Microbiol. 2008 Feb;3(1):57-66. doi: 10.2217/17460913.3.1.57.**Scabies: molecular perspectives and therapeutic implications in the face of emerging drug resistance.**Mounsey KE¹, Holt DG, McCarthy J, Currie BJ, Walton SF.

Author information

Abstract

Limited effective treatments, coupled with recent observations of emerging drug resistance to oral ivermectin and 5% permethrin, raise concerns regarding the future control of scabies, especially in severe cases and in endemic areas where repeated community treatment programs are in place. There is consequently an urgent need to define molecular mechanisms of drug resistance in scabies mites and to develop and assess alternative therapeutic options, such as tea tree oil, in the event of increasing treatment failure. Molecular studies on scabies mites have, until recently, been restricted; however, recent advances are providing new insights into scabies mite biology and genetic mechanisms underlying drug resistance. These may assist in overcoming many of the current difficulties in monitoring treatment efficacy and allow the development of more sensitive tools for monitoring emerging resistance.

PMID: 18230034 DOI: 10.2217/17460913.3.1.57

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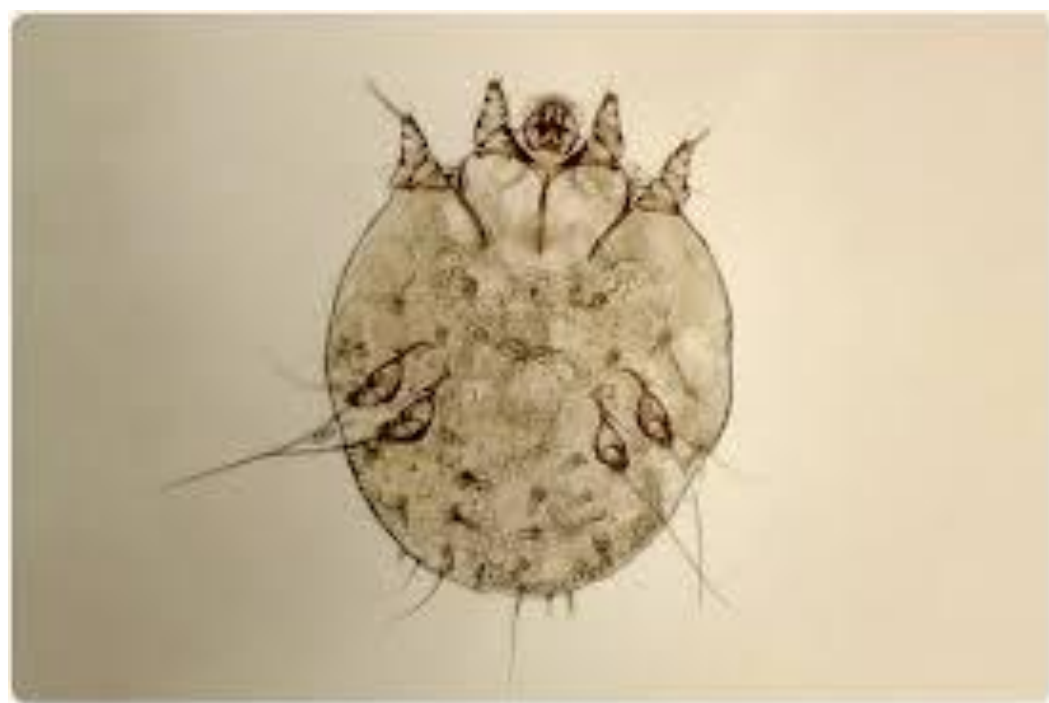
Svrab

- Indikacije za liječenje
- Karakteristični simptomi
- Visoko rizični kontakti – liječenje bez obzira na prisustvo simptoma
- Nisko rizičan kontakt – indirektan kontakt – jedino u slučaju krustoznog svraba
- Uputa izbjegavanje bliskog kontakta sa partnerima do kompletiranja liječenja
- „Traženje” seksualnih partnera unutar 6 tjedana od početka simptoma
- Detaljne informacije o liječenju najbolje na pismo
- Trudnoća/dojenje – permethrin, benzil benzoat, sumporni preparati

