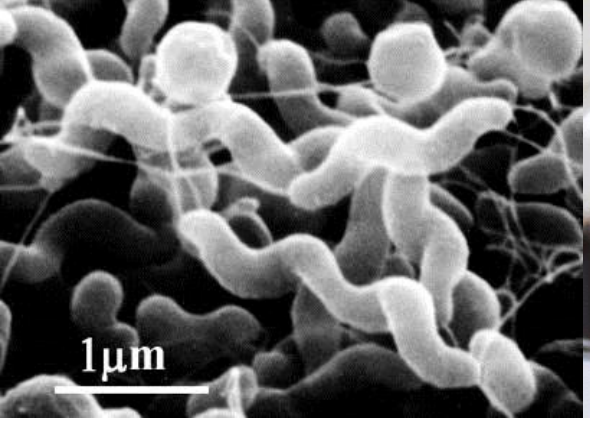
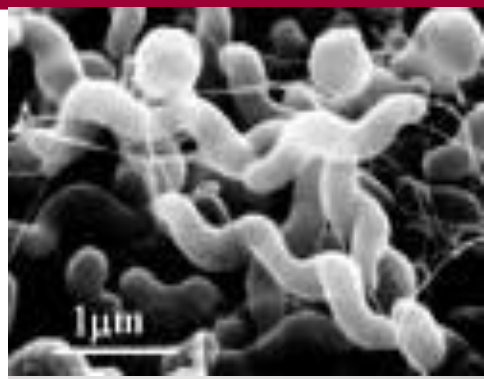


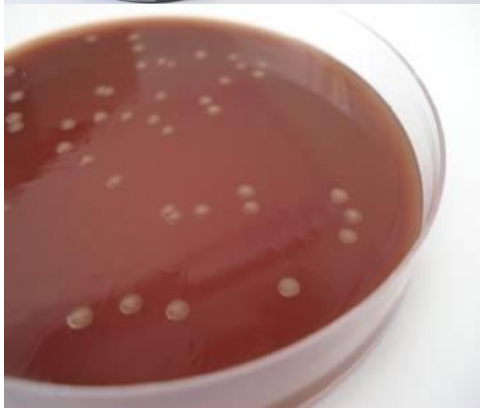

Campylobacter spp.: Characteristics, pathogenesis, distribution & detection

Eva Skřivanová

Taxonomy

- 26 species
- First mentioned on 1963 among ***Vibrio*** spp.
- In 1991, a revision of the taxonomy and nomenclature of the genus ***Campylobacter*** was proposed





Characteristics: microscopy

- Gram-negative
- Non-sporeforming rods
- Curved, S-shaped or spiral shaped bacteria with single polar flagella at one or both ends
- 0.2–0.8 μm wide and 0.5–5 μm long
- Exhibiting corkscrew motility

Characteristics: growth & metabolic activity

- Microaerophilic
- Neither ferment nor oxidise carbohydrates
- Some species (*C. jejuni*, *C. coli* and *C. lari)*, are thermophilic, growing optimally at 42°C

Characteristics: growth & metabolic activity

- **Microaerophilic**

- Neither ferment nor oxidise carbohydrates

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Characteristics: growth & metabolic activity

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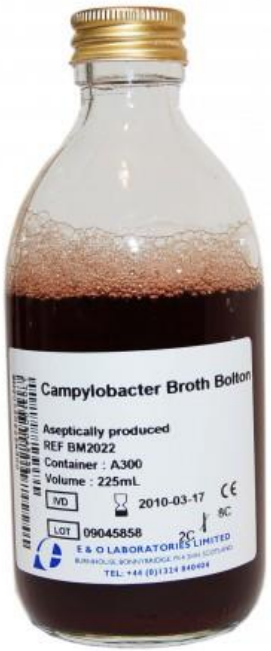


Characteristics: growth & metabolic activity

- Microaerophilic
- Neither ferment nor oxidise carbohydrates
- Some species (*C. jejuni*, *C. coli* and *C. lari)*, are thermophilic, growing optimally at 42°C

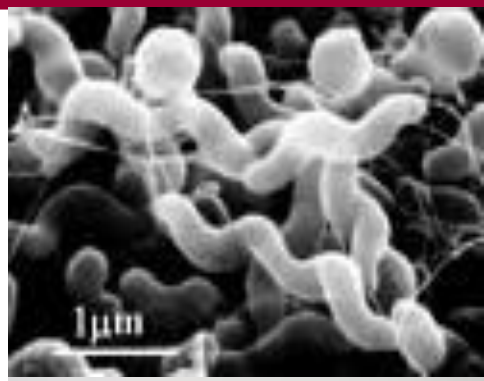
Characteristics: growth & metabolic activity

- Cultivation conditions
  - temperature: 30°C - 45°C, optimum 42°C
  - pH 5.5 – 8
  - NaCl < 2.5 %
  - atmosphere: microaerobic (5 % O_2,10% CO_2)

