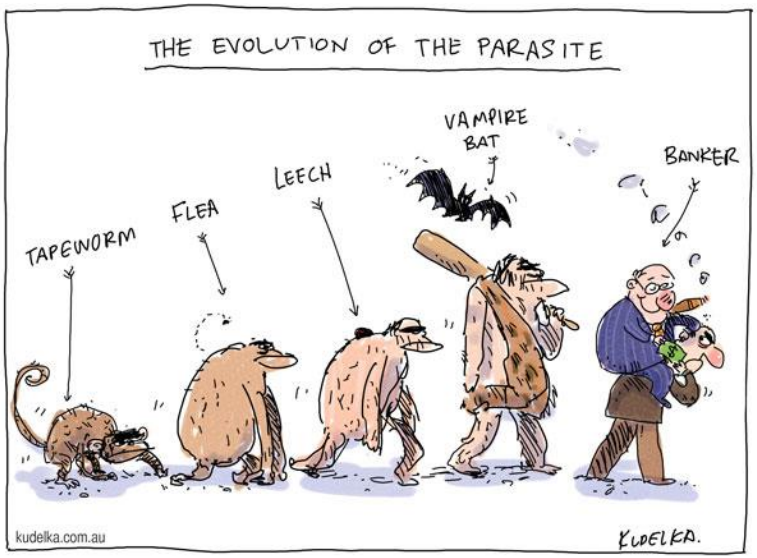
Molekularni dokaz protozoona *Cryptosporidium hominis* i bičaša *Giardia duodenalis* u uzojima kamenica (*Ostrea edulis*) i dagnji (*Mytilus galloprovincialis*) iz Hrvatske

Hengl Brigita, Zrnčić Snježana, Oraić Dražen, Gross-Bošković Andrea, Beck Relja

Paraziti i pirazitizam

Odnos između organizama različitih vrsta u kojem jedan organizam, parazit, ima koristi na štetu nositelja



MICROBIOLO

L RISK ASSESSMENT SERIES

23

Multicr
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ne parasites

Expert Meeting,
eadquarters, Rome, Italy

of the United Nations

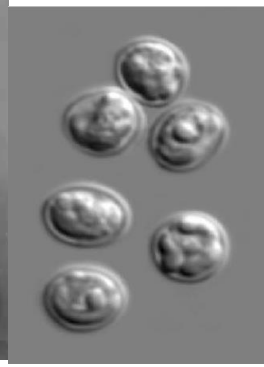
Ranking position	Parasite	Major food commodities associated with foodborne transmission
1	*Taenia solium*	Pork[b], fresh produce[c]
2	*Echinococcus granulosus*	Fresh produce
3	*Echinococcus multilocularis*	Fresh produce
4	*Toxoplasma gondii*	Meat from small ruminants, pork, beef, game meat (red meat and organs); also fresh produce
5	*Cryptosporidium* spp.	Fresh produce, fruit juice, milk
6	*Entamoeba histolytica*	Fresh produce
7	*Trichinella spiralis*	Pork
8	*Opisthorchiidae*	Freshwater fish
9	*Ascaris* spp.	Fresh produce
10	*Trypanosoma cruzi*	Fruit juices
11	*Giardia duodenalis*	Fresh produce
12	*Fasciola* spp.	Fresh produce (freshwater plants)
13	*Cyclospora cayetanensis*	Berries, fresh produce
14	*Paragonimus* spp.	Freshwater crustaceans
15	*Trichuris trichiura*	Fresh produce
16	*Trichinella* spp.[d]	Game meat
17	Anisakidae	Marine fish, crustaceans and cephalopods
18	*Balantidium coli*	Fresh produce
19	*Taenia saginata*	Beef
20	*Toxocara* spp.	Fresh produce
21	*Sarcocystis* spp.	Beef or pork depending on species
22	Heterophyidae	Freshwater/brackish water fish
23	Diphyllobothriidae	Fish (freshwater and marine)
24	*Spirometra* spp.	Frog, snake meat

Antoine van Leeuwenhoek

1681. godine

Giardia duodenalis

Edward Tyzzer



1907. godine

Cryptosporidium muris

Prije primjene molekularne tipizacije

- *C. parvum*- 1976. prvi slučaj
- *G. duodenalis* zoonoze- morfološki (WHO, 1975)
  - Dabrovi u SAD- izvor giardijoze ljude
  - Goveda izvor kriptosporidioze

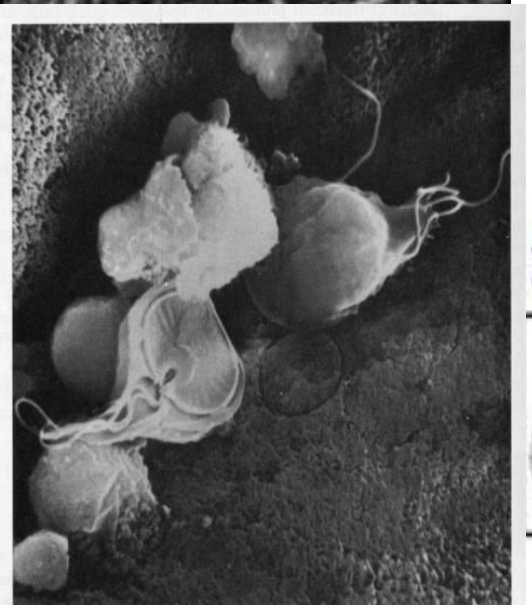
Bolesti prenosive vodom

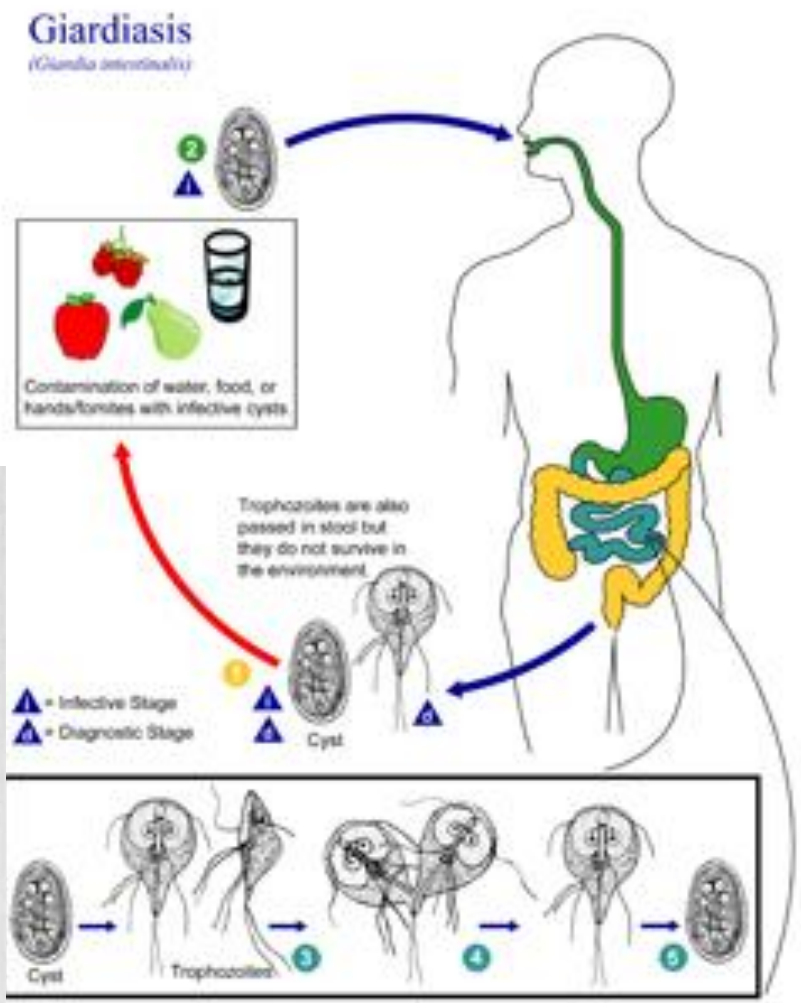
1996– WHO izvještaj- 200 milijuna slučajeva giardijoze ljudi

Primjena molekularnih metoda

- 1991.- dokazana heterogenost *C. parvum*
- 2002.- *C. hominis*
- *C. parvum* i *G. duodenalis* zoonoze- temeljem *SSU rDNA*
- Moguće odrediti izvor invazije (analiza više genskih lokusa, *Gp60*)
- Redovna dijagostika ⟹ tipizicija ⟹ epidemiologija

