VI Конгрес на микробиолозите на Македонија со меѓународно учество
VI Congress of Macedonian Microbiologists with international participation

FEMS-supported Symposium: "Emerging infections"

30.5 - 2.6.2018 год., Охрид, Р. Македонија

30.05 – 2.06.2018, Ohrid, R. Macedonia

May 31st

How Julius Richard Petri's Dishes Changed Medical History -1887-

Richard Julius Petri

(source)

Born 31 May 1852; died 20 Dec 1921 at age 69.

German physician and bacteriologist, remembered for his name given to the Petri dish. This is a shallow, cylindrical dish made of plastic or glass with a cover, used for tissue cultures and to hold solid media for culturing and sub-culturing bacteria. Petri developed it for a technique for cloning bacterial strains using an agar slope and sub-culturing onto his dish, recognizing different bacterial colonies and again sub-culturing. He was in his later days a rather vain, overweight man, who dressed in the uniform of chief army doctor whenever the opportunity presented itself. One observer remarked that the sash around his protuberant abdomen reminded him of the equator around the globe.«

May 31st

Jacques Monod

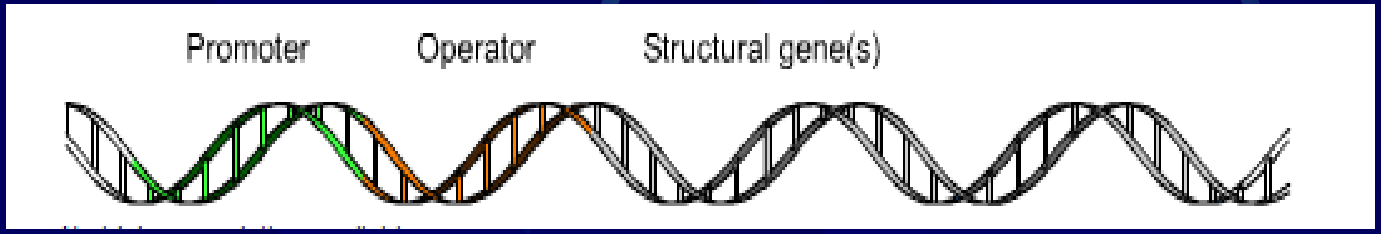
1976

(source)

Died 31 May 1976 at age 66 (born 9 Feb 1910). Quotes quotes

Jacques Lucien Monod was a French biochemist who, with François Jacob, investigated how genes regulate cell metabolism by directing the biosynthesis of enzymes. The pair shared (with André Lwoff) the 1965 Nobel Prize for Physiology or Medicine. Monod discovered the operon system that controls gene action in bacteria. In 1931, Monod joined the Pasteur Institute, and in 1971, he became its director.

Origins of Molecular Biology: A Tribute to Jacques Monod, by Agnes Ullmann (ed.). - book suggestion.



Operon

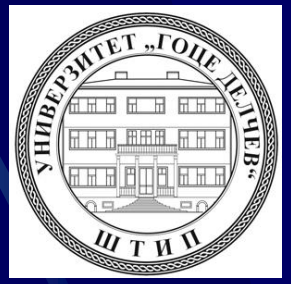
New *brucella* strains towards re-emerging trends of brucellosis

