

Gram negatív fakultatív anaerob pálcák

Enterobacteriaceae II.

Dr. Berek Zsuzsa

2009 November 11

GRAM NEGATÍV PÁLCÁK

AEROB

Bordetella

Brucella

Francisella

Pseudomonas

Acinetobacter

Legionella

FACULTATIV ANAEROB

Haemophilus

Pasteurella

Familia:

Enterobacteriaceae

Vibrionaceae

Cardiobacterium

Eikenella

Kingella

Actinobacillus

ANAEROB

Bacteroides

Prevotella

Porphyromonas

Fusobacterium

MICROAEROPHIL

Campylobacter

Helicobacter

Enterobacteriaceae

Morphologia: - Gram negatív pálcák
- csilló
(ex.: Klebsiella, Shigella)

Tenyésztés:

egyszerű táptalajon (agar, véres agar)
differentiálás: patogén - fakultatív patogén
(biokémiai tulajdonságok alapján)

- a) szelektív táptalaj
- b) differentiáló táptalaj
- c) indikátor táptalaj

emlékeztető

Enterobacteriaceae

Antigének és Virulencia faktorok:

- O (sejtfal)
- H (csilló)
- K (tok)
- felszíni fehérjék
- pilusok
- exotoxinok
- endotoxin

BACTERIALIS BÉLFERTZŐZÉSEK

I. Typus

Enterotoxin

Hypersecretio
vékonybél

vizes hasmenés

Vibrio cholerae
Escherichia coli
(ETEC)

II. Typus

Inflammatio

Invasio a mucosaba
Vastagbél

gennyes, véres, nyákos
hasmenés

Shigella
E. coli (EIEC) (EPEC, EHEC)
Salmonella
Yersinia enterocolitica
Campylobacter jejuni
Aeromonas sp.
Vibrio parahaemolyticus

III. Typus

Penetratio, Generalisatio

Intracellularis kórokozó
Ileum

Typhus, Sepsis

Salmonella typhi
S. paratyphi A, B
Yersinia enterocolitica
Y. pseudotuberculosis
Campylobacter fetus

Exogen, bejutás: per os, faeco–oralis transmissio; hygiene!

Clostridium difficile
Clostridium perfringens

Enterobacteriaceae

Fakultatív pathogen

genus

Escherichia

Klebsiella csoport

Enterobacter

Edwardsiella

Citrobacter

Proteus csoport

Serratia

Providencia

Morganella

Obligat pathogen (genera)

Escherichia coli

ETEC (enterotoxic)

EPEC (enteropathogenic)

EIEC (enteroinvasive)

EHEC (enterohemorrhagic)

EAggEC (enteroaggregativ)

Shigella

S. dysenteriae

S. flexneri

S. boydii

S. sonnei

Salmonella

S. typhi

S. paratyphi

Yersinia

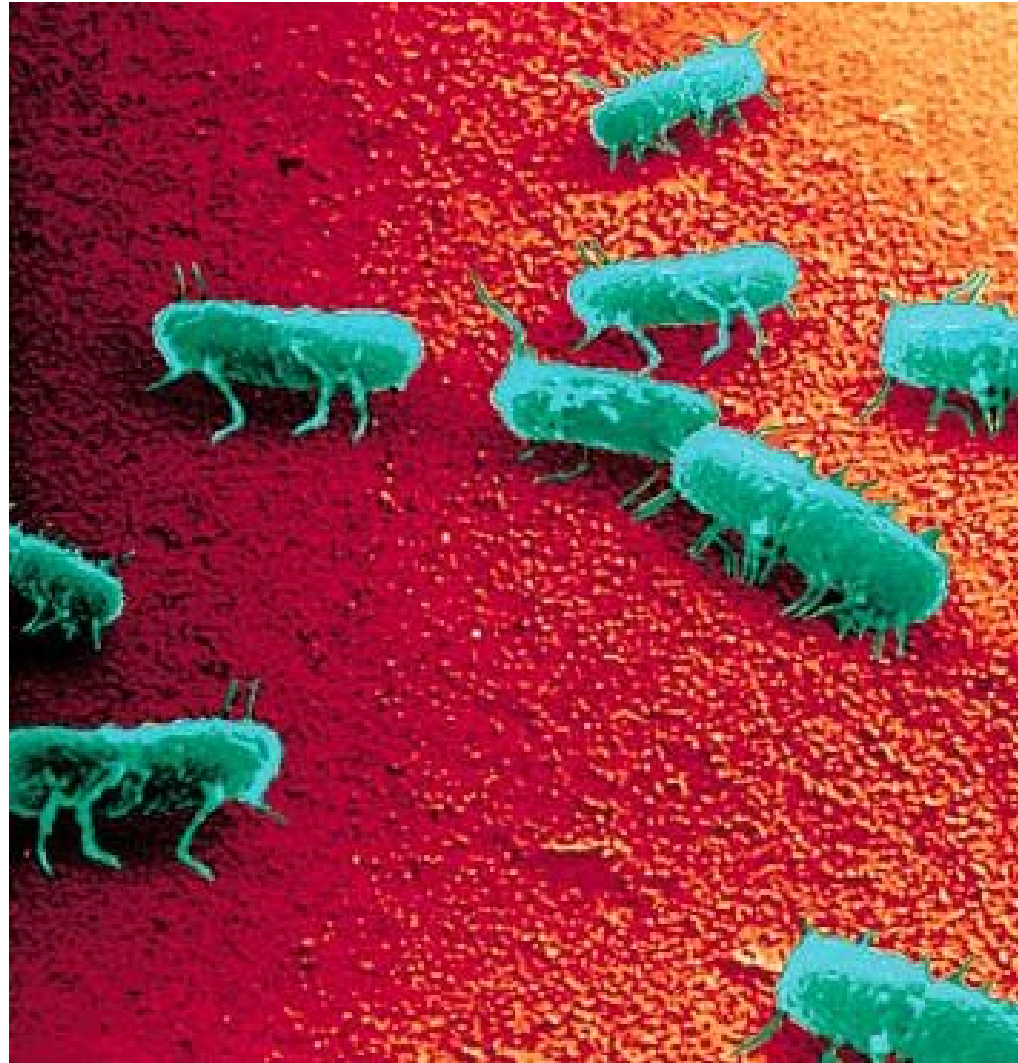
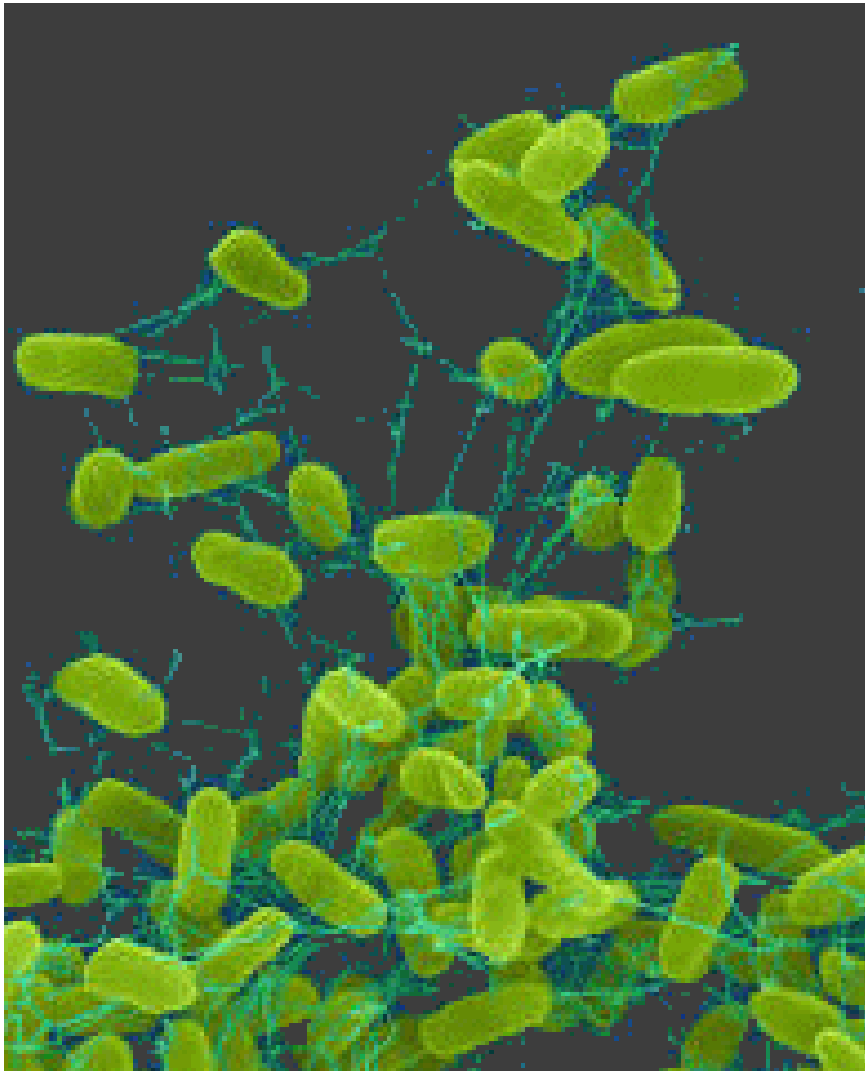
Y. pestis

Y. pseudotuberculosis

Y. enterocolitica

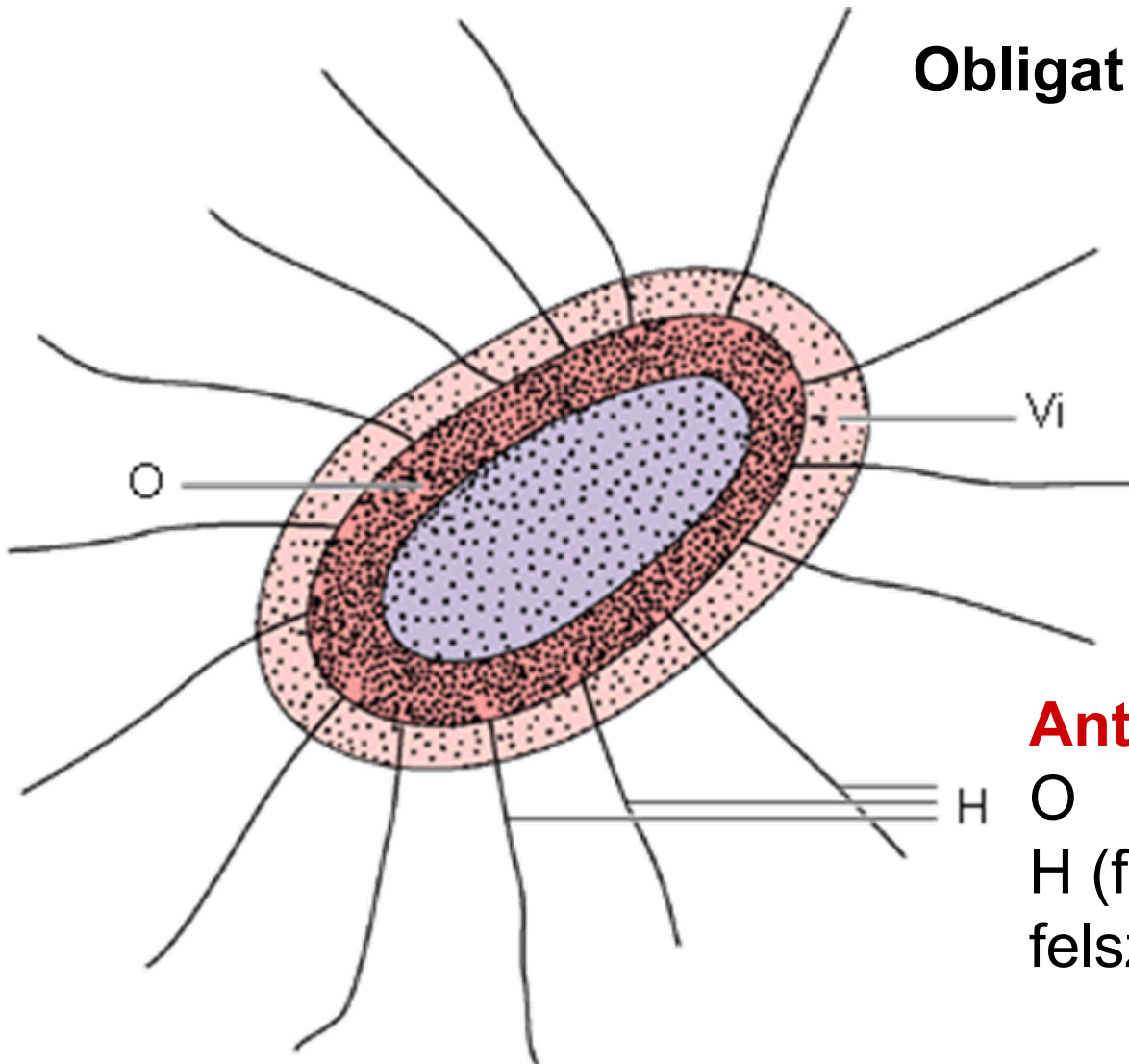
Salmoneellae

Salmonella sp.



Salmonella typhi, *S. paratyphi* A, B, C

Obligat human pathogen



Antigének

O
H (flagella)
felszíni Vi Ag = tok

Salmonella typhi, S. paratyphi A, B, C

Pathogenesis

forrás

betegek, hordozók;

Kontaminálódott élelmiszer, ivóvíz

Behatolási kapu

száj → bél → vér → szervek: lép, máj, epe(utak),
csontvelő, vese, agy

Ileum: ulceratio (vérző fekélyek, Perforatio)

kórképek:

Typhus abdominalis ***Salmonella typhi***

Paratyphus ***S. paratyphi A, B, C***

Salmonella typhi, *S. paratyphi* A, B, C



Figure 1. *Salmonella typhi*, the agent of typhoid. Gram stain. (CDC)



Figure 2. Flagellar stain of a *Salmonella typhi*. Like *E. coli*, *Salmonella* are motile by means of peritrichous flagella. A close relative that causes enteric infections is the bacterium *Shigella*. *Shigella* is not motile, and therefore it can be differentiated from *Salmonella* on the basis of a motility test or a flagellar stain. (CDC)

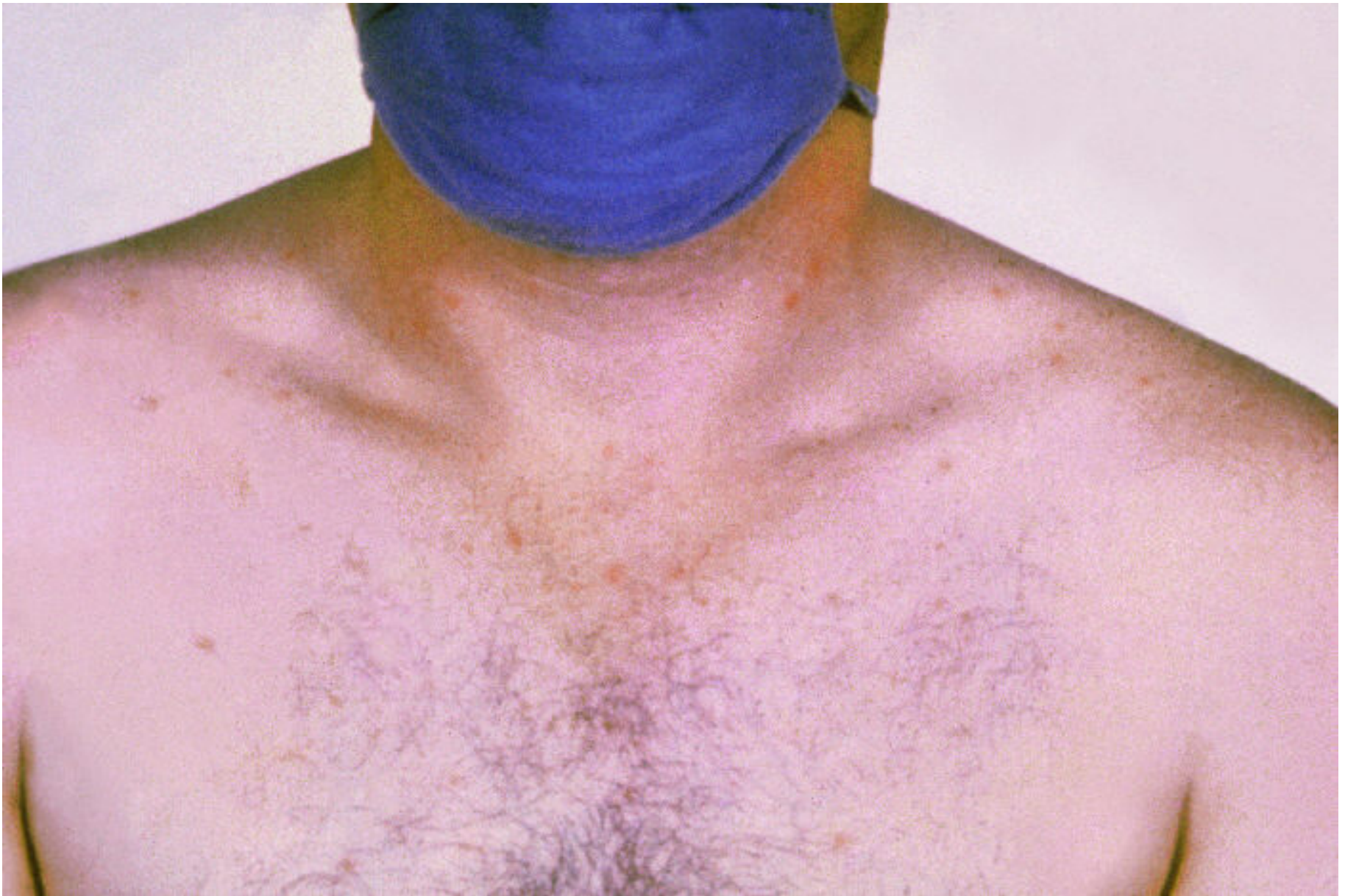
Typhus abdominalis



Roseolenartiges, makulopapulöses Exanthem bei
Typhus abdominalis



Rose spots on abdomen of a patient with typhoid fever due to the bacterium *Salmonella typhi*.