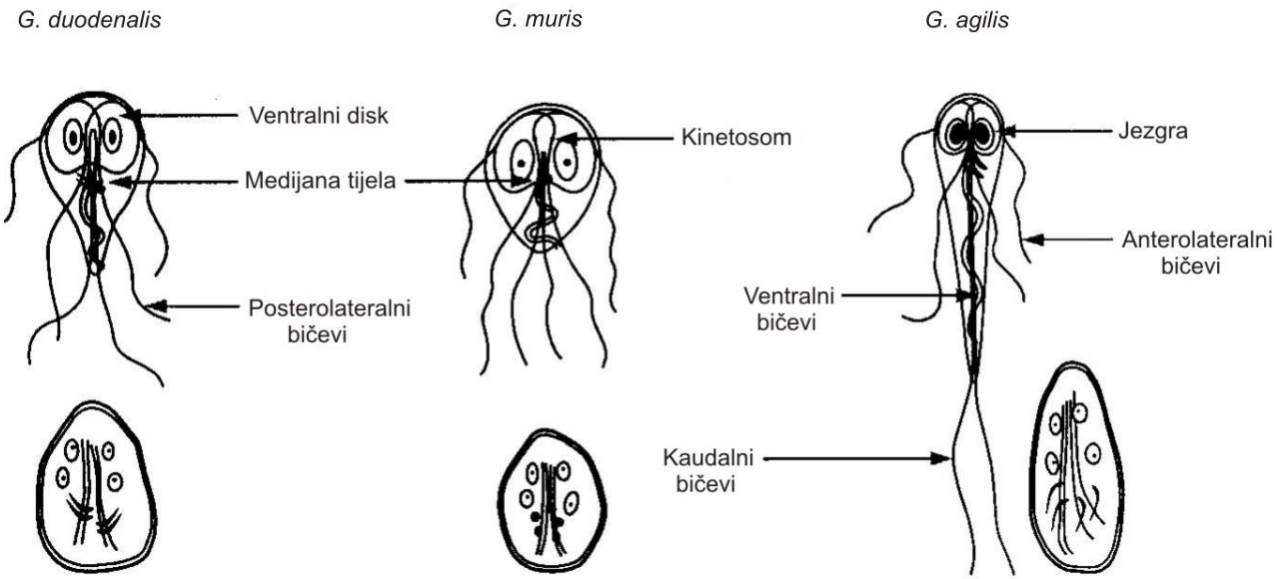
Životni ciklus- *Giardia duodenalis*





Rod *Giardia*

- *G. agilis*
- *G. muris*
- *G. ardeae*
- *G. psittaci*
- *G. microti*



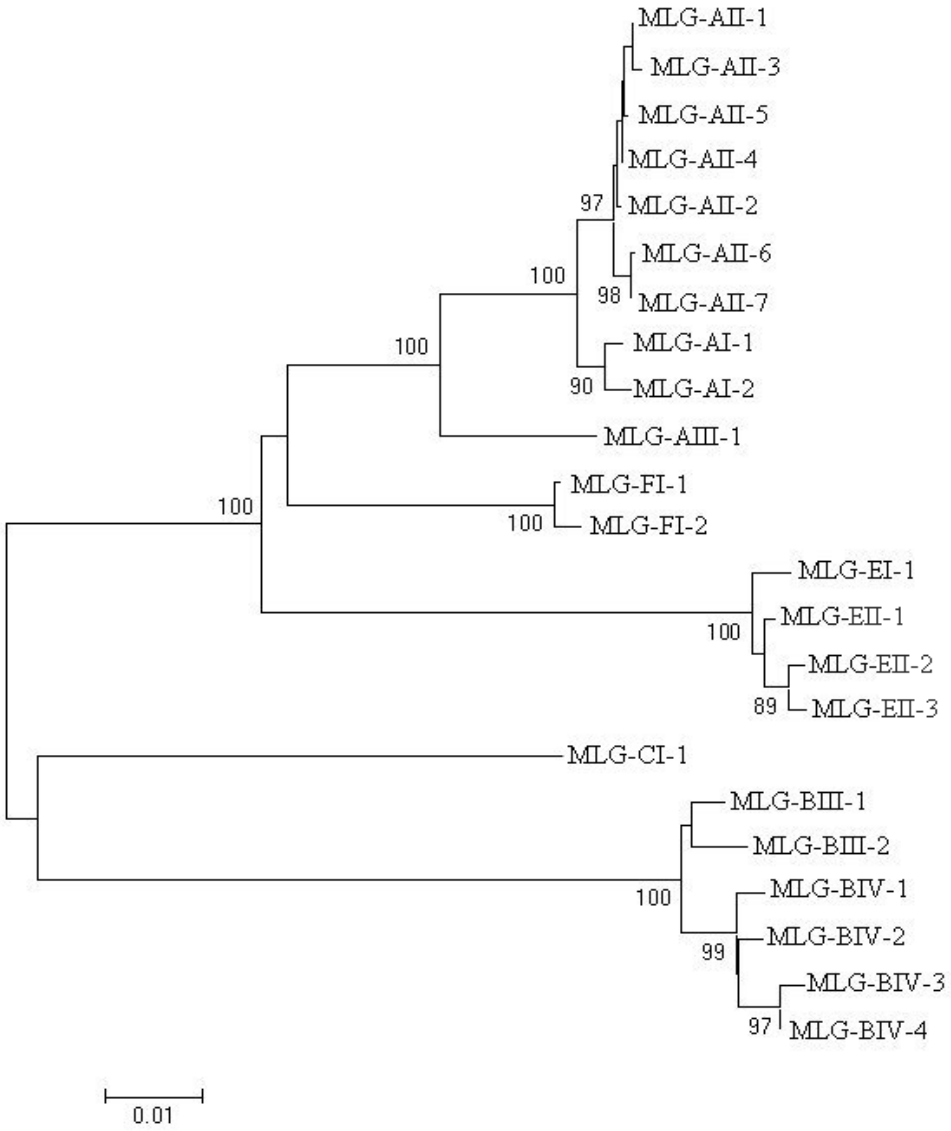
- ***G. duodenalis (intestinalis, lamblia)***

G. duodenalis- genske skupine

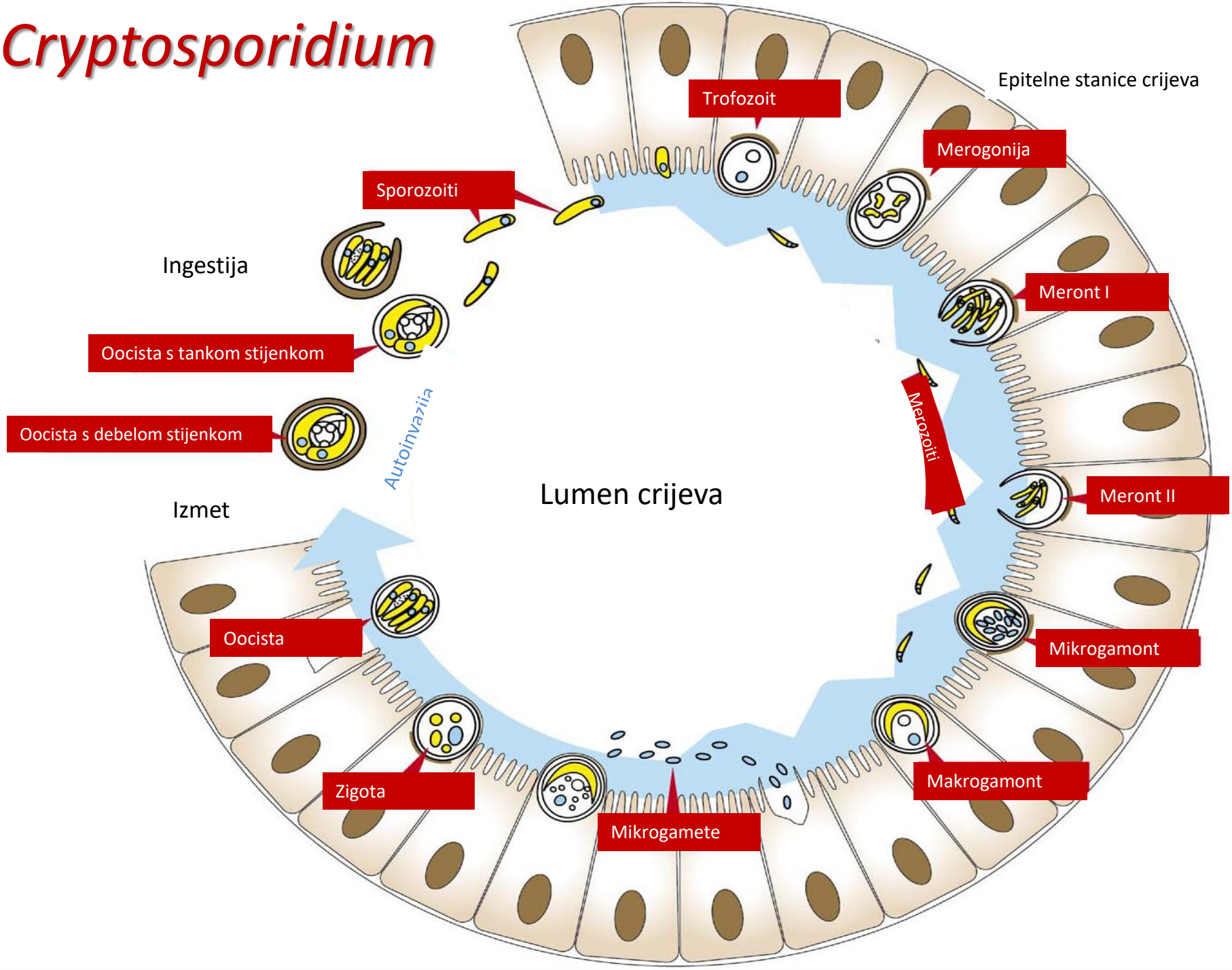
- Skupina A- Čovjek, pas, mačka, govedo, ovca...
- Skupina B- Čovjek, pas, govedo...

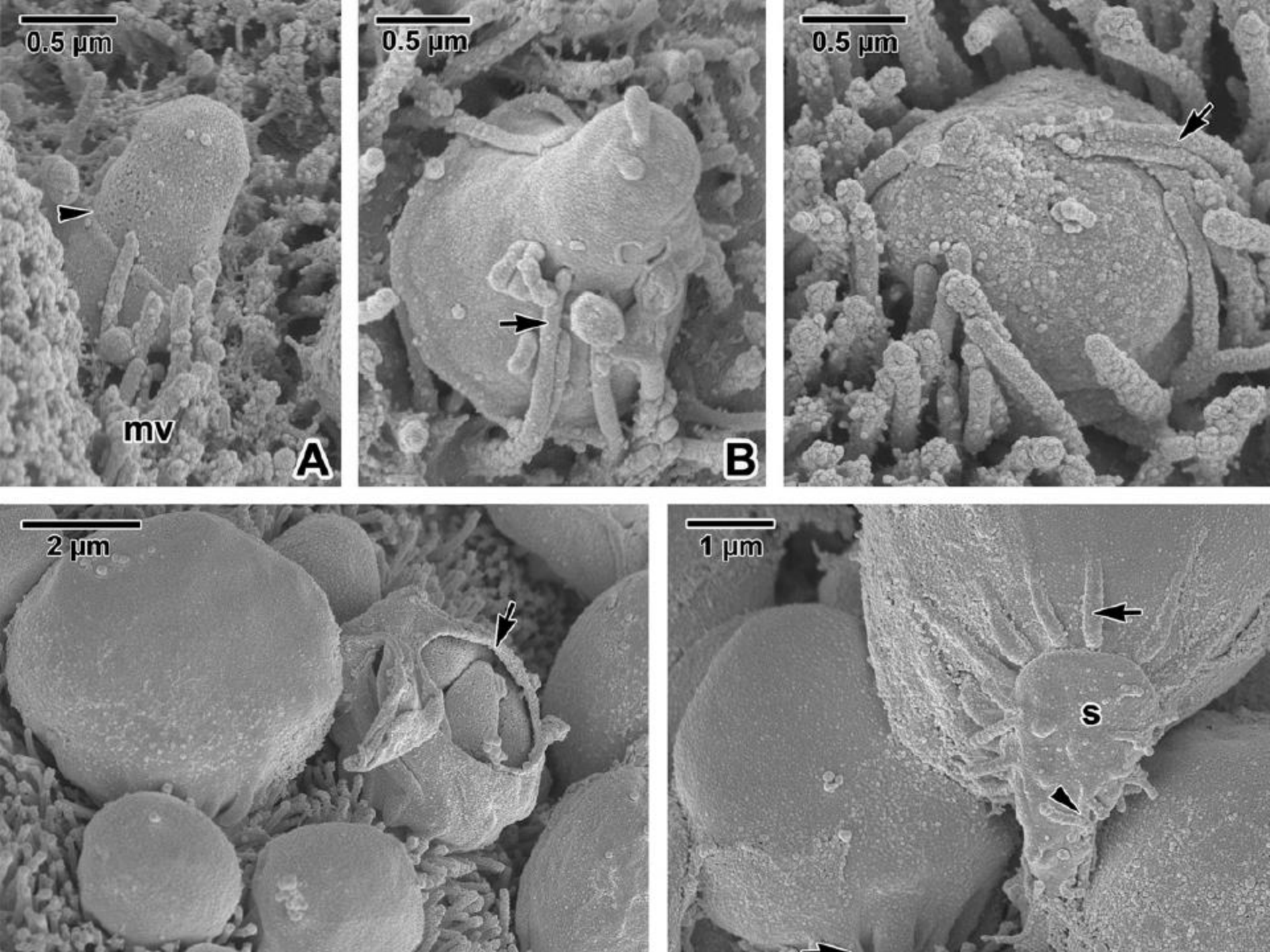
- Skupina C- Pas
- Skupina D- Pas
- Skupina E- Ovca, govedo, svinja
- Skupina F- Mačka
- Skupina G- Štakor
- Skupina H- morski kralješnjaci

