



دانشگاه اصفهان

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آشنایی با خصوصیات کلی، رنگ آمیزی و مشاهده باکتری بروسلا

رنگ آمیزی گرم و آشنایی با خصوصیات میکروسکوپی و ماکروسکوپی باکتریهای جنس اسپیتوباکتر
رنگ آمیزی گرم و آشنایی با خصوصیات میکروسکوپی و ماکروسکوپی باکتریهای جنس اسپیتوباکتر

BRUCELLA



تپسه کننده: سهیلا عباسی


INTRODUCTION

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- The genus *Brucella* consists of **Gram-negative** coccobacilli, They are strict **intracellular** parasites of animals and may also infect humans.
- Brucellosis is a zoonotic disease, primarily affecting goats, sheep, cattle, buffaloes, pigs and other animals and transmitted to humans by contact with infected animals or through ingestion of their products.
- The human diseases with various names: **Mediterranean fever, Malta fever, undulant fever/remittent fever, Gibraltar fever, Cyprus fever.**
- The diseases caused by members of this genus are characterized by a number of names based on the original microbiologists who isolated and described the organisms

TAXONOMY

- Brucella belongs to family Brucellaceae.
- Genus Brucella encompasses 9 recognized spp—6 terrestrial sp. & 3 marine spp.
- Terrestrial sp. are **B.melitensis, B.abortus, B.suis, B.canis, B. ovis, B.neotamae.**
- Marine spp are **B.delphini, B. pinnipediae, B. cetaceae.**

Species	Natural Host	Human Pathogen 
<i>B. abortus</i>	cattle	yes
<i>B. melitensis</i>	goats, sheep	yes
<i>B. suis</i>	swine	yes
	hares	yes
	reindeer	yes
	rodents	yes
<i>B. canis</i>	dogs, other canids	yes
<i>B. ovis</i>	sheep	no
<i>B. neotomae</i>	desert wood rat	no
<i>B. pinnipediae</i>	otter , seal	?
<i>B. cetaceae</i>	dolphin , porpoise	?



MORPHOLOGY

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- Brucellae species are **small, gram-negative aerobic coccobacilli**, 0.5-0.7 μm x 0.6-1.5 μm in size.
- They are nonmotile, noncapsulated, nonsporing and non-acid fast.



- **Morphology**

- Small ($0.5-0.7 \times 0.6-1.5\mu\text{m}$), gram negative, non-motile, non-capsulate, non-sporing coccobacilli
- Arranged singly, sometimes in pairs and small clusters

- **Culture**

- Strict aerobes
- Fastidious and nutritional requirement are complex
- Grow best in on tryptose soy based or other enriched media
- Optimum temperature is 37°C and pH 6.6 to 7.4
- Addition of 5-10% CO_2 improves the growth of *B. abortus* and *B. melitensis*

CULTURAL CHARACTERISTICS

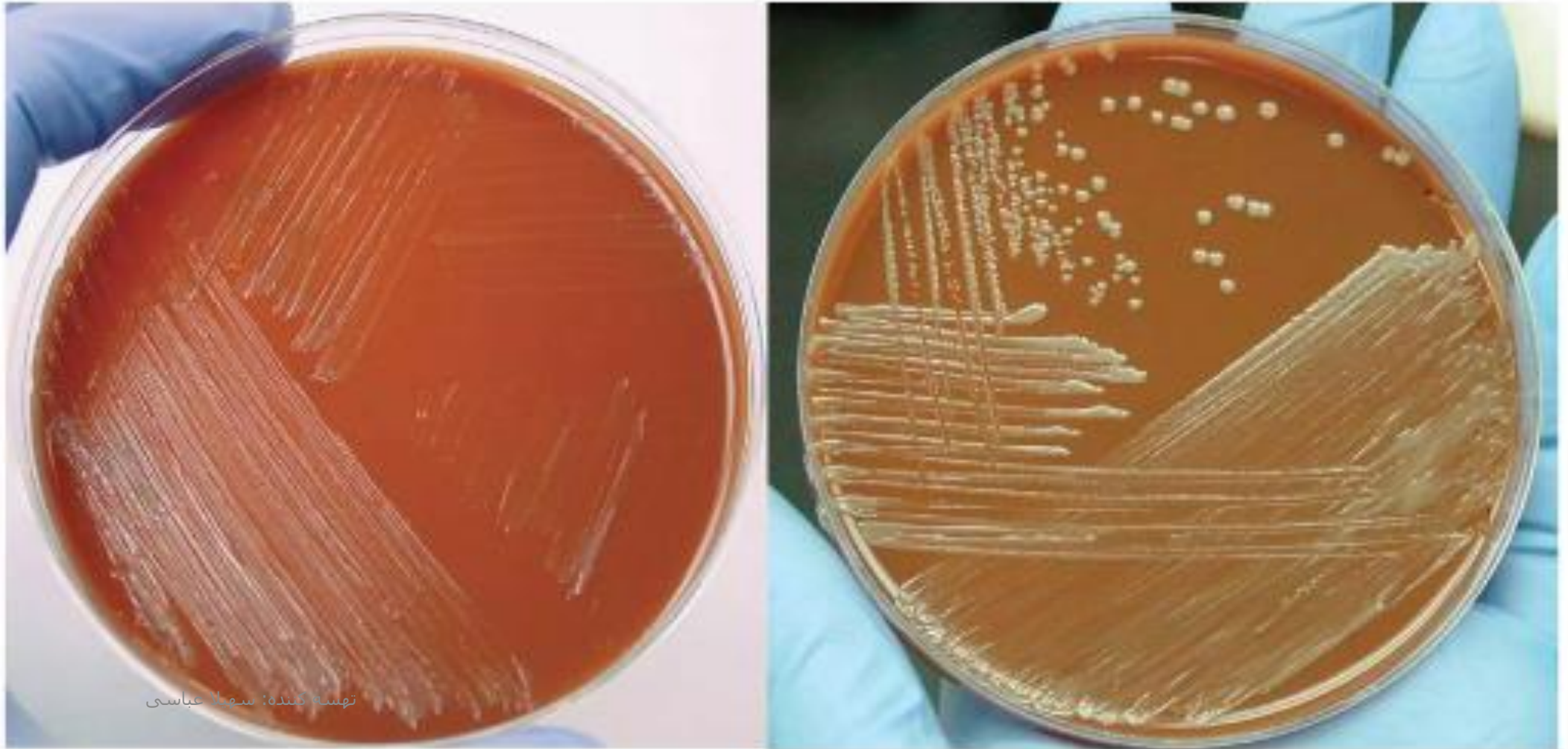
- Brucellae are strict aerobes.
- *Br. Abortus* is capnophilic, many strains requiring 5-10% CO₂ for growth.
- Optimum temperature is 37°C (range 20-40 °C) and pH 6.6-7.4.
- Grow on **simple media**, though growth is slow and scanty.
- Growth is improved by the addition of serum or liver extract.
- The media employed currently are **serum dextrose agar, serum potato infusion agar, trypticase soy agar, or tryptose agar.**