Rose spots on the chest of a patient with typhoid fever due to the bacterium *Salmonella typhi*.

www.wrongdiagnosis.com

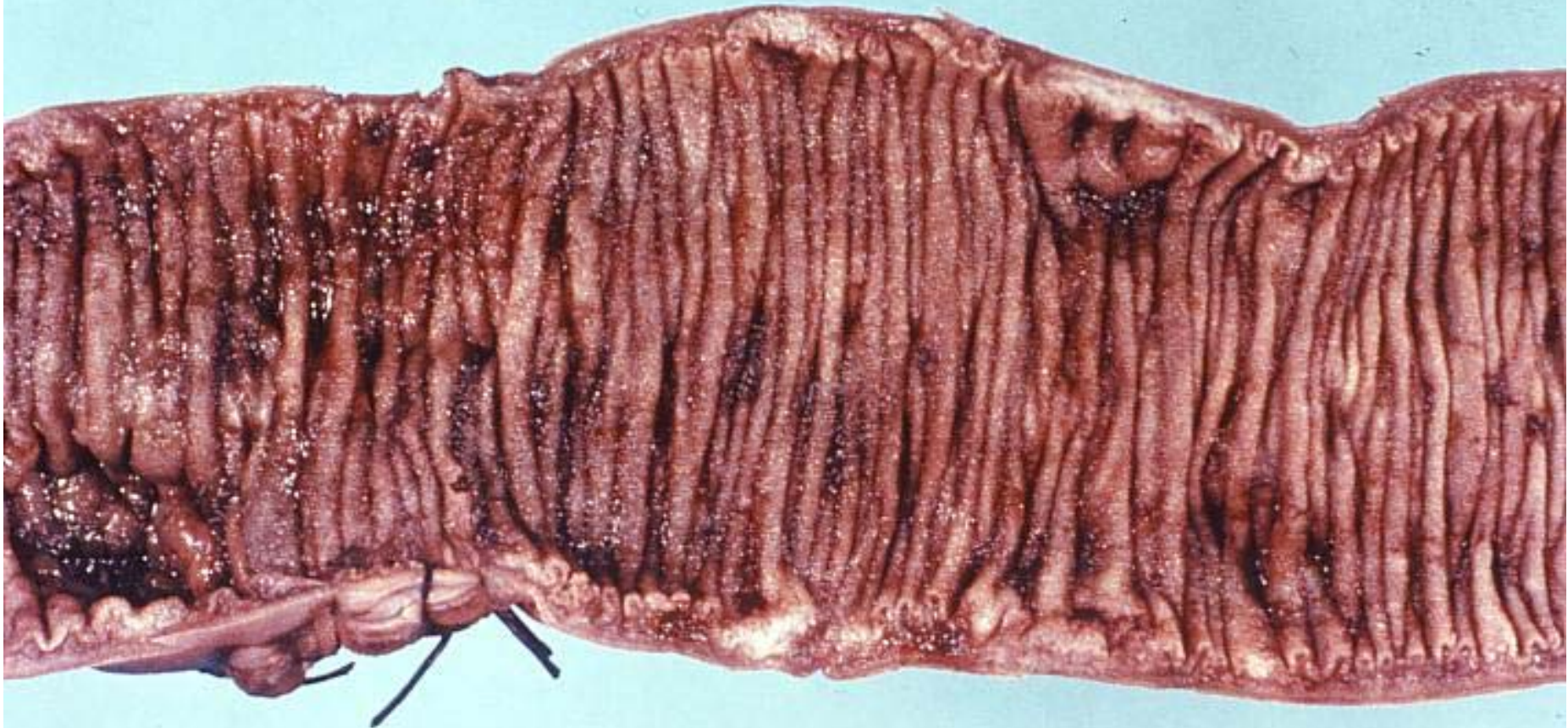


Fig. 4.37 Typhoid fever. Numerous ulcers of the small intestine overlying hyperplastic lymphoid follicles (Peyer's patches). By courtesy of Dr. J. Newman.

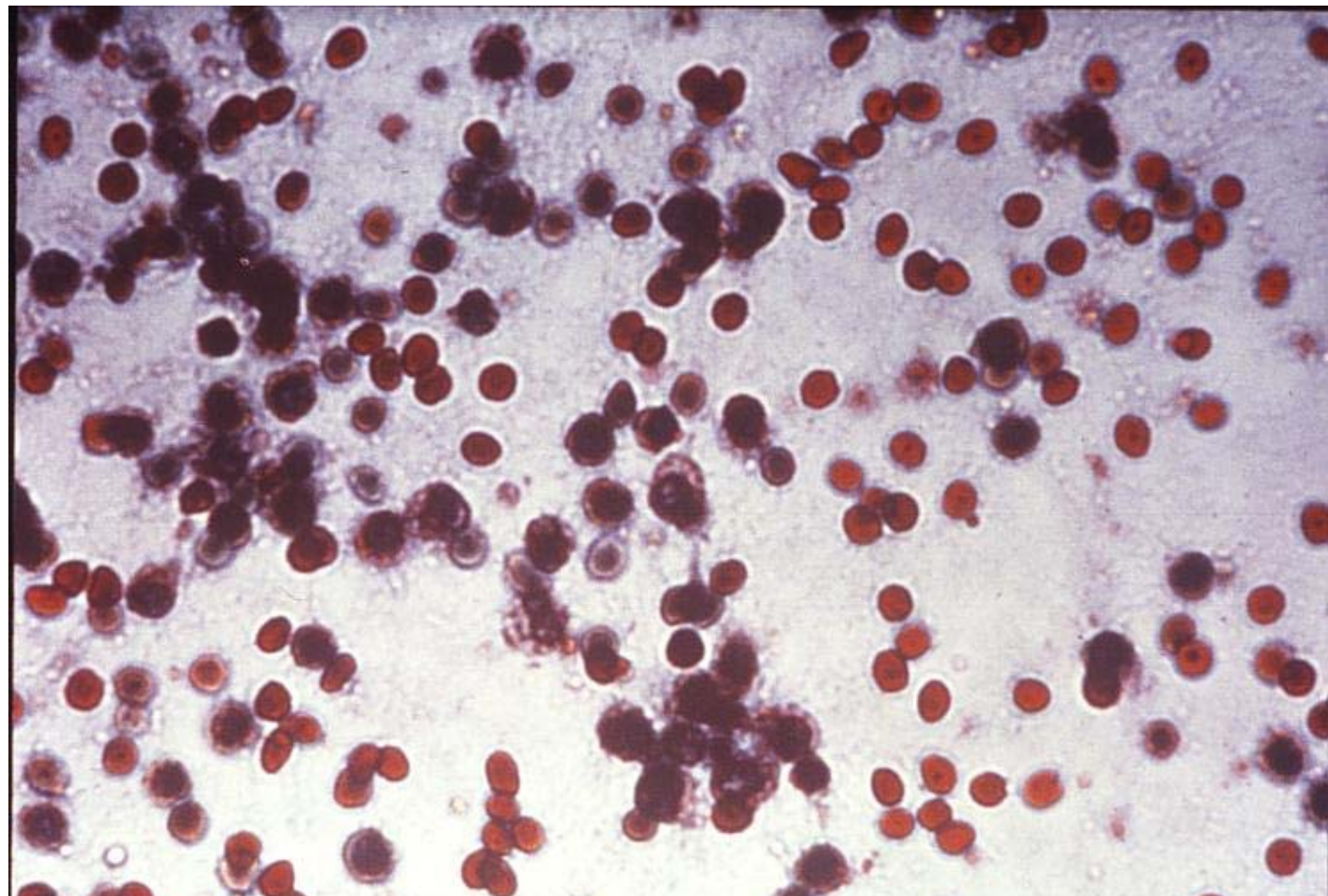


Fig. 4.39 Typhoid fever. Mononuclear cells and red blood cells in the stool. Trichrome stain. By courtesy of Dr. H.L. DuPont.

Typhus abdominalis

Diagnosis

Direct kimutatás (vér, széklet, vizelet)

Selective táptalajok
ellenanyag kimutatás
(cső-agglutináció))

Prophylaxis

Expositios prophylaxis

Immun-prophylaxis:

- 1) Aktív oral immunisatio
Ty21, egy apathogen, attenuált törzs
- 2) Parenteral injectio
Vi tok-polysaccharid
S. typhi Type-2



www.spiceisle.com

Therapia

Ampicillin

Trimethoprim

Chloramphenicol

Hordozók

meggyógyítása!

„Typhoid Mary”

Salmonella - Salmonellosis

Ubiquiter: *S. typhimurium*, *S. enteritidis* etc.

Pathogenitás: madarak (tojás!), sertés, szarvasmarha, egér, patkány és **ember**

Pathogenesis

forrás: fertőzött állatok, **kontaminált élelmiszer**

BAKTÉRIUM AZ ÉLELMISZERBEN SZAPORODIK! →

Endotoxin felszabadulás a bélben

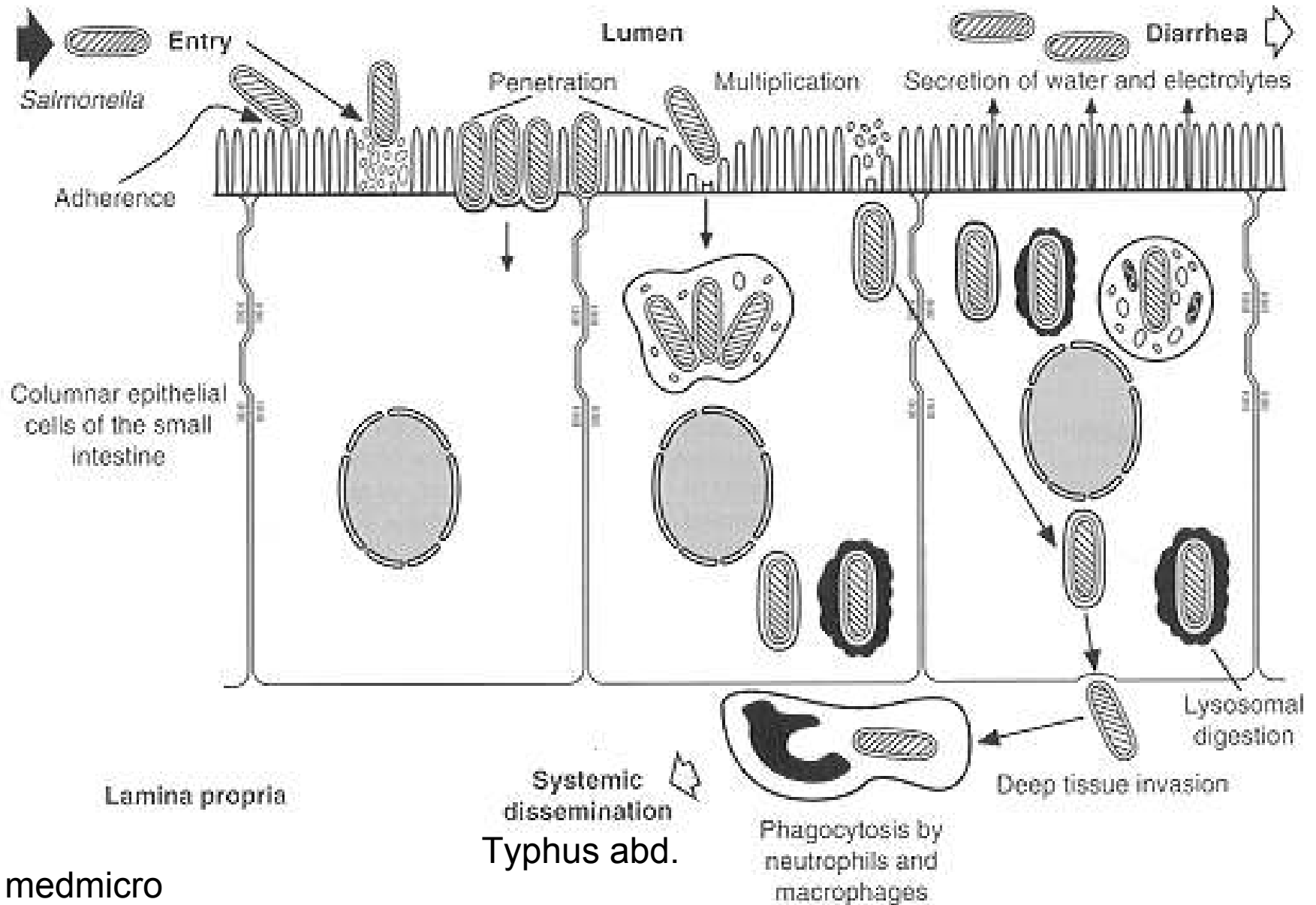
Kórkép: – Endotoxin hatás

1. **ÉTELMÉRGEZÉS** (nincs baktérium szaporodás a bélben!)
2. (Gastro)Enteritis (II. typus, min. szaporodás a bélben)

Diagnosis: direct kimutatás (élelmiszer)

Prophylaxis: élelmiszer és konyha hygiene

Figure 21-3 Invasion of intestinal mucosa by Salmonella.





Color-enhanced scanning electron micrograph showing *Salmonella typhimurium* (red) invading cultured human cells