Taleski Vaso

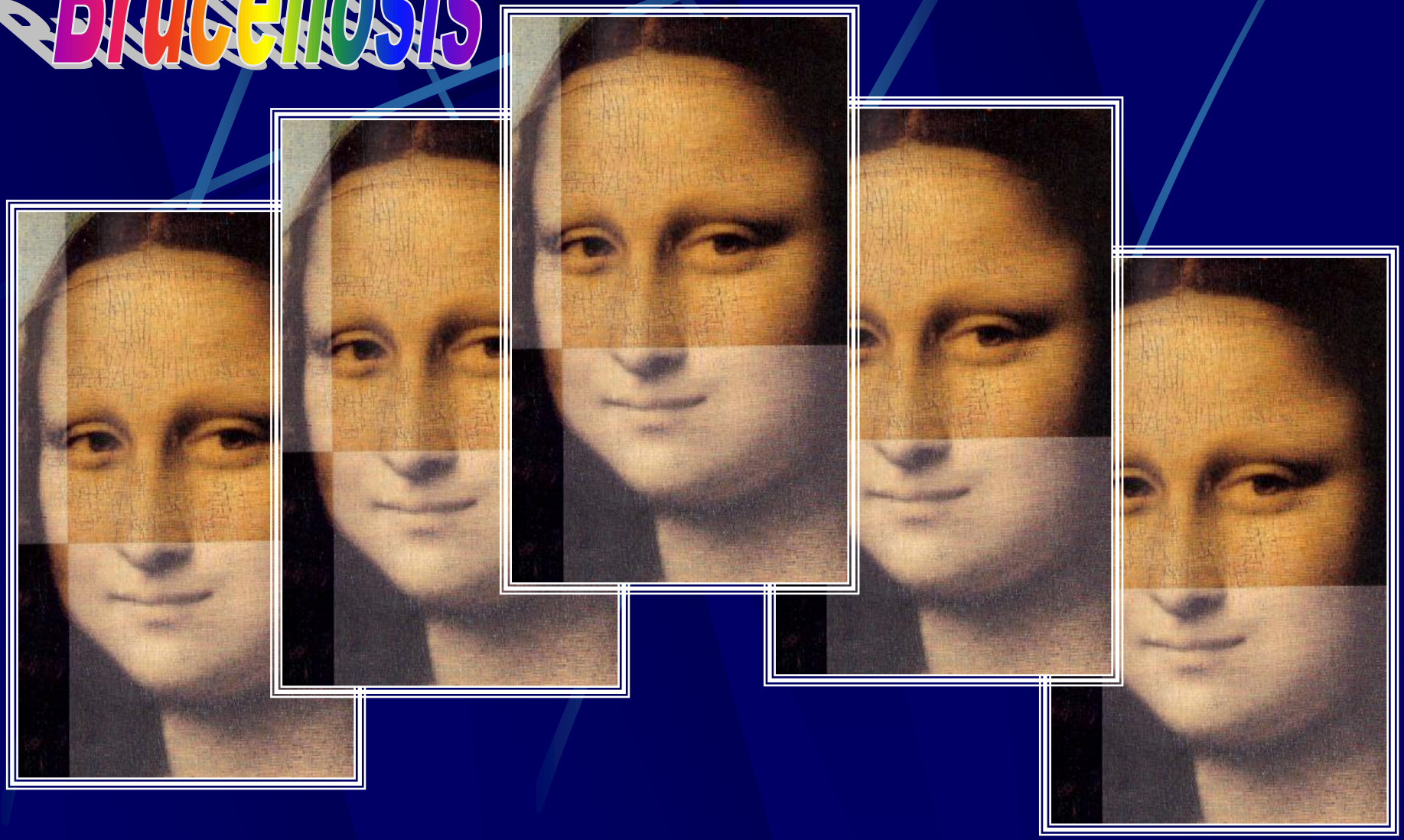
University „Goce Delchev”,

Faculty of Medical Sciences, Shtip,

Republic of Macedonia

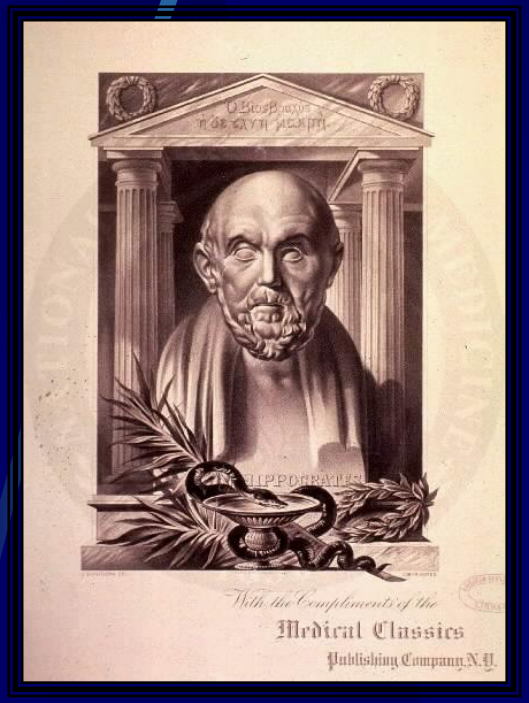


Brucellosis



Brucellosis

(Gastric Intermitent fever, Febris undulans, Malta fever, Mediterran fever, Neapolitan fever, Melitococcosis, Texas fever, Bang's disease, Febris melitensis)



In 1853, Jeffery Allen **Marston** made the **first accurate description** of the disease in British army troops serving in Malta during the Crimean war

- **Hipocrates (450 BC)**

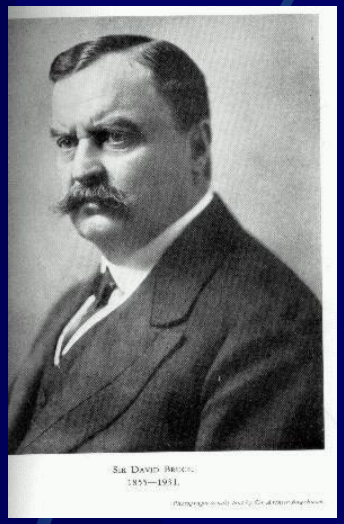


Fig. 1. Restored laboratory of Bruce and others in Malta.

• *Sir David Bruce* (1887 - Malta)
Brucella melitensis; **in spleen of death soldier**

> *B. abortus* 1897/ Kopenhagen, *Bang*
in cows with abortions
>*B. suis* 1914, *Traum* from Swine fetus,
>*B. ovis,* 1953 *Buddle &Boyes* at NewZeland,
from ships with genital diseases
>*B. neotomae,* 1957 Juta Utah USA,
Stoenner&Lackman, in woods rats
> *B. canis,* 1968, *Carmichael&Bruner,USA*
dogs with abortions

Humans become infected by:

1. **direct or indirect contact with animals or with contaminated animal products**

2. **Ingestion of unpasteurized of contaminated milk and dairy products or by**

3. **Inhalation of aerosols**

ZOONOTIC DISEASES *(75% of infectious diseases)*

Brucellosis is considered *worldwide commonest re-emerging* zoonotic disease with significantly changes of global ecological map identifying new strains, hosts and reservoirs.

Disease have been eradicated successfully in most of developed countries but remains endemic in Mediterranean region, Middle East, Asia, and Central and South America.

The new global map of human Brucellosis

Several areas traditionally considered to be endemic (eg, France, Israel (?), and most of Latin America have achieved **control** of the disease.

On the other hand, **new foci** of human brucellosis have emerged, particularly in central Asia, while the situation in certain countries of the Near East (eg, Syria) is rapidly worsening.

The disease is still present, in varying trends, both in European countries and in the USA.

Brucellosis up 83% in Israel, called a 'Third world epidemic'

Posted by Robert Herriman on August 2, 2015 // 1 Comment

The number of human cases of the zoonotic infection, brucellosis, has increased in parts of Israel by 83 percent, prompting one physician to call it a "Third world epidemic".

The Jerusalem Post reports the surge in cases in centered around Beduin inthe South and other Arabs in eastern Jerusalem, Nazareth, Acre and elsewhere in the North.

MK Ahmad Tibi, a physician said, "Just in the last six months, 217 cases were reported. There is no excuse for this negligence, because Israel has a very high level of medical and agricultural know how."