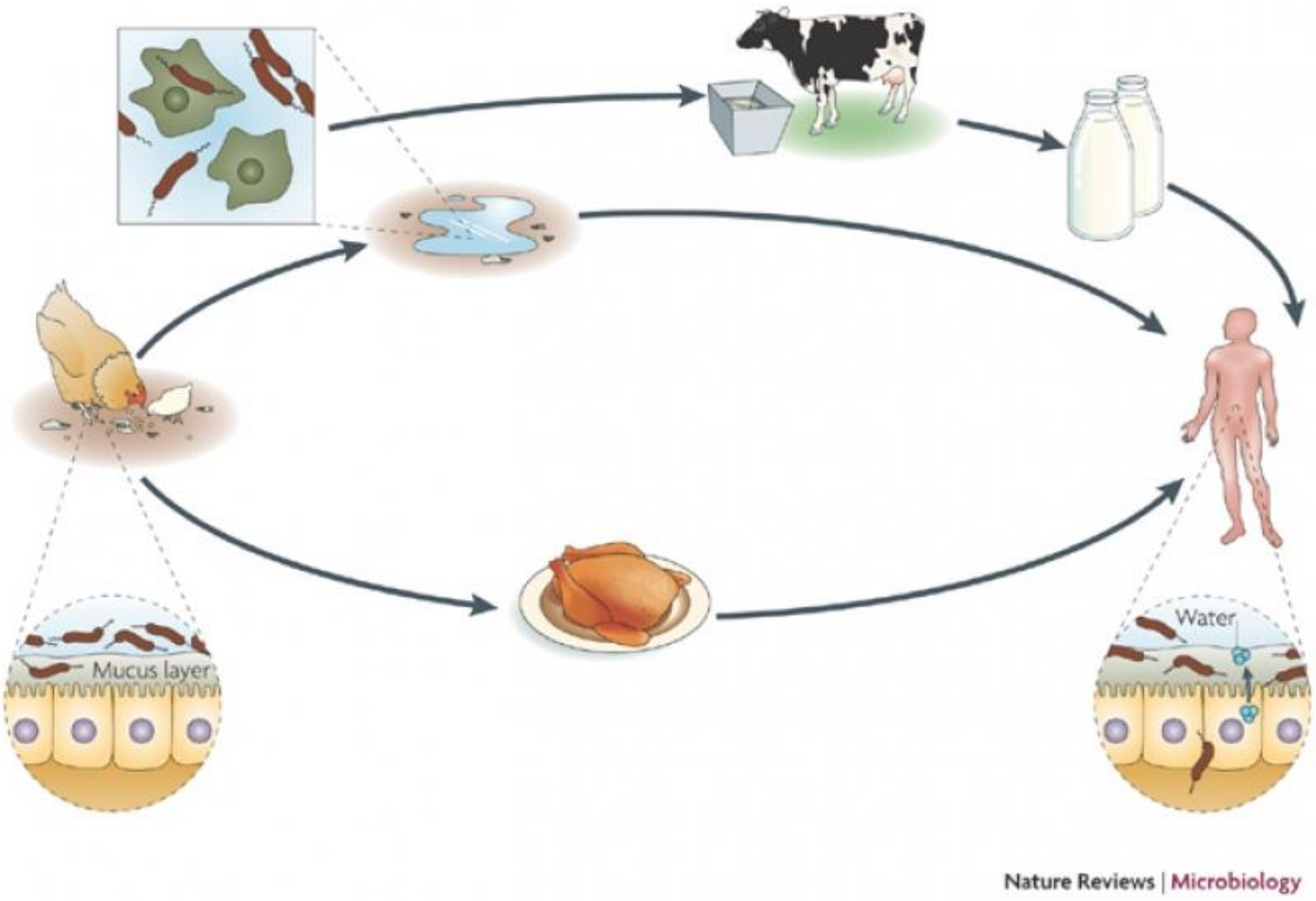
Occurrence & Distribution

- Campylobacters can colonise mucosal surfaces, usually the intestinal tract of most mammalian and avian species

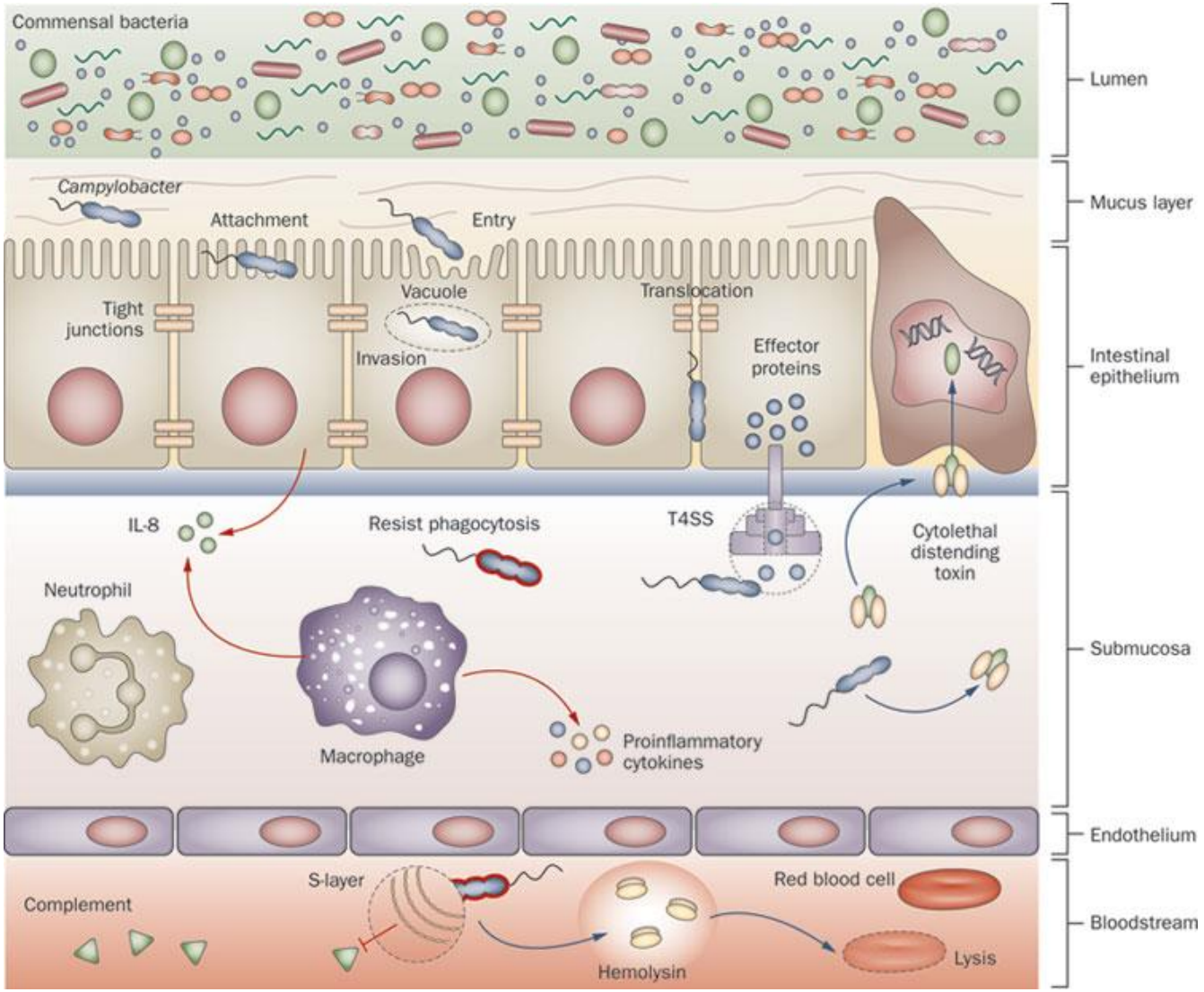
- The most frequently isolated species: ***C. jejuni*** & ***C. coli***