Salmonella

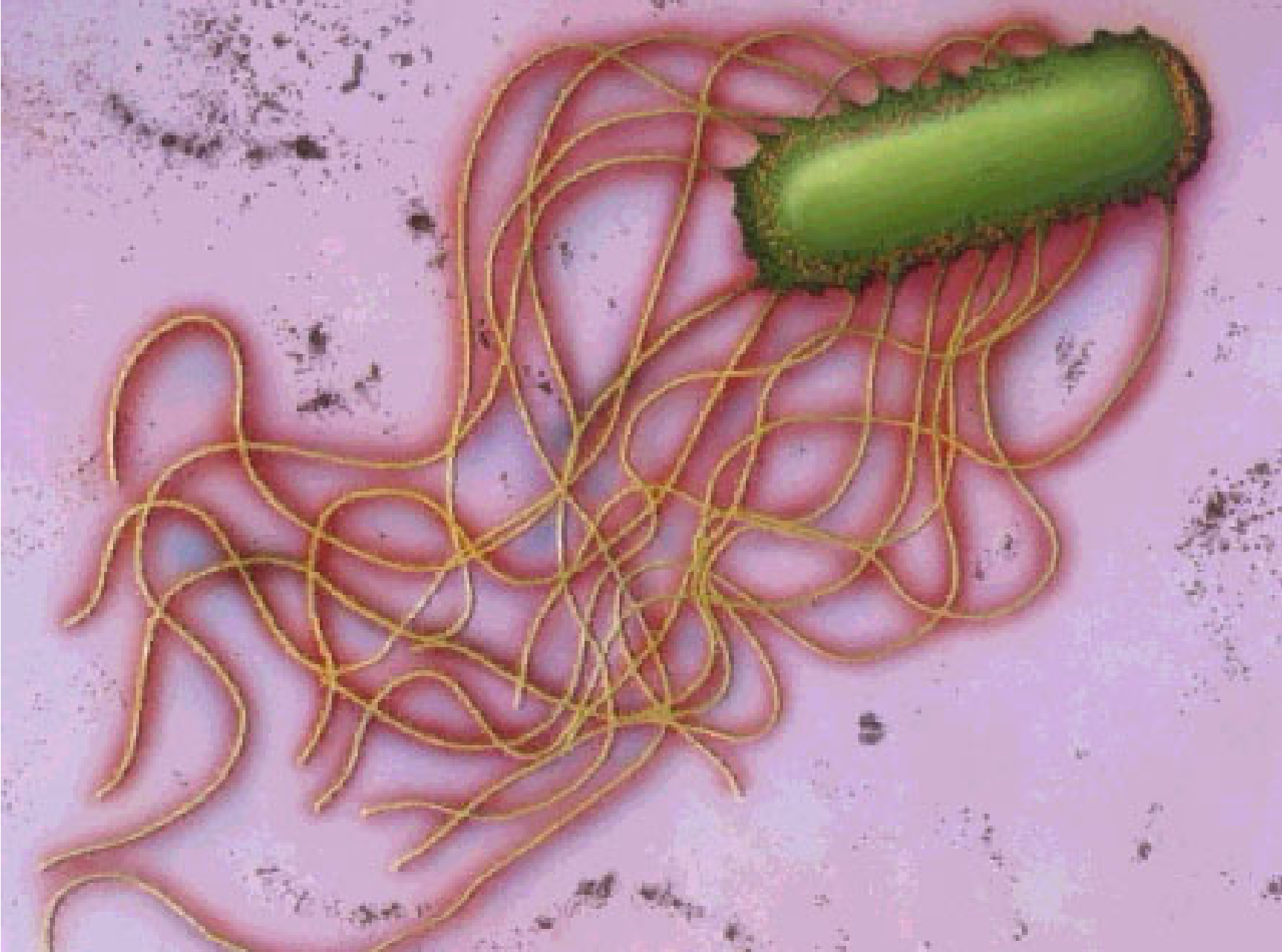
Food Poisoning



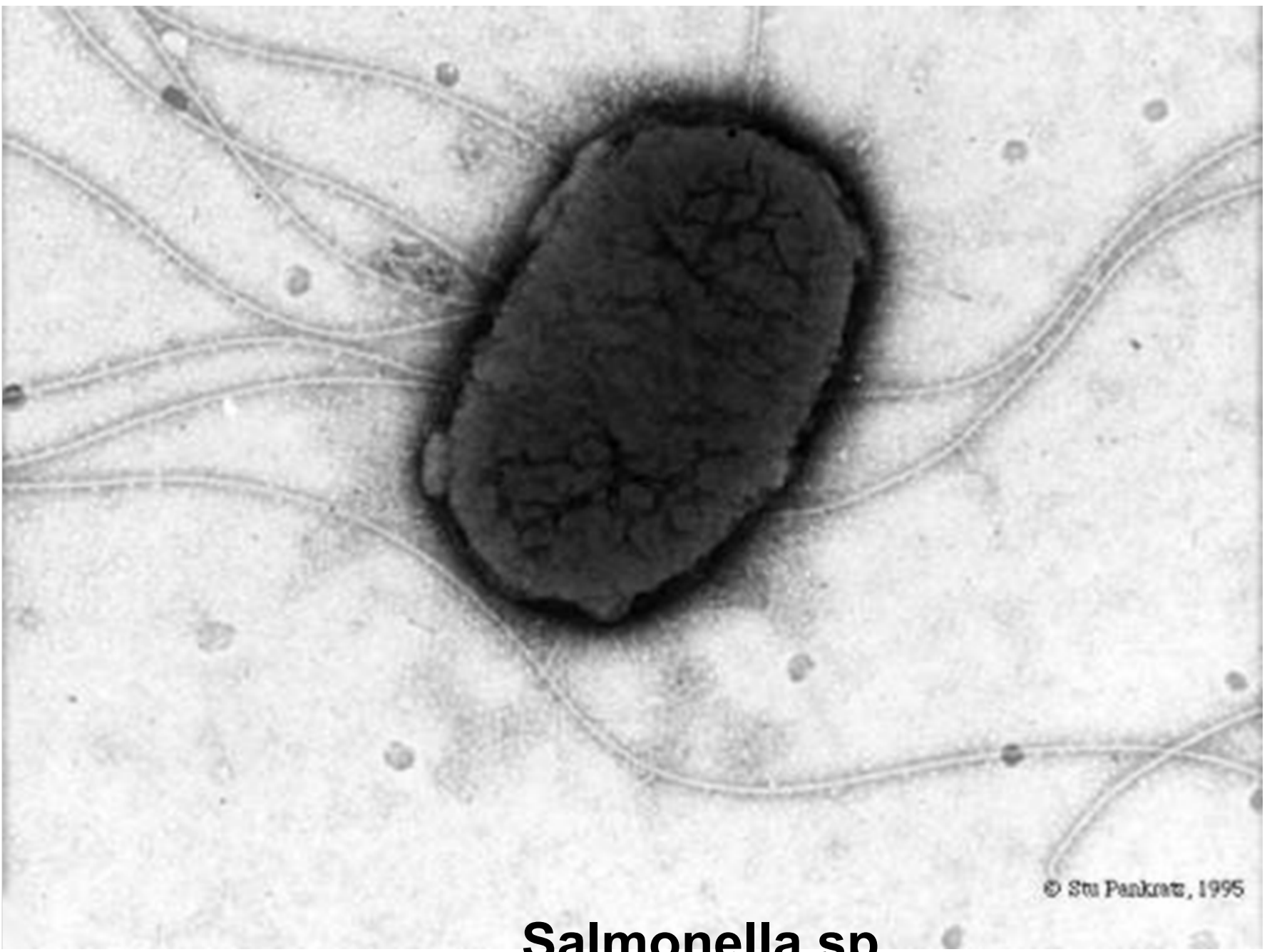
Salmonella

Food Poisoning



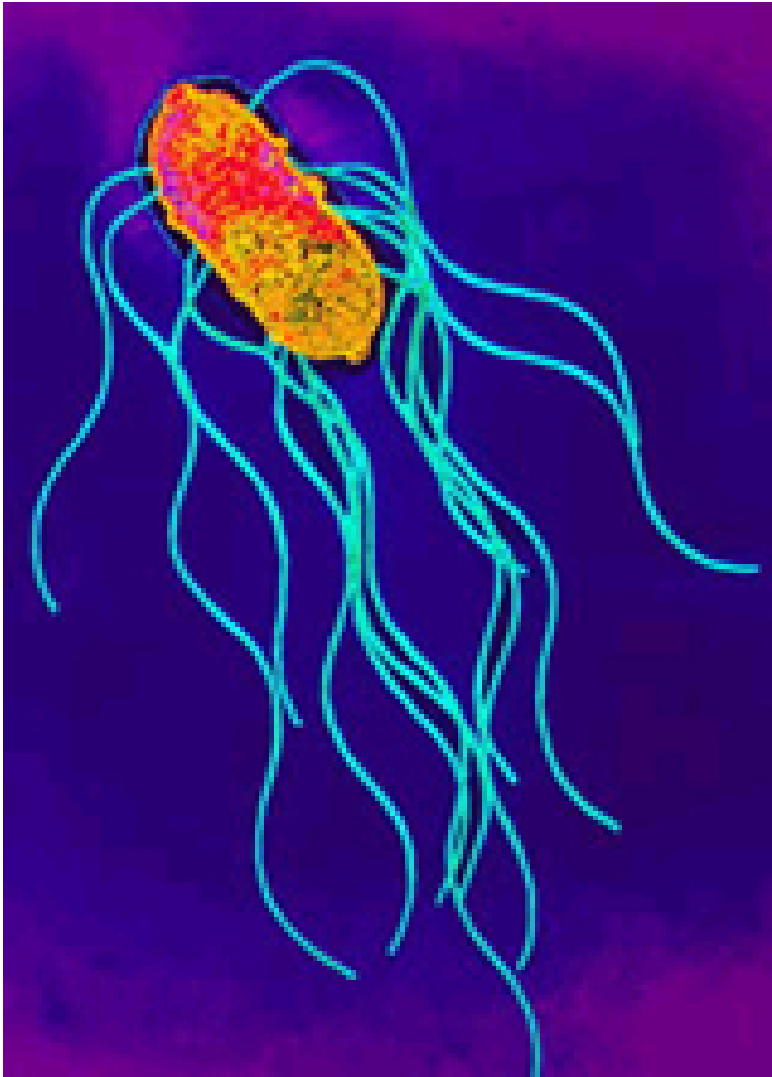


Salmonella sp.

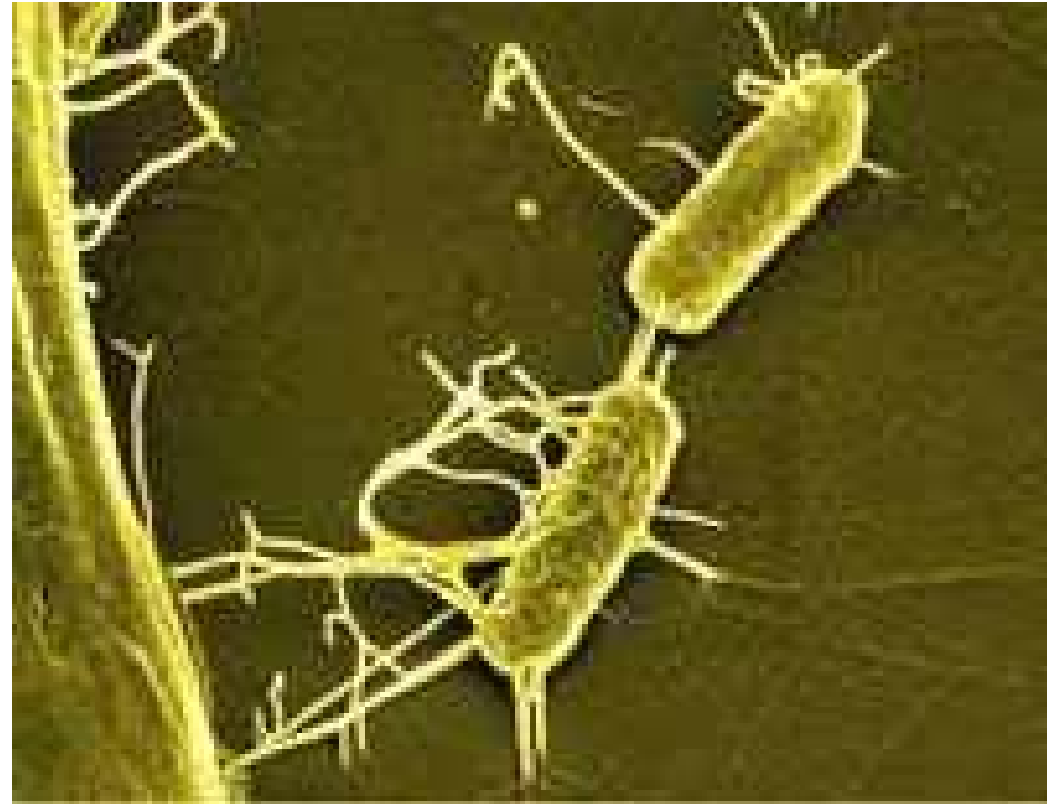


© Stu Pankratz, 1995

Salmonella sp.



Salmonella enterica



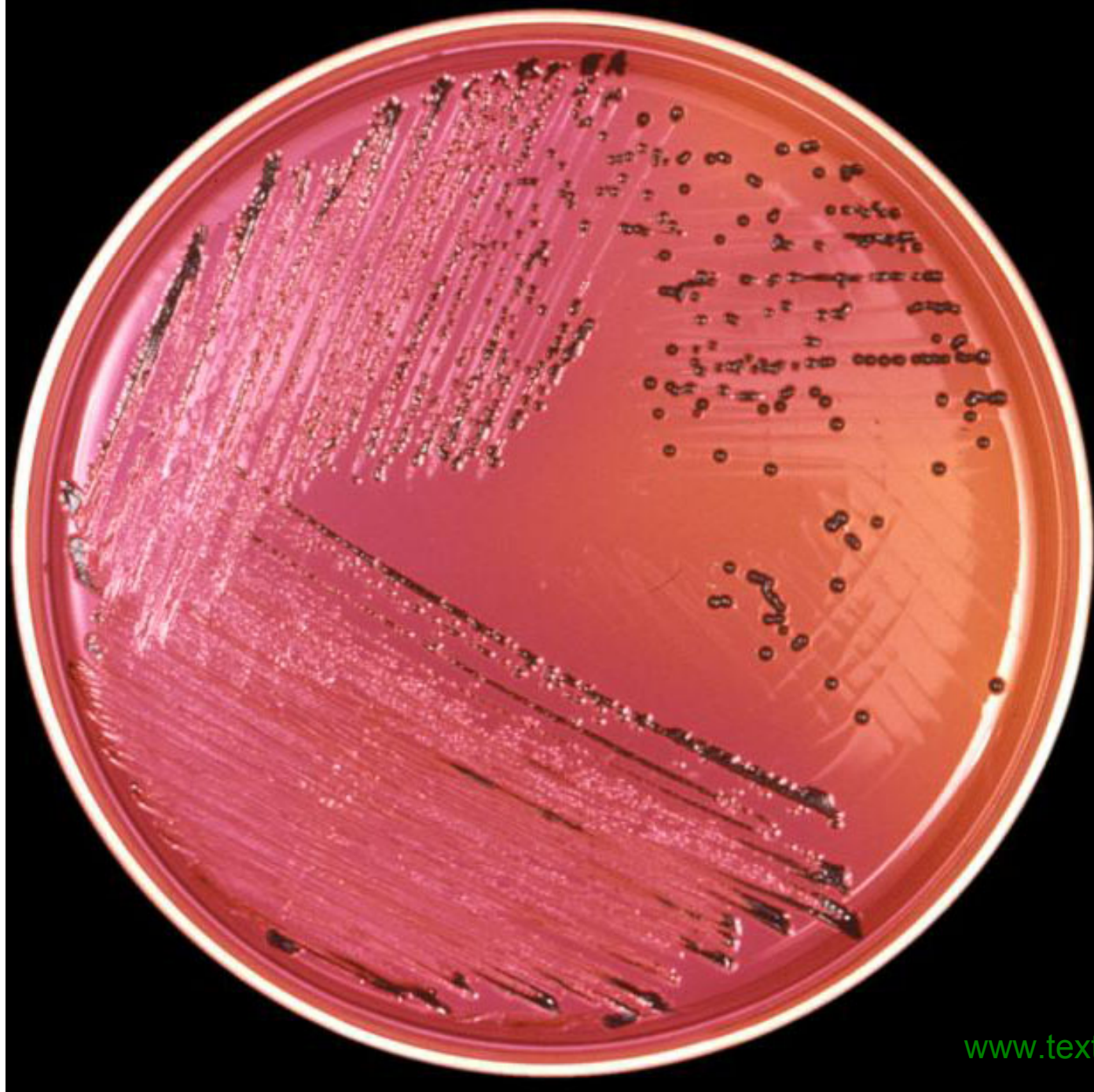


Figure 3.
***Salmonella* sp. after**
24 hours growth on
XLD agar.



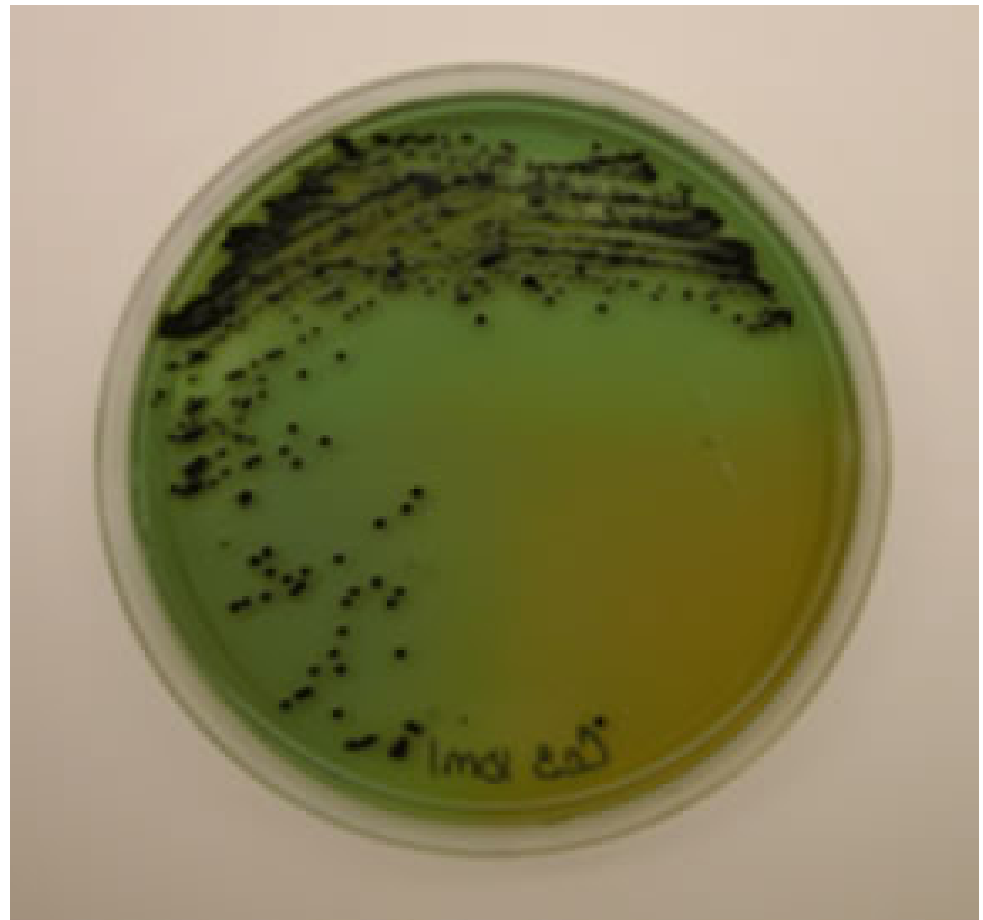
Figure 4. Colonial growth *Salmonella choleraesuis* subsp. *arizonae* bacteria grown on a blood agar culture plate. Also known as *Salmonella arizonae*, it is a zoonotic bacterium that can infect humans, birds, reptiles, and other animals. (CDC)



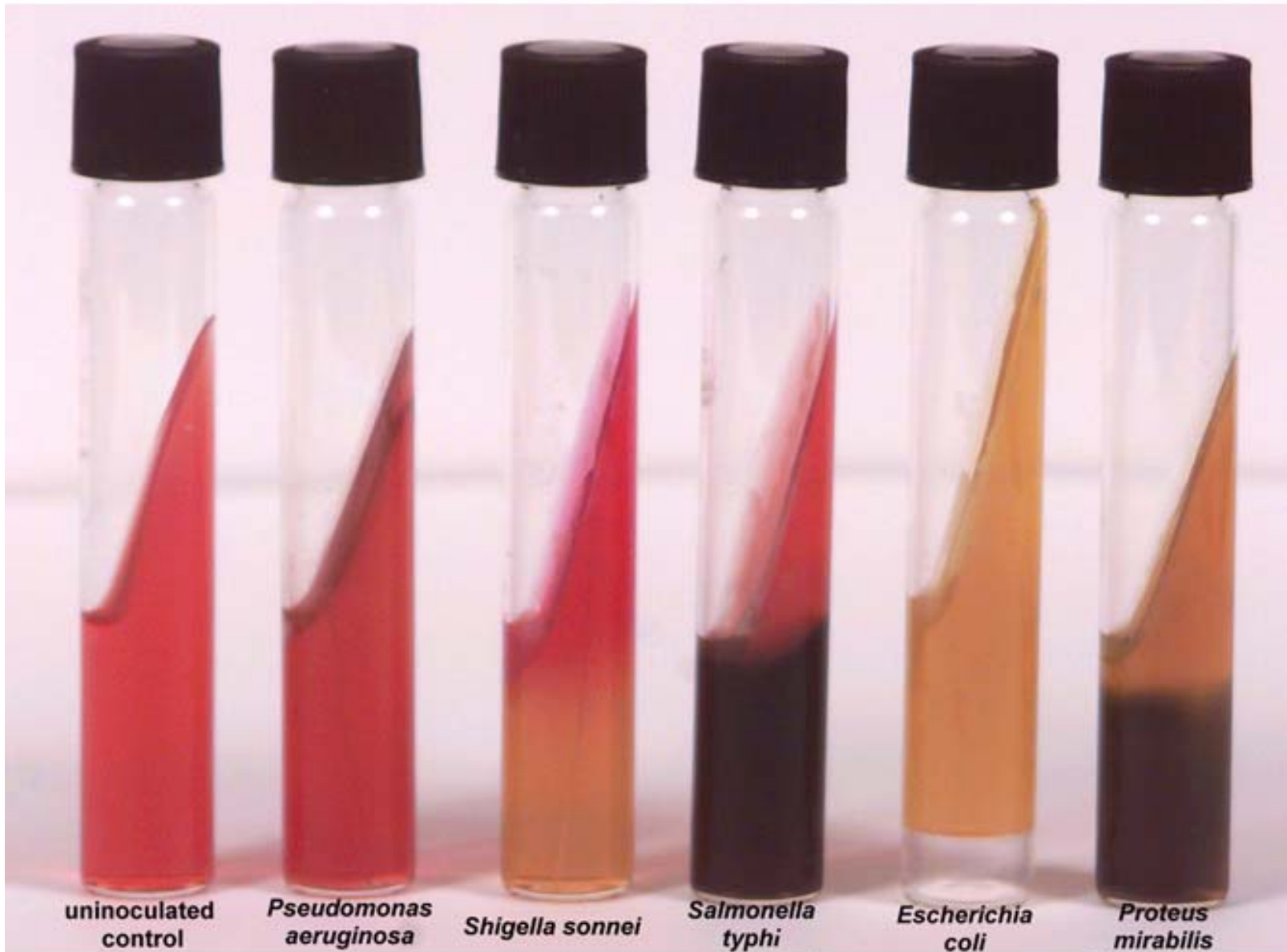
Rambach™ Agar
For detection of *Salmonella* spp.

