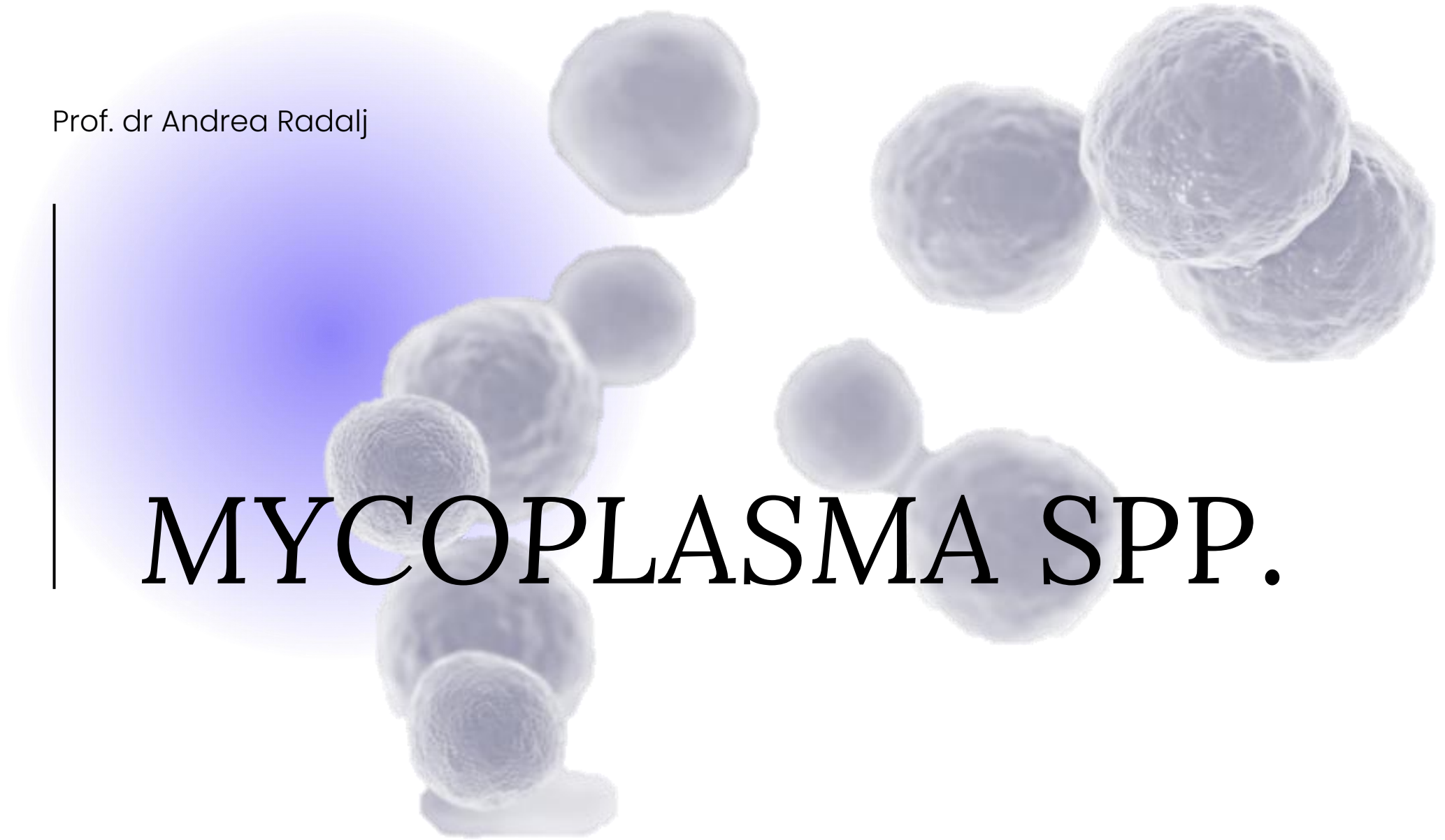
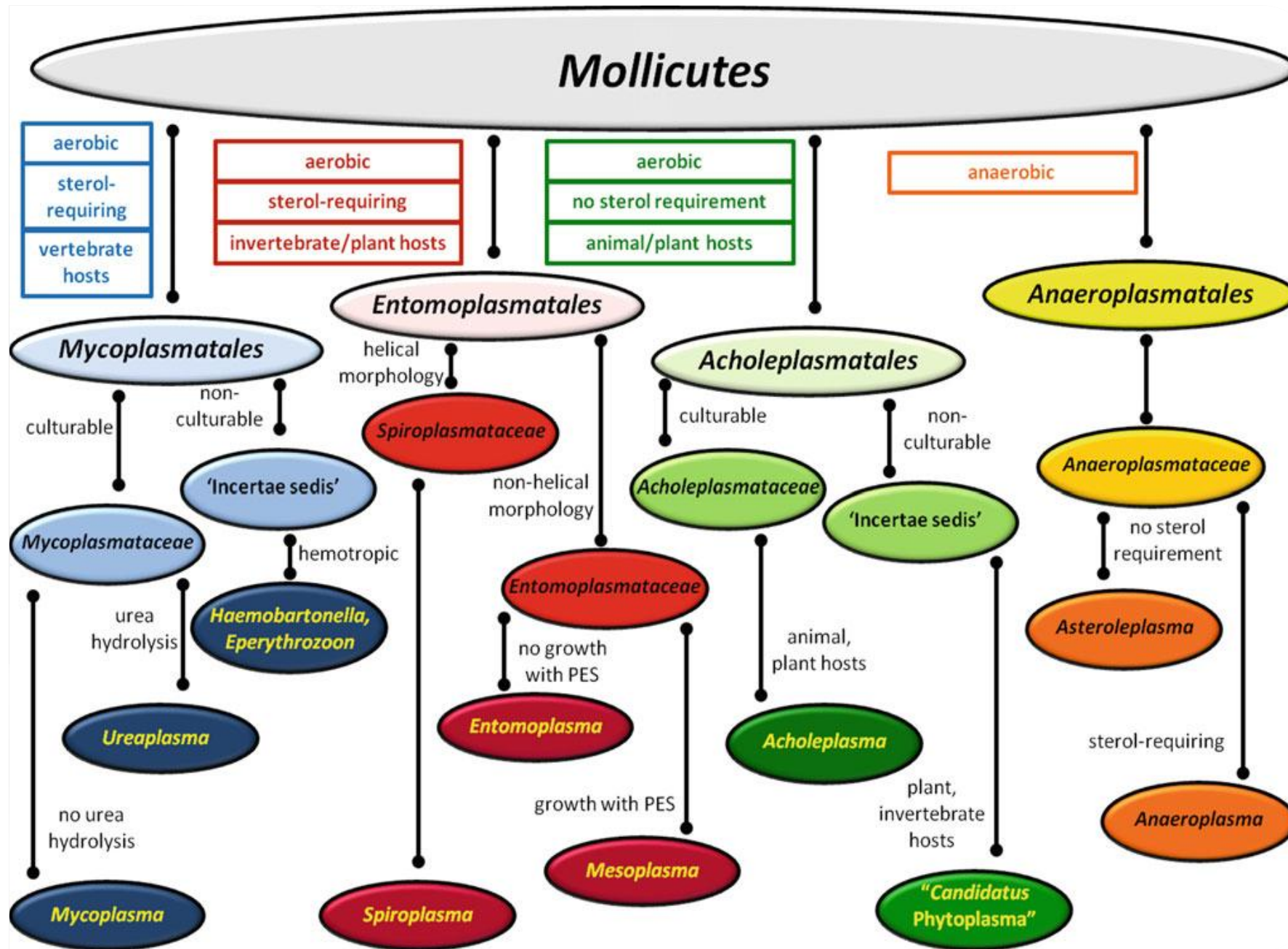


Prof. dr Andrea Radalj

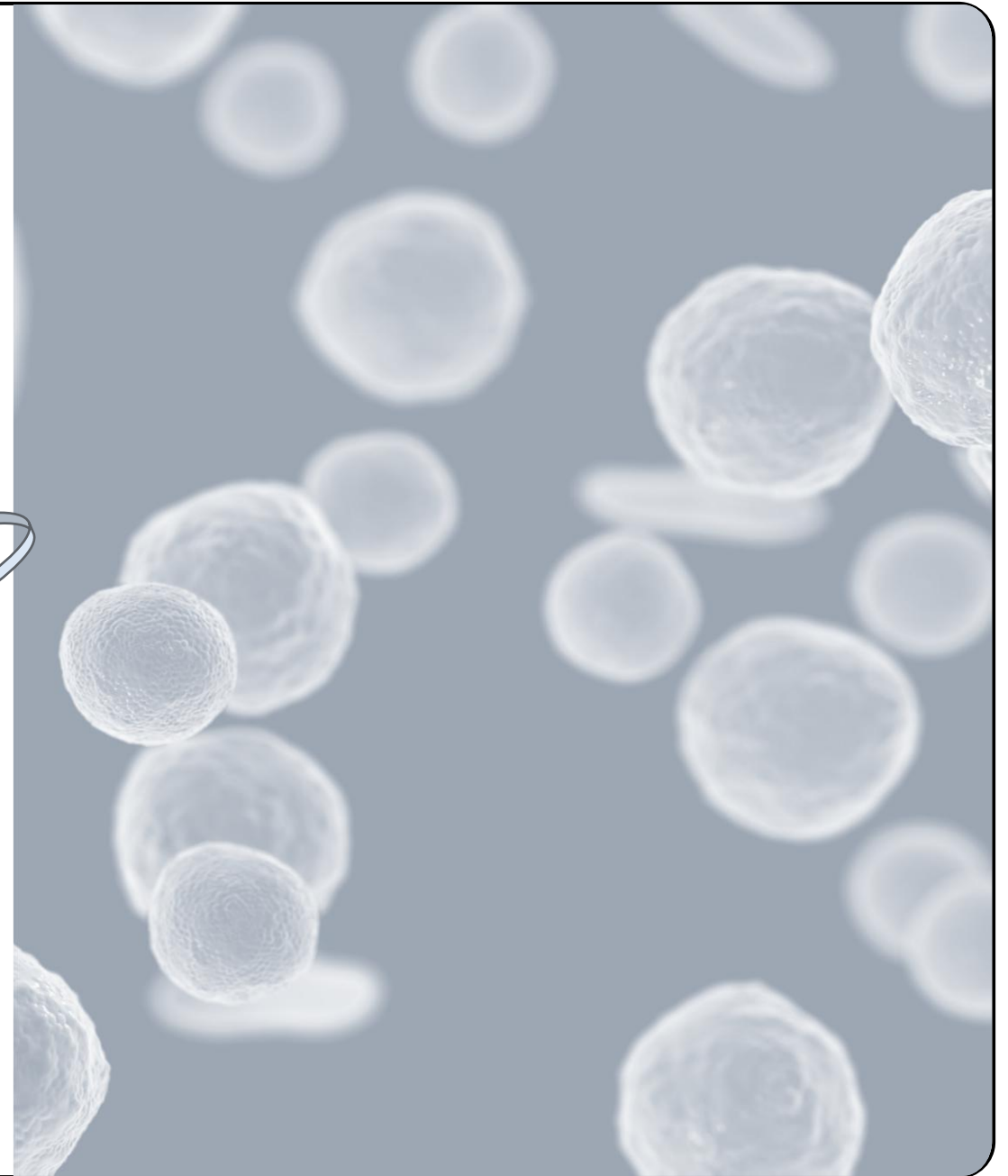
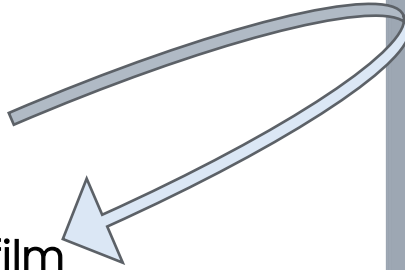
MYCOPLASMA SPP.