Različitost unutar skupina A i B



Cryptosporidium




0.5 µm
mv
A
0.5 µm
B
0.5 µm
2 µm
1 µm
s

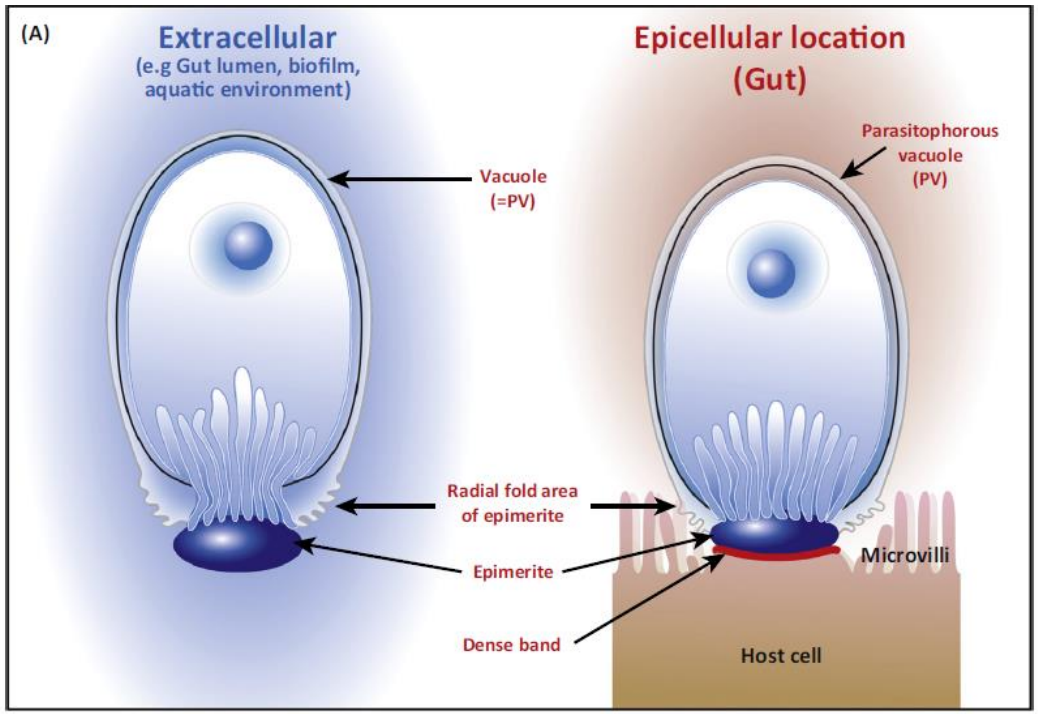
CellPress

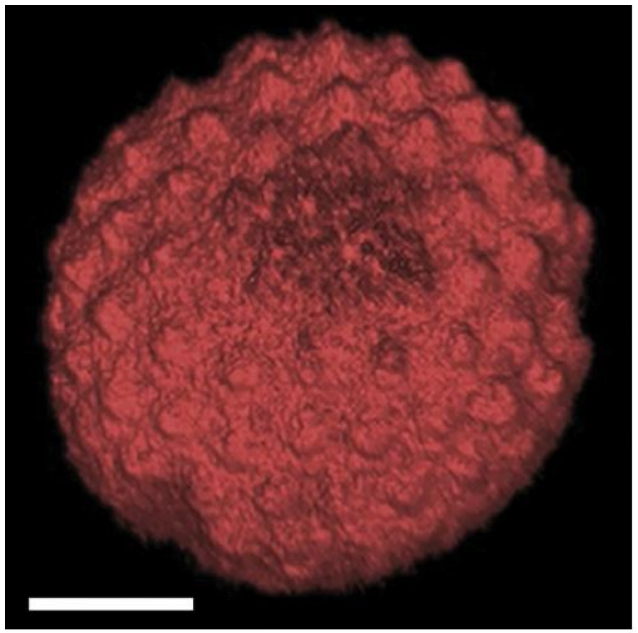
Opinion

Life without a Host Cell: What is *Cryptosporidium*?

Peta L. Clode,[1,*] Wan H. Koh,[2] and R.C. Andrew Thompson[2]

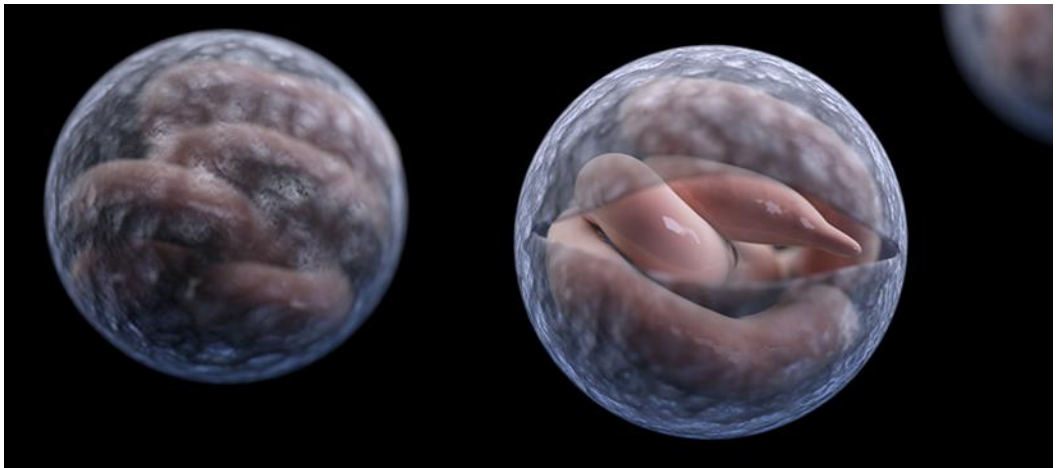
Mogu preživjeti i umnažati se bez stanica nositelja





Rod *Cryptosporidium*

- Danas službeno: 31 vrsta
- Više od 40 genotipova
- 20 vrsta u ljudi: *C. hominis*, *C. parvum*, *C. meleagridis*, *C. felis*, *C. canis*, *C. cuniculus*, *C. ubiquitum*, *C. viatorum*, *C. muris*, *C. suis*, *C. fayeri*, *C. andersoni*,*C. bovis*, *C. scrofarum*, *C. tyzzeri*, *C. erinacei*

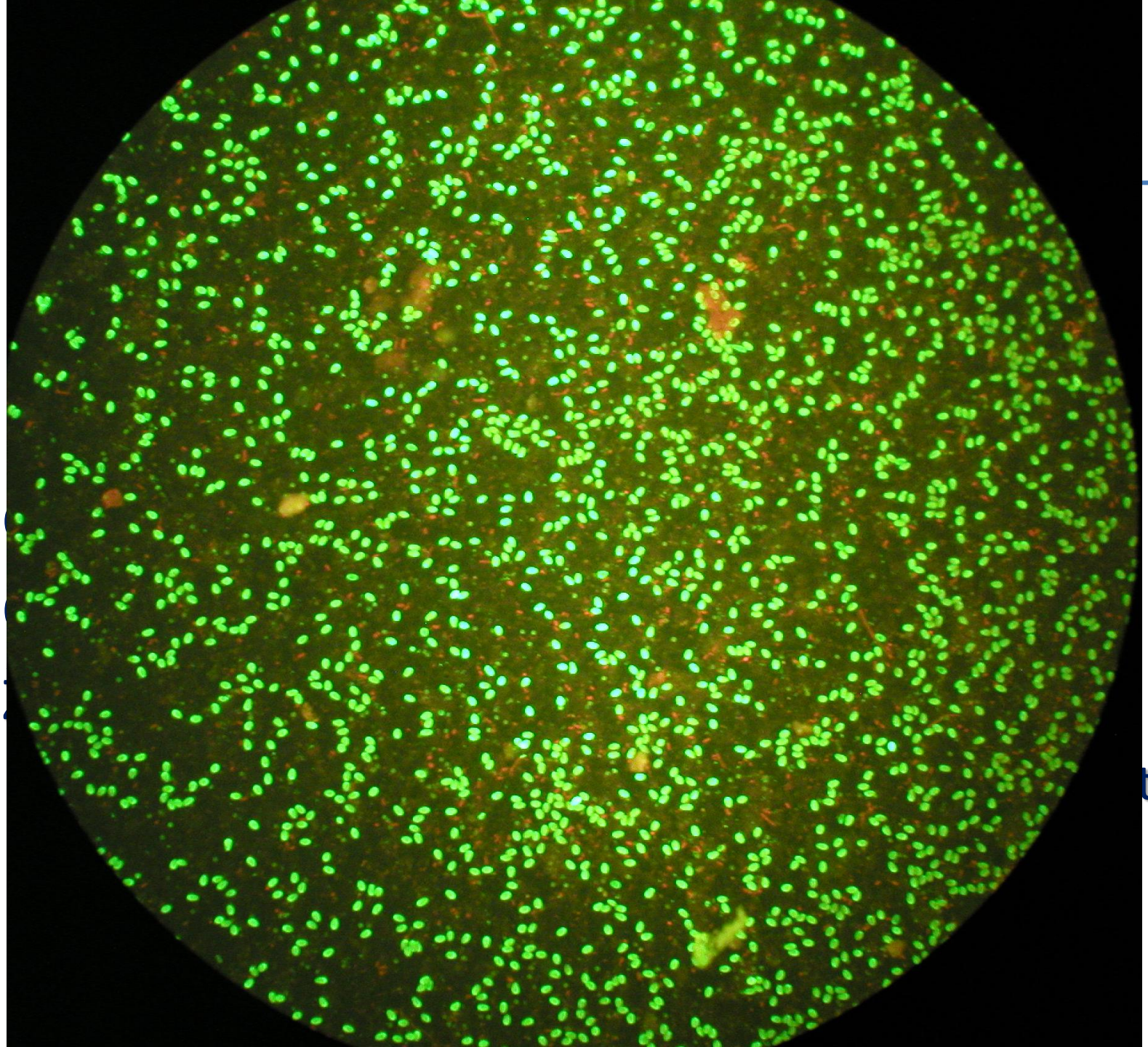


Povezanost zoonotskih izolata

GP60 subtype	Australian human	European human	Australian cattle	Canadian, European + US cattle
IaA23	1	0	0	0
IbA5G2T3	1	0	0	0
IbA9G2	1	0	0	0
IbA9G2T1	1	0	0	0
IbA10G2	21	5	0	0
IdA15G1	9	1	0	0
IdA16	1	0	0	0
IdA25	3	0	0	0
IfA11G1T1	1	0	0	0
IfA12G1	2	0	0	0
IIaA12G2R1	0	0	0	0
IIaA15G2R1	2	0	2	20
IIaA16G2R2	0	0	0	2
IIaA17G2R1	1	0	0	2
IIaA18G3R1	3	1	5	0
IIaA19G2R1	1	0	0	0
IIaA19G3R1	1	0	0	0
IIaA19G4R1	0	0	0	0
IIcA5G3a	1	0	0	0
IIdA22G1	0	1	0	0