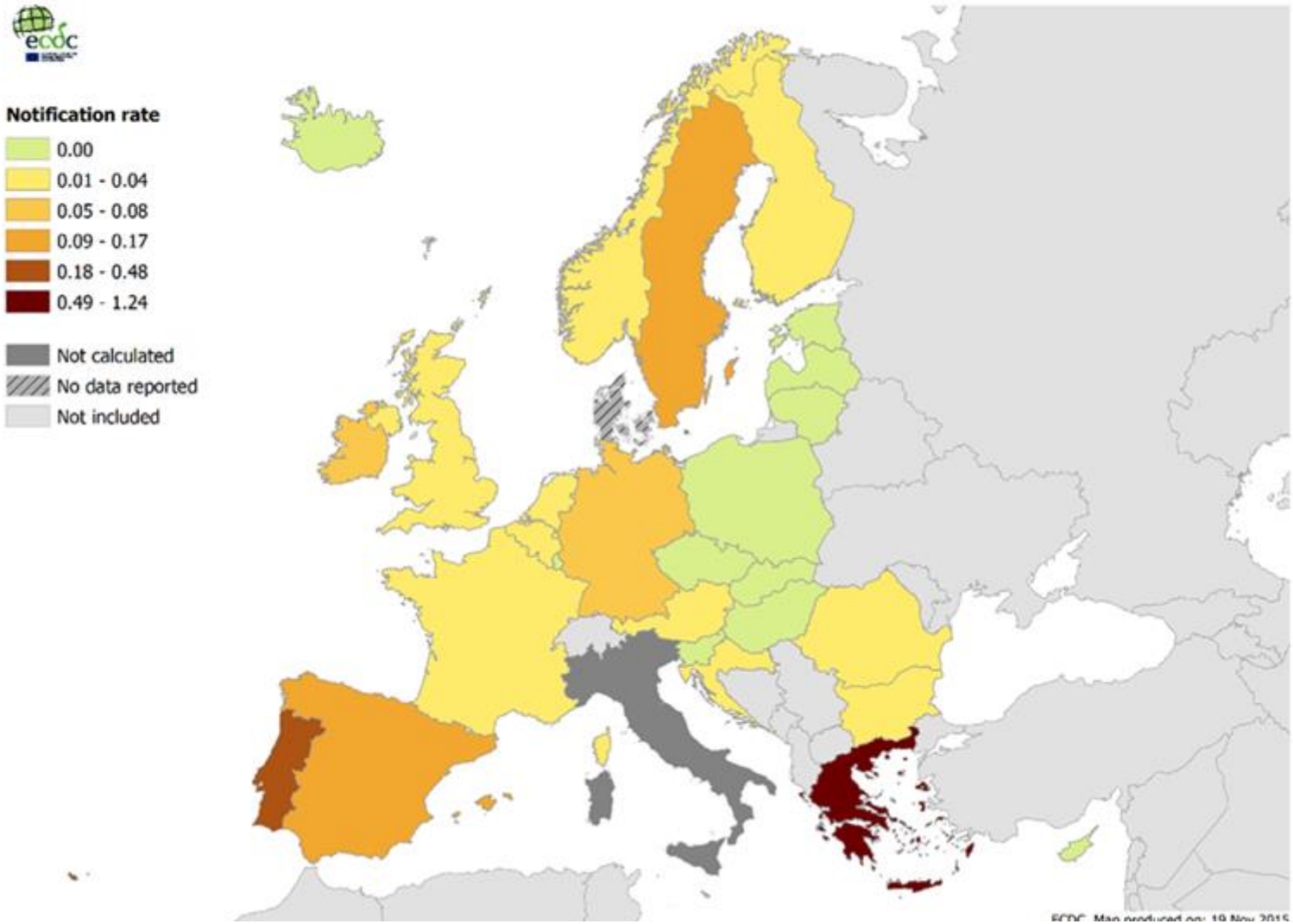
In addition, hospitalizations for the infection are up 30 percent compared to last year.

Health officials say that experts have been sent to teach the public about how to make milk safe; however, they note, "but they don't cooperate and listen, and they even hide the products from us, even though we have made it clear that they are causing themselves to get sick."

Brucellosis is one of the most serious diseases of livestock, considering the damage done by the infection in animals. Decreased milk production,weight loss, loss of young, infertility, and lameness are some of the affects on animals.

The Brucella species are named for their primary hosts: Brucella melitensis is found mostly is goats, sheep and camels, B. abortus is a pathogen of cattle, B. suis is found primarily in swine and B. canis is found in dogs.

Figure 1. Reported confirmed brucellosis cases: rate per 100 000 population, EU/EEA, 2014



Source: Country reports from Austria, Belgium, Bulgaria, Croatia, Cyprus, the Czech Republic, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, the Netherlands, Norway, Poland, Portugal, Romani Slovakia, Slovenia, Spain, Sweden, the United Kingdom.

Suggested citation: European Centre for Disease Prevention and Control. Annual epidemiological report 2015. Brucellosis. Stockholm: ECDC; 2016.

Table 1. Reported confirmed brucellosis cases: numbers and rate per 100 000 population, EU/EEA, 2010–2014