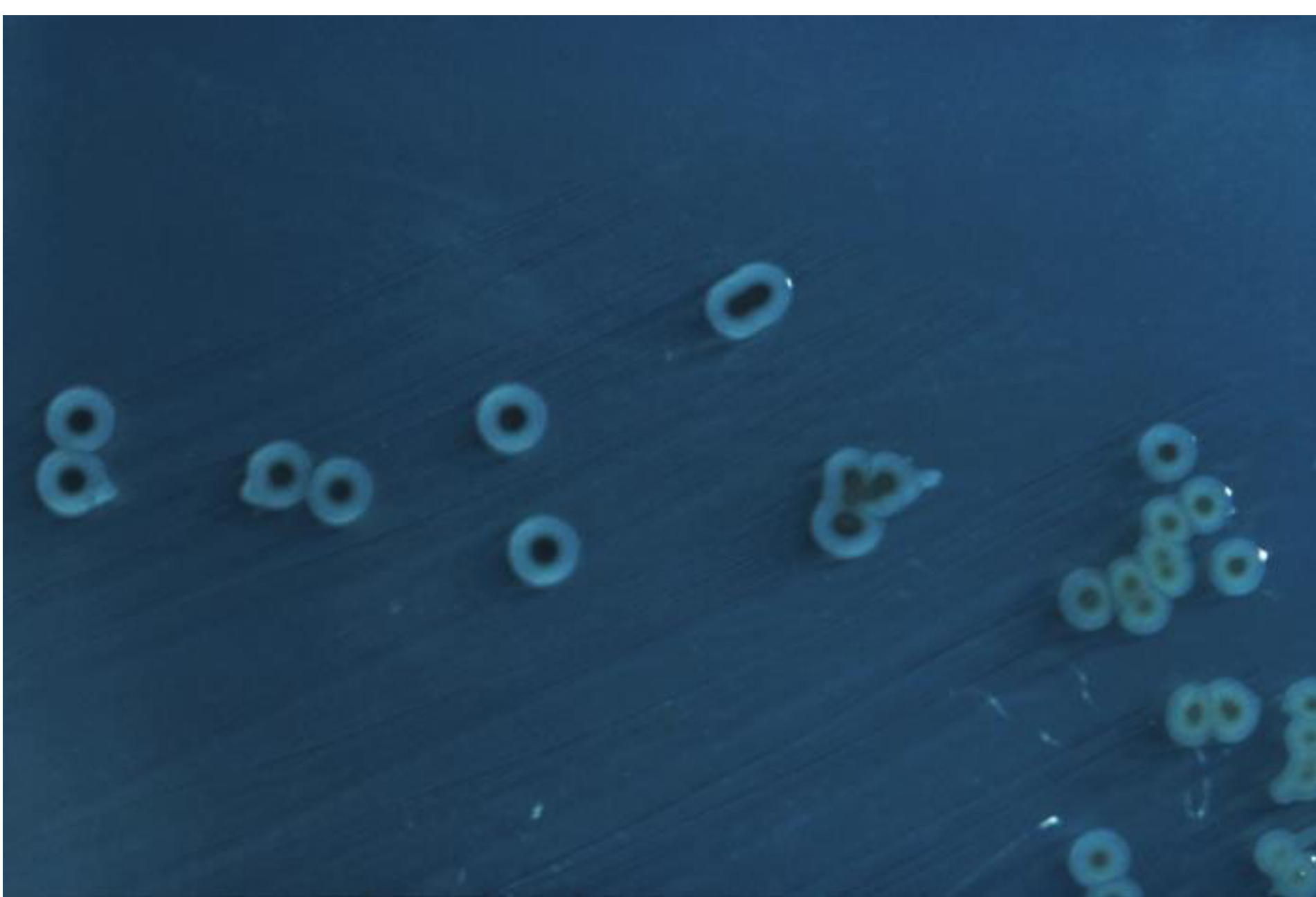
- *Salmonella* - red
- other bacteria - blue, violet, colourless, or inhibited.

www.chromagar.com



Isolation of *Salmonella*
from Environmental
Samples





***Salmonella typhimurium* HE agar**

Enterobacteriaceae

Fakultatív pathogen

genus

Escherichia

Klebsiella csoport

Enterobacter

Edwardsiella

Citrobacter

Proteus csoport

Serratia

Providencia

Morganella

Obligat pathogen (genera)

Escherichia coli

ETEC (enterotoxikus)

EPEC (enteropathogen)

EIEC (enteroinvasív)

EHEC (enterohemorragikus)

EAggEC (enteroaggregatív)

Shigella

S. dysenteriae

S. flexneri

S. boydii

S. sonnei

Salmonella

S. typhi

S. paratyphi

Yersinia

Y. pestis

Y. pseudotuberculosis

Y. enterocolitica

Shigellosis

***S. dysenteriae** 10 Serotypes**

***S. flexneri** 6 Serotypes**

***S. boydii* 15 Serotypes**

S. sonnei

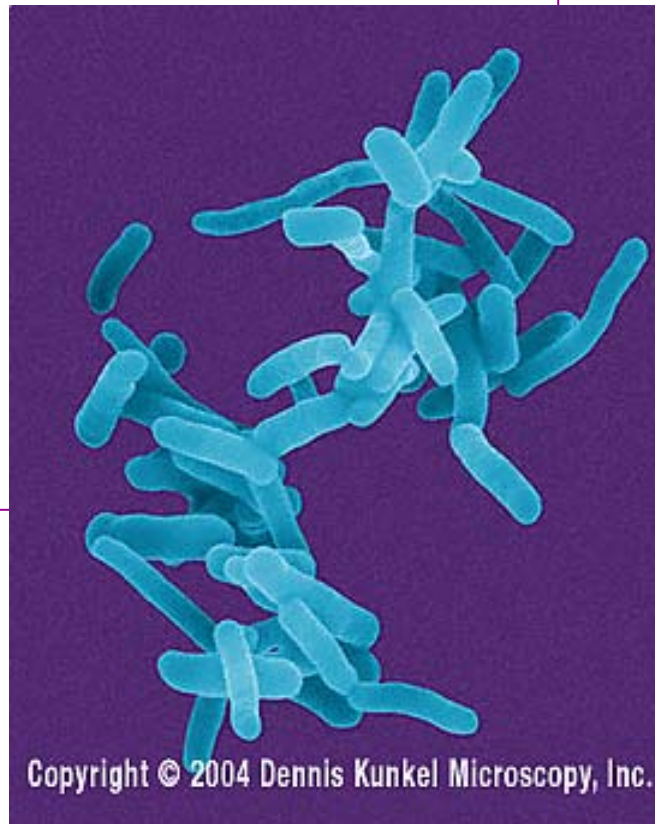
Antigen

O

* Toxin



Shigella sonnei



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Shi gel I a

Virulencia faktorok

Exotoxin

cytotoxicus (sejtlysis!);

Enterotoxikus;

Paralyticus – letalis activitas

Fragment A, B (5) – Glycolipid



Proteinsynthesis gátló – kötődés és a
60s Ribosoma alegység gátlása → sejthalál

OMP (Ipa, Ics)

Endotoxin - LPS

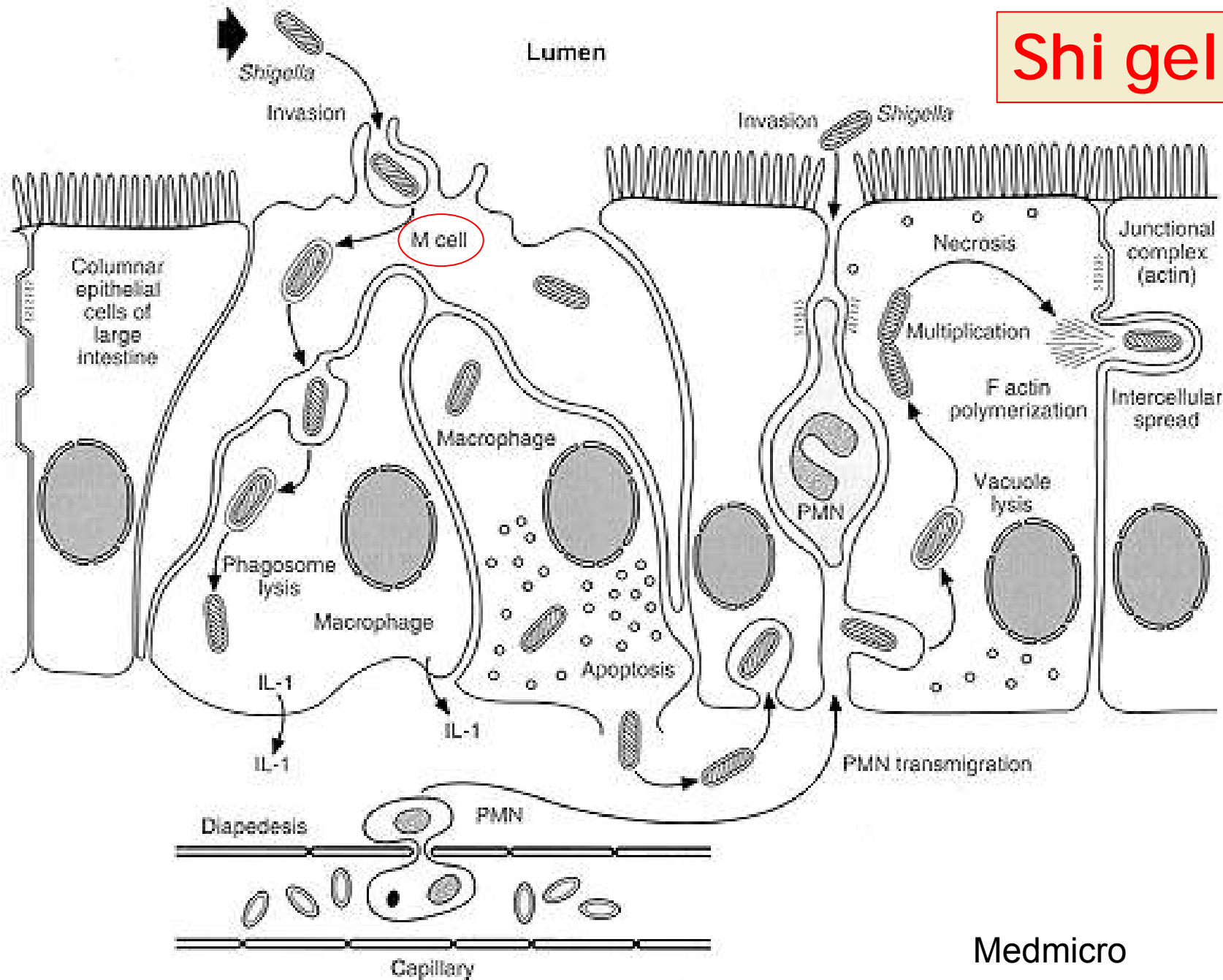
Shi gel I a

Pathogenitás, infekció – ID50: 100-200 Bacterium
Behatolás és szaporodás Epithel sejtekben:
Invasion **P**lasmid **A**ntigens – Ipa
Intercellular **S**pread – Ics

Pathogenesis, kórképek

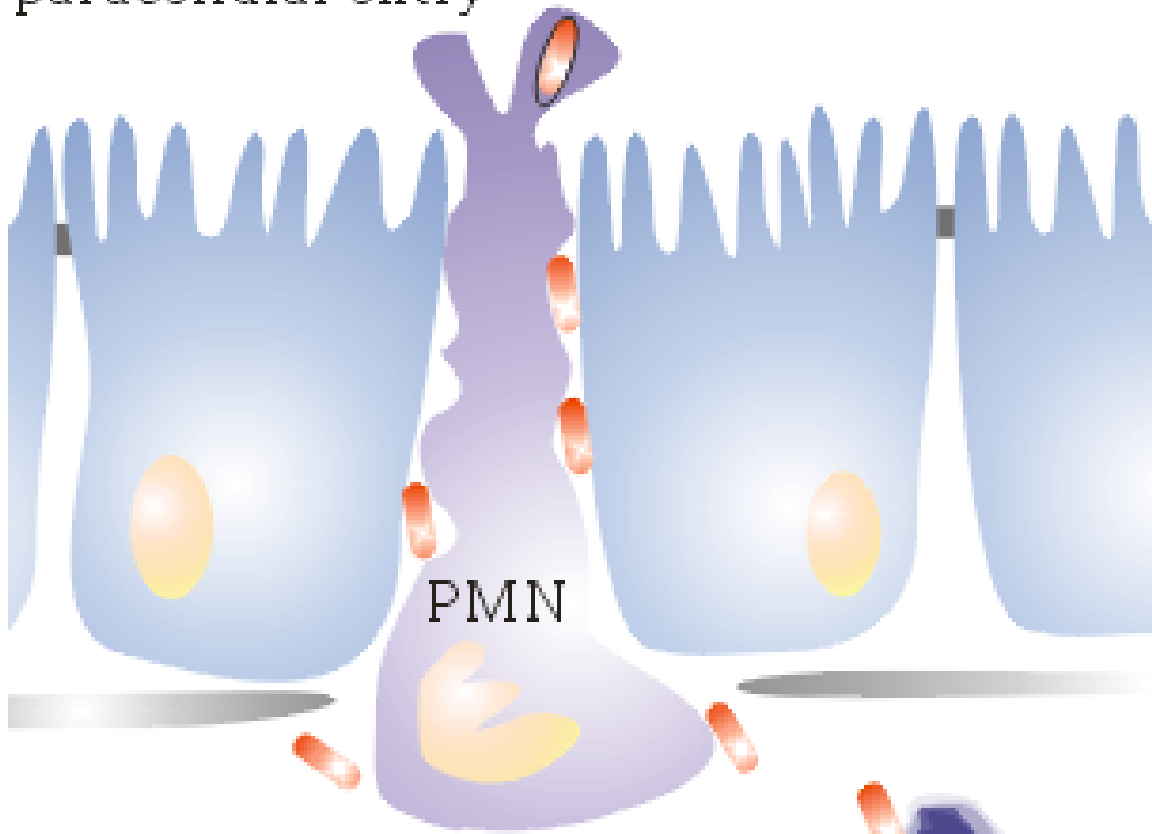
Localis Infectio
Epithel necrosis, ulceratio
Absorptio gátlás
HUS!

Shigellosis



Shigella

paracellular entry

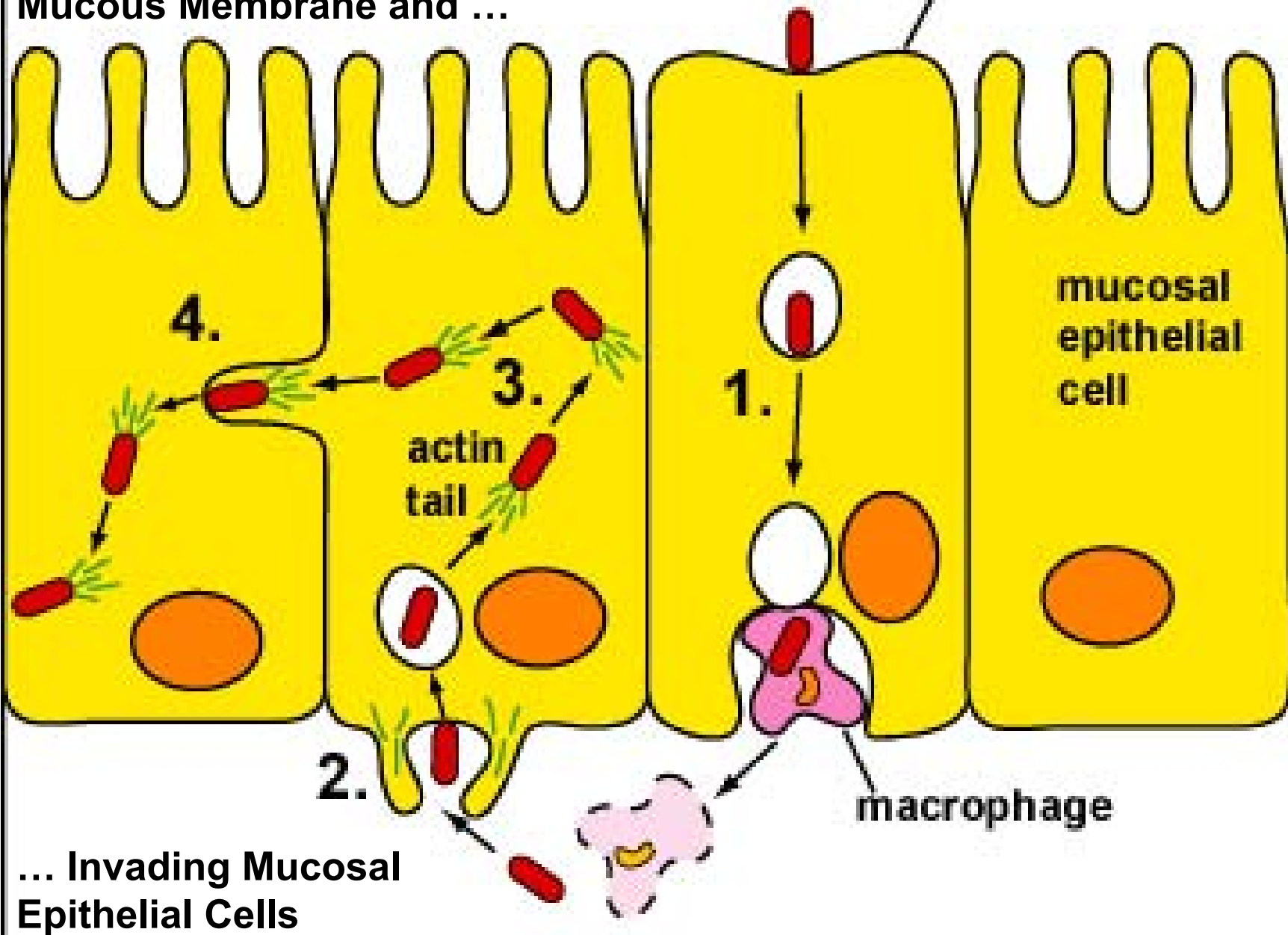


Influx of PMN destabilize the epithelial integrity
enhance bacterial invasion



Macrophage apoptosis
release of massive
amounts of cytokines

Fig. 2: *Shigella* Passing Through the Mucous Membrane and ...