Portugal

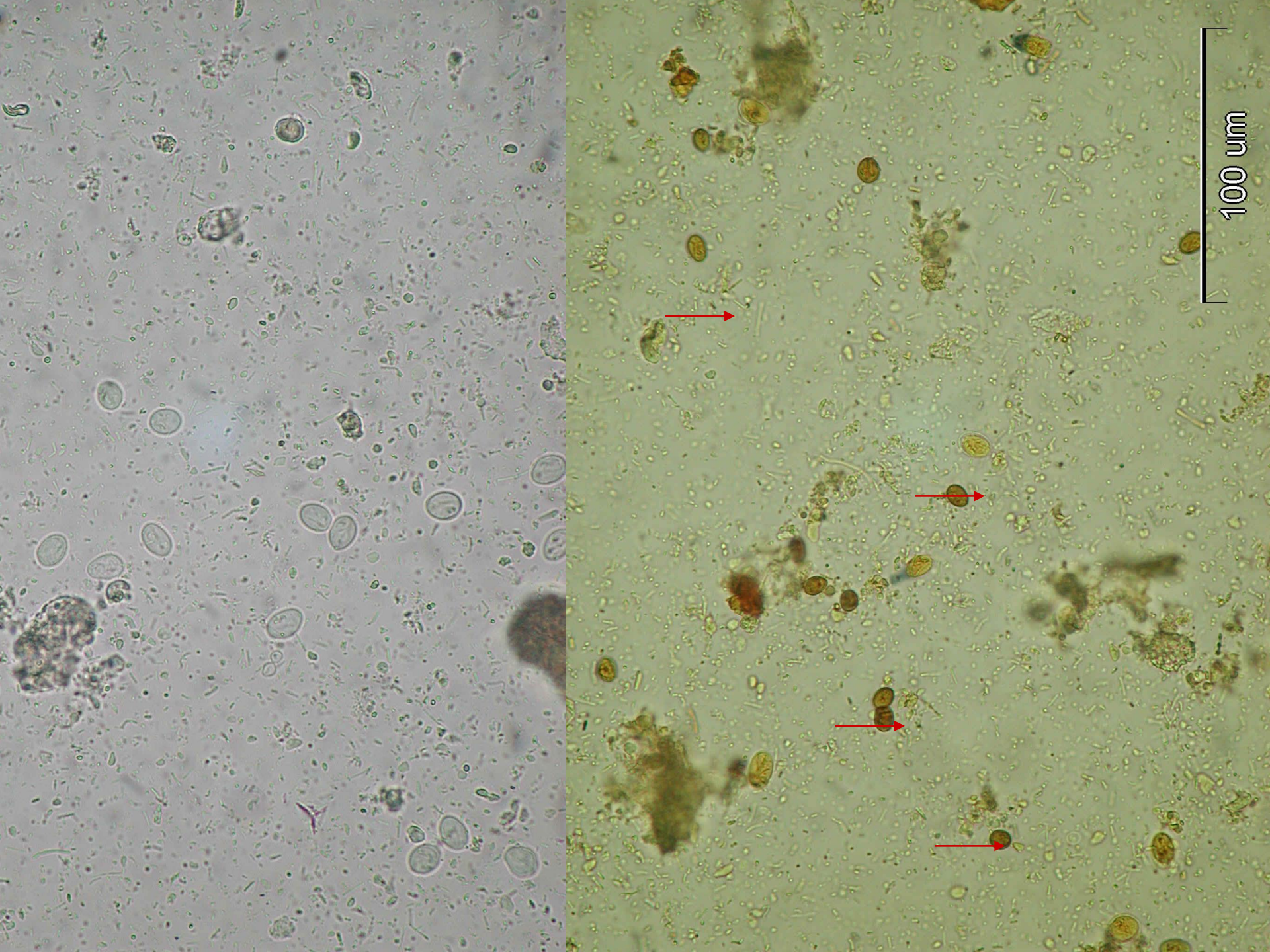
Subtype	Host				
	Human HIV+	Bovine	Sheep	Deer	Zoo ruminants
IIaA15G2R1	9	61	1	1	9
IIaA16G2R1	0	7	0	0	0
IIbA14	1	0	0	0	0
IIcA5G3a	1	0	0	0	0
IIcA5G3b	6	0	0	0	0
IIdA17G1	2	4	0	0	0
IIdA19G1	3	0	0	0	0
IIdA21G1	2	0	1	0	0
IIdA22G1	1	0	0	0	0
IaA19R3	1	0	0	0	0
IbA10G2	10	0	0	0	0
IdA15	1	0	0	0	0
IeA11G3T3	2	0	0	0	0
IfA14G1	1	0	0	0	0



tima

Zajednička obilježja

- Ciste *G. duodenalis* morfološki jednake
- Oociste kriptosporidaja gotovo jednake
- Značajnija istraživanja započinju 80-ih godina(HIV)
- Učestalost ovisi o dijagostičkoj metodi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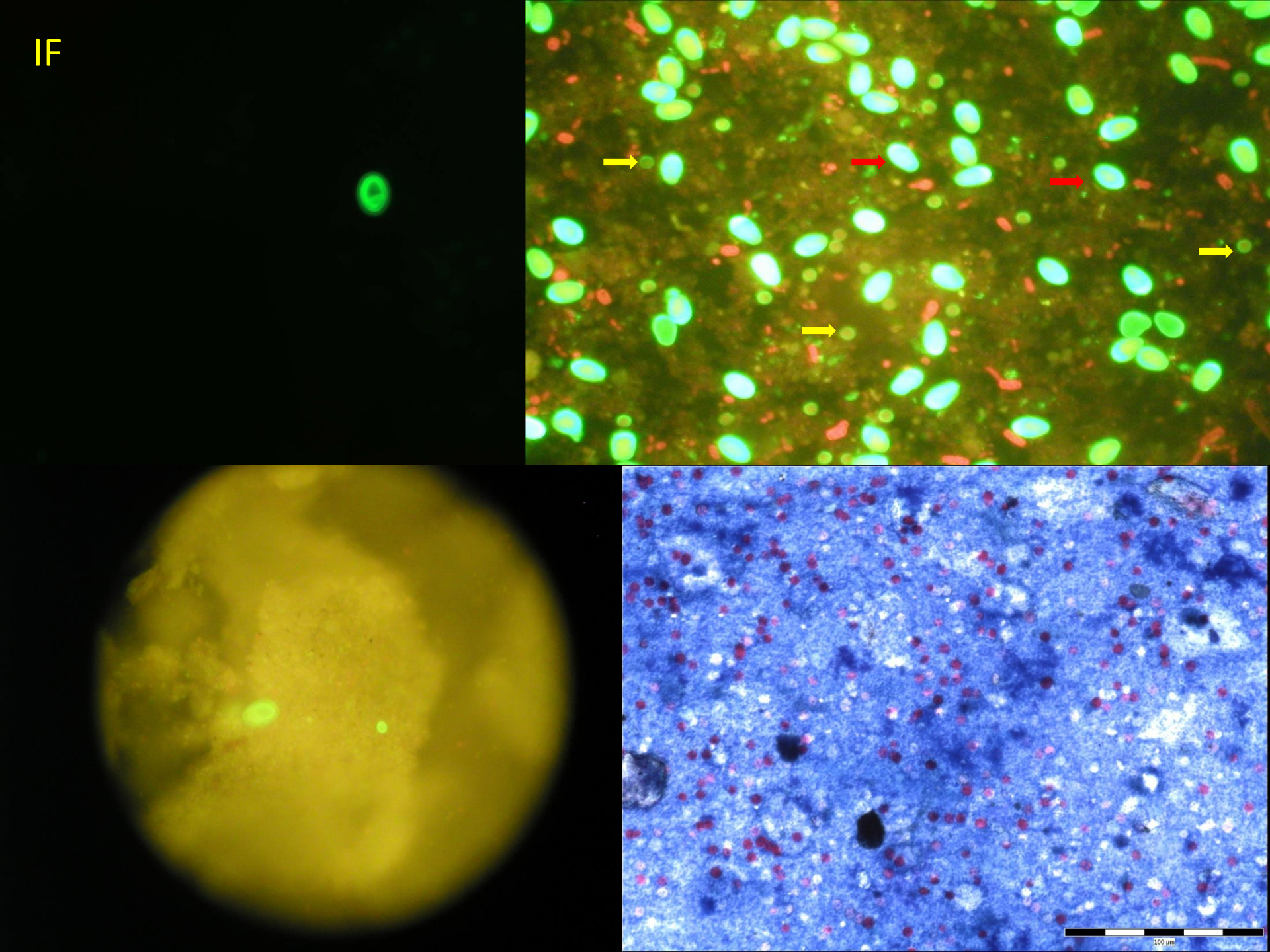
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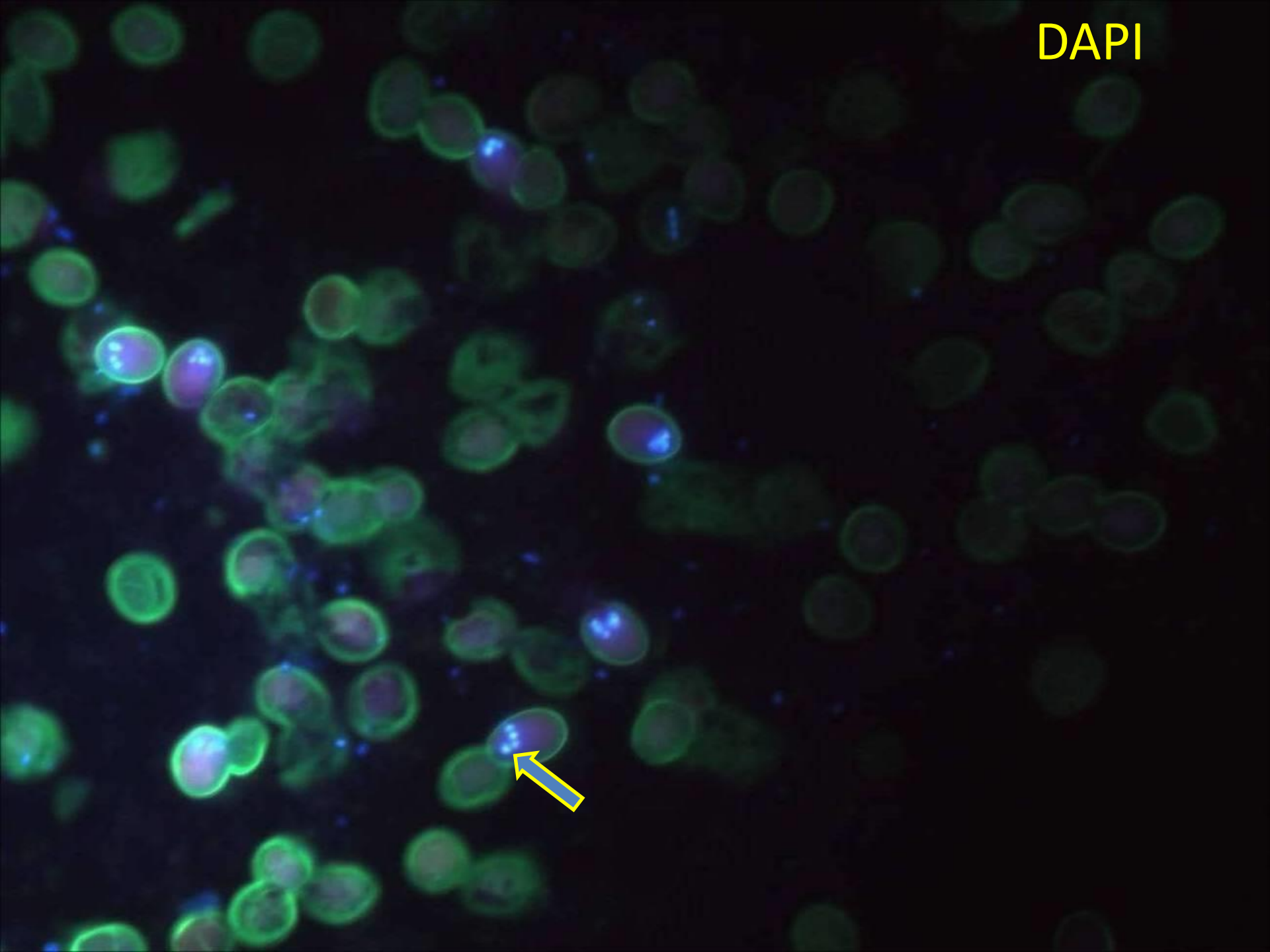