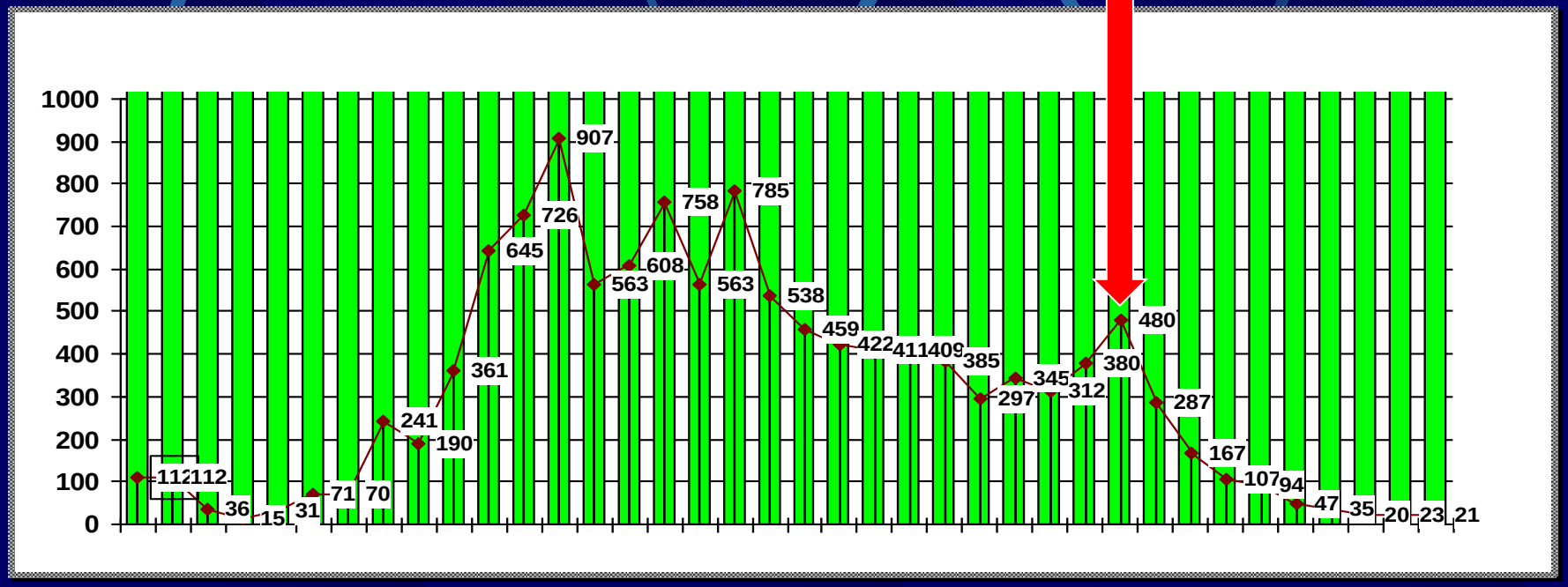
Country	2010		2011		2012		2013		2014					
	Cases	Rate	Cases	Rate	Cases	Rate	Cases	Rate	National data	Report type	Reported cases	Confirmed cases	Rate	ASR
Austria	3	0	5	0,1	2	0	7	0,1	Y	C	1	1	0	0
Belgium	0	0	5	0	4	0	0	0	Y	C	1	1	0	-
Bulgaria	2	0	2	0	1	0	0	0	Y	A	2	2	0	0
Croatia	.	.	.	.	0	0	0	0	Y	C	1	1	0	0
Cyprus	0	0	0	0	0	0	0	0	Y	C	0	0	0	0
Czech Republic	1	0	0	0	0	0	0	0	Y	C	0	0	0	0
Denmark	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Estonia	0	0	0	0	0	0	0	0	Y	C	0	0	0	0
Finland	0	0	0	0	1	0	0	0	Y	C	1	1	0	0
France	20	0	21	0	28	0	19	0	Y	C	16	14	0	0
Germany	22	0	24	0	28	0	26	0	Y	C	47	45	0,1	0,1
Greece	97	0,9	98	0,9	123	1,1	159	1,4	Y	C	135	135	1,2	1,2
Hungary	0	0	0	0	0	0	0	0	Y	C	0	0	0	0
Iceland	0	0	0	0	0	0	0	0	Y	C	0	0	0	0
Ireland	1	0	1	0	2	0	1	0	Y	C	3	3	0,1	0,1
Italy*	171	0,3	166	0,3	184	0,3	137	0,2	N	C	8	8	-	-
Latvia	0	0	0	0	0	0	1	0	Y	C	0	0	0	0
Liechtenstein	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Lithuania	0	0	0	0	0	0	2	0,1	Y	C	0	0	0	0
Luxembourg	1	0,2	1	0,2	0	0	0	0	Y	C	0	0	0	0
Malta	0	0	0	0	0	0	1	0,2	Y	C	0	0	0	0
Netherlands	6	0	1	0	3	0	5	0	Y	C	1	1	0	0
Norway	2	0	2	0	4	0,1	2	0	Y	C	2	2	0	0
Poland	0	0	0	0	0	0	1	0	Y	C	1	1	0	0
Portugal	88	0,8	76	0,7	37	0,4	22	0,2	Y	C	54	50	0,5	0,5
Romania	2	0	1	0	0	0	0	0	Y	C	2	2	0	0
Slovakia	1	0	0	0	1	0	1	0	Y	C	0	0	0	0
Slovenia	0	0	1	0	0	0	0	0	Y	C	0	0	0	0
Spain	78	0,2	43	0,1	62	0,1	87	0,2	Y	C	70	60	0,1	0,1
Sweden	12	0,1	11	0,1	13	0,1	10	0,1	Y	C	16	16	0,2	0,2
United Kingdom	12	0	25	0	14	0	15	0	Y	C	11	11	0	0
EU/EEA	**519**	**0,1**	**483**	**0,1**	**507**	**0,1**	**496**	**0,1**	.	**C**	**372**	**354**	**0,1**	**0,1**

Source: Country reports. Legend: Y = yes, N = no, C = case based, A = aggregated, · = no data reported, ASR: age-standardised rate, - = no report

** Provisional data for 2014. Notification rates not calculated.*

- **Serbia** /1980 to 2008 reported 1521 (Cekanjac at all., 2010).
- **Bosna & Hercegovina** 1980 to 2008 reported 1639 cases (Obradovic, 2010).
- **Croatia until** 1990 free from brucellosis, when epizooty between sheeps and goats appear in Istria. Proved reservoir of *B. suis* in wild boars (Taleski at all. / Cvetnic, Katalinic, 2002).
- **Bulgaria report on only two cases of human brucellosis for the period** 1996 to 2001 (Taleski at all. / Kantardziev, 2002), 37 cases in 2005 and 58 in 2007 (Nenova at all., 2013).
- **Turkey in** 2005, annual incidence of human brucellosis was 26,20 on 100.000 inhabitants.

Macedonia:
Area: ~25.000 km²
Population: ~2.000.000



Human *brucellosis* in Macedonia (1980-2017, ~12.000 human cases)

As a result of vaccination of small ruminants (Rev 1) started in 2008, number of human cases significantly decreased.