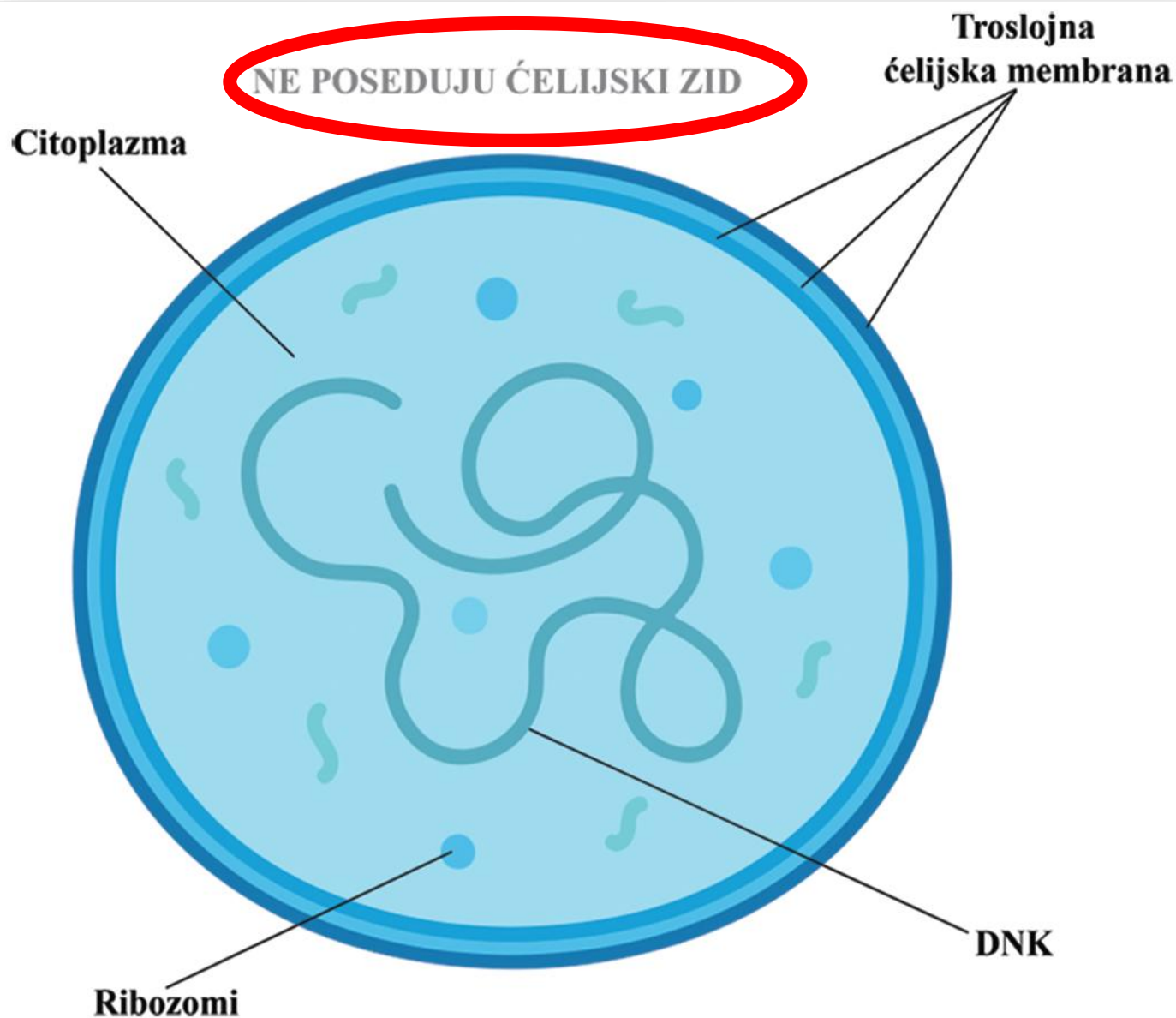




- Bakterije, familija *Mycoplasmataceae*
- Patogene vrste klasifikovane u dva roda: *Mycoplasma* i *Ureaplasma*
- Hemotrofne vrste mikoplazmi (hemoplazme) rodovi *Haemobartonella* i *Eperythrozoon*

- Najsitniji prokariotski organizmi
- Poseduju troslojnu citoplazmatsku membranu
- **Nemaju ćelijski zid**
- Ne boje se Gramu
- Pleomorfni i filterabilni, imaju plastičnu formu
- Specifično adaptirane za određenu vrstu
- **Perzistentne infekcije**
- Antigenska varijabilnost, neke vrste - biofilm
- Ne umnožavaju se u spoljašnjoj sredini
- Veoma su osetljive na isušivanje, toplotu i hemijska sredstva





MORFOLOŠKE I TINKTORIJALNE OSOBINE

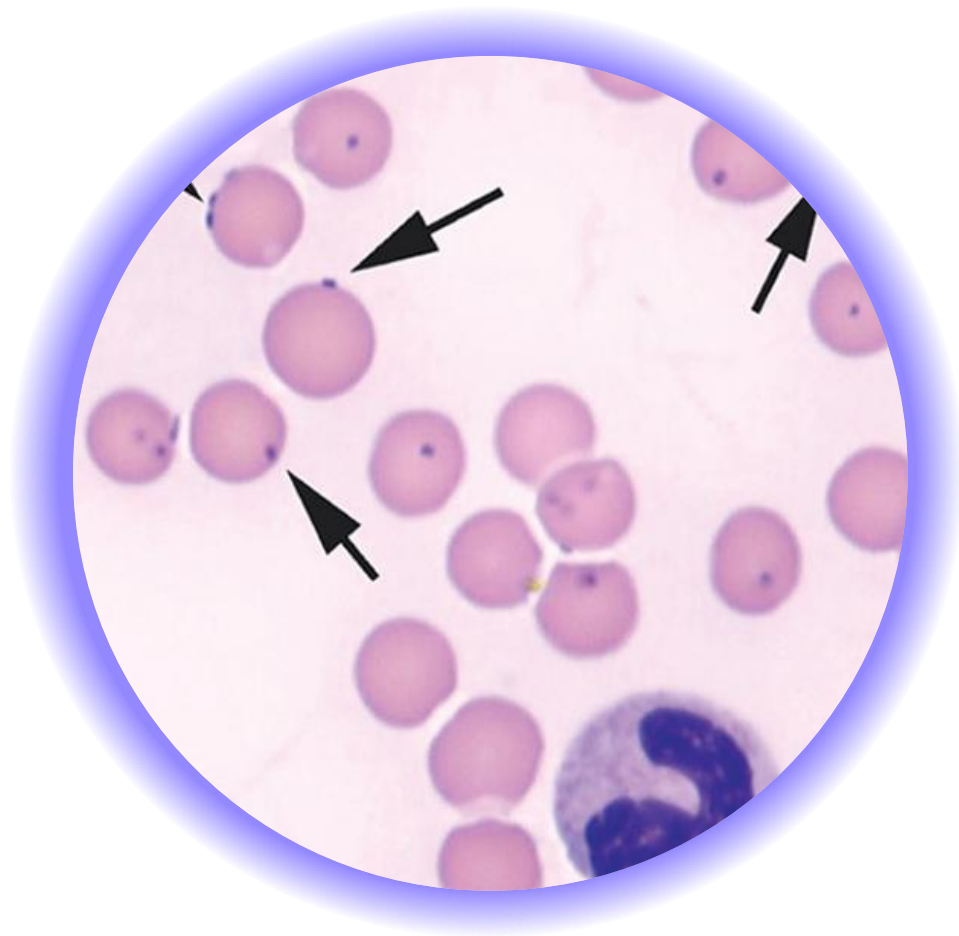
Mikoplazme – pleomorfne ili sferične

Jako sitne – $0,3\ \mu\text{m}$ do $0,8\ \mu\text{m}$ –
prolaze kroz filtere najsitnijeg
promera – problem, kontaminacija u
laboratoriji

Troslojna membrana od proteina,
glikoproteina, lipoproteina, fosfolipida
i sterola

Holesterol – osmotska stabilnost

Bojenje – metode Giemsa,
Castaneda..

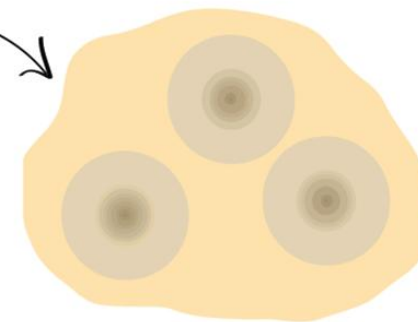
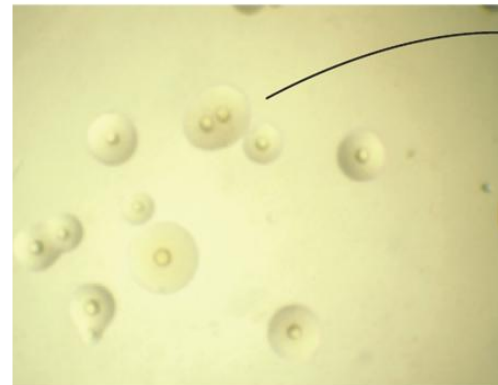


- Hemoplazme – krvni razmazi
- Ranije su bile svrstane u rodove *Haemobartonella* i *Eperythrozoon*.
- Sferične forme na površini eritrocita
- Bojenje po Giemsi, Romanovskom, Diff Quik
- Inficiraju eritrocite i izazivaju hemolitičnu anemiju
- Predisponirajući faktori: stres i imunosupresija
- Prenos putem krvi i ektoparazita
- Ne rastu na standardnim hranljivim podlogama
- Klinički znaci: ikterus, splenomegalija, hiperplazija koštane srži
- Važne vrste:

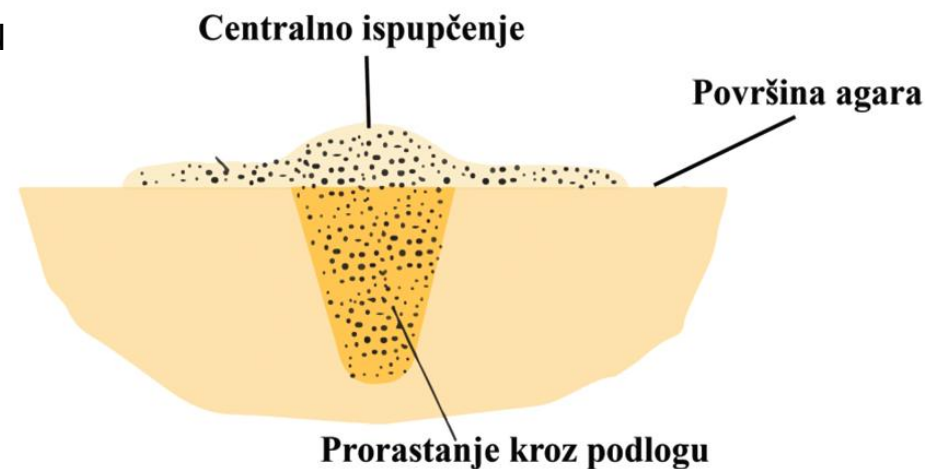
M. haemofelis, M. wenyonii, M. haemocanis