Svrab

- Nakon terapije
- Svrbež može perzistirati nekoliko tjedana, osobito kod pacijenata sa atopijom
- Moguća mikroskopija uzorka i traženje grinje u preparatu – ne preporuča se





- *Sarcoptes scabiei* – gotovo okrugla do jajolika, kratkih nogu, zdepasta i kornjačasta. Tijelo zbijeno i prilagođeno kopanju po koži
- Žitna grinja-sitnija, izdžženija, duže noge





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Ostvarite više uz

BD MAX™

sustav za molekularnu dijagnostiku



Performanse

Učinkovitost

Svestranost

BD MAX™ - Molekularna platforma po mjeri Vašeg
laboratorija



Dr. sc. Denis Vadlja, Key Account Manager OEE

Waters™

Advanced Diagnostics

Formerly BD Diagnostic Solutions

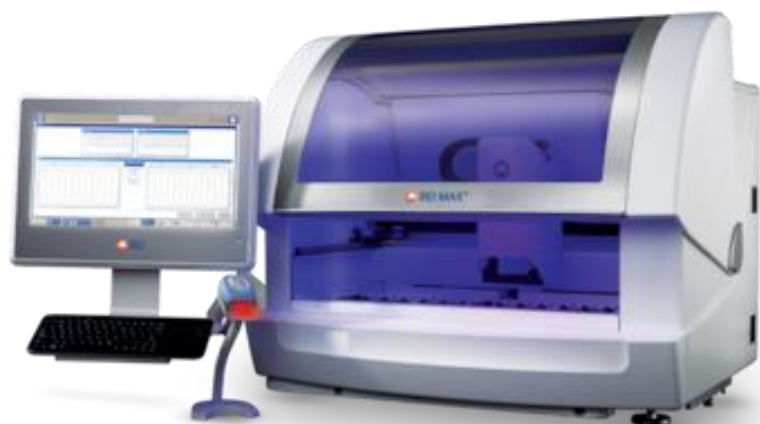
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BD MAX™ sustav za molekularnu dijagnostiku

Waters™



- **Potpuno automatizirana real-time PCR platforma** koja objedinjuje ekstrakciju, amplifikaciju i detekciju, uz širok portfelj validiranih IVD i open-system testova.



Manje od 1,5 min ručnog rada po uzorku



24 rezultata u prosjeku za 2,5 sata



96-120 uzoraka po smjeni (8 sati)

BD MAX™ sustav za molekularnu dijagnostiku

Waters™



12.06.2026. – BD MAX™ presentation

BD is now part of Waters™ Advanced Diagnostics

Sastav BD MAX™ pakiranja testova

Waters™

- Pakiranje sadrži materijal i reagense za **24 testa**.
- **Liofilizirani reagensi** spremni za upotrebu.
- Pohrana na **sobnoj temperaturi**.

- 1 Tubice za uzorke – Sample buffer tubes
- 2 Traka s reagensima – Unitized Reagent Strip
- 3 Ekstrakcijski reagens
- 4 PCR mastermiks (jedan ili dva)
- 5 Probojni čepovi (ovisno o panelu)
- 6 Potrošni materijal za transport uzorka (eze, pipete)



12.06.2026. – BD MAX™ presentation

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BD MAX™ Sample buffer tubes – Tubice za uzorke

Waters™

- **Pierceable Cap dizajn** – nema potrebe za zamjenom čepa tijekom pripreme ili pohrane uzoraka.
- **Samozatvarajući septum** – sigurno rukovanje s uzorcima nakon obrade. **Minimalna evaporacija** uzoraka uz **očuvanje kvalitete** uzoraka i do 5 dana na 2-8 °C.
- Brza, **izravna obrada uzoraka stolice** – **bez predtretmana** za bakterijske i virusne panele.
- **Optimiziran workflow za parazitologiju** – maksimalna pouzdanost i osjetljivost i kod kompleksnih uzoraka.
- Bris, LBC, aspirat, BAL, sputum, urin, stolica – i mnogo više.