YEAR	HUMAN CASES
2008	480
2009	287
2010	167
2011	107
2012	94
2013	47
2014	35
2015	20
2016	23
2017	21

Brucella species Classical (terrestrial):

1. B. melitensis*	**16M**	**biotype 1**
	63 / 9	**biotype 2***
	Ether	**biotype 3**
2. B. abortus*	**544**	**biotype 1**
	86 / 8 / 59	**biotype 2**
	Tulya	**biotype 3**
	292	**biotype 4**
	B3196	**biotype 5**
	870	**biotype 6**
	63 / 75	**biotype 7**
	C68	**biotype 9**
3. B. suis*	**1330**	**biotype 1**
	Thomsen	**biotype 2**
	686	**biotype 3**
	40	**biotype 4**
4. B. canis*	**RM6 / 66**	
5. B. ovis	**63 / 290**	
6. B.neotomae	**5K33**	

УНИВЕРЗИТЕТ „СВ.КИРИЛ И МЕТОДИЈ“ СКОПЈЕ
ФАКУЛТЕТ ЗА ВЕТЕРИНАРНА МЕДИЦИНА

Кирил Крстевски

МОЛЕКУЛАРНА ТИПИЗАЦИЈА И ФИЛОГЕНЕТСКА АНАЛИЗА НА БАКТЕРИИТЕ ОД РОДОТ *BRUCELLA*, ДЕТЕКТИРАНИ НА ТЕРИТОРИЈАТА НА РЕПУБЛИКА МАКЕДОНИЈА

1. Isolation
2. Identification determining IS711 using **rt-PCR**
3. *Brucella* species determined by **AMOS PCR**
4. Genotyping using method **MLVA – 16** **(multiple-locus variable-number tandem repeat analysis)**

2015

- Genetic similarity and phylogenetic grouping accordning programe package ***Bionumerics 7***

- Significantly bigger differences of species ***B. melitensis*** (**22 genotypes**), belongs to east-Mediterranian species, most similar as **Turkish species** (?).

- In ***B. abortus*** ***only 2 genotypes were proved (most similar with Portuguese species).***

Until recently the genus *Brucella* was considered to represent a genetically homogeneous and clonal group of bacteria associated with:

1.Terresterial mammalian hosts (Classical strains *B. melitensis*, *B. abortus*, *B. suis*, *B. canis*, *B. ovis*, *B. neotomae*)

2. Marine mammals (*B. ceti* and *B. pinnipedialis*), and

3. **„Atypical”,** more recently identified (*B. microti*, *B. inopinata*, *B. papionis* and *B. vulpis*).

All species are genetically highly related (> 99%).

Since **1994**, isolated novel *brucella* species.

The group of classical *Brucella* species was extended in **2007** to include *B. ceti* and *B. pinnipedialis*, isolated from marine mammals

-

B. ceti (marine mammals/ **whales, dolphins, porpoises**

- ***B. Pinnipedialis*** **(seals and sea lions)**

Vet Res. 2005 May-Jun;36(3):313-26.

From the discovery of the Malta fever's agent to the discovery of a marine mammal reservoir, brucellosis has continuously been a re-emerging zoonosis.

Godfroid J, Cloeckaert A, Liautard JP, Kohler S, Fretin D, Walravens K, Garin-Bastuji B, Letesson JJ.

Lancet Infect Dis. 2006 Feb;6(2):91-9.

The new global map of human brucellosis.
Pappas G, Papadimitriou P, Akritidis N, Christou L, Tsianos EV.

Int J Antimicrob Agents. 2010 Nov;36 Suppl 1:S8-11. doi: 10.1016/j.ijantimicag.2010.06.013. Epub 2010 Aug 8.

The changing Brucella ecology: novel reservoirs, new threats.

Pappas G.

“Atypical *brucella*”

- ***B. microti*** (voles in Czech Republic, red foxes in Austria, Hungarian wild boar)

- ***B. Inopinata*** (2010, isolated from breast implant and blood of woman of age of 71, host unknown)

New "Atypical *brucella*"

- B. papionis

- B. vulpis

Whatmore, A. M. *et al.* *Brucella papionis* sp. nov., isolated from baboons (*Papio* spp.). *Int. J. Syst. Evol. Microbiol.* **64**, 4120–4128 (2014).