DAPI

Simptomi u ljudi

- Asimptomatske invazije- kronični nositelji
- Akutne- proljev, mučnina, grčevi, anoreksija, groznica, zatvor, vjetrovi, bolovi u trbuhu, umor, glavobolja, stolica neugodnog mirisa i sluz u stolici
- Kronične- gubitak težine i zaostajanje u rastu, GI problemi mogu trajati i mjesecima
- Samolimitrajući gastroenteritis u zdravih ljudi

Kriptosporidiji, giardije i voda

- 1984.- Texas- prvo izbijanje bolesti- 79 oboljelih
- 1993.- Milwaukee- 400 000 oboljelih
  - *C. hominis* Ib
- Od 325 izbijanja bolesti povezanih s vodom i protozoima- 51% *Cryptosporidium*
- 2004.- *G. duodenalis* (BIII)- norveška, Bergen
  - 1300 dokazanih slučajeva, procjena oko 2500

Hrana

- Hrana može biti kontaminirana tijekom proizvodnje, sakupljanja, prijevoza i pripreme (mlijeko, voće, povrće, svježi sokovi) te tijekom obrade

- Najčešći izvor kontaminacije hrane su izmet ili kontaminirana voda i zemlja

Giardije i hrana

- 1979.- lososovim mesom opisana je godine su
- 1985. i 1986.- tri epidemije s više od 100 oboljelih (voćna salata, salata od tjestenine i sendviči)
- 1990. - kontaminirano voće
- 1992. - u 27 osoba nakon konzumacije sladoleda.
- 1996.- 26 ljudi nakon konzumacije svježeg povrća. (inkriminirane su osobe koje su pripremile hranu)

Primjeri kriptosporidija u hrani

Vrsta hrane	Značaj	Izvor hrane	Zemlja
Voće i povrće	4% salata, klice graha	Komercijalne veletrgovine	Norveška
	2% zelena salata	Tržnica	Sj. Irska
	7% zelena salata	Tržnica	Poljska
Školjke	30/43 mjesta uzorkovanja	Uzgajalište kamenica	USA
	55.2% ,56%, 34%	Uzgajalište- razne školjke	Španjolska
	7% *C. hominis*, dagnje	Uzgajalište	Sj. Irska
	13% i 6.7% kamenice	Uzgajalište, slobodne	Nizozemsaka
Meso	33%, koze	Klaonice	Indija

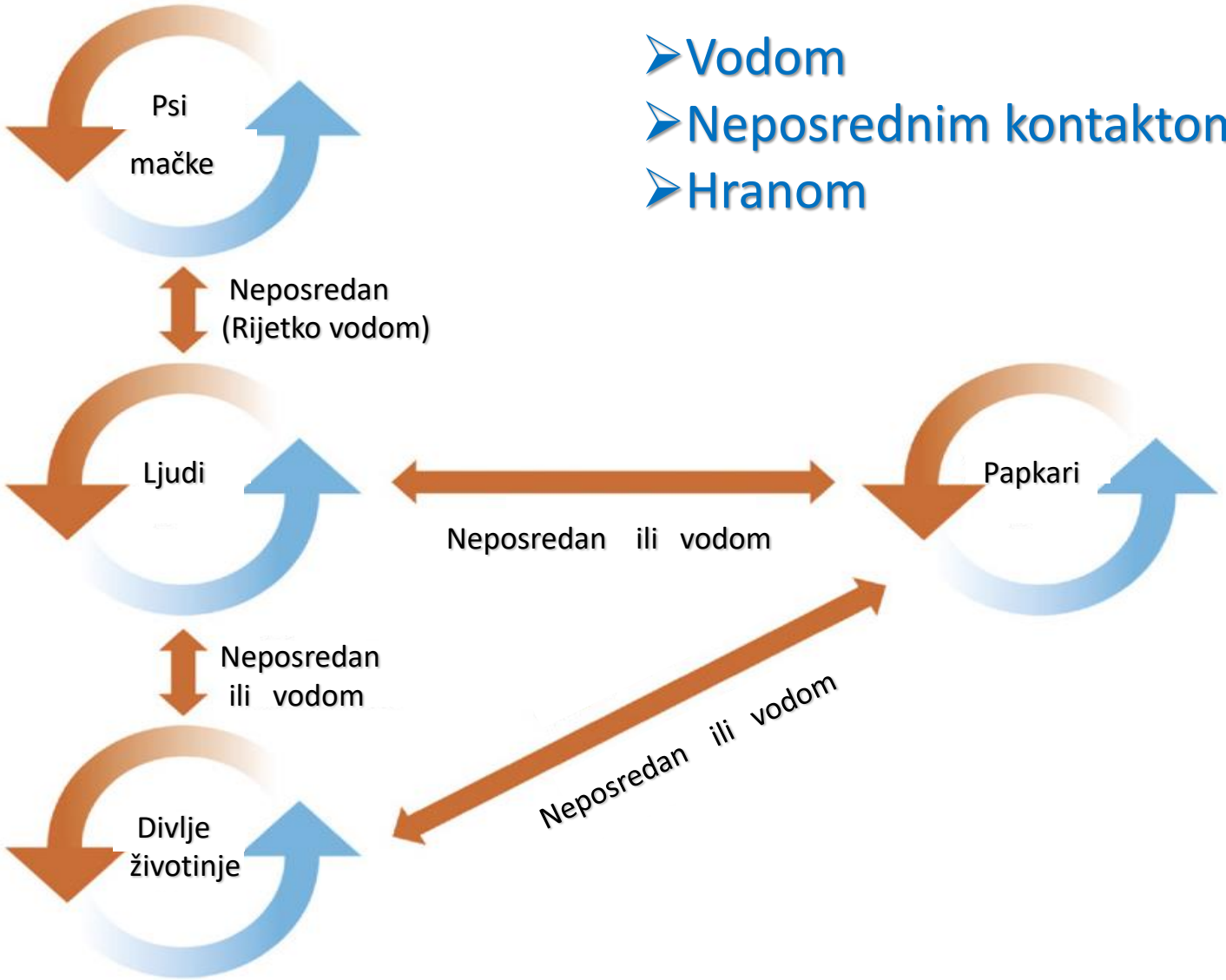
G. duodenalis (B) i *C. parvum* – dokazani u sirovom mesu: pileća prsa, svinjski odresci, mljevena govedina

Primjeri izbijanja kriptosporidioze

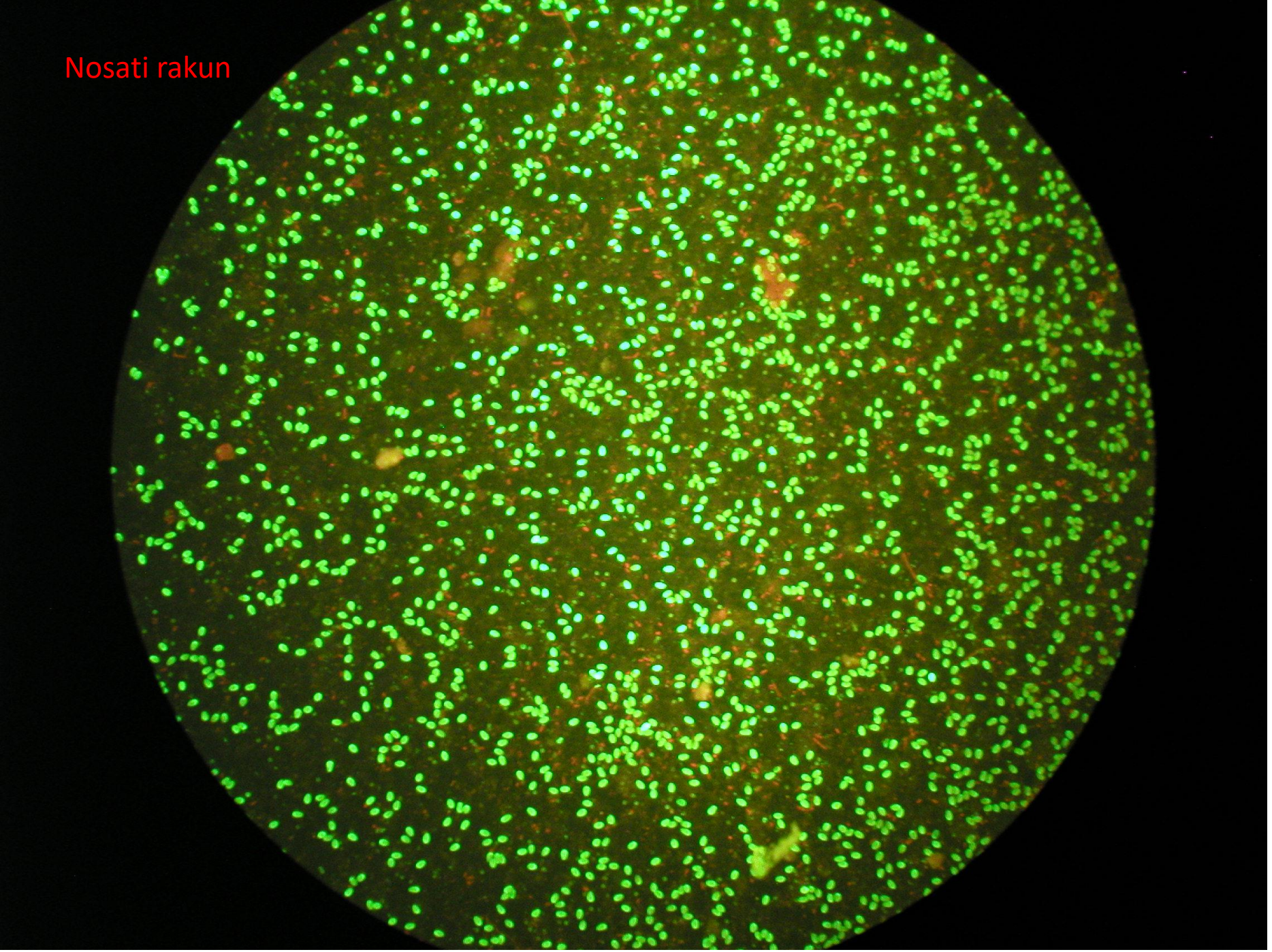
Br. oboljelih	Vrsta hrane	Izvor invazije	*Crypto*
160	Svježi sok od jabuke	Kontaminirane jabuke	/
25	Svježi sok od jabuke	Voda za pranje jabuka	/
15	Salata od piletine	Osoba koja je pripremala	/
48	Svježe školsko mlijeko	Školsak krava	/
54	/	? Različita hrana	*C. hominis*
152	Kantina u sveučilištu	Osoba koja je pripremala	*C. hominis*
8	Kravlje mlijeko		/
12	Svježi sok od jabuke	Sok- ozoniran	IIaA15G2R1 IIaA17G2R1
15	Bernise umak- restoran	Svježi peršin	/
250	Ready-to -eat salata		IIdA17G1