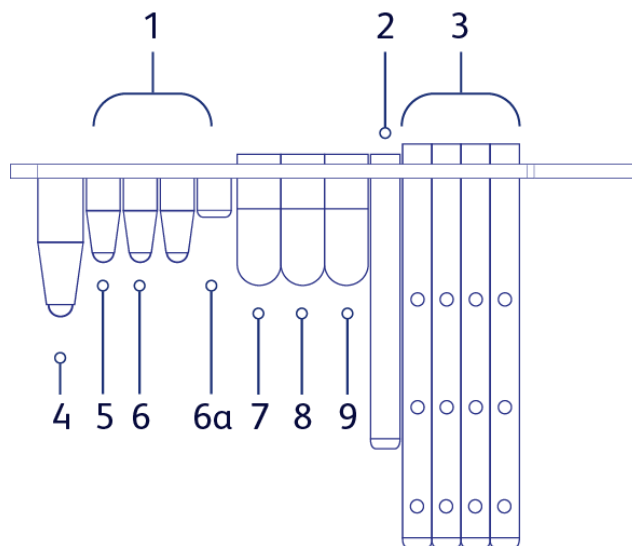


Pojednostavite pripremu uzoraka i upravljanje otpadom

Waters™

Unitized Reagent Strips

- 1 Snap-in Tubes
- 2 Waste Reservoir
- 3 Pipette Tips
- 4 Lysis Tube
- 5 Extraction Tube
- 6 Master Mix 1
- 6a Master Mix 2 if required
- 7 Wash Buffer
- 8 Elution Buffer
- 9 Neutralization Buffer



- Ekstrakcijski i PCR reagensi, zajedno s nastavcima za pipetiranje, integrirani su u **jedinstvenu traku s reagensima (Unitized Reagent Strip)**.
- Svi reagensi su stabilni na **sobnoj temperaturi**.
- Brza priprema uzoraka uz **manje od 1,5 minute praktičnog rada** po uzorku.
- Po završetku analize, sav **otpad** iz procesa ekstrakcije **ostaje unutar trake** i može se zbrinuti u skladu s laboratorijskim standardima.

Provedba analiza po zaprimanju uzoraka u laboratorij, bez čekanja Waters™

- **Kombiniranje testova (Mix-and-Match)** za istovremenu **obradu različitih testova** unutar **istog radnog ciklusa**.
- **Ispreplitanje ciklusa** skraćuje vrijeme do rezultata.
- Od uzorka do rezultata za **2-3 sata**.
- Obrada do **120 uzoraka** unutar **8-satne smjene**.



Ukupno 24 neovisne PCR reakcije na jednoj mikrofluidnoj kartici

Waters™

- **Reakcijske komore se zagrijavaju neovisno jedna o drugoj** – različiti testovi s jedinstvenim temperaturnim profilima mogu se izvoditi zajedno.
- **Bez rizika od kontaminacije** – voštani ventili zatvaraju svaku komoru u kojoj se provodi PCR reakcija.
- **Interna kontrola prati kompletan postupak**, od prikupljanja uzorka, preko ekstrakcije do PCR-a.
- **Apsolutna iskoristivost** reagenasa i potrošnog materijala.



Pojednostavljen, učinkovit i optimiziran workflow

Waters™



Snap

Sastavite trake s gotovim reagensima, pripremite uzorke i postavite ih na stalke.



Load

Postavite stalke s uzorcima i reagensima te PCR kartice na uređaj.