KULTURELNE OSOBINE

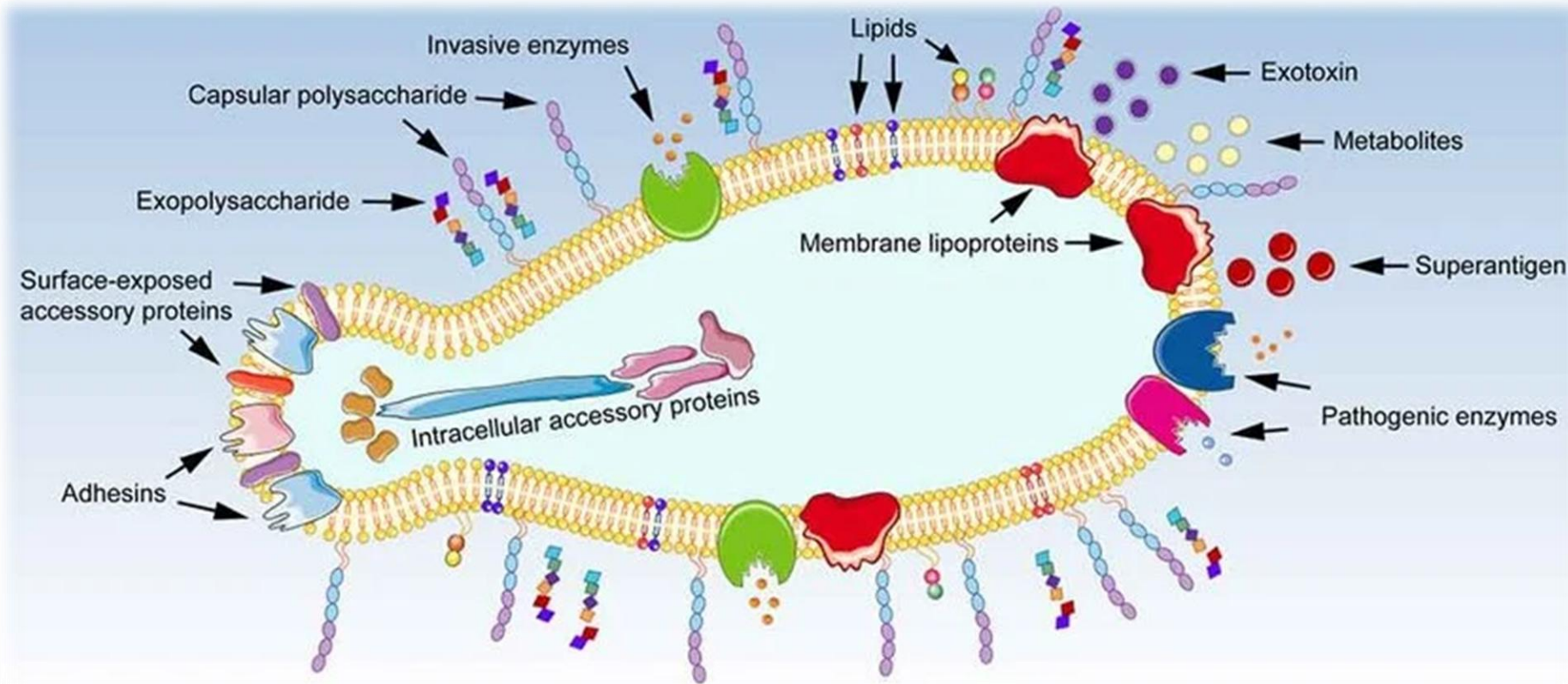
- Mikoplazme su veoma zahtevni mikroorganizmi
- Za izolaciju zahtevaju specifične faktore rasta i izotoničnu sredinu
- Dodaju se konjski serum, ekstrakt kvasca, DNK i nukleotidi
- Selektivnost se postiže penicilinom i talijum-acetatom
- Optimalan pH podloge je 7,2–7,6
- Inkubacija se vrši na 37°C, uz visoku vlažnost
- Većina vrsta raste aerobno, a neke zahtevaju 5–10% CO₂
- **Kolonije se pojavljuju za 2–4 dana i liče na „jaje na oko”**
- Uzorak je negativan ako nema rasta nakon 14 dana



Kolonije izgledaju kao jaje “na oko”



FAKTORI VIRULENCIJE



Yiwen, C., Yueyue, W., Lianmei, Q., Cuiming, Z., & Xiaoxing, Y. (2021). Infection strategies of mycoplasmas: Unraveling the panoply of virulence factors. *Virulence*, 12(1), 788–817. <https://doi.org/10.1080/21505594.2021.1889813>

Izražena specifičnost prema domaćinu

Teže infekcije kod imunokompitovanih jedinki: klinička slika: od blage do septikemične

Kolonizacija sluznica je prvi korak infekcije

Ciliostaza pogoduje širenju infekcije

Pogoduju sekundarnim bakterijskim i virusnim infekcijama

Izbegavaju imunski odgovor domaćina

Dovode do oštećenja tkiva – delovanjem metabolita, enzima i zapaljenske reakcije

INFEKCIJE GOVEDA

- Kontagiozna pleuropneumonija goveda – zarazno respiratorno oboljenje
- Afrika
- M. mycoides subsp. mycoides*
- Prenosi se aerosolom i direktnim kontaktom
- Visoka temperatura, anoreksija, otežano disanje, kašalj i iscedak iz nosa
- Na obdukciji pluća imaju karakterističan **mramoriran** izgled – eksudata u interlobularnim septama
- Supklinički inficirane životinje – važan izvor infekcije u stadu



INFEKCIJE GOVEDA

Table 1: Major signs of Mycoplasma infection

Cows	Calves
Mastitis	Pneumonia
Often in multiple quarters	Acute and severe
Not treatable	Poor response to treatment
Higher cull rate of mastitis cows	May affect very young calves (<2 weeks of age)
Lameness	Lameness
Diffuse swelling of joints/leg	Diffuse swelling of joints/leg
One or more joints/limbs affected	One or more joints/limbs affected
Pneumonia	Head tilt

Source: Dairy Australia

- *M. bovis* – mastitis
- Dramatičan pad mlečnosti, često vodi do potpunog prestanka laktacije
- Često se javlja zajedno s upalom zglobova (artritisom) ili upalom pluća u stadu
- *M. bovis* i *M. dispar* respiratorne infekcije teladi

MYCOPLASMA

DESCRIPTION



Gram-negative, mostly facultative anaerobic, pleomorphic bacteria

Mycoplasma bovis - most pathogenic species to affect cattle, causes bovine mastitis

DISEASE



Mycoplasma mastitis - decreases milk production, requires culling

Highly contagious with few clinical signs and asymptomatic carriers

Transmitted through milk, bodily fluids, direct contact, sometimes airborne

DIAGNOSIS



From milk samples; PCR 4 hours or culture 10 days

FOODS AFFECTED



milk and dairy products

INFECTION CONTROL



Routine testing of milk, animals, and the bulk tank

If detected, isolate animals, maintain hygiene, avoid overcrowding, screen replacement cows, consider culling

No effective treatment

INCIDENCE

6.8-20% of dairy bulk tanks are *Mycoplasma*-positive

COST

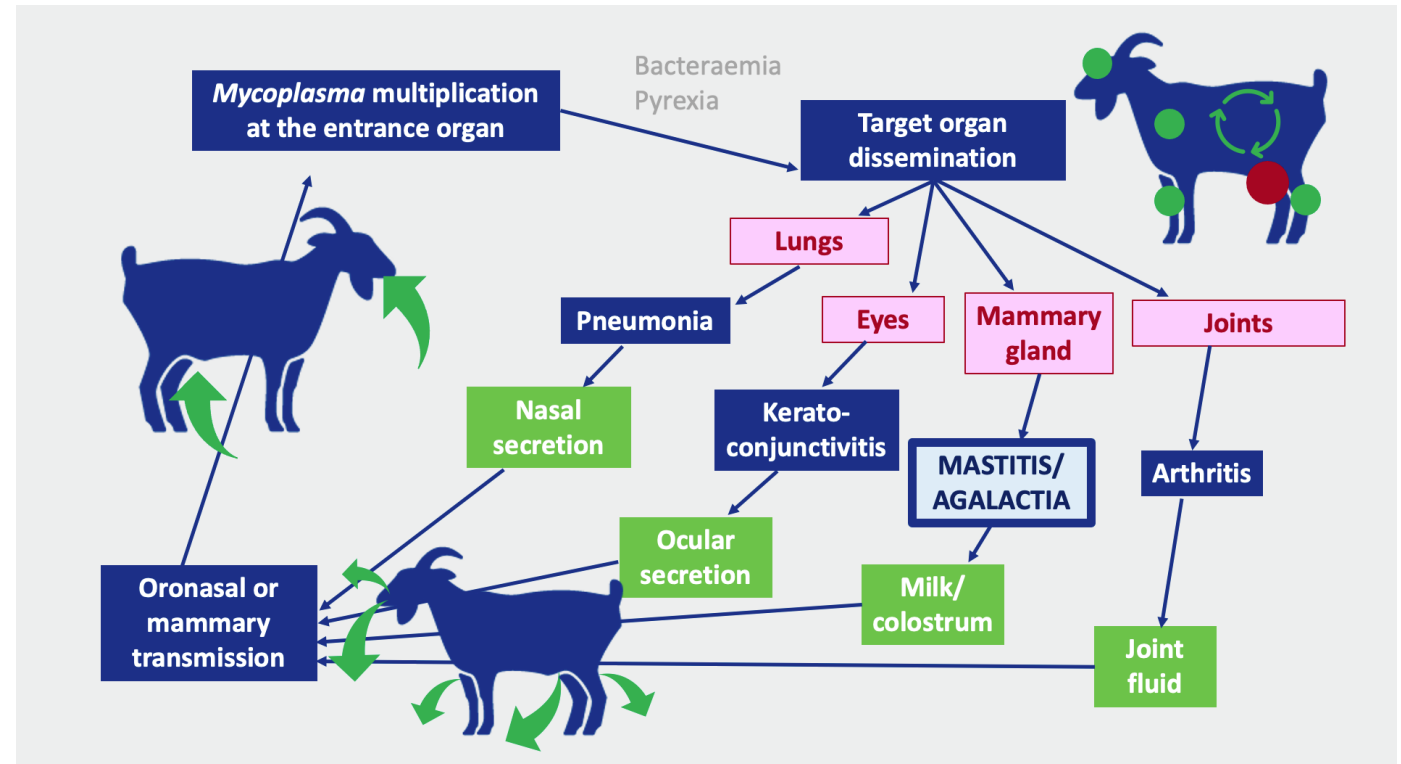


costs US dairy industry >\$100M/year

Source: shutterstock, "Rosengarten R. Citti C. The role of ruminant mycoplasmas in systemic infection. In: Stipkovits L, Rosengarten R. Frey J, eds. Mycoplasmas of Ruminants: Pathogenicity, Diagnostics, Epidemiology and Molecular Genetics. Brussels: European Commission;1999:14-17