C. jejuni



Proposed mechanisms of pathogenesis used by emerging *Campylobacter* species to colonize the intestinal tract or to spread to systemic sites



Man, S. M. (2011) The clinical importance of emerging *Campylobacter* species
Nat. Rev. Gastroenterol. Hepatol. doi:10.1038/nrgastro.2011.191

Table ES-1: Annual Disease Burden Caused by 14 Foodborne Pathogens

Pathogen	Combined Rank*	QALY loss	Cost of Illness ($ mil.)	Illnesses#	Hospital-izations#	Deaths#
Salmonella spp.	1	16,782	3,309	1,027,561	19,336	378
Toxoplasma gondii	2	10,964	2,973	86,686	4,428	327
Campylobacter spp.	3	13,256	1,747	845,024	8,463	76
Listeria monocytogenes	3	9,651	2,655	1,591	1,455	255
Norovirus	5	5,023	2,002	5,461,731	14,663	149
E.coli 0157:H7	6	1,565	272	63,153	2,138	20
Clostridium perfringens	6	875	309	965,958	438	26
Yersinia enterocolitica	8	1,415	252	97,656	533	29
Vibrio vulnificus	8	557	291	96	93	36
Shigella spp.	10	545	121	131,254	1,456	10
Vibrio other+	11	341	47	57,616	210	4
Cryptosporidium parvum	12	149	107	52,228	183	12
E.coli non-O157 STEC	13	327	26	112,752	271	0
Cyclospora cayetanensis	14	10	2	11,407	11	0
Total		**61,461**	**14,114**	**8,914,713**	**53,678**	**1,322**

* Combined rank is the rank order when QALY rank and COI rank are averaged
# Incidence estimates are mean estimates reported in Scallan et al. (2011a).
+ includes *Vibrio parahaemolyticus* and other non-choleric *Vibrio* species

Ranking by pathogen – food pair

TABLE ES-2: THE TOP 10 PATHOGEN-FOOD COMBINATIONS IN TERMS OF ANNUAL DISEASE BURDEN, BY COMBINED RANK

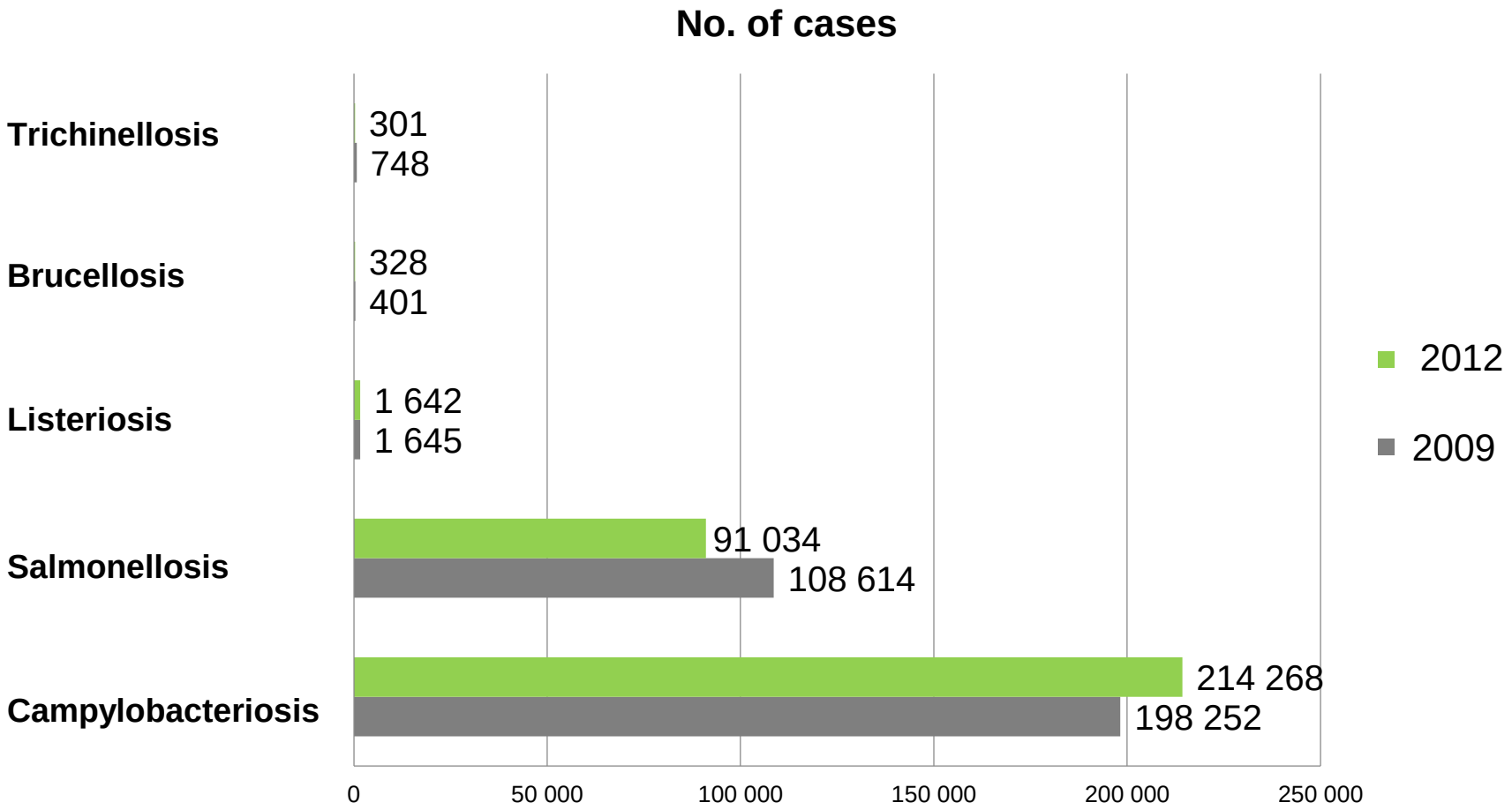
PATHOGEN-FOOD COMBINATIONS	COMBINED RANK	QALY LOSS	COST OF ILLNESS ($ MIL.)	ILLNESSES	HOSPITAL-IZATIONS	DEATHS
Campylobacter – Poultry	1	9,541	1,257	608,231	6,091	55
Toxoplasma – Pork	2	4,495	1,219	35,537	1,815	134
Listeria – Deli Meats	3	3,948	1,086	651	595	104
Salmonella – Poultry	4	3,610	712	221,045	4,159	81
Listeria – Dairy products	5	2,632	724	434	397	70
Salmonella – Complex foods	6	3,195	630	195,655	3,682	72
Norovirus – Complex foods	6	2,294	914	2,494,222	6,696	68
Salmonella – Produce	8	2,781	548	170,264	3,204	63
Toxoplasma – Beef	8	2,541	689	20,086	1,026	76
Salmonella – Eggs	10	1,878	370	115,003	2,164	42
TOTAL		36,915	8,151	3,861,128	29,830	765