Brucella evolution & taxonomy

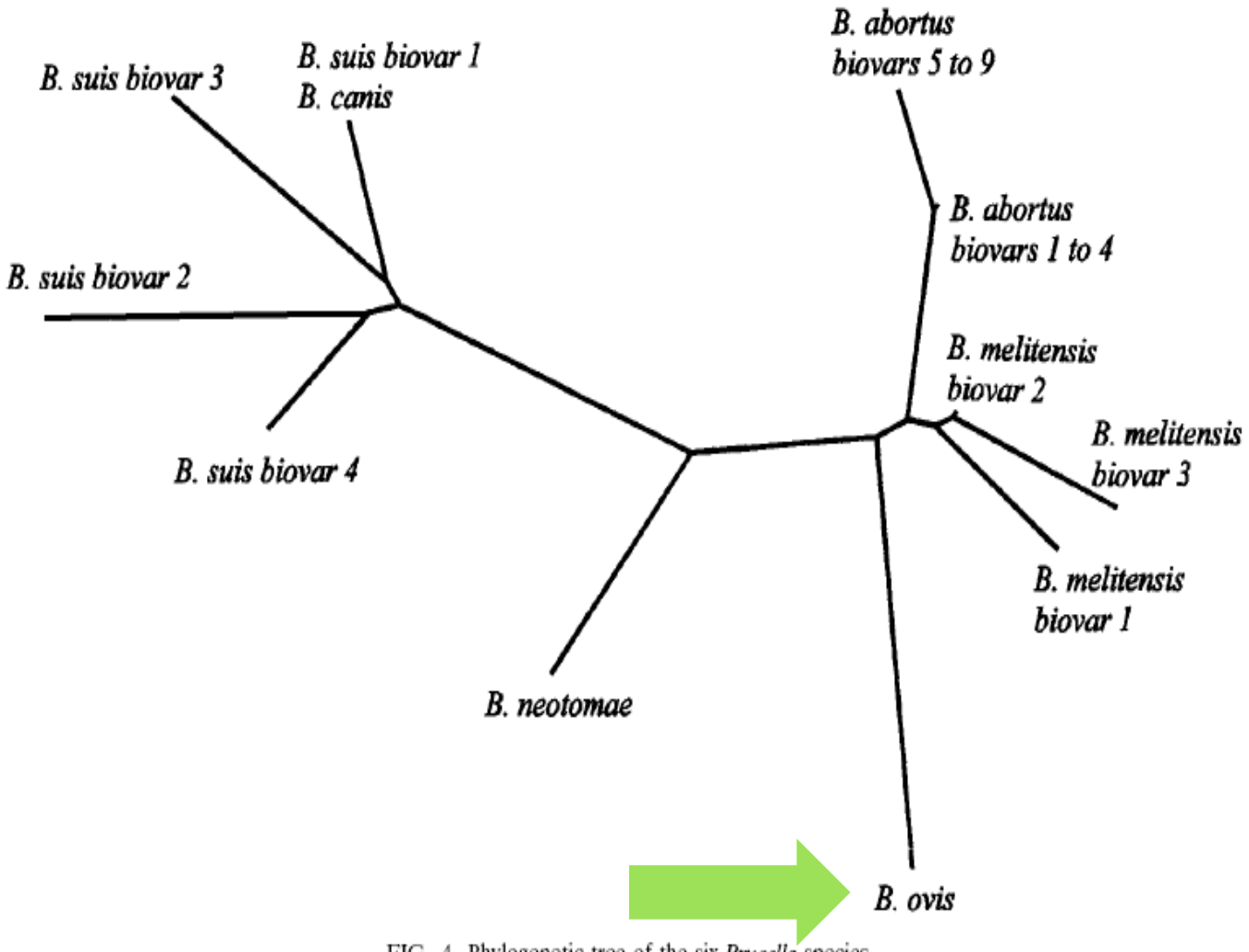
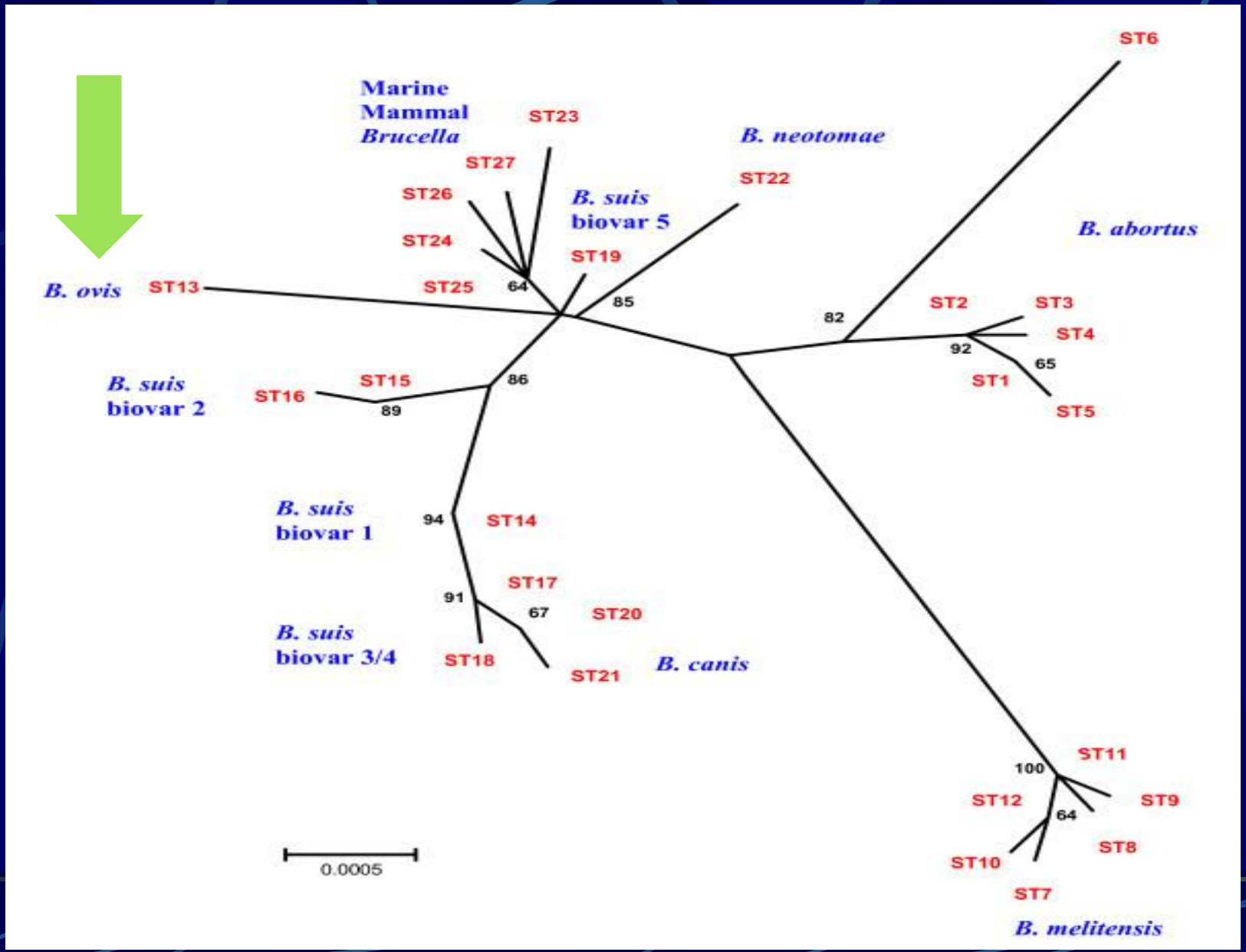
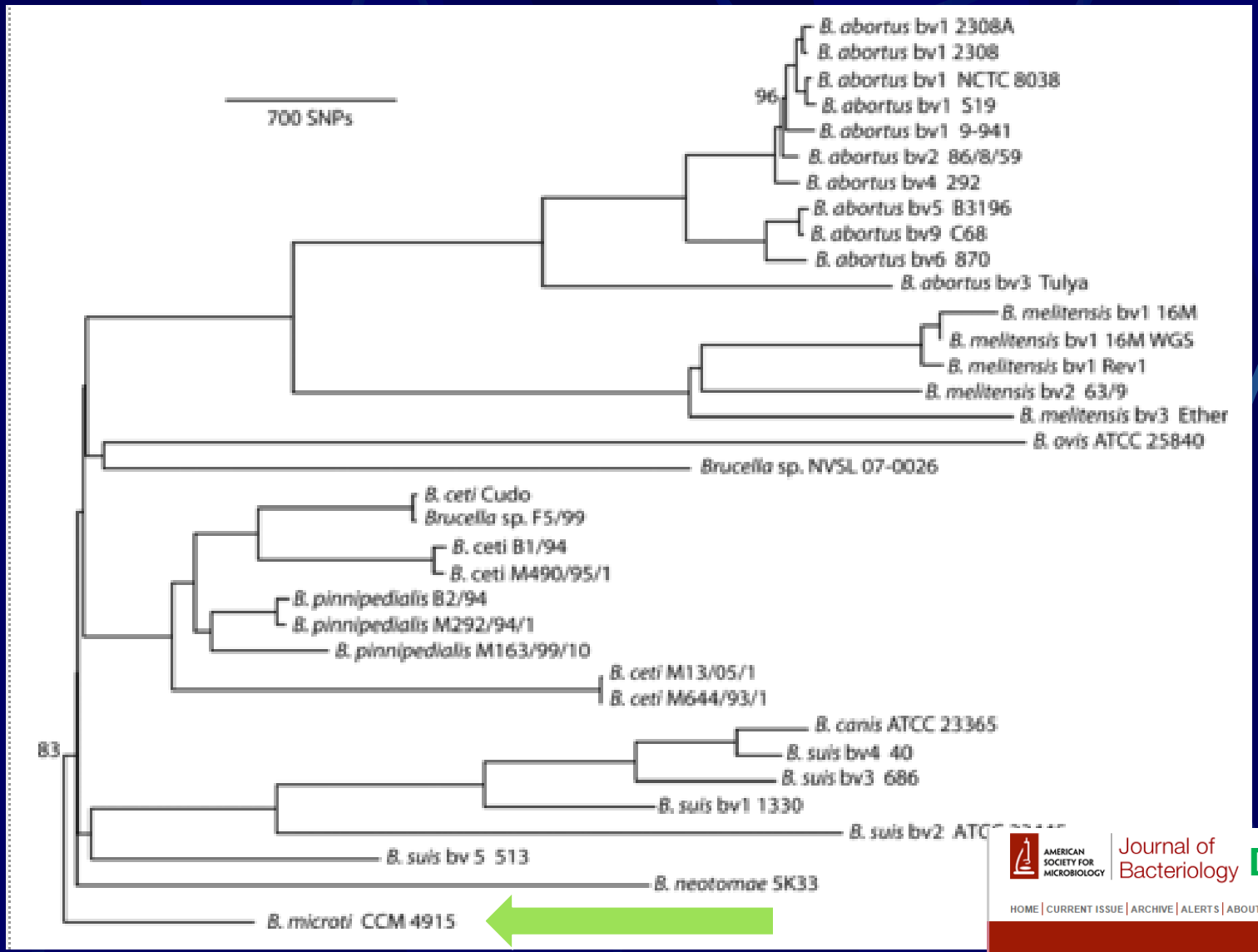