Prijenos giardija i kriptosporidija

- Vodom
- Neposrednim kontaktom
- Hranom



Nosati rakun



Istraživanja u RH???

VECTOR-BORNE AND ZOONOTIC DISEASES
Volume 12, Number 3, 2012
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DOI: 10.1089/vbz.2011.0751

Genotyping *Giardia duodenalis* Isolates from Dogs: Lessons from a Multilocus Sequence Typing Study

Relja Beck,[1] Hein Sprong,[2] Edoardo Pozio,[3] and Simone M. Cacciò[3]

Identification of *Giardia* species and *Giardia duodenalis* assemblages by sequence analysis of the 5.8S rDNA gene and internal transcribed spacers

SIMONE M. CACCIÒ[1]*, RELJA BECK[1,2], ANDRE ALMEIDA[1,3], ANNA BAJER[4] *and* EDOARDO POZIO[1]

VECTOR-BORNE AND ZOONOTIC DISEASES
Volume 00, Number 00, 2010
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DOI: 10.1089/vbz.2010.0113

VBZ-2010-0113-Beck_1P
Type: research-article

ORIGINAL RESEARCH

A Large Survey of Croatian Wild Mammals for *Giardia duodenalis* Reveals a Low Prevalence and Limited Zoonotic Potential

Relja Beck,[1] Hein Sprong,[2] Snjezana Lucinger,[3] Edoardo Pozio,[4] and Simone M. Cacciò[4]

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Available online at www.sciencedirect.com
ScienceDirect
International Journal for Parasitology 38 (2008) 1523–1531
www.elsevier.com/locate/ijpara

Multilocus genotyping of *Giardia duodenalis* reveals striking differences between assemblages A and B ☆,☆☆

S.M. Cacciò [a,*], R. Beck [a,b], M. Lalle [a], A. Marinculic [b], E. Pozio [a]

Veterinary Parasitology 175 (2011) 40–46

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Contents lists available at ScienceDirect
Veterinary Parasitology
journal homepage: www.elsevier.com/locate/vetpar

Prevalence and molecular typing of *Giardia* spp. in captive mammals at the zoo of Zagreb, Croatia

Relja Beck [a], Hein Sprong [b], Ingeborg Bata [c], Snjezana Lucinger [d], Edoardo Pozio [e], Simone M. Cacciò [e,*]

Case Report

Wien Klin Wochenschr (2006) 118/15–16: 485–487
DOI 10.1007/s00508-006-0637-7

WIENER KLINISCHE WOCHENSCHRIFT
The Middle European Journal of Medicine
Printed in Austria

A family outbreak of cryptosporidiosis: probable nosocomial infection and person-to-person transmission

Nenad Pandak[1], Kristof Zeljka[2], and Ante Cvitkovic[3]

DISTRIBUCIJA GENSKIH SKUPINA

G. duodenalis

Zoološki vrt Zagreb

Divlje životinje u Hrvatskoj

Host	No of animals	No of infected animals (percentage)	*G. duodenalis* assemblages
Deer (*Cervus elaphus*)	285	3 (0.9%)	Assemblage A (AI) Assemblage D
Roe deer (*Capreolus capreolus*)	15	4 (27%)	Assemblage D Assemblage A (AI)
Wild boar (*Sus scrofa*)	144	2 (1.4%)	Assemblage A (AIII)
Fox (*Vulpes vulpes*)	66	3 (4.5%)	Assemblage A (1)
Bear (*Ursus arctos*)	19	0	
Wolf (*Canis lupus*)	127	13 (10%)	Assemblage A (AI) (5) Assemblage C (2) Ass A+C (1) *G. microti* (1)
Jackal (*Canis aureus*)	8	1 (12.5%)	
Hare (*Lepus europaeus*)	73	0	

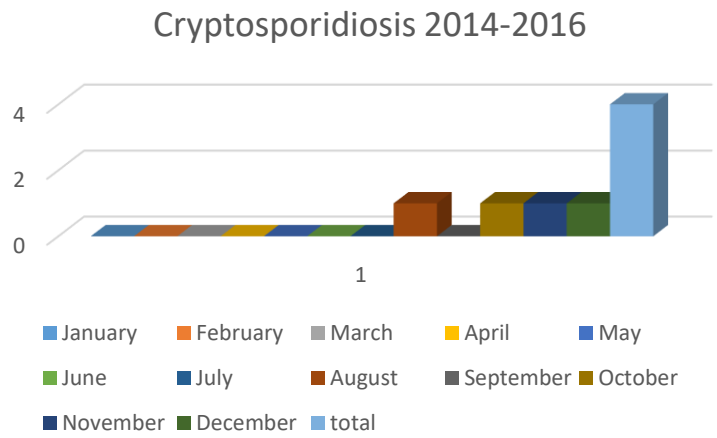
Prijava bolesti

- Obavezno u Irskoj, Finskoj, Njemačkoj i Švedskoj
- Velika Britanija – na dobrovoljnoj osnovi od 1983
  - Preko 70% od svih u EU; 151 epidemija od 1983
  - 96% *C. parvum* i *C. hominis* od 13 112 izolata od 1989.
- SAD – dobrovoljno od 1971.

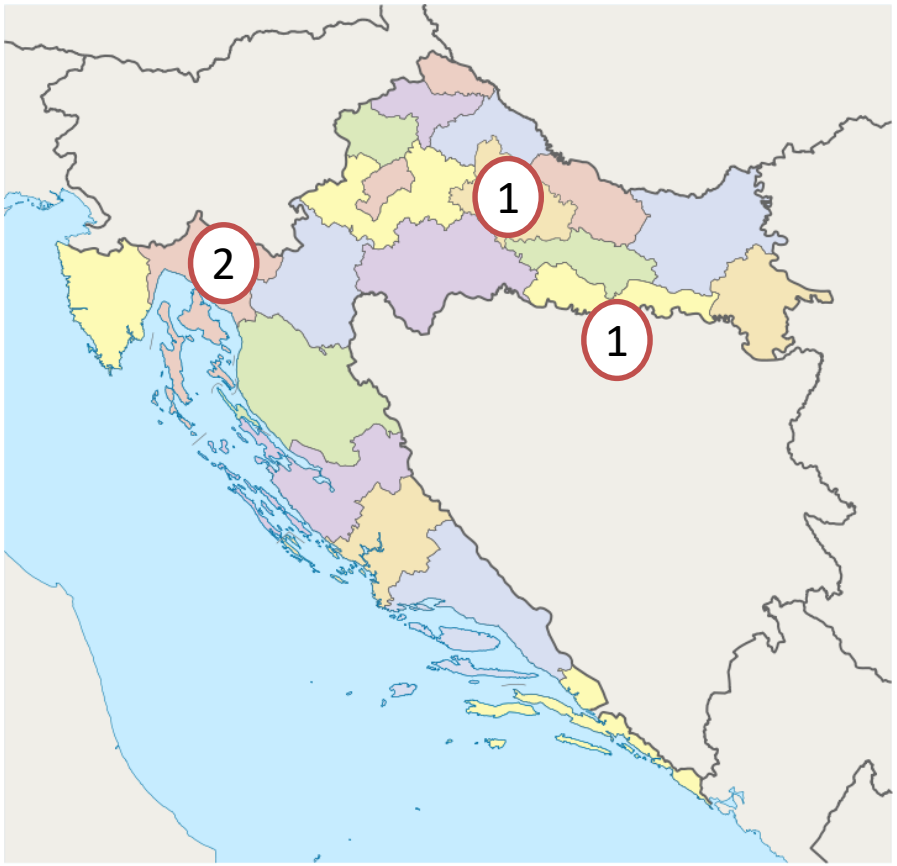
- Hrvatska obavezno od 2007 ?!?