CULTURE CHARACTERISTICS



- The addition of **bacitracin**, **polymyxin** and **cycloheximide** to the above media makes them selective.
- **Erythritol** has a specially stimulating effect on the growth of Brucellae.
- On solid media, colonies are small, moist, translucent and glistening after 3 or more days of incubation.
- In liquid media growth is uniform.

CULTURE CHARACTERISTICS



توسعه کننده: مهلا عباسی

BIOCHEMICAL REACTIONS



- No carbohydrates are fermented.
- Catalase and oxidase positive (except for Br. Neotomae and Br. ovis which are negative).
- Nitrite reduction positive,
- IMViC- All negative & Urease positive.

RESISTANCE



- Brucellae are destroyed
 - by heat at 60 °C in 10 minutes
 - by 1% phenol in 15 minutes.
 - are killed by pasteurization.
- They may survive in soil and manure for several weeks.
- The organism survives for 10 days in refrigerated milk, for months in butter, one month in ice cream.
- They are sensitive to direct sunlight and acid.
- **They are resistant to penicillin but are susceptible to streptomycin, tetracycline, chloramphenicol and ampicillin.**

CLASSIFICATION



- Brucellae may be classified into different species, based on
 - 1. Lysis by specific Bacteriophage.
 - 2. CO₂ requirements,
 - 3. H₂S production,
 - 4. Agglutination by monospecific sera,
 - 5. Sensitivity to dyes (basic fuchsin and thionin).
- **BIOTYPES:**
 - Br. abortus-7 biotypes
 - Br. melitensis-3 biotypes
 - Br. suis-5 biotypes

- **Biochemical reactions**

- Catalase positive, oxidase positive (expect for *B. ovis* and *B. neotomae*) and urease positive
- Indole negative, MR-VP negative, citrate negative,
- Do not grow on MA.

- Localize in the reproductive organs of the host animals, causing abortions and sterility.
- Shed in large no. in the animals urine, milk, placental fluid and others fluids.

America, Africa, Asia, Arab peninsula, Indian subcontinent and the Middle East.

- Source of infection and responsible organism vary according to geographic area.
- Brucellosis is predominantly an occupational diseases of those working with infected animals especially farm workers, veterinarians and abattoir workers. Hence more frequent among males.
- Sporadic cases and outbreaks occur among consumers of raw milk and milk products (especially unpasteurized soft cheese) from cow, sheep and goat.

Mode of transmission



Transmitted to humans by following ways:

- a. **Ingestion:** Primarily by consuming raw milk or milk products (such as unpasteurized soft cheese), meat of infected animals. Also be transmitted by drinking water or eating raw vegetables contaminated with feces or urine of infected animals.

- b. **Direct contact:** Infection is acquired by direct contact with infected animals. Inoculation through cuts and skin abrasions from handling infected animals carcasses, placentas or contact with animal vaginal secretion or urine.

- c. **Inhalation:** Infection is transmitted by inhalation of dust from wool or other dried material of infected animals. Infection by inhalation is important among veterinarians and laboratory workers

- d. **Accidental inoculation:** Accidental needle stick puncture with *Brucella* B19 vaccine while vaccinating animals. Also a serious risk in laboratory workers who handle culture of the organism.

Pathogenesis

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a. Virulent brucellae

Brucella organisms



Entry via lesions or cuts, ingestion or inhalation




Phagocytosed by macrophages




Survive and replicates with phagocytes and monocytes (much of the pathogenesis of brucellosis is associated with intracellular survival)





Infected macrophages localize in reticuloendothelial system namely lymph nodes, liver, spleen and bone marrow



Results to formation of granuloma with lymphocytes and epitheloid gaints cells, which can progress to form focal abscesses and caseation

b. Avirulent strain

Ingested by mononuclear or polymorphonuclear leucocytes



No symptoms of disease

- Placentas and fetal membranes of cattle's, sheep contains erythritol, a growth factor for brucellae. The proliferation organism in pregnancy animals leads to placentitis and abortion.
- No erythritol in human placentas thus abortion is not part of *Brucella* infection of humans