INFEKCIJE OVACA I KOZA

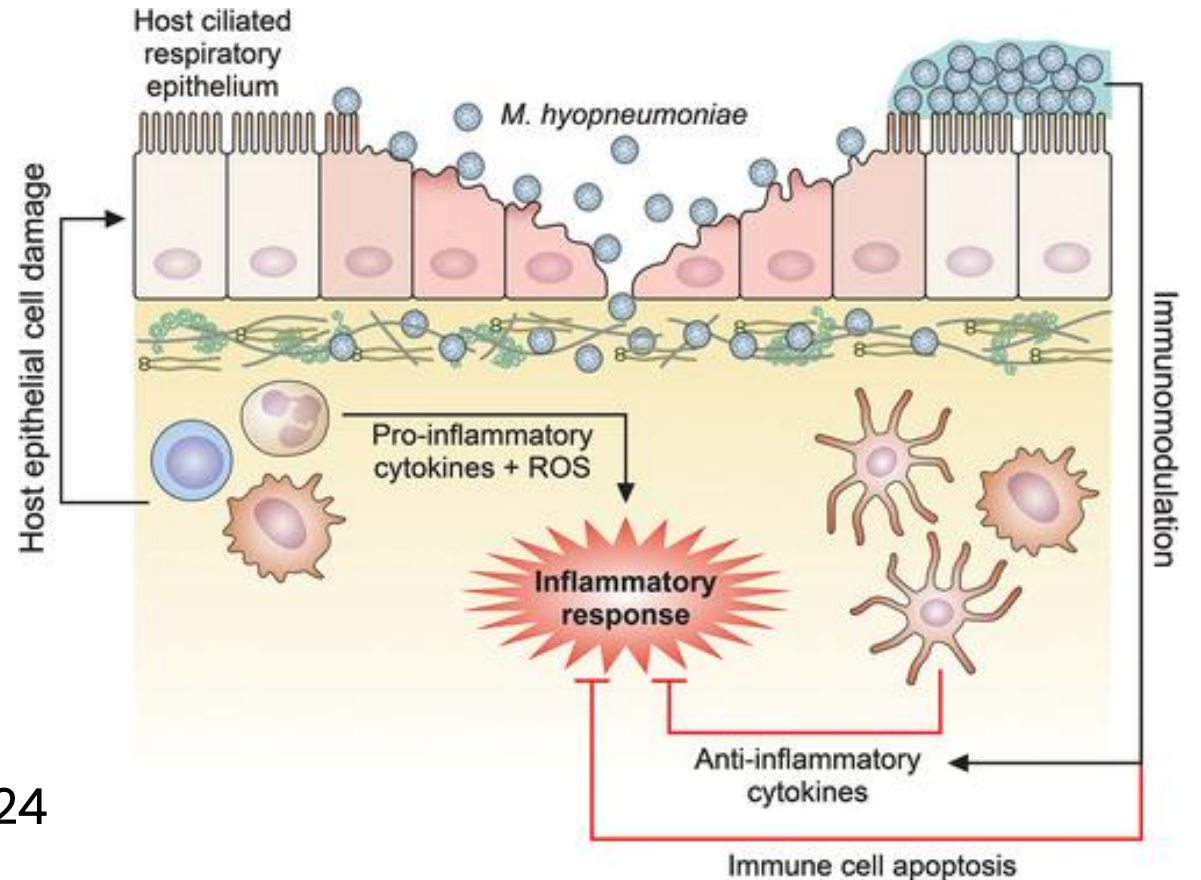
- **Koze:** pneumonija, pleuropneumonija, mastitis, artritis, septikemija
- *M. mycoides*, *M. capricolum subsp. capripneumoniae*, *M. agalactiae*
- Teže infekcije prate visok mortalitet, naročito kod mladih
- **Ovce:** obično blaža klinička slika
- *M. ovipneumoniae* – pneumonija
- *M. capricolum subsp. capripneumoniae*
- *M. agalactiae*



<https://aboutsmallruminants.com/contagious-agalactia-udder/>

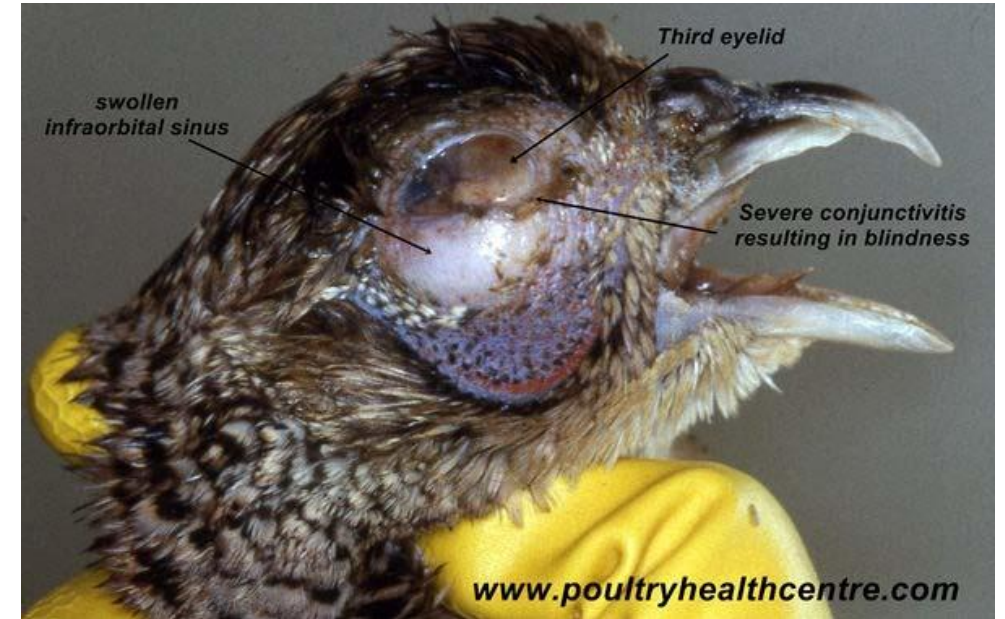
INFEKCIJE SVINJA

- *M. hyopneumoniae* je uzročnik enzootske pneumonije svinja.
- Oboljenje je praćeno visokim morbiditetom i niskim mortalitetom.
- Klinički – hronični neproduktivni kašalj
- Prisutan je i smanjen dnevni prirast.
- *M. hyosynoviae* izaziva artritis svinja.
- Artritis se najčešće javlja kod svinja uzrasta 12–24 meseca.



INFEKCIJE ŽIVINE

- *M. gallisepticum* – respiratorne infekcije kokošaka i sinuzitis čuraka
- Klinički znaci: kašalj, nosni iscedak, otok sinusa
- Mogući su pad nosivosti i promene na zglobovima
- Vertikalna transmisija (preko jaja).
- *M. synoviae* – infekcije zglobova, tetivnih ovojnica i vazdušnih kes

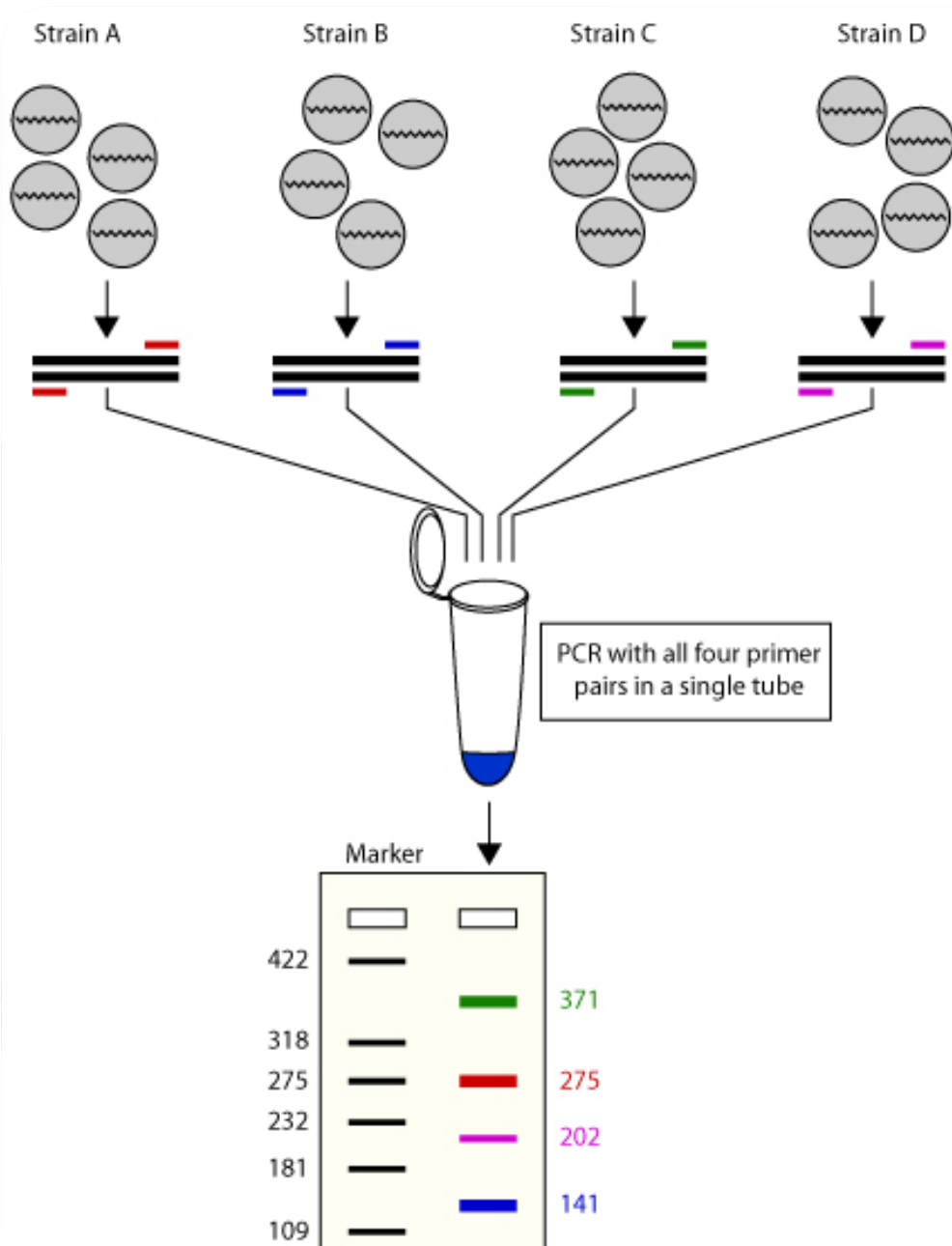


A photograph showing a white, rectangular package of Amies transport medium. The text "Amies transport medium" is printed in blue on the package. Below the package, a blue and white swab is visible, with a label that includes fields for "NAME", "DATE", "HOUR", and "USE".