... Invading Mucosal Epithelial Cells

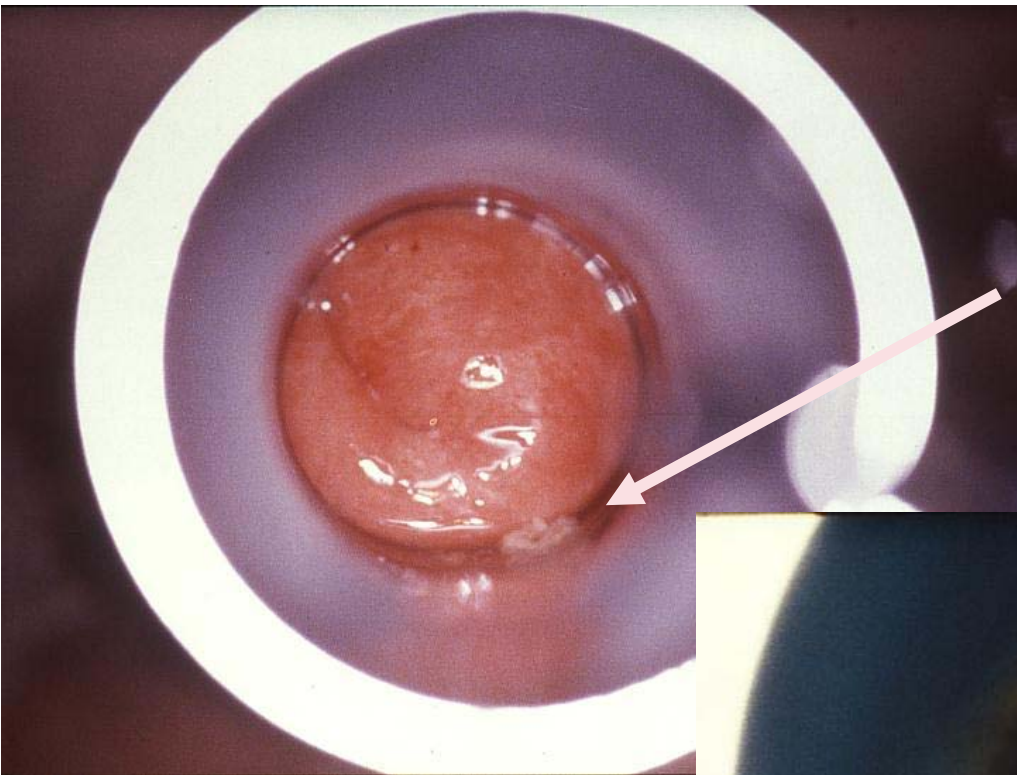
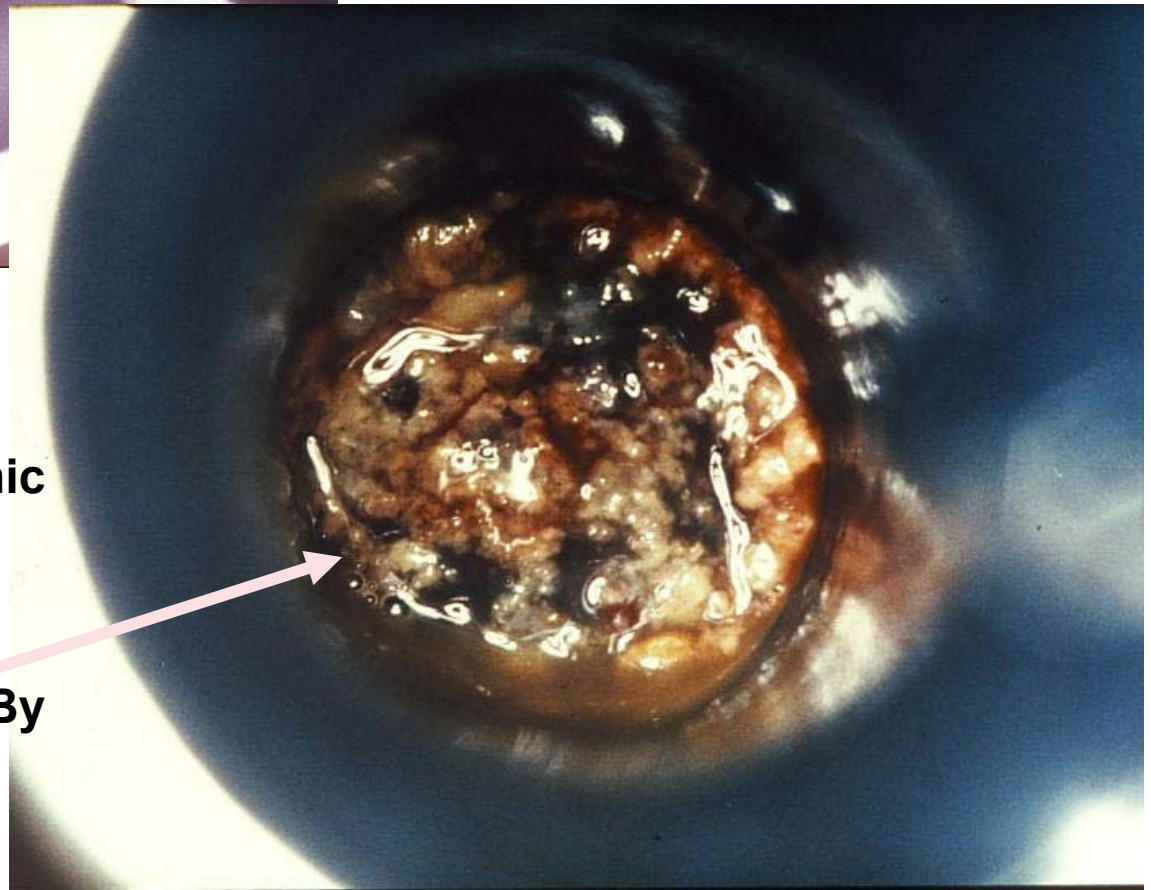


Fig. 4.33 Shigellosis. Sigmoidoscopic view of colonic mucosa in a mild case of infection due to *S. flexneri*. Note the thin whitish exudate, which is made up of fibrin and polymorphonuclear leucocytes. By courtesy of Dr. R.H. Gilman.

Fig. 4.34 Shigellosis. Sigmoidoscopic view of colonic mucosa in a fatal case of infection with *S. dysenteriae* type 1 showing extensive pseudomembranous colitis. By courtesy of Dr. R.H. Gilman and Dr. F. Koster.



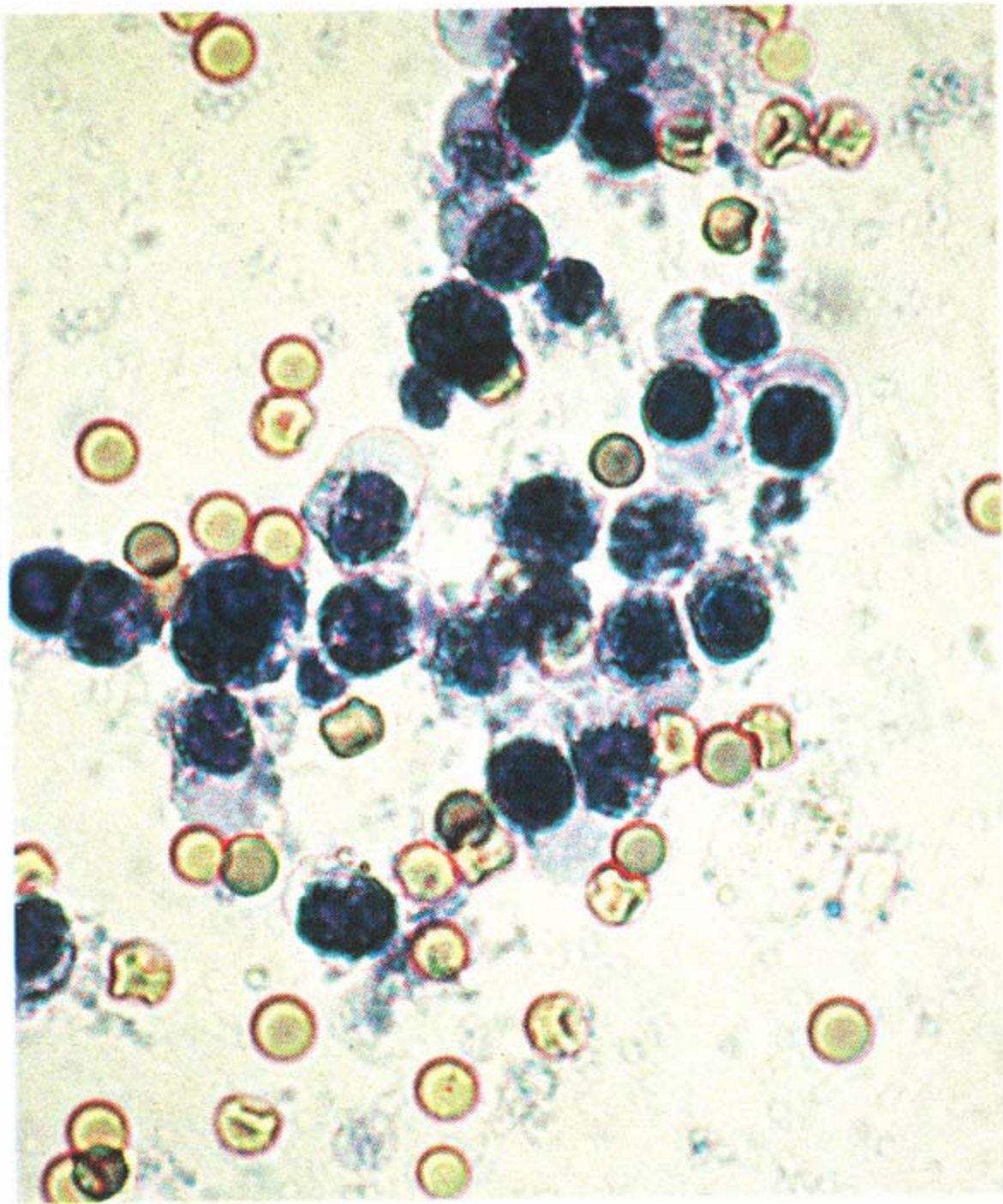


Fig. 49 Shigellosis.

Polymorphonuclear and mononuclear leucocytes and red blood cells in the stool of a patient with shigellosis. Presence of inflammatory cells in faeces is characteristic of infections due to invasive microorganisms. Methylene blue wet mount under cover slip. Courtesy of Dr H. L. DuPont.



Fig. 4.18 Positive Serény test. Keratoconjunctivitis in the rabbit produced by the instillation of shigella microorganism. By courtesy of Dr. H.L. DuPont.

Shigella

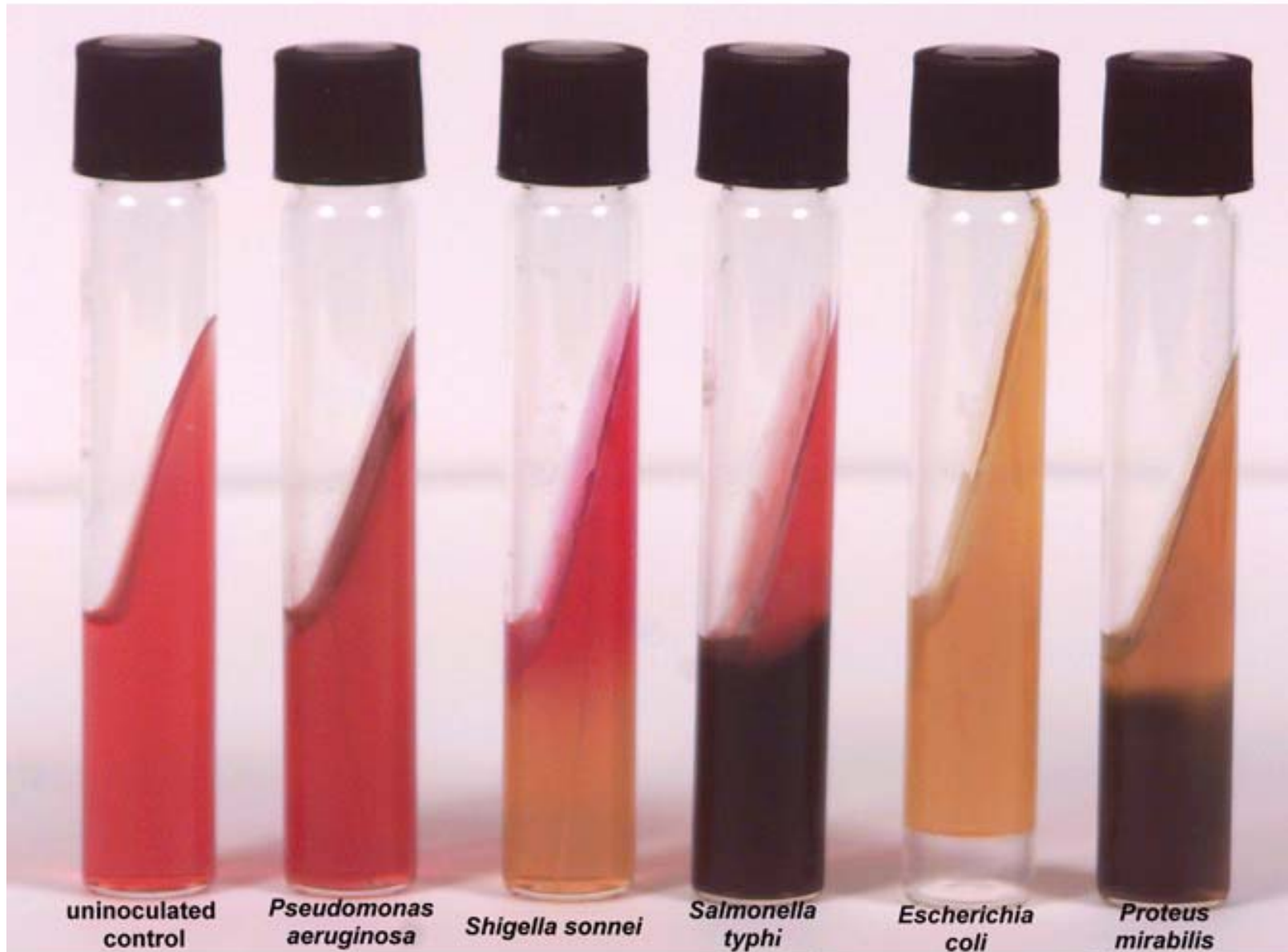


Diagnosis

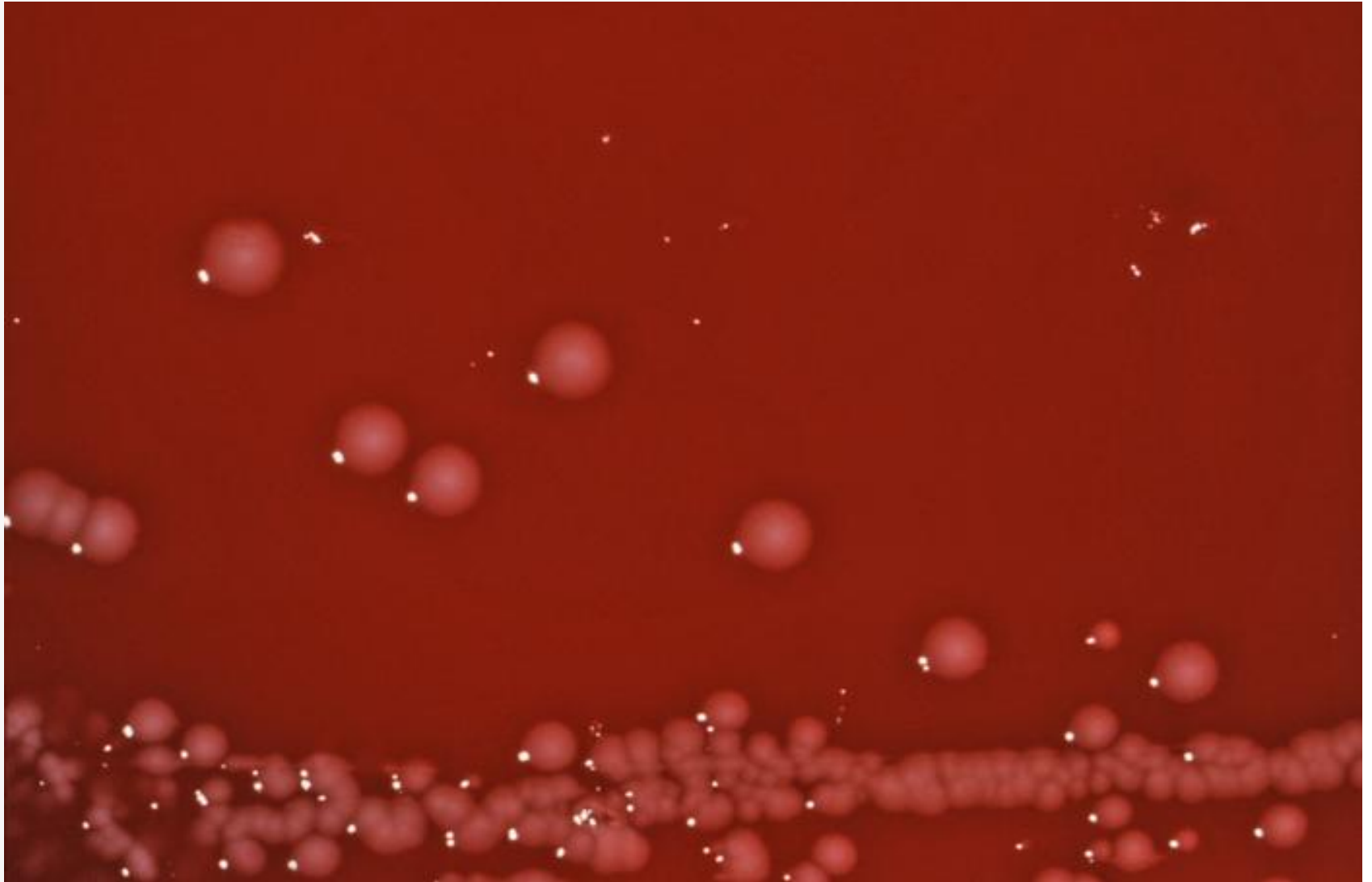
Direct kimutatás
(széklet)