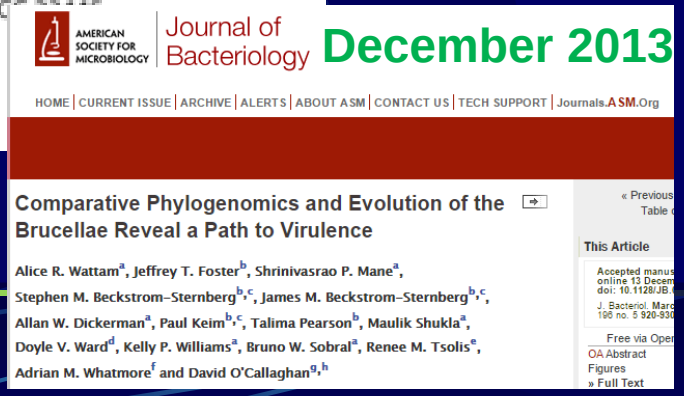
FIG. 4. Phylogenetic tree of the six *Brucella* species.

Brucella evolution & taxonomy





Phylogenetic analysis, showing a rapid radiation following the divergence of ***B. Microti***



Taxonomy of *brucella* is still controversial and debating is still going on.

It has been suggested that divergence of species *brucella* was a resultat of divergence of their hosts, 60 million years ago.

But, divergence of hosts of *B. ceti* and *B. pinnipedialis* happened:

before about 35 million years for seals and sea lions,
before 55 million years for whales and dolphins

Biggest number of known, classical brucella had divergence from their common ancestor *B. ovis, probably about 86.000 to 296.000 years ago.*

=> Divergence of *brucella* of marine mammals is incompatible with divergence of their hosts.

Following the evolution of *B. microti,* first evolution was divergence to two following speciae:
***B. suis* and *B. neotomae* (Audic, 2011).**

QUESTIONS:

1. Infection of marine mammals happened through food chain from terrestrial mammals?

2. Marine mammals *brucella* infected terrestrial hosts?

Infections occur among various *warm-blooded animal* species, marine mammals, and humans.

***Brucella hots*:**

Mammals

- marine
- terresterial

Rodents

Amfibija (frogs)?

Recently reported *brucellae* from amphibians (worldwide-distributed exotic frogs) are ***genetically highly diverse*** and might represent several new *Brucella* species ***or link*** between free living soil saprophytes and the pathogenic *Brucella.*

Amphibian *brucellae* are capable of causing disease in different frog species ranging from localized manifestations to generalized infections.