Okvirno 17000 humanih slučajeva gijardioze i 7000 human slučajeva kriptosporidioze u Europi (ECDC)

Kriptosporidioza ljudi u RH

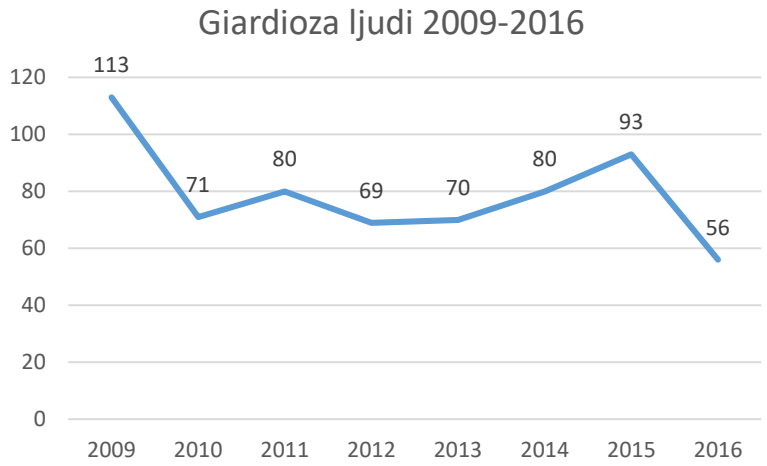


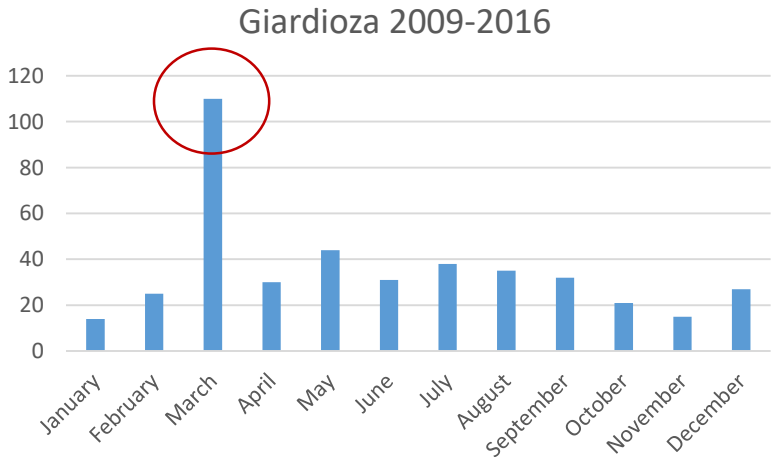
2017 - 4 SLUČAJA

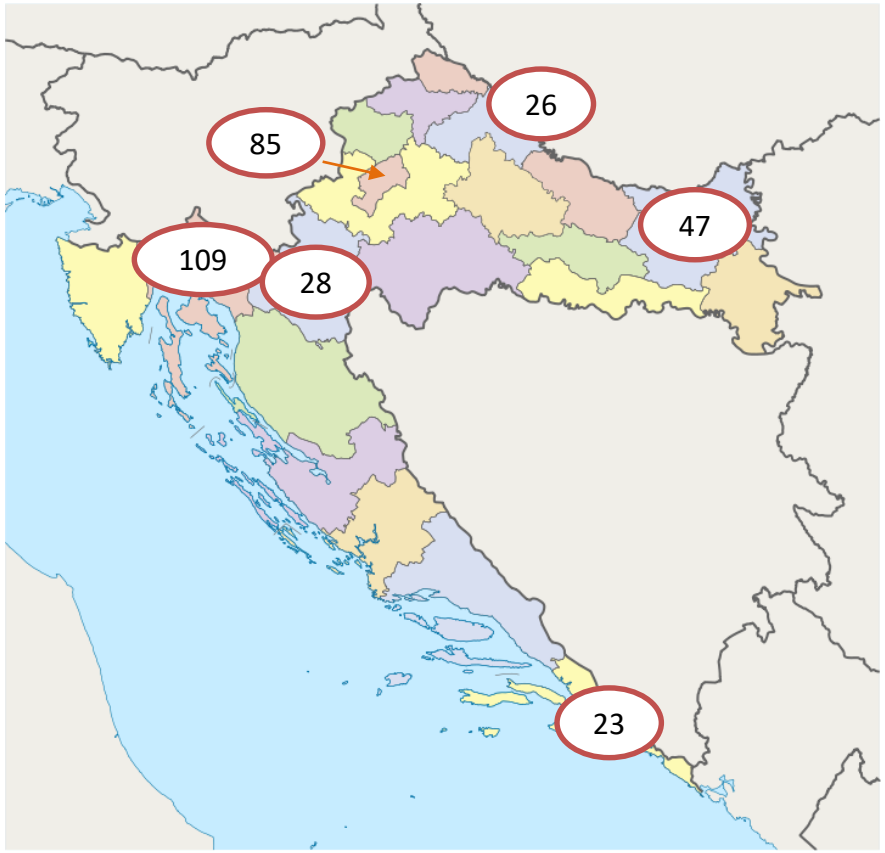


Source: Epidemiological Journal, Croatian Institute of Public Health, Division for Epidemiology of Communicable Diseases

Giardioza ljudi u RH







Source: Epidemiological Journal, Croatian Institute of Public Health, Division for Epidemiology of Communicable Diseases

Review TRENDS in Parasitology Vol.20 No.11 November 2004

Zoonotic protozoa: from land to sea

Ronald Fayer[1], Jitender P. Dubey[2] and David S. Lindsay[3]

Table 1. *Cryptosporidium* detected in contaminated shellfish

Location	Species (common name)	No. positive/no. examined	Detection method[a]	Ref.
Sligo area, Ireland	*Mytilus edulis* (Common mussel)	1/3 sites	IFA	[57]
Chesapeake Bay, MD, USA	*Crassostrea virginica* (Virginia or Eastern oyster)	6/6 noncommercial sites; 5-18/30 oysters per site	IFA	[34]
Chesapeake Bay, USA	*Ischadium recurvum* (Bent mussel)	2/2 sites	IFA	[58]
Chesapeake Bay, USA	*C. virginica*	5/7 commercial sites; 0-29/30 oysters/site	IFA	[59]
Galicia, Spain	*Dosinia exoleta* (Rayed artemis) *Venerupis (Ruditapes) rhomboideus* (Carpet shell clam) *Venerupis (Ruditapes) pullastra* (Venus clam) *Venus verrucosa* (Mediterranean common clam) *Mytilus galloprovincialis* (Mediterranean mussel) *Ostrea edulis* (Native, common or flat oyster)	15/29 shellfish	IFA	[60]
Italy	*Venerupis (Ruditapes) phillipinarum* (Japanese littleneck)	5/8 clams	IFA	[60]
UK	*O. edulis*	1/1 oyster	IFA	[60]
Galicia, Spain	*M. galloprovincialis*	2/9 sites	IFA, B, M	[61]
	Cerastoderma edule (Common cockle)	1/9 sites	IFA, B, M	[61]
St Lawrence River, Canada	*Dreissena polymorpha* (Zebra mussel)	514 pooled mussels; positive finding	IFA, M	[62]
Chesapeake Bay, USA	*C. virginica*	7 sites, 8 collections/site; all sites positive at each collection	IFA, B, M	[1]
Atlantic coast, USA	*C. virginica*	24/37 sites	IFA, B, M	[38]
Portugal	*C. edule*	4/26 cockles	IFA	[63]
	Scrobicularia plana (Soft shell clam)	1/10 clams	IFA	[63]
	M.edulis	3/32 mussels	IFA	[63]
	Venerupis (Tapes) decussates (Carpet shell clam)	3/18 clams	IFA	[63]
	Donax sp. (Donax clam)	2/5 clams	IFA	[63]

International Journal of Food Microbiology 171 (2014) 62–67

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Cryptosporidium parvum genotype IIa and *Giardia duodenalis* assemblage A in *Mytilus galloprovincialis* on sale at local food markets

Annunziata Giangaspero [a,*], Roberto Papini [b], Marianna Marangi [a], Anson V. Koehler [c], Robin B. Gasser [c]

- *C. parvum* IIa (subgenotypes IIaA15G2R1, IIa15G2 and IIaA14G3R1)- 66.7%
- *G. duodenalis* assemblage A -60%

Cilj istraživanja

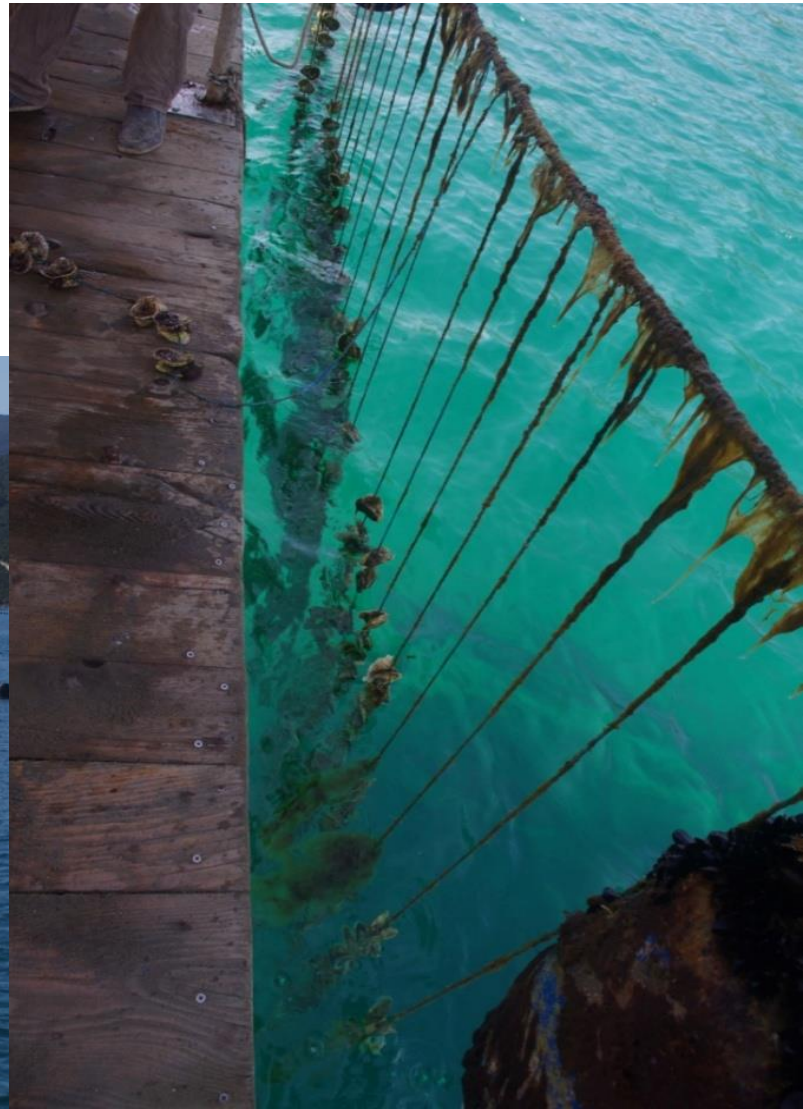
- Odrediti prisutnost gijardija i kriptosporidija u komercijalnim uzgojima školjaka
- Istražiti prisutnost zoonotskih vrsta/izolata
- Procijeniti rizik

Uzorci

- *G. duodenlis*- u 1050 uzoraka (500 dagnji 550 kamenica)
- *Cryptosporidium* spp.-693 uzorka (310 dagnji i 381 kamenica)
- Uzorak- 3 do 5 probavnih žlijezda