Differenciálás –
Selectiv
táptalajok

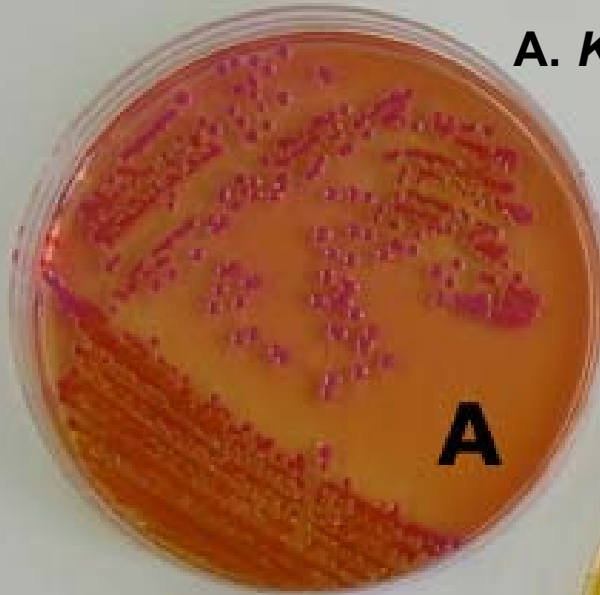
Identificatio
Szerotipizálás



Shigella boydii colonies on blood agar



Appearance of Colonies on *Salmonella-Shigella* Agar



A. *Klebsiella pneumoniae*

Klebsiella pneumoniae & *Escherichia coli* are positive for acid production from fermentation of the carbohydrate(s) present.

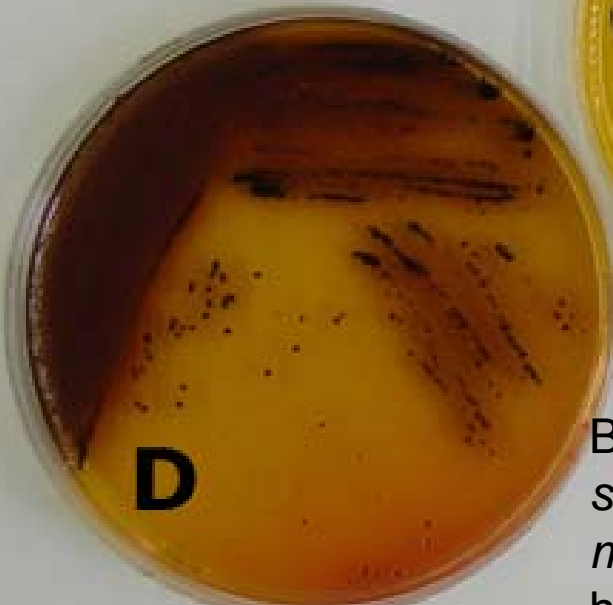


B. *Escherichia coli*



C: *Salmonella* sp.

Both *Salmonella* sp. & *Proteus mirabilis* product hydrogen sulfide.



D: *Proteus mirabilis*



E: *Pseudomonas aeruginosa*

The *Pseudomonas* colonies are nearly colorless.

Shigellosis

Prophylaxis

Expositio prophylaxis – Hygiene, rendszabályok

Therapia

Antibiogram

Tetracyclin, Ampicillin, Chloramphenicol, Sumetrolim

Enterobacteriaceae

Fakultatív pathogen

genus

Escherichia

Klebsiella csoport

Enterobacter

Edwardsiella

Citrobacter

Proteus csoport

Serratia

Providencia

Morganella

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EIEC (enteroinvasive)

EHEC (enterohemorrhagic)

EAggEC (enteroaggregativ)

Shigella

S. dysenteriae

S. flexneri

S. boydii

S. sonnei

Salmonella

S. typhi

S. paratyphi

Yersinia

Y. pestis

Y. pseudotuberculosis

Y. enterocolitica

Verisimilitudo

Yersinia enterocolitica

Morphologia Gram negativ,
bipolaris pálcák



www.wadsworth.org



Yersinia enterocolitica

tenyésztés

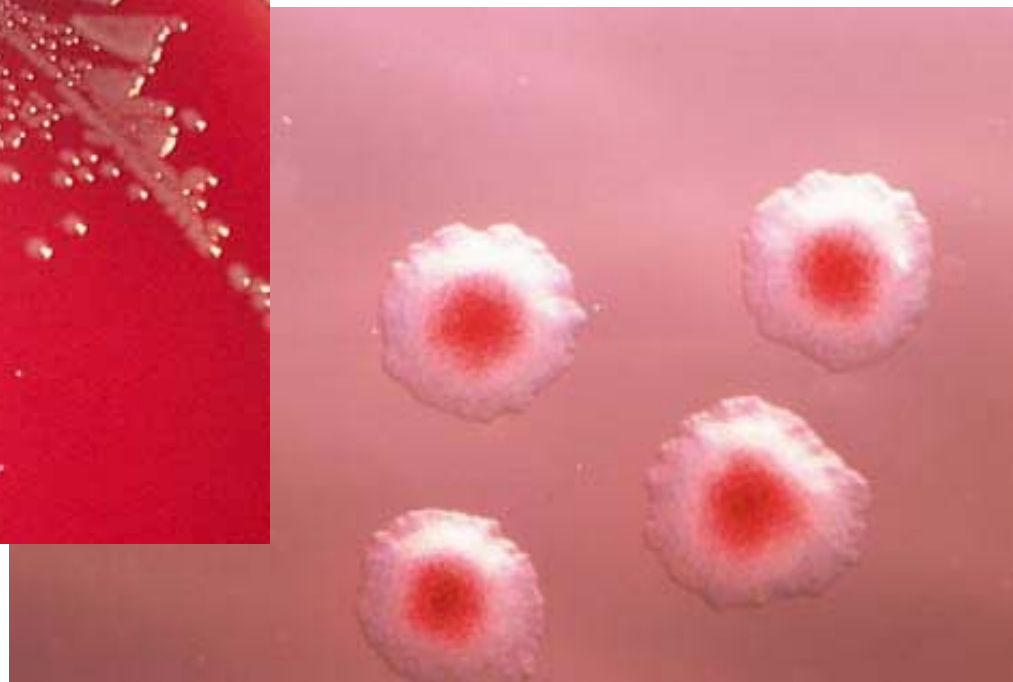
optimum 28°C, motilitás 28°C



Antigenek

O és H

Y. enterocolitica
O:5,27 CIN-ag



Blood agar



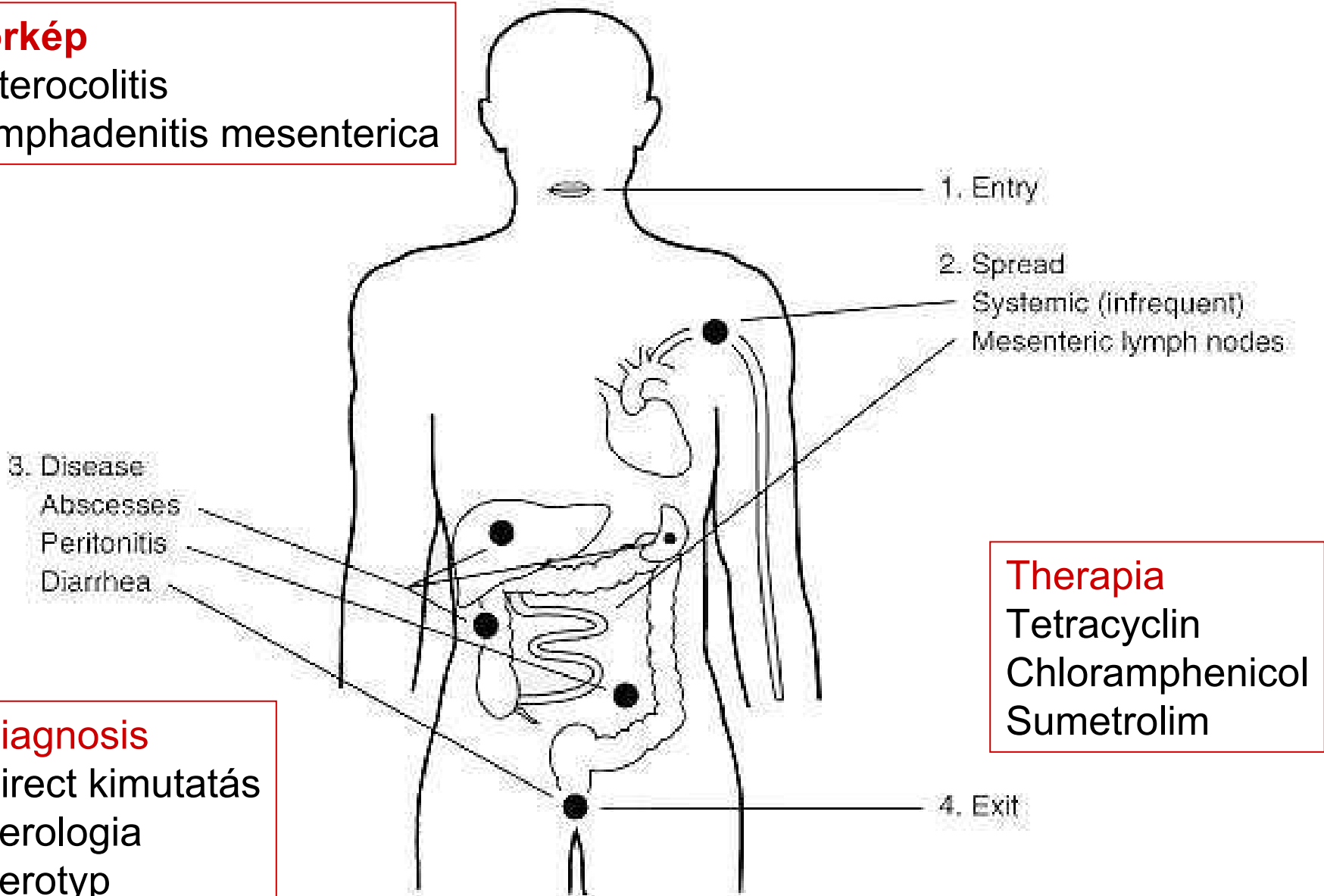
Y. enterocolitica

Figure 29-7 Pathogenesis of *Y. enterocolitica*.

Kórkép

Enterocolitis

Lymphadenitis mesenterica



Diagnosis

Direct kimutatás

Serologia

Serotyp

Therapia

Tetracyclin

Chloramphenicol

Sumetrolim

Yersinia pseudotuberculosis

Morphologia

Gram negatív, bipolaris pálca
flagellae

tenyésztés

könnyű, 37°C és 20°C; 4°C
motilitás 20°C

Pathogenitás

Pseudotuberculosis
rágcsálók

forrás:

Beteg állatok

behatolás: száj, mucosa

www.microbes-edu.org



Y. pseudotuberculosis

Yersinia pseudotuberculosis

Kórképek

Lymphadenitis mesenterica

Septicus-typhous Forma

Enteritis

Diagnosis

Direct kimutatás

Szerotipizálás (O3 és O9; újabban O8) – Agglutinatio

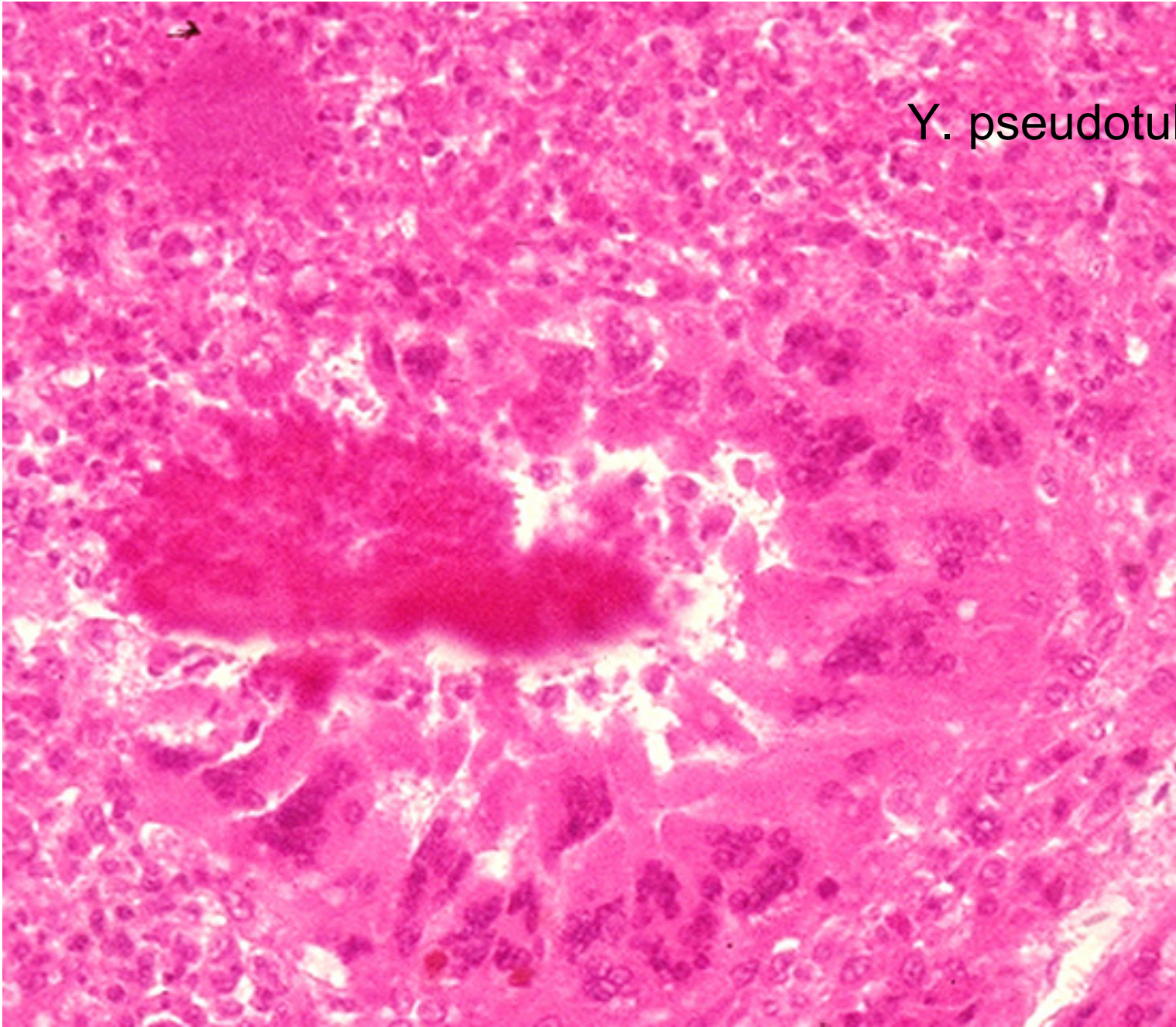
Serologia

Therapia

Tetracyclin



Fig. 4.50 Yersinia infection. Gross specimen of ileum, showing superficial necrosis of the intestinal musosa with several well-defined deep and superficial ulcers.



Y. pseudotuberculosis

**Microaerophil Gram
negatív pálcák –
Campylobacter és
Helicobacter**

BACTERIALIS BÉLFERTZŐZÉSEK

I. Typus

Enterotoxin

Hypersecretio
vékonybél

vizes hasmenés

Vibrio cholerae
Escherichia coli
(ETEC)

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Inflammatio

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gennyes, véres, nyákos
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Shigella
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Salmonella
Yersinia enterocolitica
Campylobacter jejuni
Aeromonas sp.
Vibrio parahaemolyticus

III. Typus

Penetratio, Generalisatio

Intracellularis kórokozó
Ileum

Typhus, Sepsis

Salmonella typhi
S. paratyphi A, B
Yersinia enterocolitica
Y. pseudotuberculosis
Campylobacter fetus

Exogen, bejutás: per os, faeco–oralis transmissio; hygiene!