Campylobacter in poultry is ranked first in both QALYs and dollars. While *Campyolobacter* is only the third (tied) ranked pathogen overall, these impacts are estimated to be primarily focused in a single food commodity, based on our expert elicitation.

Ranking the Risks: The 10 Pathogen-Food Combinations with the Greatest Burden on Public Health

Incidence

of major food-borne pathogens related diseases (EU 2009 and 2012)

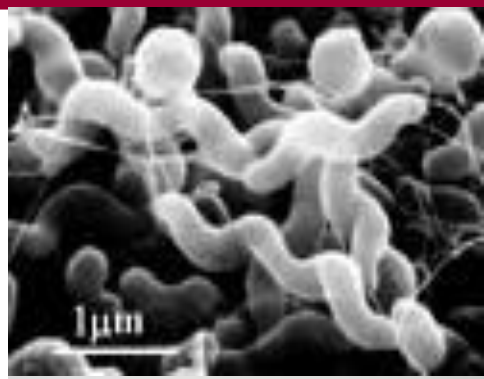


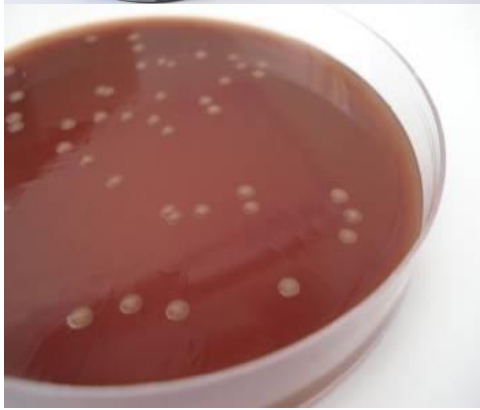
Campylobacter jejuni

- Isolated mostly from poultry, young livestock, including piglets, lambs and calves with enteritis

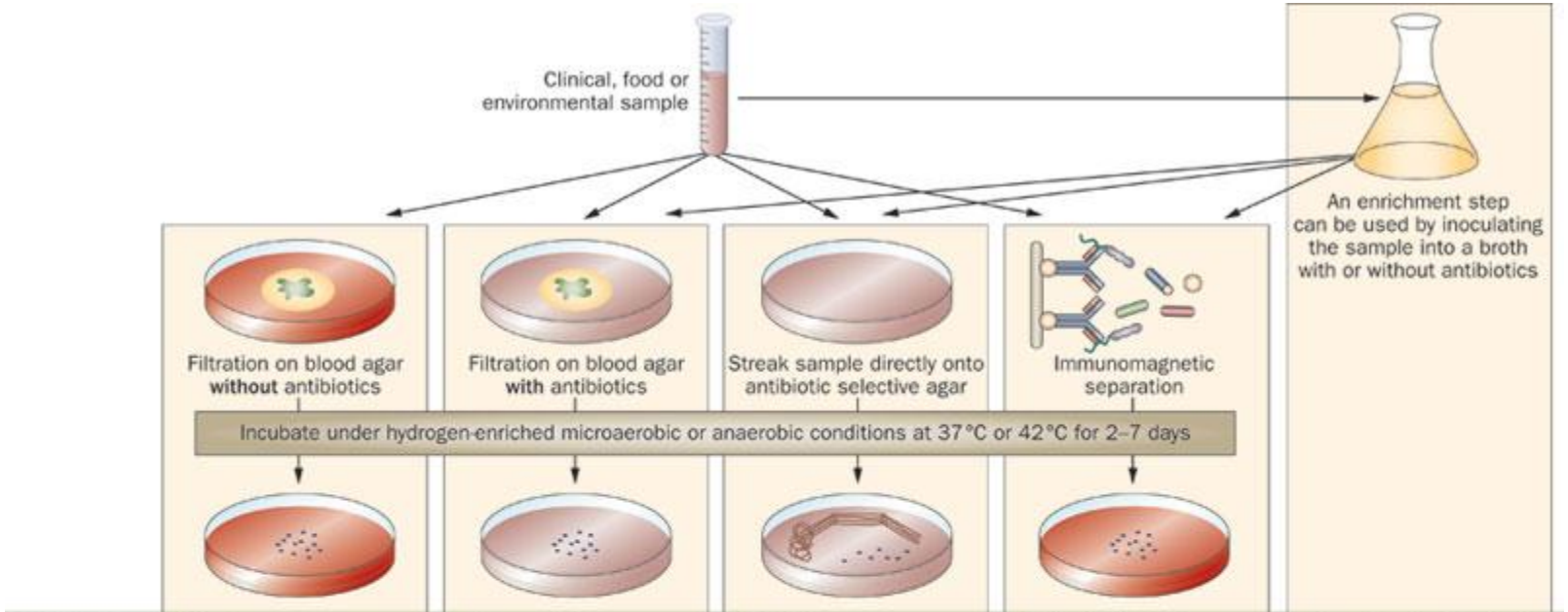
- *C. jejuni/coli* infection in humans: acute enteritis and abdominal pain lasting for 7 days or more