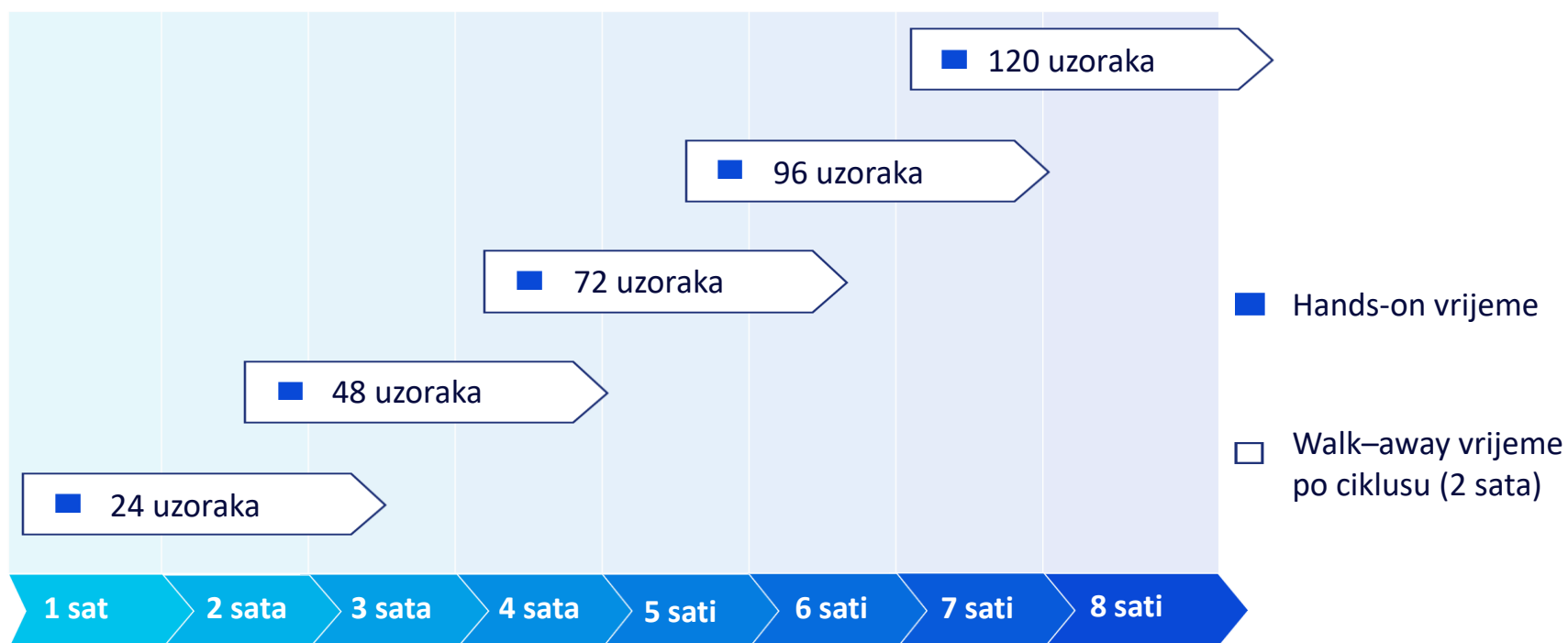


Go

Rezultate provjerite za 2–3 sata, ovisno o testu i broju uzoraka koje ste analizirali.

Pojednostavite i ubrzajte rad laboratorija uz fleksibilan workflow

- Izvođenje više testova u jednom runu — uz “walk-away” vrijeme za druge zadatke u laboratoriju.



- **BD (Becton Dickinson) – vlastiti CE-IVDR i FDA portfolio**
 - BD razvija i proizvodi vlastite testove za rutinsku dijagnostiku na BD MAX™ sustavu.
- **Partnerski paneli za proširenje portfelja**
 - BD MAX™ je otvorena platforma, pa značajan dio testova dolazi kroz sljedeće partnere:
 1. CerTest Biotec VIASURE CE-IVD paneli
 2. BioGX partnerski CE-IVD i RUO paneli
 3. Biolegio ReadyMax primarno RUO paneli
- **Mogućnost razvoja vlastitog molekularnog testa na BD MAX™ sustavu.**