Clostridium difficile
Clostridium perfringens

**Spiralis & hajlott
Gram-negatív bacteriumok**

Spirillaceae

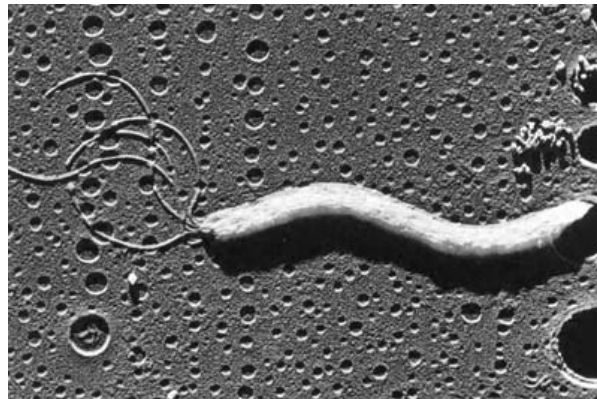
Campylobacter

Helicobacter

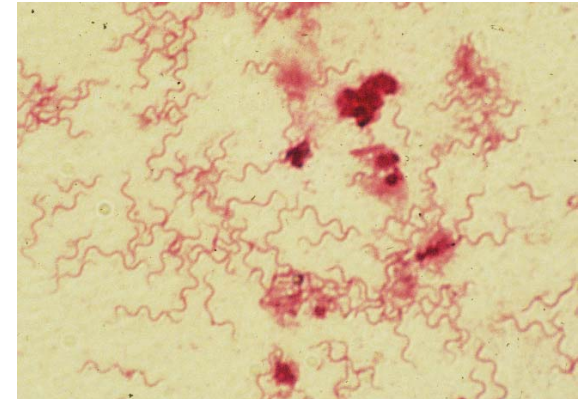
Spirillum



C. jejuni



H. pylori



S. minus

Legfontosabb *Campylobacter* fajok

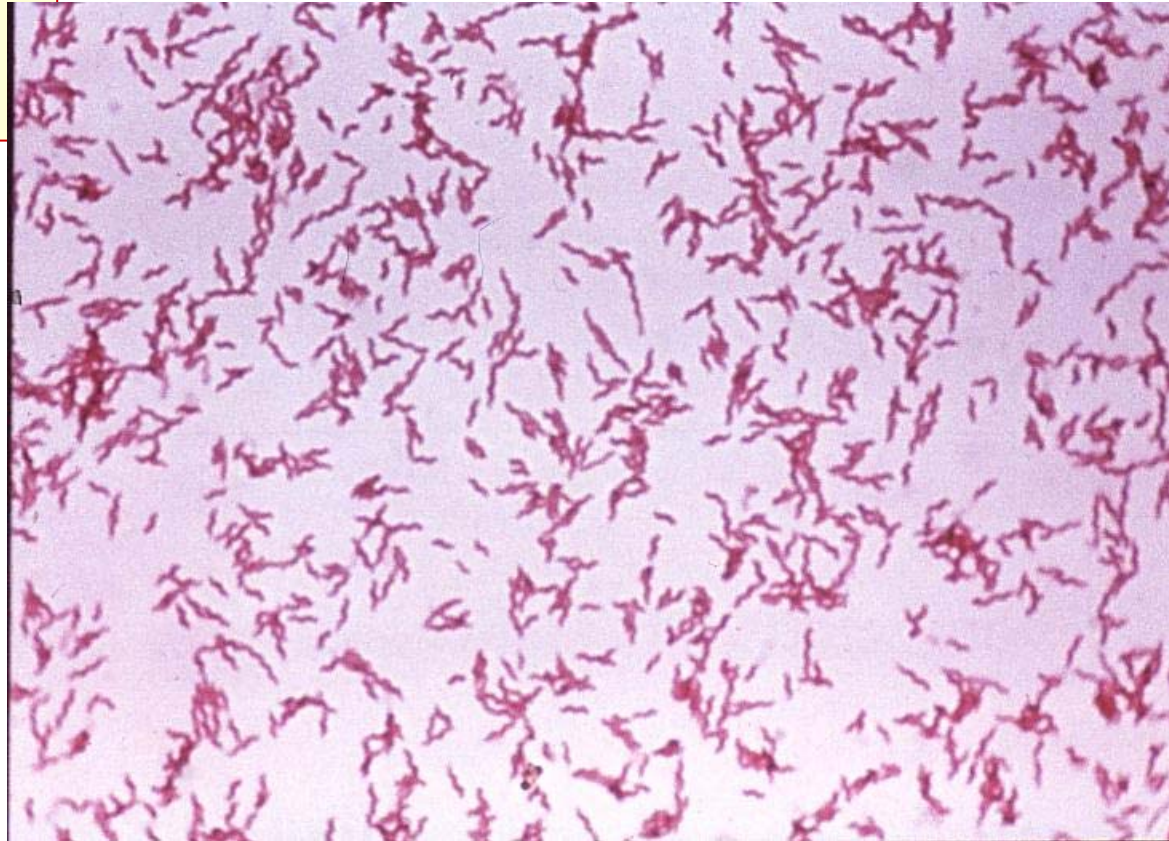
Species	Reservoir	kórképek	előfordulás
<i>C. jejuni</i>	szárnyas, sertés, szarvasmarha, nyúl	Gastroenteritis, sepsis, meningitis, Guillan-Barré	gyakori
<i>C. coli</i>	sertés, szárnyas, szarvasmarha, birka	Sepsis, gastroenteritis, meningitis	Uncommon
<i>C. fetus</i>	szarvasmarha, birka	Sepsis, gastroenteritis, meningitis	Uncommon
<i>C. lari</i>	szárnyas, sertés, macska, majom, ló	Gastroenteritis, sepsis	ritka
<i>C. upsalensis</i>	kutya, macska	Gastroenteritis, sepsis, abscess	?

Campylobacter

Campylobacter

Morphologia

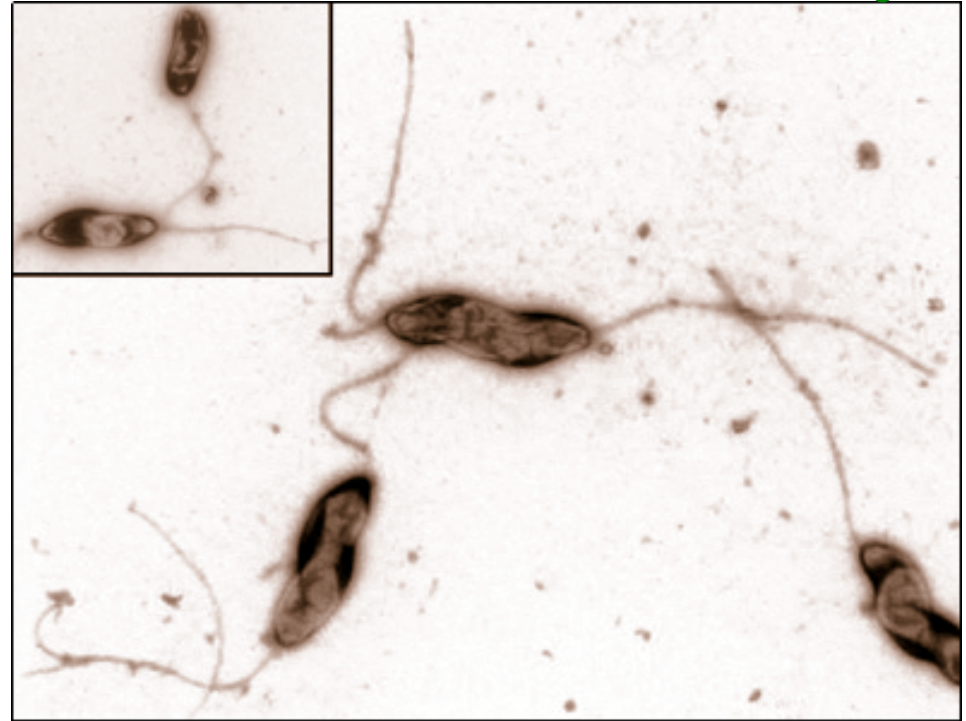
Gram negatív hajlott
pálca (0,3–0,6 μm)



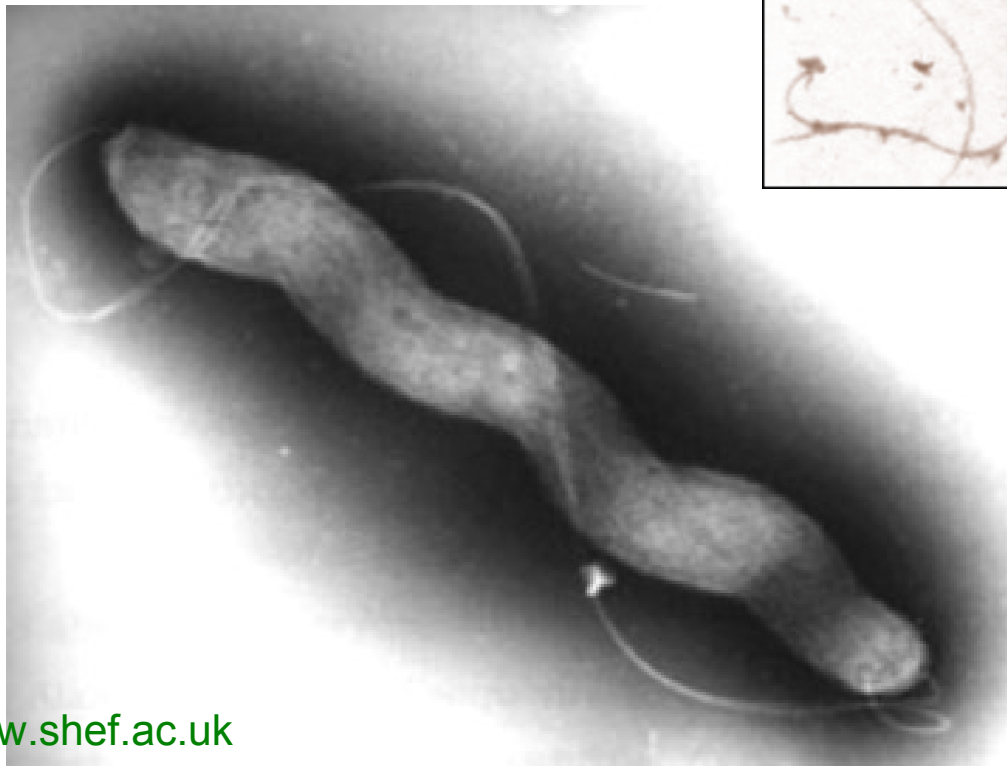
Campylobacter

www.wadsworth.org

Polaris flagellum



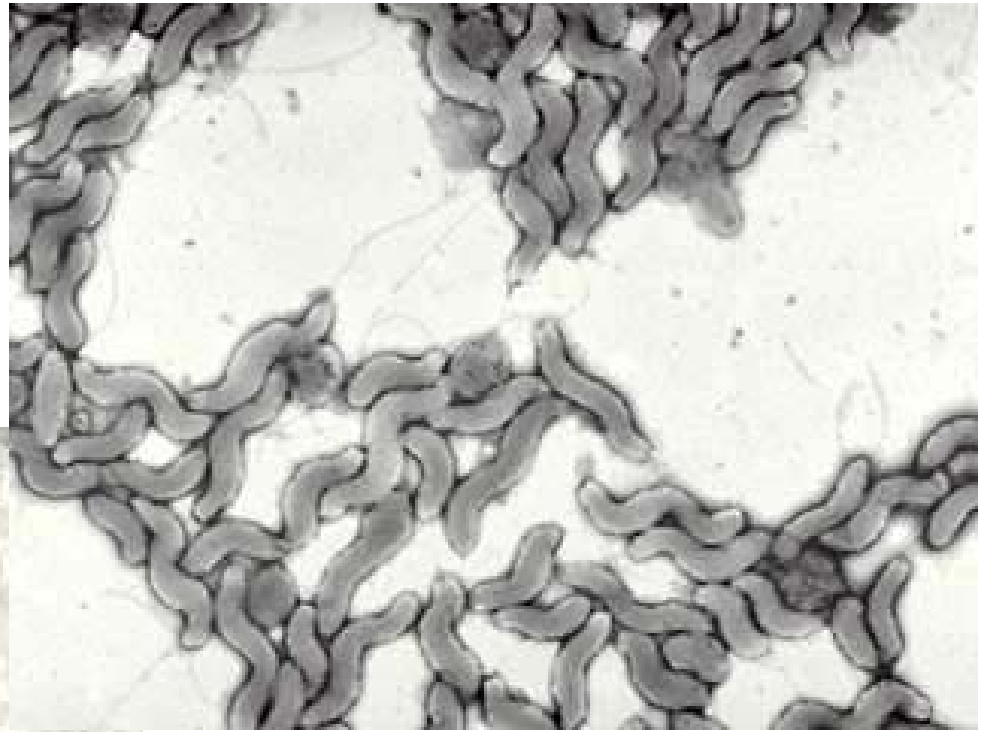
Transmission electron micrographs of *Campylobacter jejuni*, negatively stained to enhance contrast.



www.shef.ac.uk

Campylobacter jejuni

Campylobacter jejuni



Campylobacter

tenyésztés

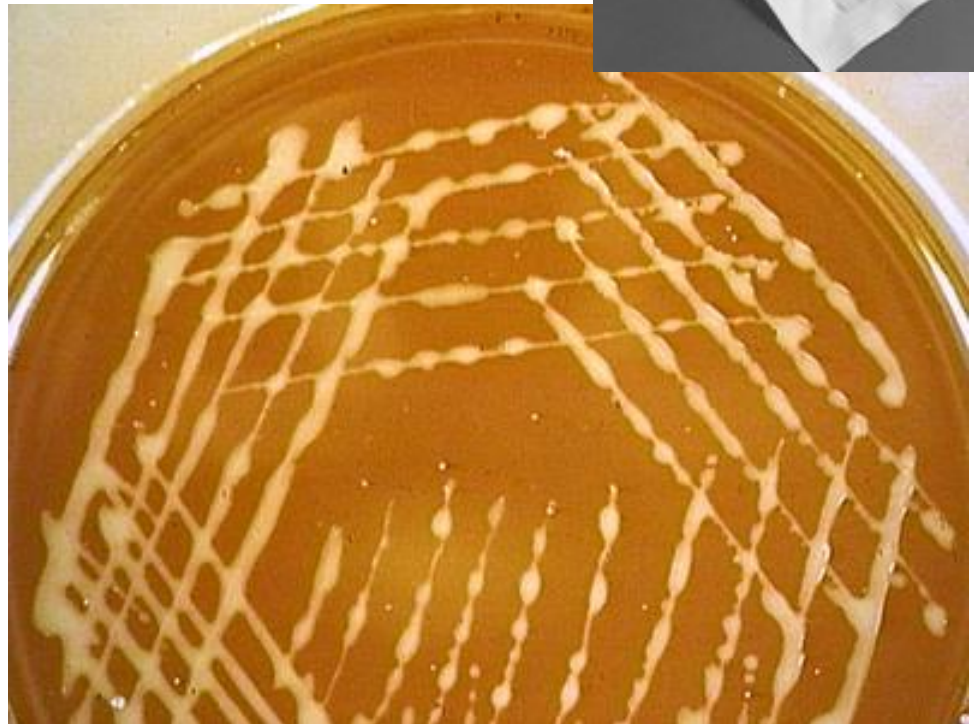
Microaerophil

5–7% O₂

5–10% CO₂

Thermophil: 42°C

Campy-blood-agar



Special media



Campylobacter coli ATCC 43478-24h

Campylobacter coli ATCC 43478-48h

Campylobacter jejuni ATCC 11322-48h

Campylobacter jejuni ATCC 11322-24h



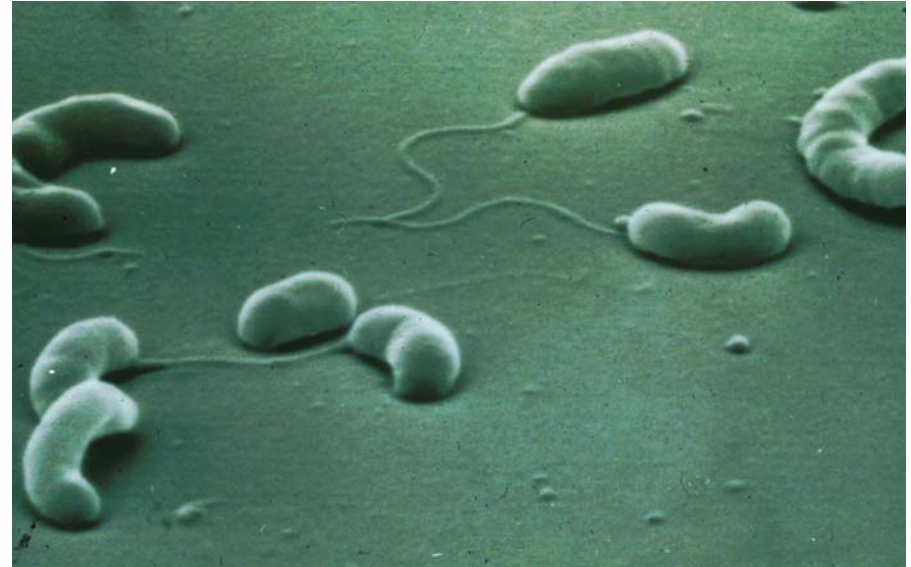
Campylobacter

Biokémia reakciók

Non-fermenting

- Catalase +, Oxidase +
- Nitrate reductio +
- Antigen szerkezet
 - O, H, K (Serotyping)