Amies transport medium

UZORKOVANJE MATERIJALA

- Veoma osetljive u spoljašnjoj sredini
- Uzorke čuvati na 4°C
- Brz transport do laboratorije
- Uzorci: mleko, brisevi, aspirati, trahealni eksudat ..krv (hemoplazme)
- Kod uginulih: pluća i zglobovi
- Brisevi u Amies podlozi



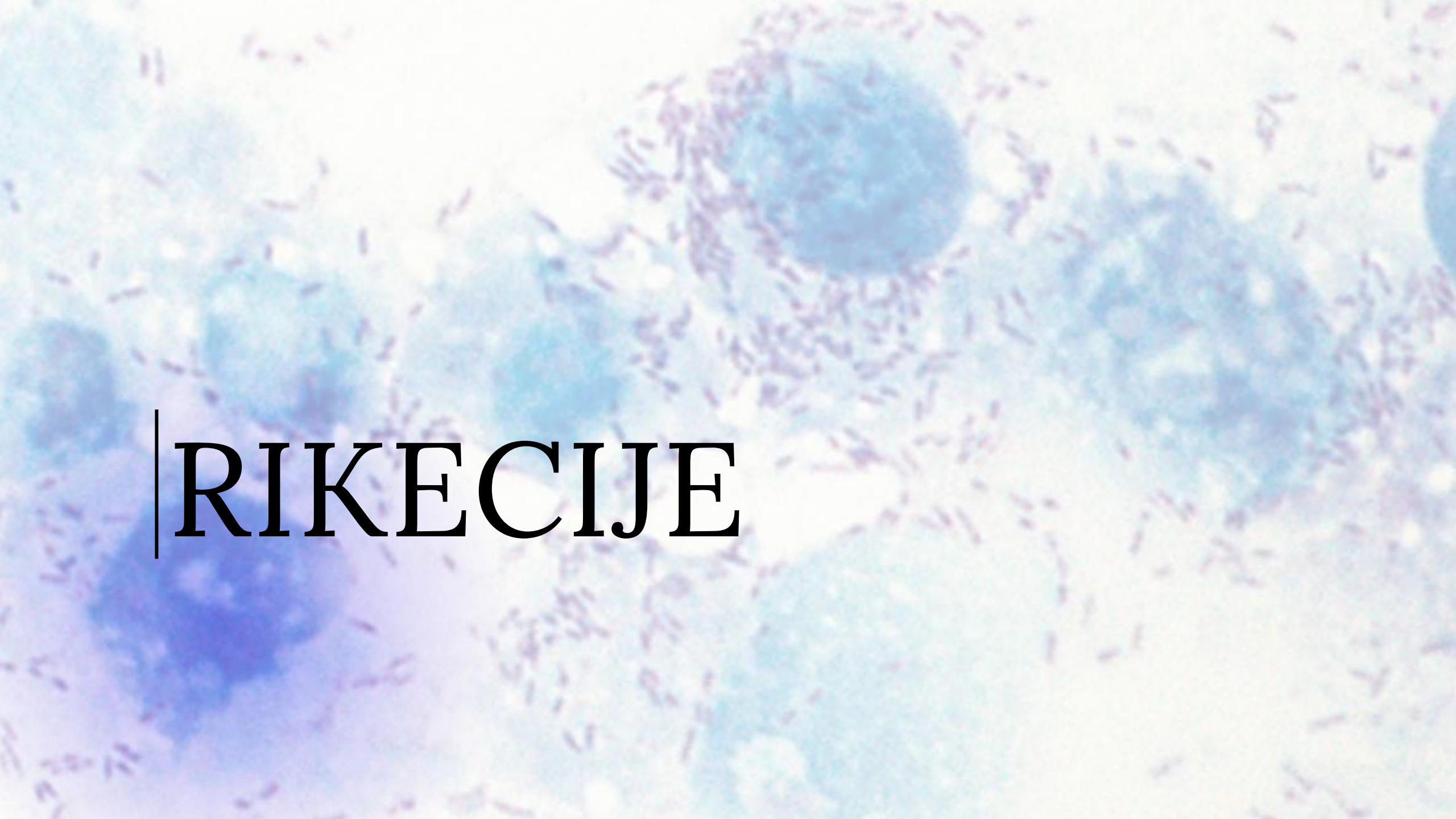
LABORATORIJSKA DIJAGNOSTIKA

- Klinička slika i vrsta domaćina ukazuju na mogućeg uzročnika
- Precizna identifikacija zahteva laboratorijske metode
- Klasične metode: biohemijska ispitivanja
- Izolacija čiste kulture je dugotrajna i zahtevna
- **Brže metode: direktna imunofluorescencija i PCR**
- Serološke metode: aglutinacija, HI, AGID, RVK, ELISA
- Multiplex PCR otkriva više vrsta istovremeno

TERAPIJA ANTIBIOTICIMA

- Nemaju ćelijski zid
- Intrinzično su rezistentne na β -laktame i glikopeptide
- Osetljive su na antibiotike koji deluju na sintezu proteina i nukleinskih kiselina
- Terapija: tetraciklini, makrolidi, linkozamidi, aminoglikozidi, fluorohinoloni
- Mogu razviti i stečenu rezistenciju





|RIKECIJE

Rickettsiales

Sitne, pleomorfne Gram-negativne bakterije

Uzorci krvnih razmaza - Giemsa bojenje

Peptidoglikan - ćelijski zid

Anaplasmataceae - citoplazmatična
membrana bez ćelijskog zida

Tropizam:

-*Rickettsiaceae* vaskularni endotel ili leukociti

-*Anaplasmataceae* eritrociti i leukociti

Obligatni intracelularni patogeni

Sistemska oboljenja životinja i ljudi

Najčešće se prenose putem vektora-artropoda

Inokulacija u embrionirana jaja i kulture tkiva,
imunofluorescencija, PCR



Rickettsia

R. rickettsii

Tačkasta groznica

Stenovitih planina

Anaplasma

A. bovis

A. marginale

A. ovis

A. phagocytophilum

A. platys

Ehrlichia

E. canis

E. ewingii

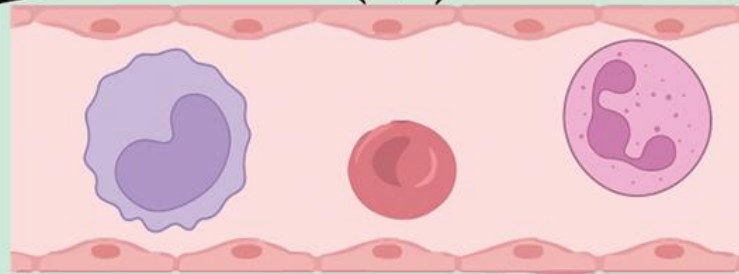
E. ruminatum

Neorickettsia

N. risticii

Aegyptianella

A. pullorum



RIKECIJE

- Prirodni rezervoari rikecija su životinje i artropode (krpelji), ponekad metilji
- U artropodama se rikecije razmnožavaju u epitelu digestivnog trakta.
- Zatim se šire u pljuvačne žlezde i reproduktivni sistem.
- Održavaju se u populaciji vektora transovarijalnim i transstadijalnim prenošenjem.
- **Transovarijalna transmisija** je prenos patogena sa ženke na potomstvo putem jaja.
- **Transstadijalna transmisija** je održavanje patogena kroz razvojne stadijume artropode
- Rikecije se prenose ujedom ili ubodom artropoda
- Rasprostranjenost bolesti zavisi od geografske rasprostranjenosti vektora.

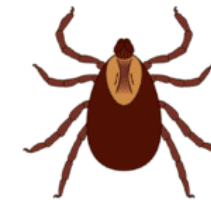
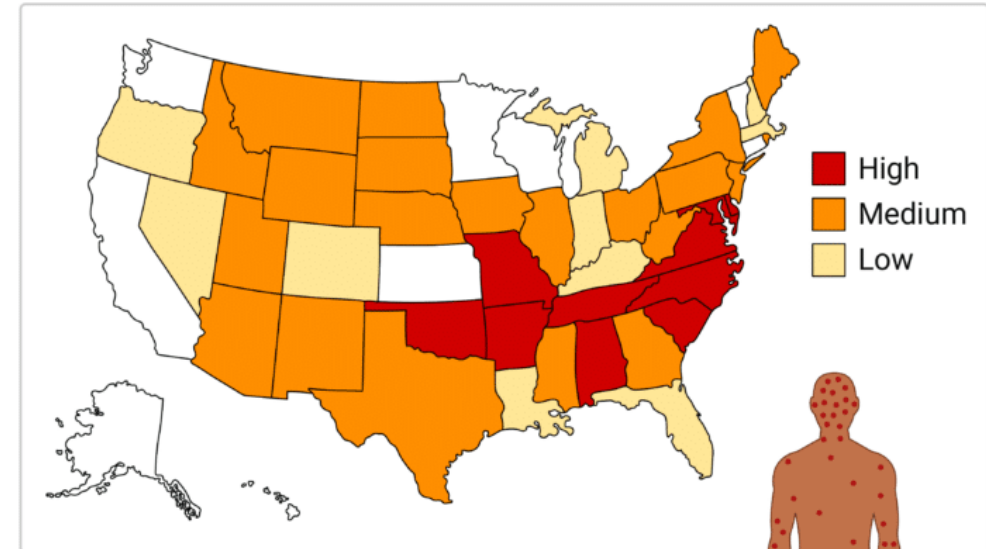
VIRULENCIJA - VRSTE

Rikecije poseduju različite faktore virulencije (toksine, hemolizine i endotoksine)

- ***Rickettsia rickettsii*** je uzročnik tačkaste groznice Stenovitih planina (ljudi i psi)
- U Severnoj i Južnoj Americi, vektori su krpelji (*Dermacentor* spp.)
- Proizvodi fosfolipazu – izlazak iz fagozoma
- Tropizam prema **endotelu krvnih sudova**: osip, temperatura, neurološki simptomi
- .Oštećenje endotela dovodi do krvarenja, tromboze, edema i nekroze krvnih sudova

Rocky Mountain Spotted Fever

Rickettsia rickettsii



Dermacentor
(dog tick, wood tick)



Rumpel-Leede phenomenon
Petechiae formation
after blood pressure
cuff inflation



Centripetal rash
(from ankles/wrists
to trunk)

Clinical

- Tick bite
- Abrupt onset of symptoms
- Fever
- Headache
- Myalgias (calf tenderness)
- Rash (palms and soles)

Antibiotics

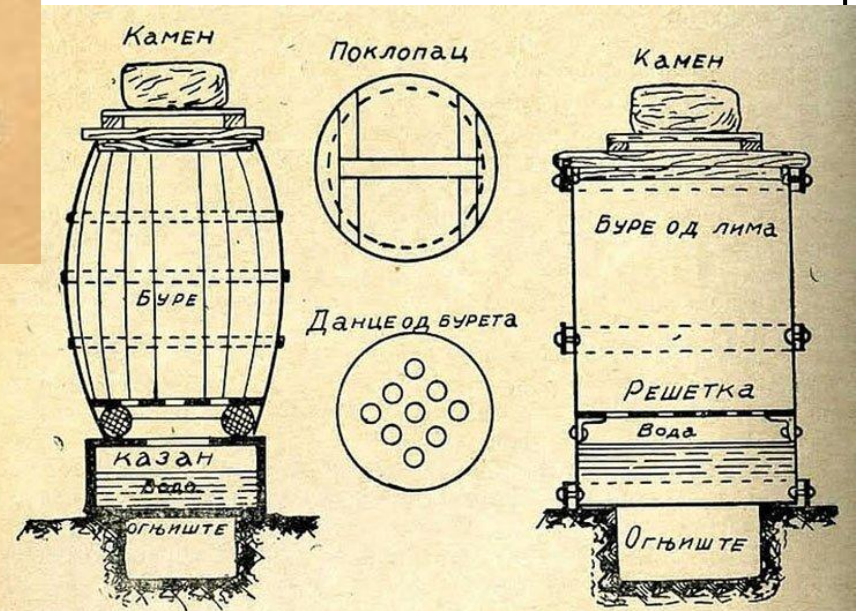
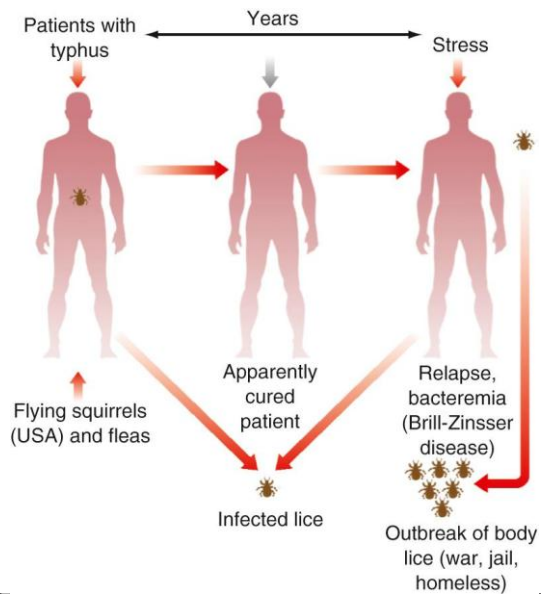
- Doxycycline
- Chloramphenicol



Levin ML, Killmaster LF, Zemtsova GE, Ritter JM, Langham G (2014)
Clinical Presentation, Convalescence, and Relapse of Rocky
Mountain Spotted Fever in Dogs Experimentally Infected via Tick
Bite. PLoS ONE 9(12): e115105.
<https://doi.org/10.1371/journal.pone.0115105>

<https://www.cdc.gov/rocky-mountain-spotted-fever/about/index.html>

R. prowazekii – pegavi tifus



Слика бр. 1.