- Possible complications: bacteraemia, Guillain–Barré syndrome, reactive arthritis, abortion





Methods of detection



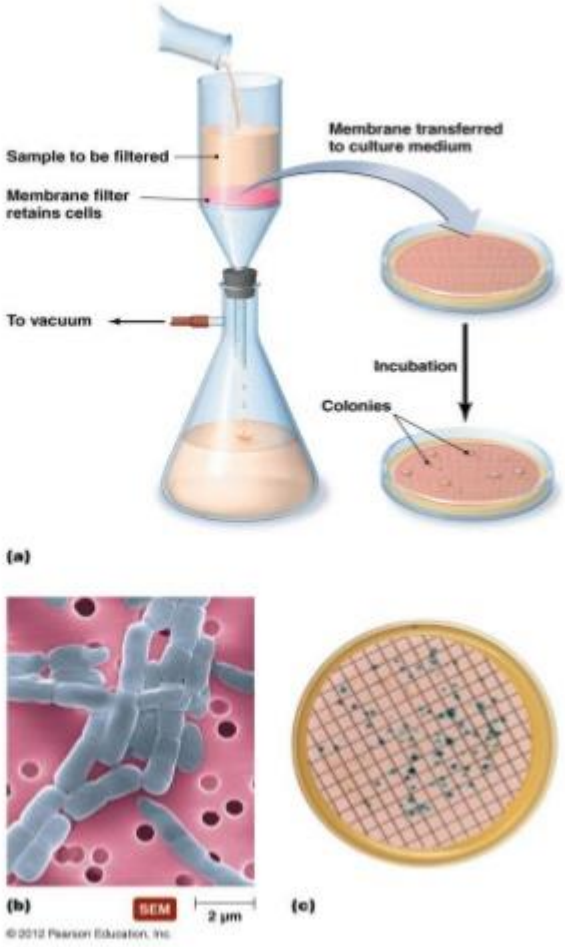
	Filtration on blood agar without antibiotics	Filtration on blood agar with antibiotics	Streak sample directly onto antibiotic selective agar	Immunomagnetic separation	An enrichment step can be used by inoculating the sample into a broth with or without antibiotics
Advantages	▪ Removes large or nonmotile organisms during filtration ▪ The lack of antibiotics in media supports the growth of all *Campylobacter* species	▪ Removes large or nonmotile organisms during filtration ▪ Inhibits the growth of a range of commensal bacteria with the use of antibiotics in media	▪ Simple ▪ Cost-effective ▪ Inhibits the growth of a range of commensal organisms with the use of antibiotics in media	▪ Selectively captures target bacteria with the use of antibodies ▪ Removes other nonspecific bacteria by several washing steps	▪ Promotes the growth of *Campylobacter* species present in low numbers ▪ Increases the motility of *Campylobacter* species for use in filtration methods
Disadvantages	▪ Labor-intensive ▪ Blocks the passage of nonmotile *Campylobacter* species ▪ Some small nonfastidious organisms will traverse the filter membrane ▪ Not all filter membrane materials are equally effective	▪ Labor-intensive ▪ Blocks the passage of nonmotile *Campylobacter* species ▪ Inhibits the growth of *Campylobacter* species sensitive to the antibiotics used ▪ Not all filter membrane materials are equally effective	▪ Inhibits the growth of *Campylobacter* species sensitive to the antibiotics used ▪ Not all commensal bacteria can be inhibited by the antibiotics used	▪ Relatively costly compared with other methods ▪ Labor-intensive	▪ Increases the number of other nonfastidious bacteria present in the sample