Bolničke infekcije i rezistencija

- *C. difficile*
- **MRSA XT** i **StaphSR** (MRSA, mecA i mecC)
- **CPO** (KPC, NDM, OXA-48, VIM, IMP, GES), **colistin** (mcr-1) i **vankomicin** (vanA, vanB, vanC1, vanC2/3)



CNS infekcije i infekcije kod imunokompromitiranih pacijenata

- NSH i ELGBS meningitis paneli
- HSV-1, HSV-2, VZV encefalitis panel
- **Imunokompromitirani pacijenti** (EBV)

▣ Respiratorne infekcije

- **Osnovni virusni panel** (SARS-CoV-2, FluA, FluB, RSV)
- **Prošireni virusni panel** (SARS-CoV-2, FluA, FluB, RSV, PIV, MPV, ADV, HCV)
- **MDR-TB** (TB + rezistencije na RIF i INH) – **WHO Guidelines**
- **Ostale respiratorne infekcije** (*Bordetella*, *Pneumocystis*, atipične pneumonije)

Spolno prenosive infekcije i žensko zdravlje

- **Spolno prenosive infekcije i rezistencija** (CT/GC/TV i *Mycoplasma/Ureaplasma*, rezistencija na makrolide)
- **Genitalne ulceracije** (*HSV-1*, *HSV-2*, *Treponema pallidum*, *Haemophilus*)
- **Bakterijska vaginoza, vaginitis i kandidijaze**
- **Screening u trudnoći** (Group B *Streptococcus*)

Gastrointestinalne infekcije (bakterije i virusi)

- **Osnovni bakterijski panel** (*Salmonella*, *Shigella*, EIEC, *Campylobacter*, STEC)
- **Prošireni bakterijski panel** (*Yersinia enterocolitica*, *Plesiomonas shigelloides*, *Vibrio* spp., ETEC)
- **Patotipovi *E. coli*** (O157, EHEC, EIEC, EPEC, ETEC i dr.)
- **Virusni panel** (NoV, RV, AdV, SaV, AstV)

□ **Gastrointestinalne (parazitske) infekcije - crijevni paraziti**

- *Giardia lamblia* / *intestinalis*, *Entamoeba histolytica*, *Cryptosporidium parvum* / *hominis*, *Dientamoeba fragilis*,
Cyclospora cayetanensis, Microsporidia (*Encephalitozoon*, *Enterocytozoon*)

Krvne (protozoarne) infekcije

- *Plasmodium* spp., *Plasmodium falciparum*, *Plasmodium vivax*
- *Babesia microti*

□ **Vektorske bakterijske infekcije**

- *Anaplasma phagocytophilum*, *Ehrlichia* spp.

Održavanje ove radionice pomogli su:

A & B

Axon Lab

Biogen

JANAF

Jasika

Kefo

Roche

Tehmed

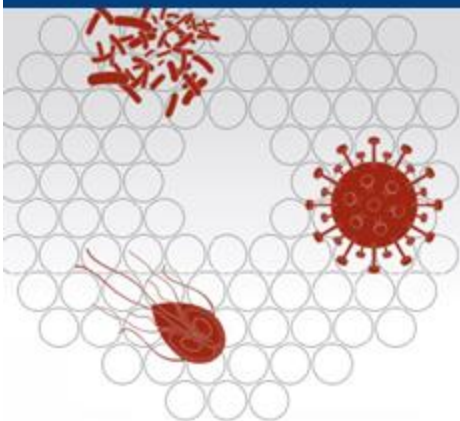
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Vaši partneri u mikrobiologiji



FilmArray®



The BioFire® FilmArray® Gastrointestinal (GI) Panel

Sample Type: Stool in Cary Blair

BACTERIA

Campylobacter (*C. jejuni*/*C. coli*/*C. upsaliensis*)
Clostridioides (*Clostridium*) difficile (toxin A/B)*
Plesiomonas shigelloides
Salmonella
Vibrio (*V. parahaemolyticus*/*V. vulnificus*/*V. cholerae*)
Vibrio cholerae
Yersinia enterocolitica
Diarrheagenic Escherichia coli/Shigella
Enteraggregative *E. coli* (EAEC)
Enteropathogenic *E. coli* (EPEC)
Enterotoxigenic *E. coli* (ETEC)/*st*
Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2*
E. coli O157
Shigella/Enteroinvasive *E. coli* (EIEC)