Слика бр. 2.

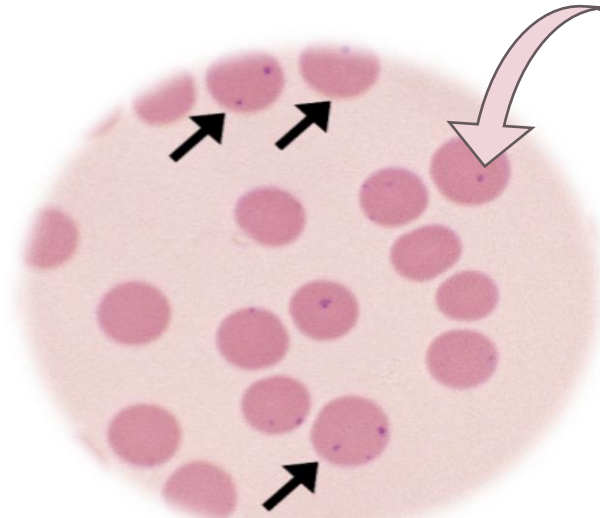
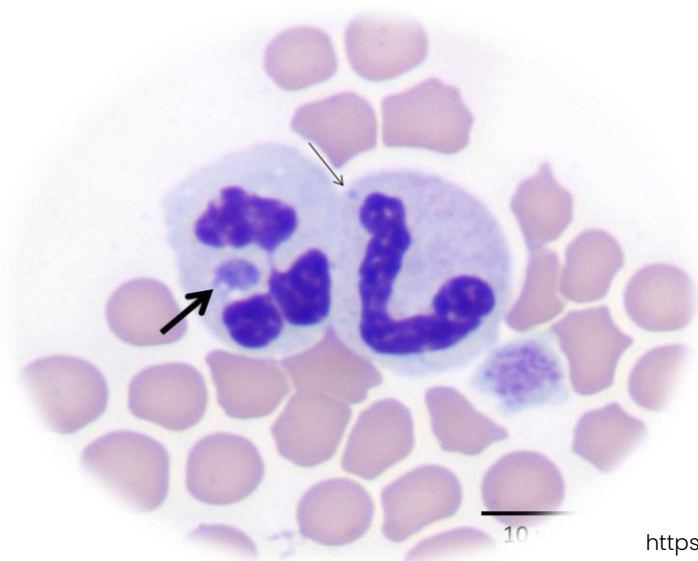
- *Anaplasma marginale* anaplazmoza preživara,

(*Ixodes*, *Rhipicephalus* – *Boophilus* i *Dermacentor* spp.) – **ERITROCITI**

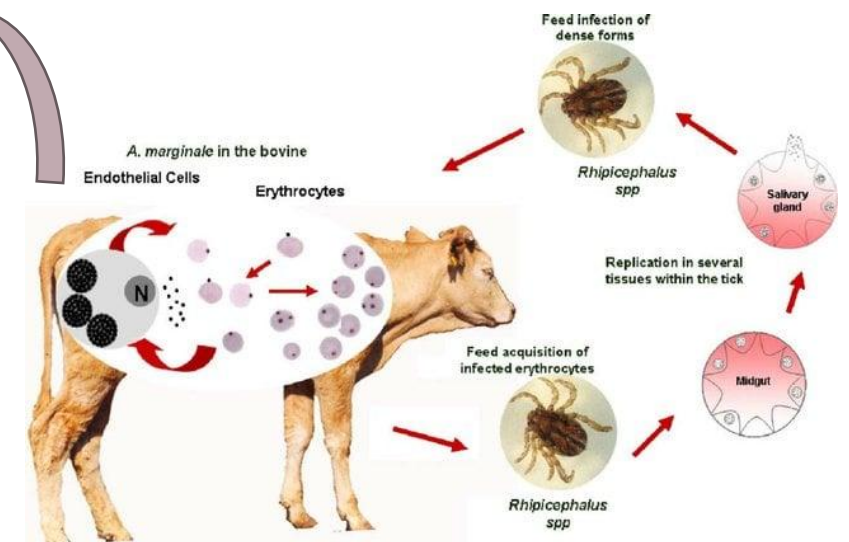
- *Anaplasma phagocytophilum* krpeljska groznica goveda i

granulocitna anaplazmoza konja i ljudi, (*Ixodes* spp.) – **GRANULOCITI**

Anaplasma ovis anaplazmoza malih preživara, (*Rhipicephalus* spp.) – **GRANULOCITI**



<https://vetclinpathimages.com/2018/01/08/anaplasma-marginale/>





OPEN ACCESS

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First detection of *Anaplasma phagocytophilum* and enzootic focus of *Theileria orientalis* in cattle from Bosnia and Herzegovina

Ana Vasić^{1*}, Goran Vasilčić², Andrea Radalj³, Isidora Prošić³, Jasna M. Kureljusić¹, Ratko Sukara⁴, Snežana Tomanović⁴ and Oliver Stevanović³

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Theileria orientalis is a tick-borne intraerythrocytic protozoan parasite of cattle causing oriental or benign theileriosis. It has a worldwide distribution and is now considered a zoonotic agent, while the disease symptoms range from subclinical to severe. *Theileria* spp. are very common co-infecting agents with other tick-borne pathogens such as *Babesia* spp., *Anaplasma* spp. including zoonotic *Anaplasma phagocytophilum* with rising prominence. This study is an exploratory investigation of the occurrence of *Anaplasma* spp. and other blood parasites in cattle from Gacko and Bileća, Herzegovina. Blood samples from 35 clinically healthy cattle were selected based on veterinary reports of tick infestation and examined microscopically, by PCR (*Babesia/Theileria*; *Anaplasmataceae* and qPCR (*A. phagocytophilum*, *A. platys*, *A. marginale*). Microscopy revealed intraerythrocytic bacterial forms in multiple samples. *A. phagocytophilum* DNA was detected in one and *Babesia/Theileria* DNA in 34 samples. Based on 16S rRNA gene sequencing, the *A. phagocytophilum* isolate was grouped with isolates from Europe, suggesting regional pathogen circulation. *AnkA* gene analysis placed the isolate in European group I, but outside the zoonotic subcluster, indicating a ruminant-associated lineage maintained primarily in wildlife–*Ixodes ricinus* cycles. The 18S rRNA gene sequencing showed subtle regional clustering of *T. orientalis* isolates. This is the first molecular confirmation of *A. phagocytophilum* in cattle from Eastern Herzegovina and identification of possible enzootic foci of *T. orientalis* infection, deeming further investigation into tick vectors and reservoir hosts in the region.

Molecular Biology Reports (2024) 51:936
https://doi.org/10.1007/s11033-024-09869-9

ORIGINAL ARTICLE



High genetic diversity of *Anaplasma ovis* in sheep from Bosnia and Herzegovina

Oliver Stevanović¹ · Tamara Ilić² · Nemanja Jovanović² · Branislav Vejnović³ · Andrea Radalj⁴

Received: 21 June 2024 / Accepted: 15 August 2024
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Abstract

Background Ovine anaplasmosis (*sensu stricto*) is a rickettsial blood disease caused by the tick-borne species *Anaplasma ovis*. The disease is characterized by mild anemia, fever, and icterus. A more severe clinical presentation is possible in non-endemic areas. There is no existing data on the presence of *Anaplasma ovis* in Bosnia and Herzegovina. However, given the country's location within the Mediterranean Basin and the recent molecular detection of *Babesia ovis*, it is plausible that sheep in the region could naturally be infected with this tick-borne pathogen.

Methods and results Blood samples from 81 sheep in the Podrinje and Herzegovina areas were examined by PCR. PCR positivity was found in 38 (46.9%) cases indicating a high number of infected sheep. Mixed infections with *Babesia ovis* and *A. ovis* were observed in 63.3% of cases. A higher number of positive sheep was recorded in the area of Herzegovina. Phylogenetic analysis of the *gltA*, *groEL*, and *msh4* genes of *A. ovis* revealed numerous genotypes and significant genetic variability. This diversity was not related to geographic origin, tick-borne infection status, or sheep breeding practices in Podrinje and Herzegovina.

Conclusions The data obtained in this study suggest that the emergence of new genotypes and the high genetic variability of *A. ovis* are driven by specific local and micro-environmental factors.

Keywords Ovine anaplasmosis · *Anaplasma ovis* · *gltA*, *groEL*, and *msh4* partial sequences · Molecular prevalence · Phylogeny · Bosnia and Herzegovina

- *Ehrlichia* vrste inficiraju leukocyte i opstaju u fagozomima.
- *Ehrlichia canis* – monocitna erlihioza pasa, (*Rhipicephalus* spp.) – **MONOCITI**

Psi mogu da budu kliconoše i preko dve godine nakon izlečenja akutne infekcije

Visoka temperatura, letargija, febra, splenomegalija, limfadenopatija, krvarenjatrombocitopenija, leukopenija i anemija

- *Ehrlichia ruminantium* – bolest vodenastog srca preživara, (*Ambylomma* spp.) i **MAKROFAGI**, **ENDOTEL**



FIGURE 1: *E. canis* affected dog – pale conjunctiva



FIGURE 2: bleached gingival mucosa – *E. canis* dog



FIGURE 3: Icteric gingival mucosa – *E. canis* dog



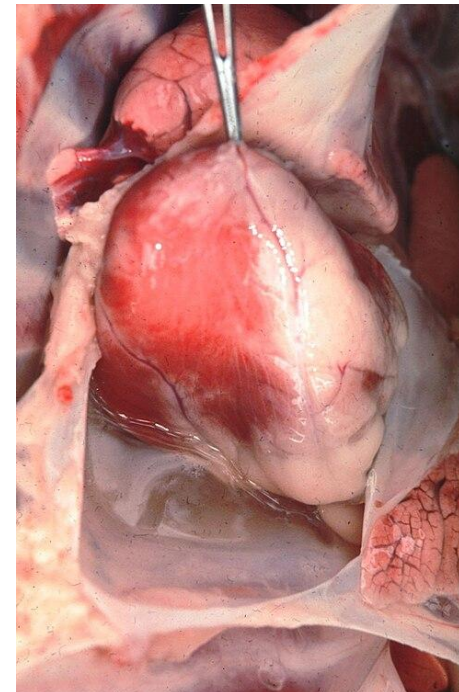
FIGURE 4: Icteric abdomen – *E. canis* dog