Nature.com

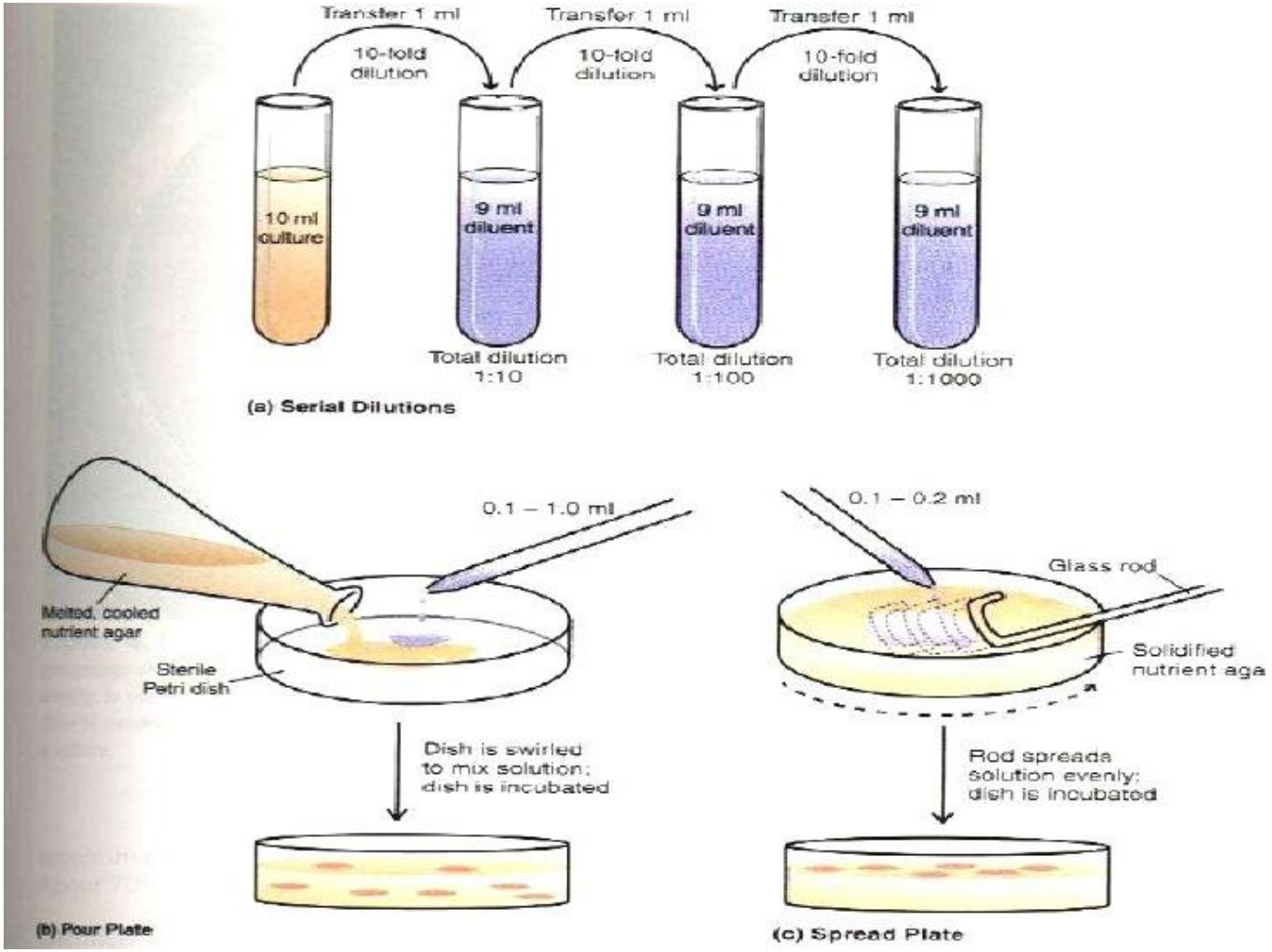
Methods of detection

Direct Method

Counting Bacteria by Membrane Filtration



Methods of detection



Methods of detection: food and water

- Two ISO procedures:
  - 1. Horizontal method for detection of thermotolerant *Campylobacter* in food and animal feeding stuffs (ISO 10272-1:2006)
  - 2. Procedure for the isolation of *Campylobacter* from water (ISO 17995:2005)

Methods of detection

- **Collection of samples**
  - *At the slaughter*
  - *From food*
  - *From feed*
    - Aseptically
    - Representative sample
    - Transport media
    - Fast transport to the laboratory
    - Ideally at 4 °C in microaerophilic conditions

Methods of detection: animals

- **Collection of samples**
  - *Poultry at the farm*
    - ***C. jejuni*** (65–95%), ***C. coli*** and rarely other *Campy.* species
    - **Colonisation is age-related** (> with age)
    - Samples should be taken close to the slaughter date
    - Fresh faeces/caecal droppings or cloacal swabs
    - Prevented from drying out

Methods of detection: animals

- **Collection of samples**
  - *Cattle, sheep and pigs at the farm*
    - ***C. jejuni***, ***C. coli***, ***C. hyointestinalis***, ***C. fetus***
    - Samples should be taken repeatedly (intermittent shedding)
    - Fresh faecal/rectal samples
    - Prevented from drying out

Methods of detection

- **Transport and treatment of samples**
  - *Transport*
    - Campylobacters are sentitive to environmental conditions (O_2, dehydratation, sunlight, t°)
    - Prevent of sunlight, high and low temperatures and its fluctuations, store at 4°C
    - Transport media: Amies, Cary-Blair, Stuart medium
    - In laboratory: process asap. (same day: RT, 2-3 days: 4°C + equilibrate to RT before processing)

Methods of detection

- **Isolation of Campylobacter**
  - *Selective media*
    - *Blood containing media*
    - *Charchoal containing media*

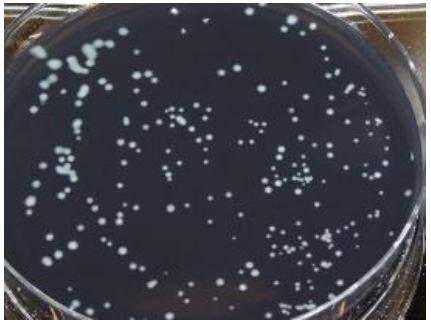
    → Remove O_2 derivatives
    - *Antibiotics*
      - *Cefalosporins*
      - *Vancomycin*
      - *Trimethoprim*
      - *Amphotericin B*

      → Selectivity

Methods of detection: animals

- *Blood containing media*
  - *Preston agar*
  - *Skirrow agar*
  - *Butzler agar*

- *Charchoal containing media*
  - *mCCDA agar (modified charcoal cefoperazone deoxycholate agar)*
  - *Karmali agar*
  - *CAT agar (cefoperazone, amphotericin and teicoplanin)*



Methods of detection: animals

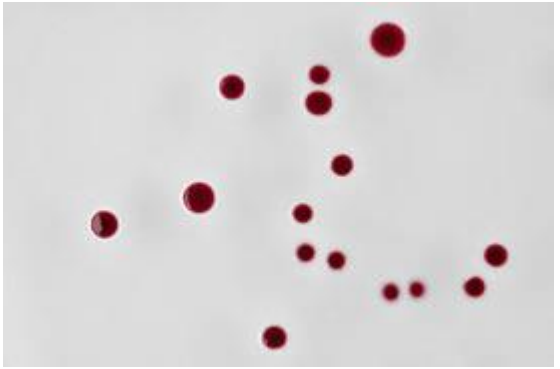
- **Incubation**
  - *Atmosphere*
    - *5 % O_2,10 % CO_2*
  - *Temperature*
    - *37°C*
    - *42°C*
  - *Time*
    - *24 – 48 h*

Methods of detection: animals

- **Confirmation**

Methods of detection: animals

- **Confirmation**
  - *Identification on solid medium, typical colonies for each agar type*
  - *Microscopic examination of morphology and motility*
  - *Biochemical confirmation*
  - *Immunological confirmation*
  - *Molecular biology (PCR)*



Methods of detection: animals

- **Identification of *Campylobacter* to the species level:**
  - **Biochemical detection**

Table 2. *Basic phenotypic characteristics of selected thermophilic* Campylobacter *species*

Characteristics	*C. jejuni*	*C. coli*	*C. lari*
Hydrolysis of hippurate	+	–	–
Hydrolysis of indoxyl acetate	+	+	–

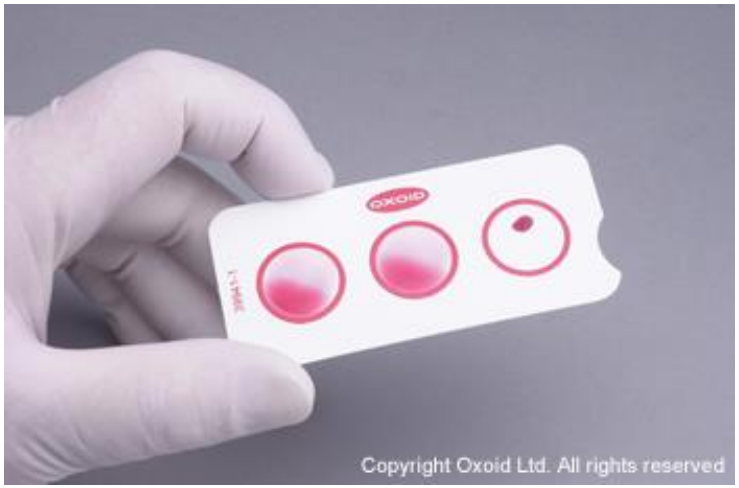
Key: + = positive; – = negative.