Istraživanje

- Uzorci su prikupljeni sa više različitih lokacija



Molekularna

TPI gen
Giardia

18S rRNA
Cryptosporidium

DNAekstrakcija

PCR

Kapilarna elektroforeza

Pročišćavanje

sekvenciranje

BLAST

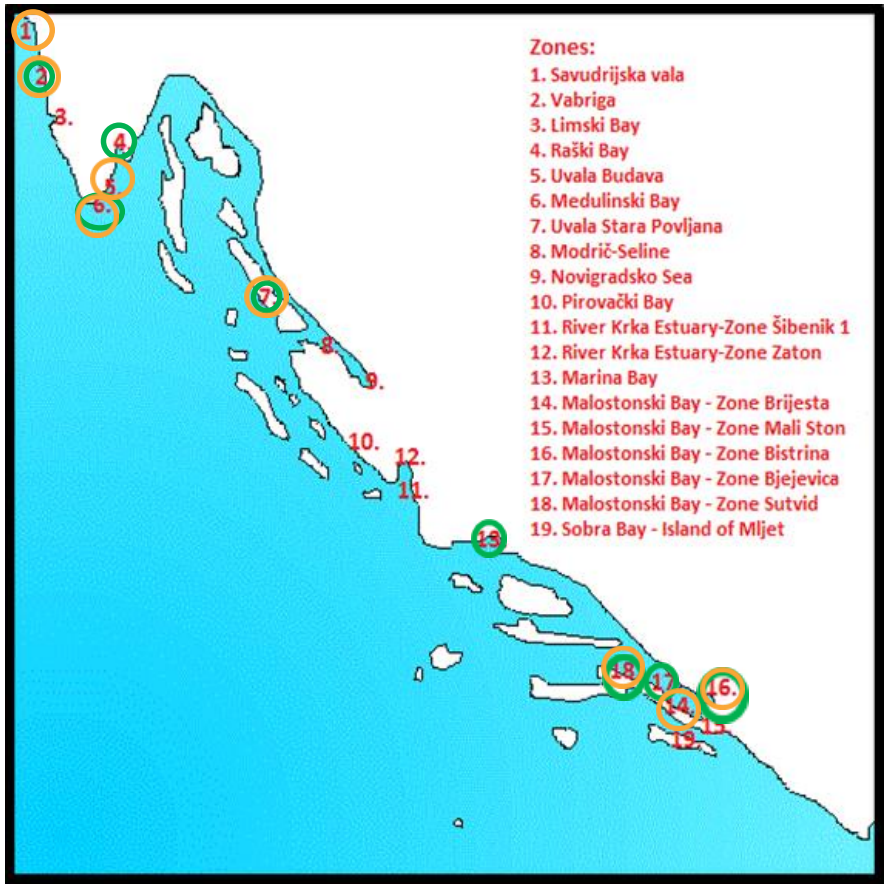
Rezultati- *G. duodenalis* 7%

Skupina AI	• 1.4% dagnji • 2.7% kamenicama
Skupina B	• 0.8% dagnji • 0.4% kamenica
Skupine C i E	• 0,8% i 0,2% dagnji • 0,4% i 0,7% kamenica

- Kamenice učestalije invadirane (4,2%) od dagnji (3,2%)

Rezultati- *G. duodenalis*

Dagnje	Oznaka	Number of samples	*G. duodenalis* +	% prevalence
Vabriga	2	50	1	2
Raški zaljev	4	50	2	4
Medulinski zaljev	6	50	1	2
Uvala Dinjiška, Pag	7	50	1	2
Marinski zaljev/Uvala Maslinova Brač	13	100	5	5
Bistrina, Malostonski zaljev/Mali Ston	16	100	3	3
Bjejevica, Malostonski zaljev	17	50	1	2
Sutvid, Malostonski zaljev	18	50	2	4
Ukupno		500	16	3,2
Kamenice				
Savudrijska vala	1	50	1	2
Vabriga	2	50	1	2
Uvala Budava, Pula	5	50	2	4
Medulinski zaljev	6	50	3	6
Lukar projekt, Pag/Pag Stara Povjana/	7	150	4	3
Brijesta, Malostonski zaljev	14	50	3	6
Bistrina, Malostonski zaljev/Mali Ston	16	100	6	6
Sutvid, Malostonski zaljev	18	50	3	6
Ukupno		550	23	4,2

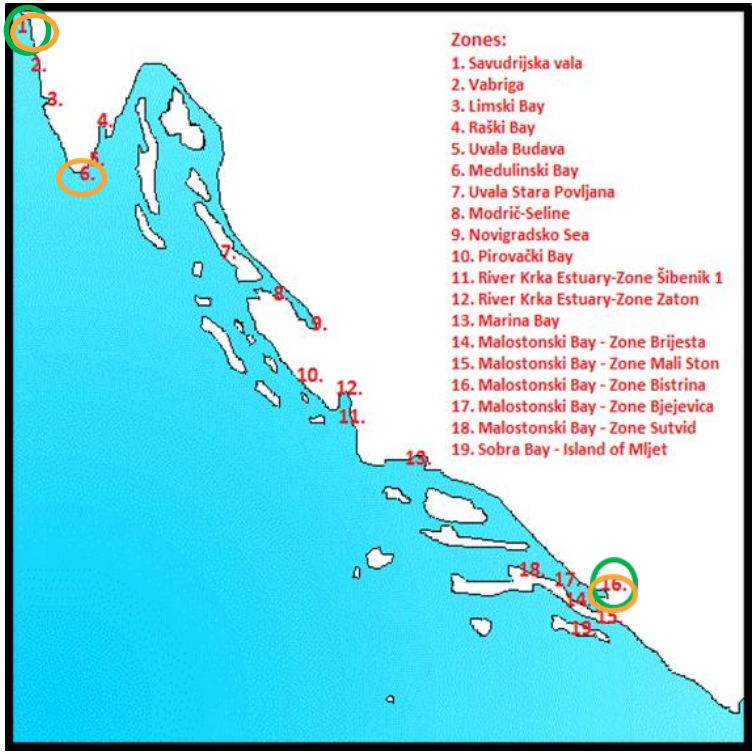


Positive mussel samples – green; Positive oyster samples – yellow;
Nr. 7 – one sampling area but 4 sampling places

Rezultati - *C. hominis* 2%

Dagnje	Sampling place mark	Number of samples	*C. hominis* +	%
Savudrijska vala	1	40	1	2,5
Limski zaljev	3	16	0	0
Marčana	4	8	0	0
Istočna obala Istre/Uvala Budava	5	33	0	0
Medulinski zaljev	6	8	0	0
Pag (Stara Povljana)	7	32	0	0
Modrič Seline	8	16	0	0
Novigrad/Zaton	9	16	0	0
Pirovački zaljev	10	32	0	0
Ušće rijeke Krke	12	8	0	0
Marinski zaljev	13	8	0	0
Malostonski zaljev	16	93	1	1,08
Ukupno		310	2	0,65

Kamenice	Sampling place mark	Number of samples	*C. hominis* +	%
Savudrijska vala	1	64	2	3,13
Limski zaljev	3	38	0	0,00
Istočna obala Istre/Uvala Budava	5	16	0	0,00
Medulinski zaljev	6	39	3	7,69
Ušće rijeke Krke	12	8	0	0,00
Marinski zaljev	13	16	0	0,00
Malostonski zaljev	16	194	7	3,61
Ukupno		375	12	3,2



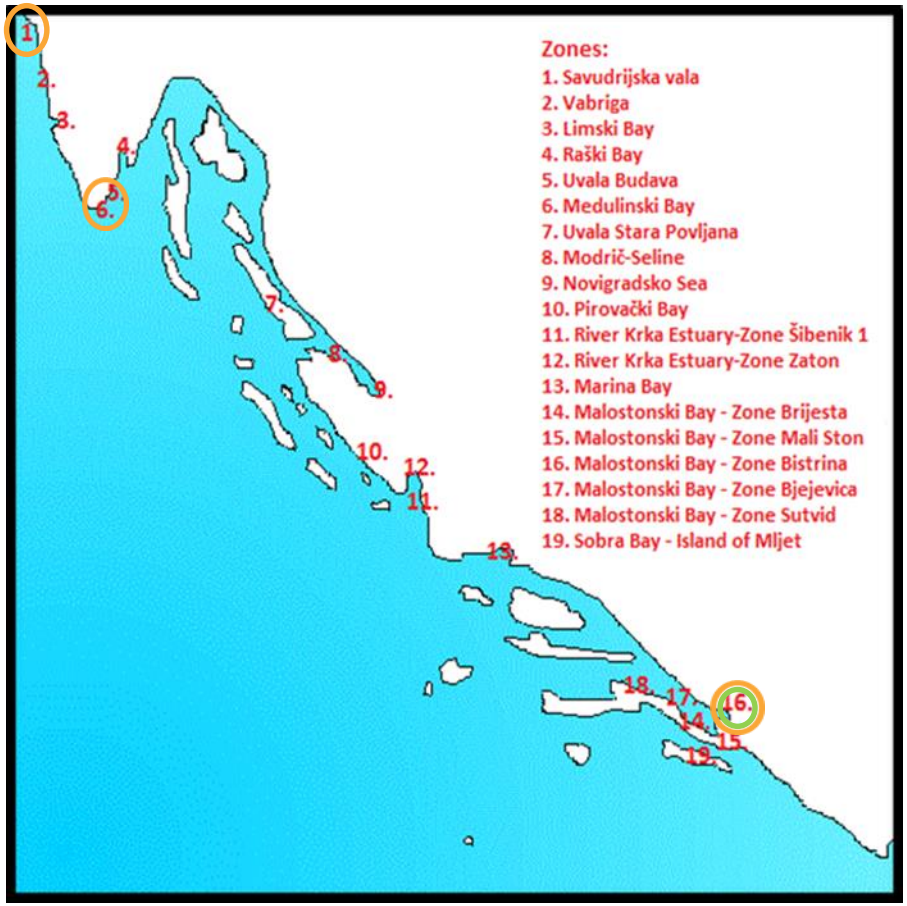
Positive mussel samples – green; Positive oyster samples – yellow; Nr. 7 – one sampling area but 4 sampling places

3,1% (12/383) kamenica i 0,6% (2/310) dagnji

Oba parazita

Dagnje	Sampling place mark	Number of samples	*G. duodenalis & C. hominis poz.*	%
Bistrina, Malostonski zaljev/Mali Ston	16	193	3	1,55

Kamenice	Sampling place mark	Number of samples	*G. duodenalis & C. hominis poz.*	%
Savudrijska vala	1	114	3	2,63
Medulinski zaljev	6	89	6	6,74
Malostonski zaljev	16	244	10	4,10



Positive mussel samples – green; Positive oyster samples – yellow

Različiti izvori “kontaminacije”

- Ljudi- *Cryptosporidium homis*, *G. duodenalis* AI
- Preživači- *G. duodenalis* (E)
- Kanidi – *G. duodenalis* (C)
- Učestalije u kamenica (4.2%:3.2%- *Giardia*; 3.2%:0.65%- *Cryptosporidium*)

Zaključak

- Školjke su potencijalni izvor invazije za ljude, no koliko često ljudi oboljevaju tek treba istražiti
- Kamenice učestalije invadirane
- Provesti molekularna istraživanja gijardija u ljudi, a kriptosporidija u ljudi i životinja
- Koristiti osjetljivije metode dokazivanja

Purifikacija školjki 48h

Hvala na pažnji