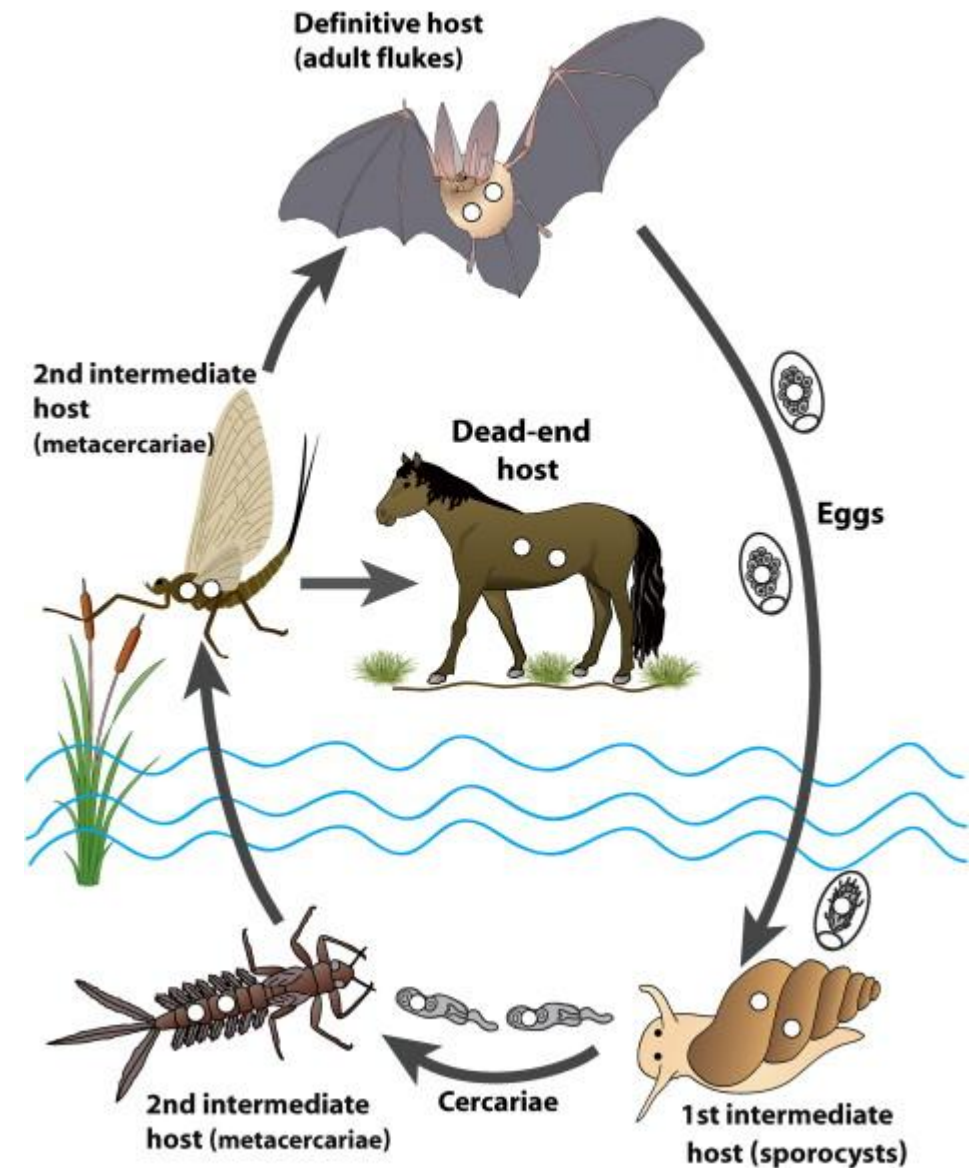
FIGURE 5: Canine ehrlichiosis – bilateral nasal bleeding



FIGURE 6: Canine ehrlichiosis – bilateral petechial hemorrhages on abdomen

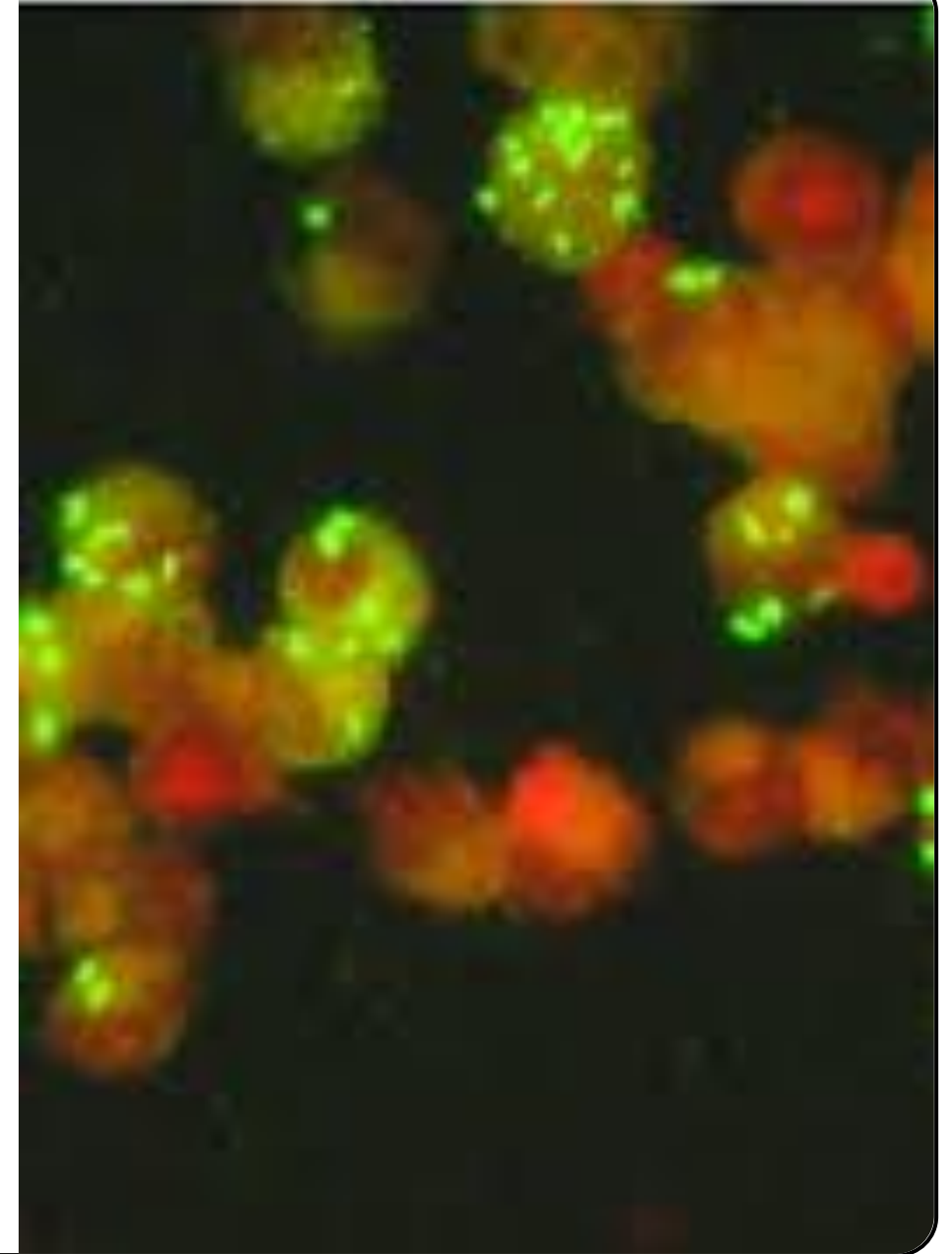


- ***Neorickettsia risticii*** monocitna erlihioza kopitara (*Potomac Horse Fever*), vektori su metilji i tropizam – **MONOCITI, EPITEL KOLONA, MAKROFAGI**
- Najčešće tokom leta
- U životnom ciklusu metilja učestvuje i vodeni puž
- Vodeni insekti prenose infektivne stadijume metilja
- Konji se inficiraju ingestijom inficiranih insekata
- Visoka temperatura, kolike, leukopenija i otežano hodanje
- Mortalitet može dostići 30%.



DIJAGNOSTIKA

- Uzorci: nezgrušana krv i promenjeno tkivo
- Metode: krvni razmaz, imunofluorescencija, PCR, izolacija (retko)
- Serologija: parni serum i titar antitela
- Rikecije se **ne boje po Gramu** – Giemsa i Romanovski
- **Konačna identifikacija: imunofluorescencija, imunohistohemija ili PCR**
- Izolacija rikecija u *in vitro* uslovima je izuzetno zahtevna i nije pogodna za rutinsku dijagnostiku – KULTURA TKIVA ILI EMRIONIRANA JAJA – ZAHTEVA ŽIVE ĆELIJE





| COXIELLA BURNETII

Coxiella burnetii

Gram-negativna bakterija

Obligatno intracelularna

Dve forme: metabolički aktivna i metabolički neaktivna (otporna forma)

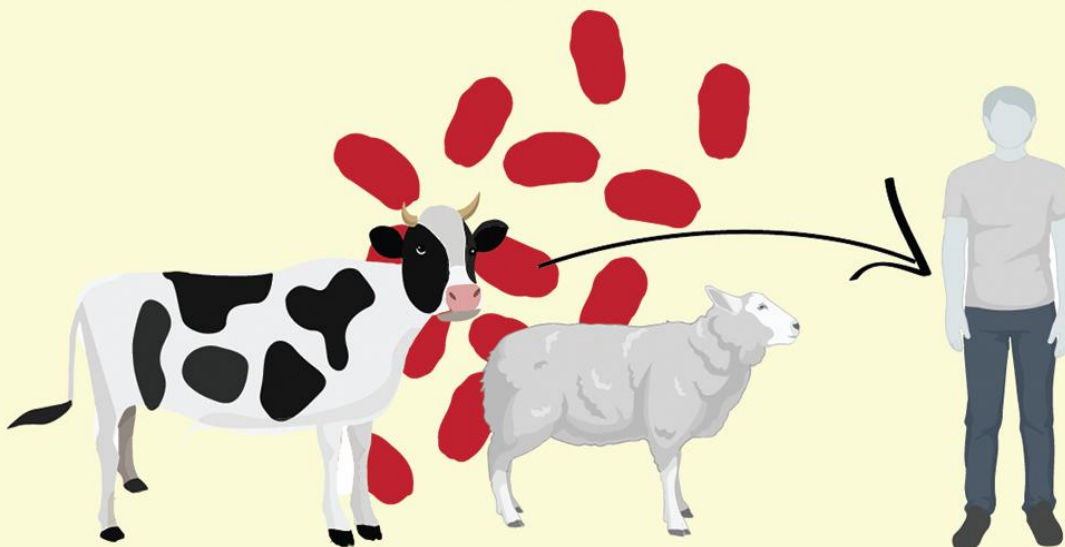
Izlučivanje: urin, feces, mleko, vaginalni iscedak, placenta i plodove vode (pobačaj)

Infekcija - aerosol i kontakt sa pobačenim sadržajem, nalazi se u i fecesu krpelja

Umnožava se u monocitima i makrofagima, krvlju se širi po celom organizmu

Inaparentna infekcija domaćih preživara, jedini simptom mogu biti sporadični pobačaji

Uzročnik Q groznice - zoonoza



Veoma otporna u spoljašnjoj sredini

Dijagnostika:

**serološke metode, PCR,
izolacija u embrioniranim
jajima ili kulturama tkiva**

Razmazi:

- Giemsa bojenje**
- Ziehl-Neelsen bojenje**



Foto: Shutterstock/Rhian Mai Hubbart

Agro Biz | Pojavio se "balkanski grip" u Nikšiću i Danilovgradu: Do sada eutanazirano 14 grla - Telegraf Biz

Pojavio se "balkanski grip" u Nikšiću i Danilovgradu: Do sada eutanazirano 14 grla

Q GROZNICA

C. burnetii se primarno umnožava u **monocitima i makrofagima**

Replikacija u kiseloj sredini fagolizozoma

Infekcija se hematogeno širi kroz organizam

Jetra, srce, reproduktivni trakt i mlečna žlezda

Perzistira u mlečnoj žlezdi i u uterusu

Kod domaćih životinja infekcija je najčešće blaga ili inaparentna

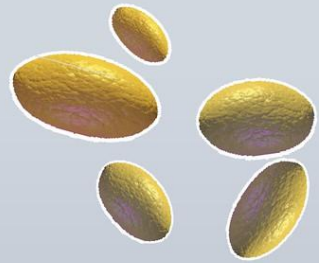
Najvažniji klinički znak sporadični abortusi kod preživara.