Frogs represent new and ecologically significant natural host and reservoir.

The Change of a Medically Important Genus: Worldwide Occurrence of Genetically Diverse Novel *Brucella* Species in Exotic Frogs

- Holger C. Scholz ,
- Kristin Mühldorfer,
- Cathy Shilton,
- Suresh Benedict,
- Adrian M. Whatmore,
- Jochen Blom,
- Tobias Eisenberg

PLOS

2016

- Published: December 30, 2016

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2011

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Isolation of Potentially Novel *Brucella* spp. from Frogs

Tobias Eisenberg[a], Hans-Peter Hamann[a], Ute Kaim[a], Karen Schlez[a], Helga Seeger[a], Nicole Schauerte[b], Falk Melzer[c], Herbert Tomaso[c], Holger C. Scholz[d], Mark S. Koylass[e], Adrian M. Whatmore[e] and Michael Zschöck[a]

Author Affiliations

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Accepted manus
online 9 March
10.1128/AEM.075
Appl. Environ. Mi
2012 vol. 78 no.
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Brucella spp. of amphibians comprise genomically diverse motile strains competent for replication in macrophages and survival in mammalian hosts

- Sascha Al Dahouk, Stephan Köhler, Alessandra Occhialini, María Pilar Jiménez de Bagüés, Jens Andre Hammerl, Tobias Eisenberg, Gilles Vergnaud, Axel Cloeckaert, Michel S. Zygmunt, Adrian M. Whatmore, Falk Melzer, Kevin P. Drees, Jeffrey T. Foster, Alice R. Wattam& Holger C. Scholz

- *Scientific Reports* 7, Article number: 44420 (2017)

African Bullfrog

First publication report (Eisenberg T, Hamann HP, Kaim U, Schlez K, Seeger H, Schauerte N, et al. Isolation of potentially novel *Brucella* spp. from frogs. Appl Environ Microbiol. 2012; 78:3753–3755.)

Wild-caught African bullfrogs (*Pyxicephalus edulis*) imported from Tanzania in a quarantine centre of a zoo in Germany, isolated from a granulomatous / purulent skin lesion

Big-eyed tree frog (Leptopelis vermiculatus)

The second publication (Fischer D, Lorenz N, Heuser W, Kämpfer P, Scholz HC, Lierz M. Abscesses associated with a *Brucella inopinata*-like bacterium in a big-eyed tree frog (*Leptopelis vermiculatus)*. J Zoo Wildl Med. 2012; 43:625–628).

Reports the isolation of a *Brucella inopinata*-like strain from subcutaneous abscess material of a big-eyed tree frog (*Leptopelis vermiculatus*) bought from a pet shop in Germany

White's Tree Frog (*Litoria caerulea*)

Third case
was reported from the UK in a White's tree frog (*Litoria caerulea*) with fluid-filled skin lesions.

Isolation of Brucella from a White's tree frog (Litoria caerulea)

- Authors: Adrian M. Whatmore[1], Emma-Jane Dale[1], Emma Stubberfield[1], Jakub Muchowski[1], Mark Koylass[1], Claire Dawson[1], Krishna K. Gopaul[1], Lorraine L. Perrett[1], Matthew Jones[2], Alistair Lawrie[3]
- **First Published Online:** 01 February 2015, JMM Case Reports , 2015 2, doi:

2016

Pacman frog (Ceratophrys ornate)

The most recent case of *Brucella* infection was described in a Pac-Man frog (*Ceratophyrus ornate*) at a veterinary hospital in Texas; USA

Soler-Lloréns PF, Quance CR, Lawhon SD, Stuber TP, Edwards JF, Ficht TA, Robbe-Austerman S, O'Callaghan D, Keriel A. A Brucella spp. Isolate from a Pac-Man Frog (*Ceratophrys ornata*) Reveals Characteristics Departing from Classical Brucellae. Front Cell Infect Microbiol. 2016; 28:116.

Meanwhile ‘atypical’ *brucellae* were also identified in :

Tomato frog (Dyscophus antongilii)

Red-eyed tree frog (Agalychnis callidryas)

Amazonian milk frogs (*Trachycephalus resinifictrix*)

Cane toads (*Chaunus marinus*)

International Journal of Veterinary Science and Medicine

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

Brucellosis: Evolution and expected comeback

Amr El-Sayed*, Walid Awad

Faculty of Veterinary Medicine, Department of Medicine and Infectious Diseases, Cairo University, Giza, Egypt

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Vet. Sci. **2018**, *5*(1), 28; https://doi.org/10.3390/vetsci5010028

Open Access Communication

Isolation of *Brucella abortus* and *Brucella melitensis* from Seronegative Cows is a Serious Impediment in Brucellosis Control

Mohamed El-Diasty [1], Gamal Wareth [2,3,*], Falk Melzer [2], Shawky Mustafa [3], Lisa D. Sprague [2] and Heinrich Neubauer [2]

1 Animal Health Research Institute-Mansoura Provincial Laboratory, 35516 Mansoura, Egypt

2 Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Institute of Bacterial Infections and Zoonoses, 07743 Jena, Germany

3 Faculty of Veterinary Medicine, Benha University, Moshtohor, 13736 Toukh, Egypt

* Author to whom correspondence should be addressed.

Received: 22 January 2018 / Revised: 6 March 2018 / Accepted: 7 March 2018 / Published: 9 March 2018

CONCLUSIONS

New *brucella* strains, hosts and reservoirs makes control of Brucellosis more complicated.

Identification of “atypical” *Brucella* strains and new, amphibian, *Brucella* species and new hosts and reservoirs, have significant contribution to understanding of evolution of the genus *Brucella* from a soil-associated motile bacterium to a host-adapted pathogen.

To date, there is no evidence that frog’s isolates represent a zoonotic threat, but precaution to avoid contacts with potentially infected amphibians until the zoonotic potential is better investigate and understood is useful advice.

Thank you very much for your attention