C. jejuni, SEM



C. jejuni, SEM

Campylobacter

Virulencia faktorok

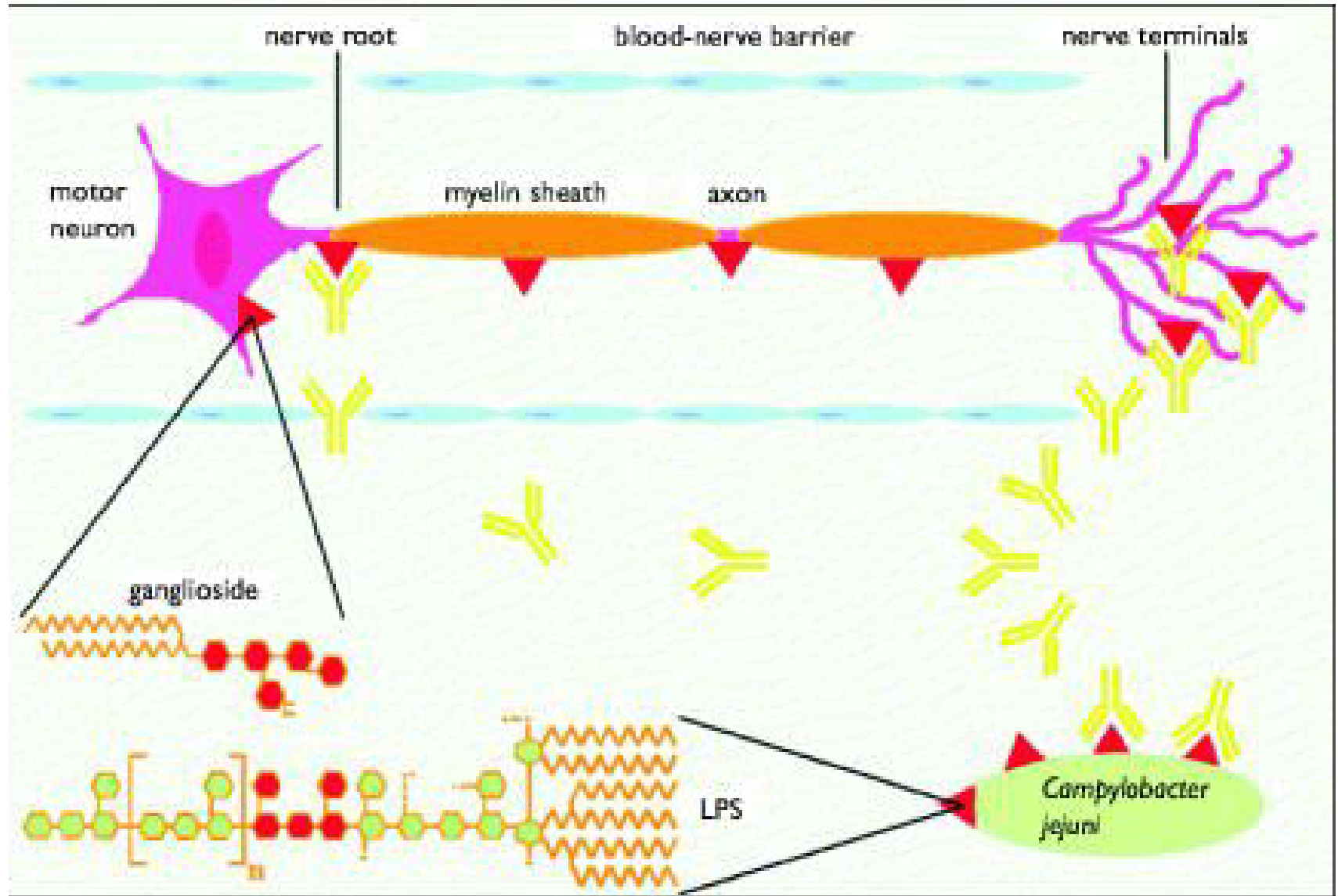
- flagellum \Rightarrow motilitás
- Adhaesio-s faktorok
- Invasio-s faktorok (?)
- Cytotoxin
- *C. fetus*: S-Protein \Rightarrow C3b kötődés gátlása \Rightarrow antiphagocyter hatás
- LPS

Complicatio:

- Guillan-Barré Syndroma

Szerkezeti hasonlóság: LPS-core és glycosphingolipid of Gangliosids (GM1, GM2) \Rightarrow Antibodies against GM1 \Rightarrow Autoimmun folyamat \Rightarrow **Demyelinisatio**

C. coli & Guillan-Barré syndrome



Epidemiologia

- Zoonosis!
- forrás:
kontaminált élelmiszer & víz

Nem megfelelő hőkezelés

- Emberről emberre: Faeco-oralis úton (gyermekek).
- ID: 500 (low)
- gyakori (fejlődő országokban a hasmenések 80%)



Campylobacteriosis: klinikai kép

- Incubatio: 1–2 nap
- Véres széklet
- láz
- Hasi fájdalom, görcsök
- Spontan gyógyulás: 1–7 nap alatt
- Complicatio
 - Időbeli elhúzódás
 - Systemás infectio
 - Reactive arthritis
 - Guillan-Barré syndroma



Diagnosis

- minta
 - széklet
 - haemokultura, liquor
 - élelmiszer
- tenyésztés (microaerophil, thermophil)
- Identificatio
- antibiogram



Campylo-agar
culture



Biochemical identification

Therapia & Preventio

- Supportiv (folyadék, electrolyt)
- Antibiotikum therapia
 - Gastroenteritis
 - Erythromycin, Doxycycline, Ciprofloxacin, Amoxicillin/clavulanic acid
 - Systemás infectio
 - Carbapenem, Aminoglycoside, Chloramphenicol
- Preventio: ételmiszer hygiene

Helicobacter

Helicobacter pylori

Morphologia

- Gram negativ, spiralis pálca

Polaris flagellae: Motilitas

tenyésztés

- Microaerophil

Biokémiai reakciók

- Non-fermenting
- Catalase +
- Oxidase +
- **Urease + (!)**



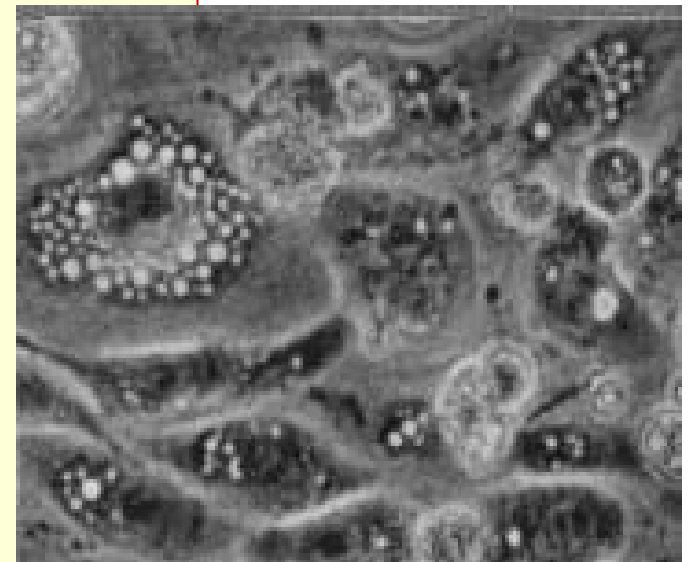
H. pylori

Virulencia faktorok

- Adhaesinek (HOP)
 - Flagellae (Motilitas)
 - Mucinase, phospholipase
 - Urease activitas
 - Vacuolizáló cytotoxin
 - Protein CagA
- Translocation via
Type IV. Secretion-system
changing cytoskeleton
IL-8, IL-1, TNF α Stimulation



Urease positive



Cytotoxic effect in HeLa cells

H. pylori

Epidemiologia

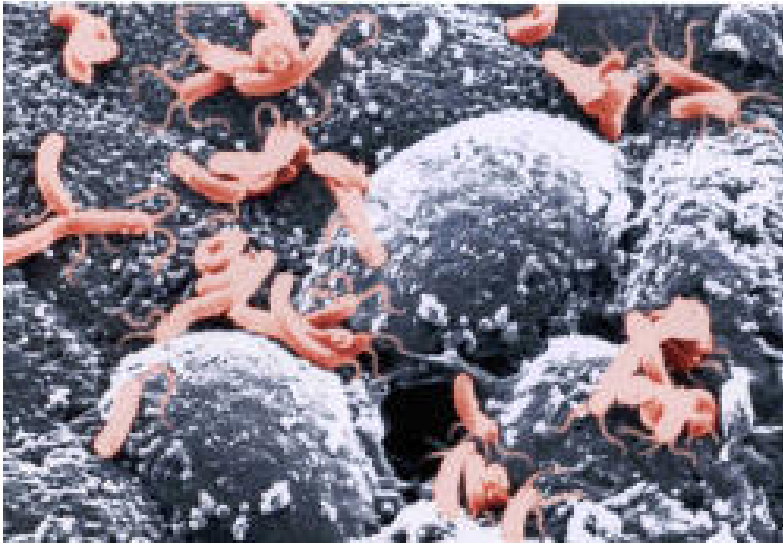
- Világ szerte
- Reservoir: emberek
- Transmissio
 - Faeco-oralis
 - Oro-oralis (nyál)
 - Endoscope!

Klinikai kép

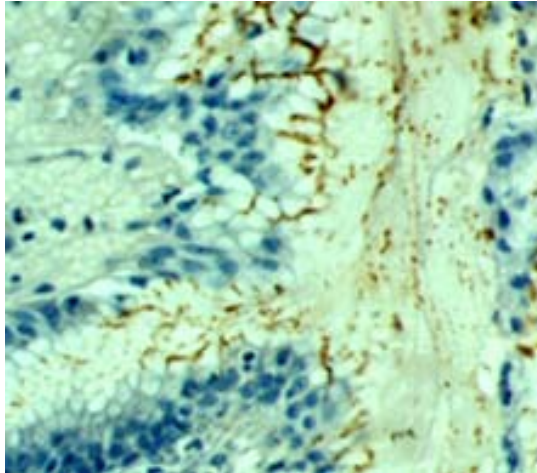
Acut Gastritis
Chronic-active Gastritis
Gastroduodenal Ulcus
Tumor



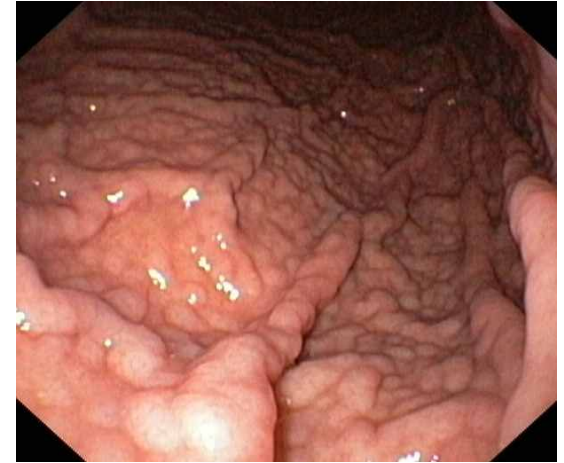
H. pylori & pepticus fekély



H. pylori on gastric mucosa, SEM



H. pylori histology, silver impregnation

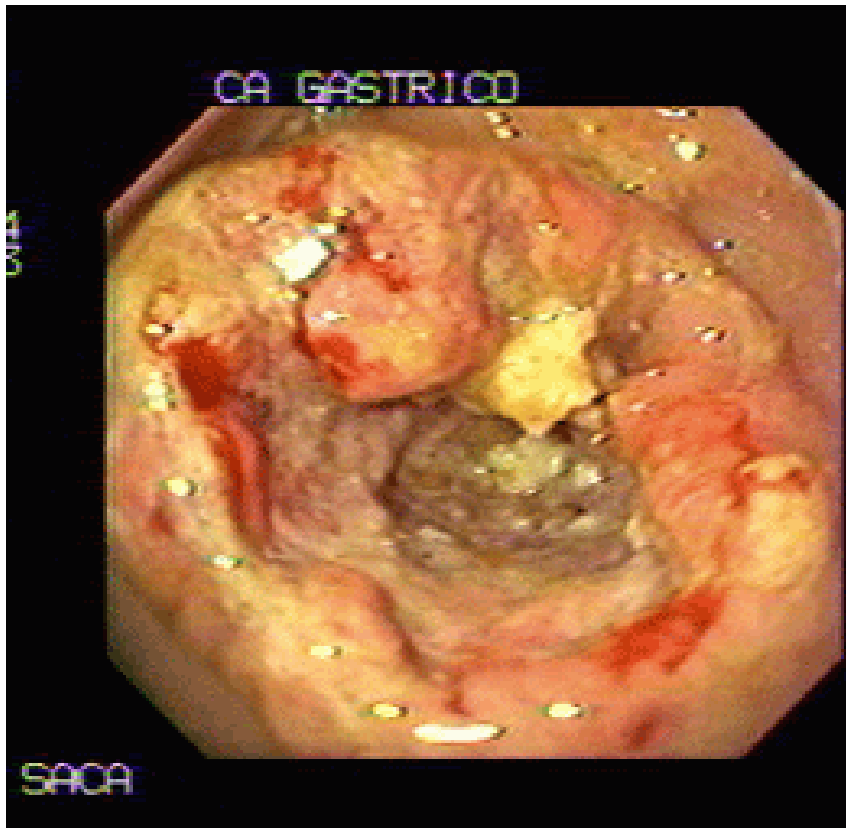


Chronic gastritis



Duodenal ulcer

H. pylori & gyomor carcinoma



Antral adenocarcinoma
gastrosopic finding

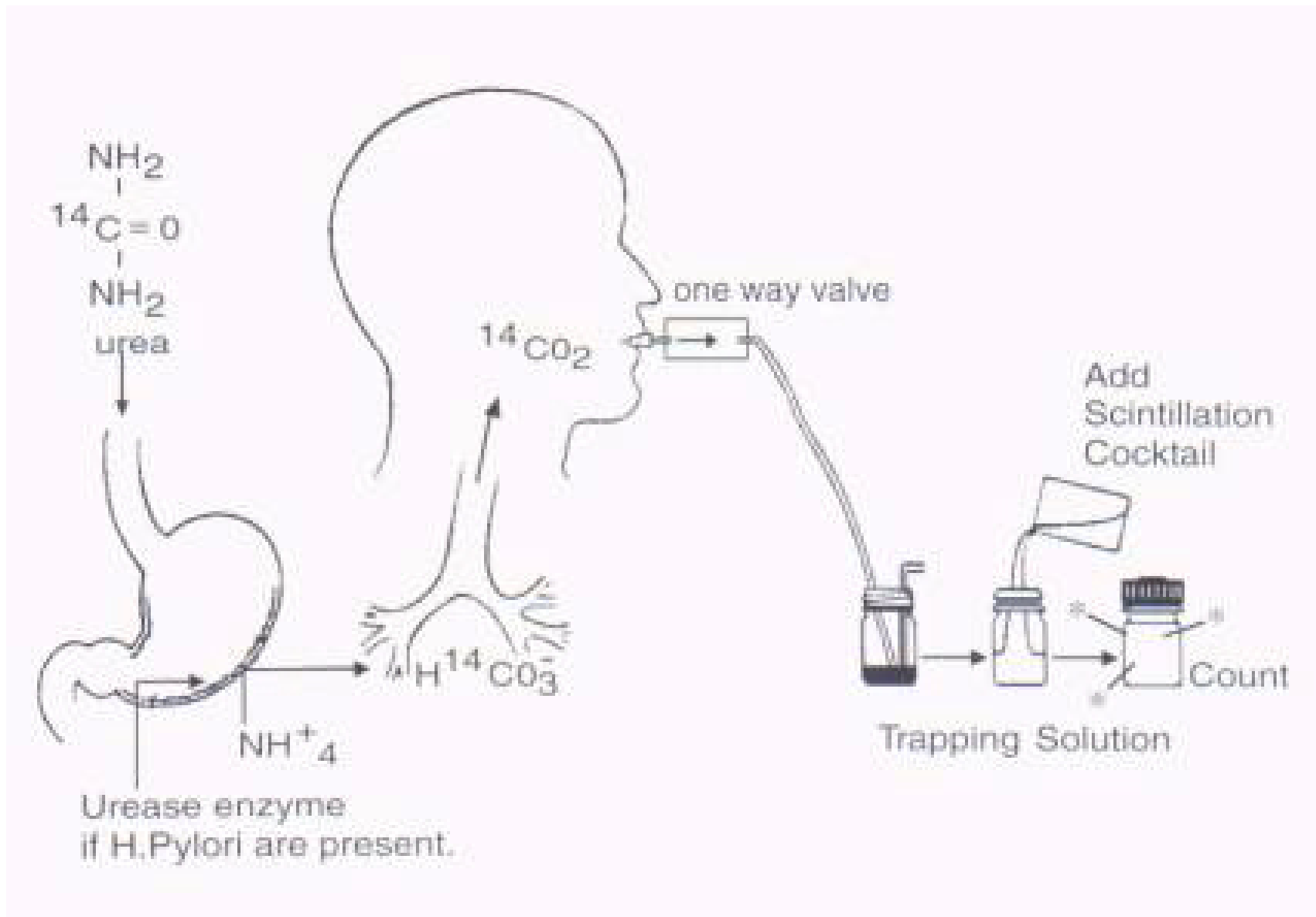


Gastric adenocarcinoma with
liver metastasis & ascites

Diagnosis *H. pylori*

- direct kimutatás: Helicobacter Antigen székletmintából
- biopsia: Histopathology, tenyésztés, molecularbiologia (PCR)
- radioactive Urea expiration test
- serologia: AT kimutatás

Radioactive Urea kilégzési test

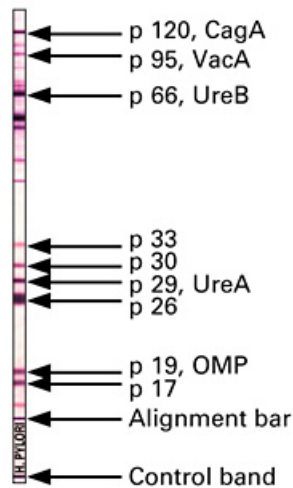


Microbiologiai Diagnosis *H. pylori*

ellenanyagok – serum

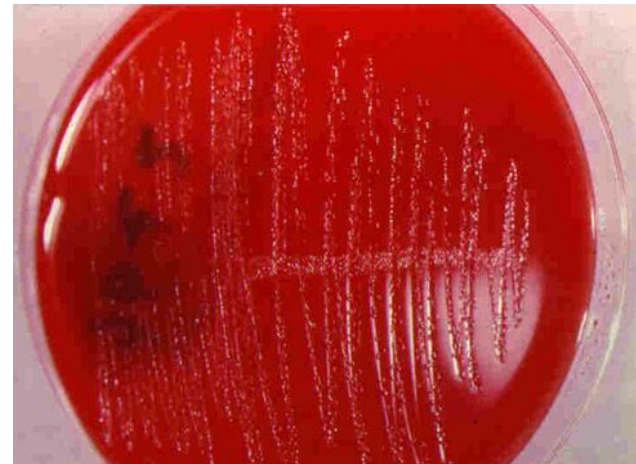


H. pylori ELISA (IgG, IgA)



H. pylori Western blot

tenyésztés –
Biopsia Material



H. pylori culture



Biochemical identification

H. pylori Eradicatio

- Combinatio - Therapia
 - Proton pumpa inhibitorok
 - Antibioticumok
 - Clarithromycin + Metronidazole
 - Amoxicillin + Metronidazole
 - Doxycycline + Metronidazole
 - Eradicatio: 90%

Santorini, 2009

THE END