U slučajevima pobačaja masovno izlučivanje uzročnika u spoljašnju sredinu.

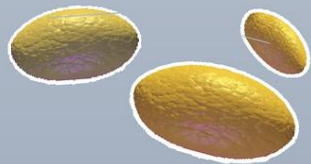
Rizik postoji i za druge životinje i za čoveka



JAKO OTPORNA U SPOLJAŠNJOJ SREDINI



**Transmission
of
Q-fever**



Birthing fluids
and mucus



Urine



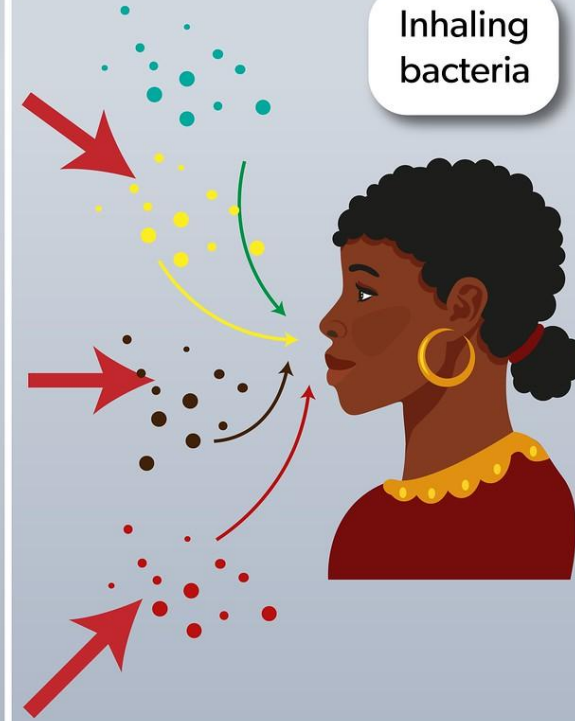
Feces



Blood



Milk and
dairy



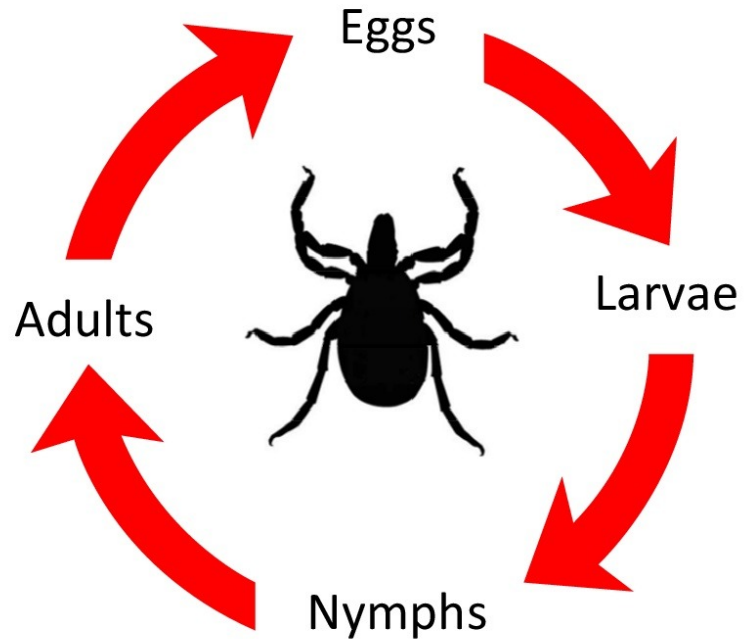
Inhaling
bacteria



Consuming
unpasteurised
dairy

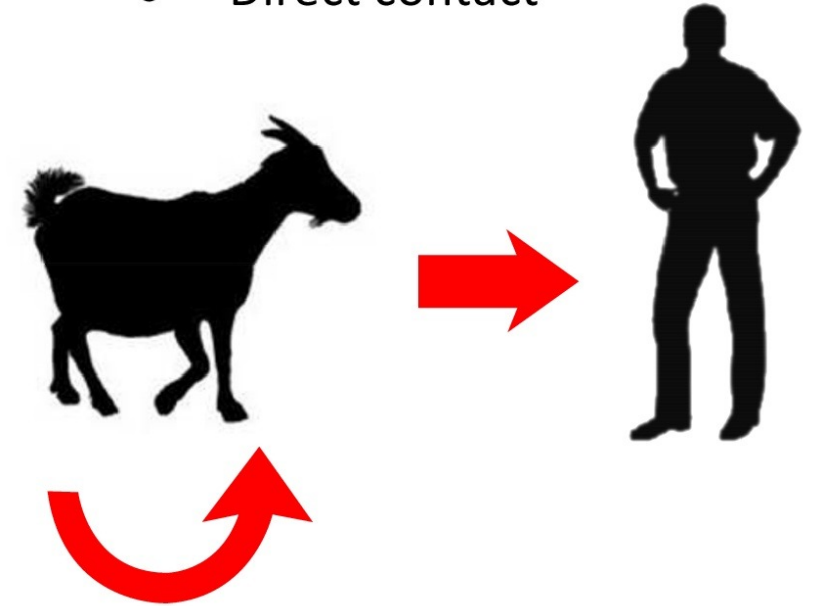
Transmission among ticks

- Transovarial
- Trans-stadial



Transmission among vertebrates

- Aerial
- Direct contact

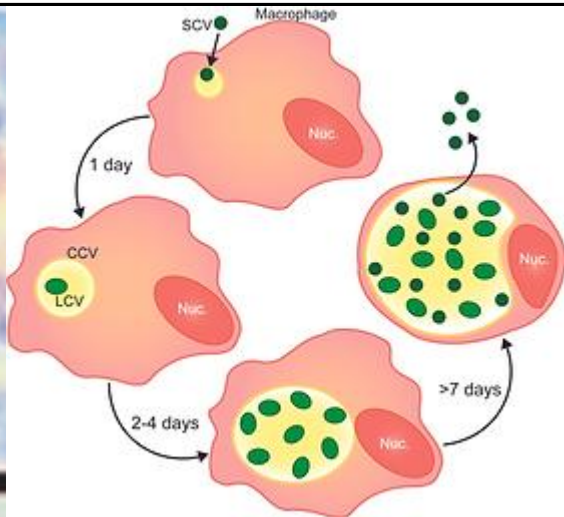


Transmission between ticks and vertebrates

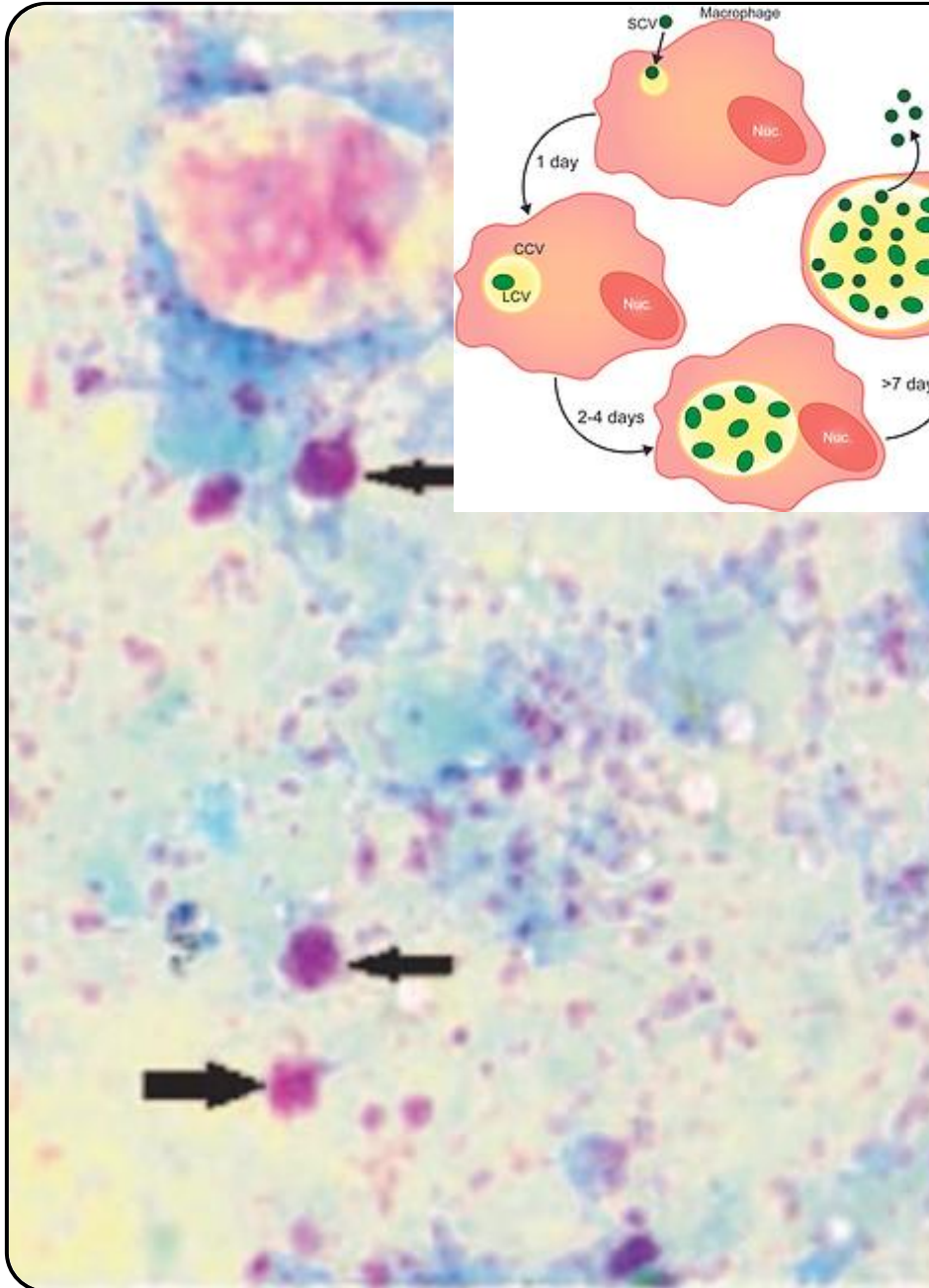
- Tick bite
- Tick feces

***Coxiella burnetii* (Q fever)**

MORFOLOŠKE I TINKTORIJALNE OSOBINE



- Gram-negativna, obligatno intracelularna, pleomorfna bakterija
- Javlja se u dve forme: SCV i LCV
- **SCV** je metabolički neaktivna forma van ćelije domaćina
- **LCV** je metabolički aktivna forma unutar ćelije domaćina
- Direktna mikroskopija se može raditi iz organa, placente i vaginalnog iscedka
- **Ne boji se po Gramu**–Giemsa i Ziehl–Neelsen
- **Izolacija – samo žive ćelije! BSL3!**



DIJAGNOSTIKA

- Direktna mikroskopija je nespecifična
- Može ukazati samo na sumnju na *C. burnetii*
- Pouzdana metode: PCR i direktna imunofluorescencija
- Serologija otkriva serokonverziju i specifična antitela
- IgG ukazuje na prethodni kontakt
- Najčešće metode: ELISA, IFA, RVK