VIRUSES

Adenovirus F40/41
Astrovirus
Norovirus GI/GII
Rotavirus A
Sapovirus (I, II, IV, and V)

PARASITES

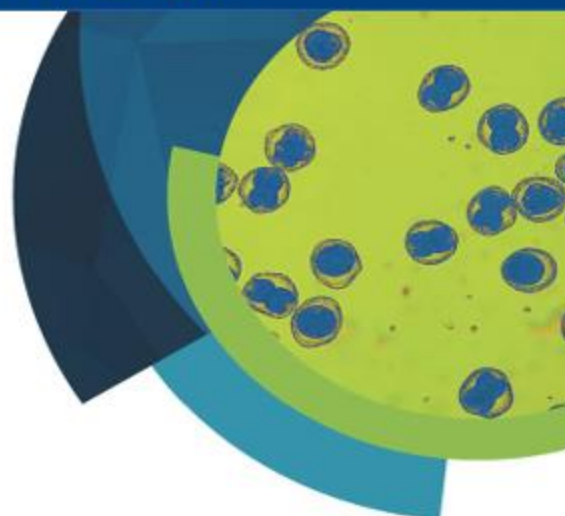
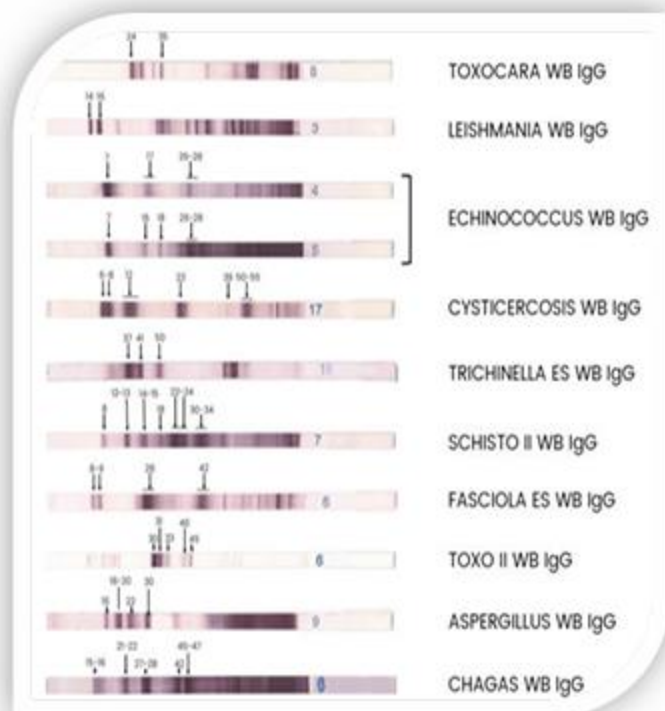
Cryptosporidium
Cyclospora cayentanensis
Entamoeba histolytica
Giardia lamblia



IMUNOBLOT TESTOVI ZA PARAZITOLOGIJU I MIKOLOGIJU



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Products	12 tests	24 tests	96 tests
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ECHINOCOCCUS WB IgG €€	ECH-WB12G	ECH-WB24G	ECH-WB96G
CYSTICERCOSIS WB IgG €€	CYS-WB12G	CYS-WB24G	CYS-WB96G
TRICHINELLA ES WB IgG €€	TRI ES-WB12G	TRI ES-WB24G	TRI ES-WB96G
SCHISTO II WB IgG €€	SCH II-WB12G	SCH II-WB24G	SCH II-WB96G
FASCIOLA ES WB IgG €€	FAS ES-WB12G	FAS ES-WB24G	FAS ES-WB96G
TOXO II WB IgG €€D459	TOXO II 12G	TOXO II 24G	TOXO II 96G
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OD X800 TESTOVA