OIE Terrestrial Manual, 2008

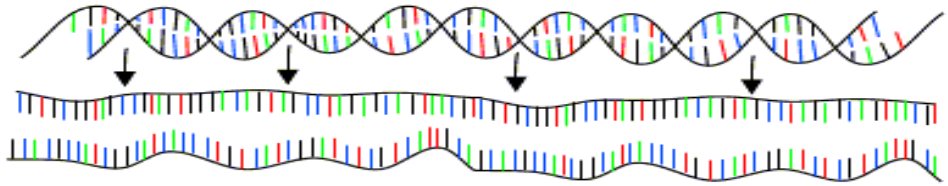
Methods of detection: animals

- **Identification of *Campylobacter:***

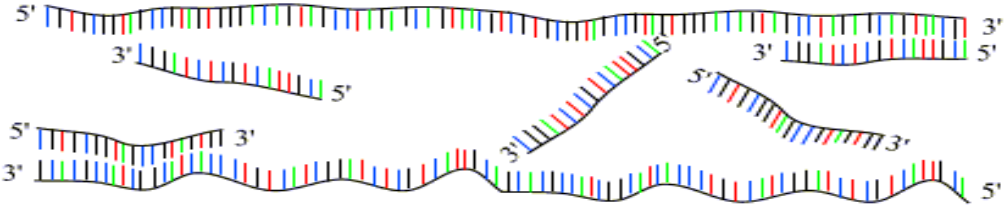
  Immunological detection



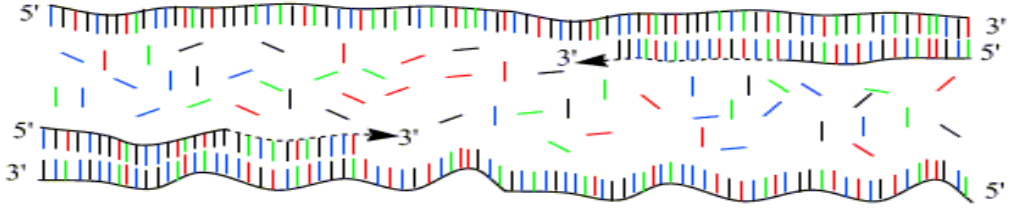
PCR



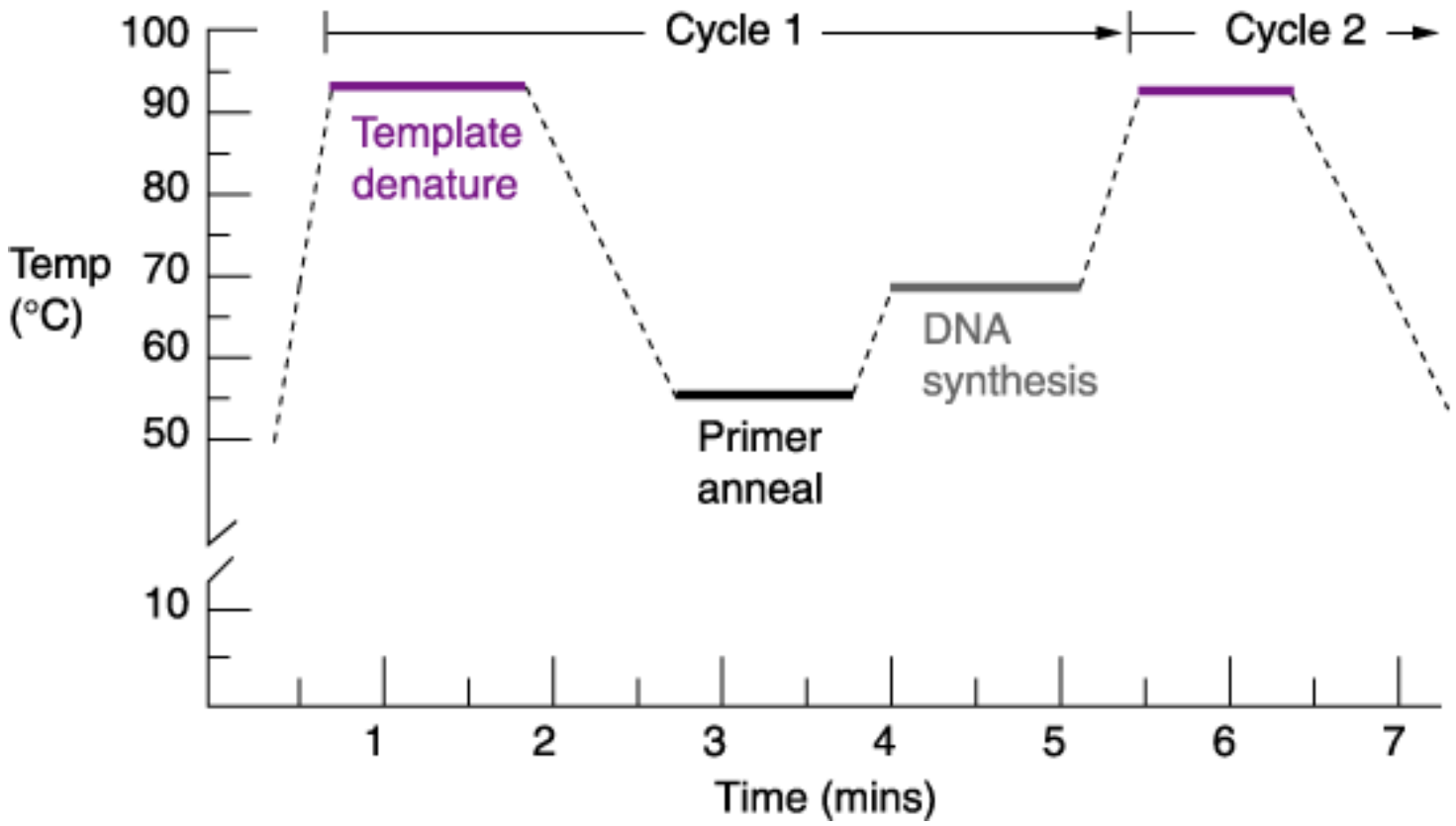
Denaturation



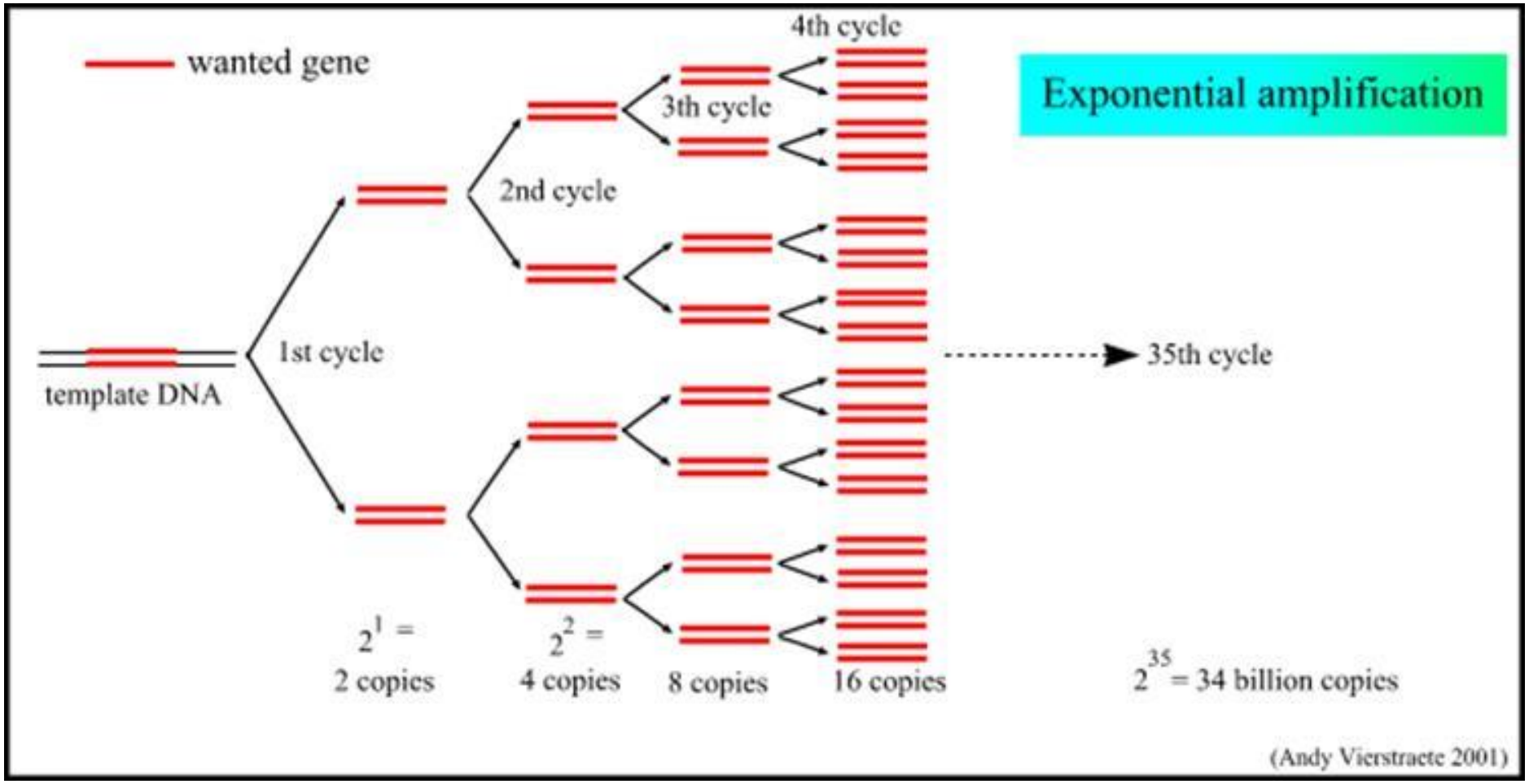
Annealing

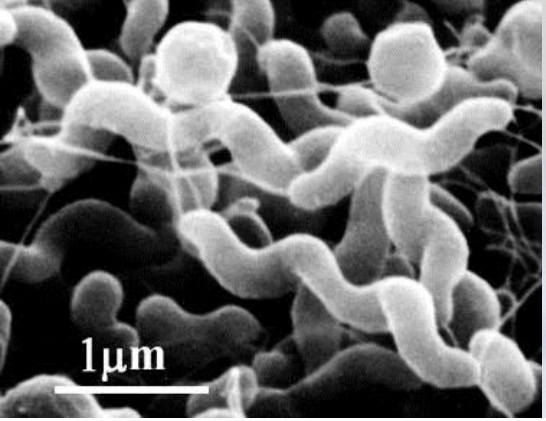


Extension


Cycle 1
Cycle 2
100
90
80
70
60
50
10
Temp (°C)
Template denature
Primer anneal
DNA synthesis
1
2
3
4
5
6
7
Time (mins)

PCR


wanted gene
4th cycle
3th cycle
2nd cycle
1st cycle
template DNA
Exponential amplification
35th cycle
2^1 =
2 copies
2^2 =
4 copies
8 copies
16 copies
2^{35} = 34 billion copies
(Andy Vierstraete 2001)




- **Antibiotics**
  - Treatment (gentamicin, ampicillin, 3rd gen. cephalosporins, chloramfenicol)
  - Prevention (not recommended!)
  - Selective agent in media
  - Resistance

Thank you for your attention