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RUSSIA

Advanced diagnostic methods and Epidemiology of Human *Brucellosis* in the Republic of Macedonia

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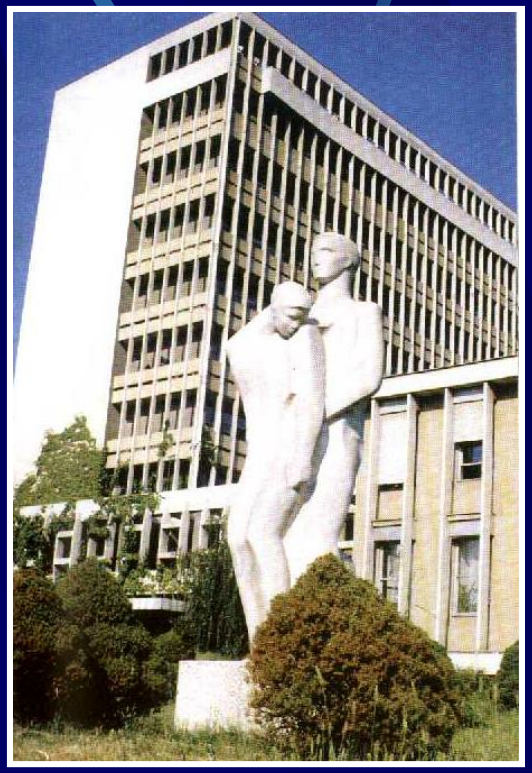
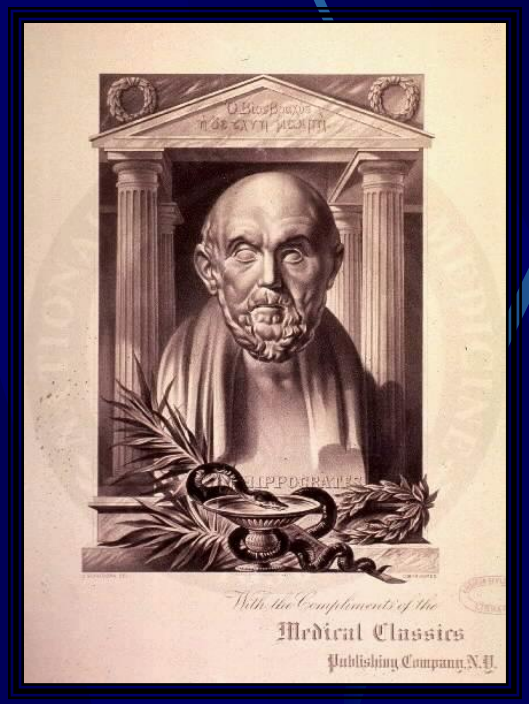


Brucellosis

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



Marston (1861)

- **Hipocrates (450 BC)**

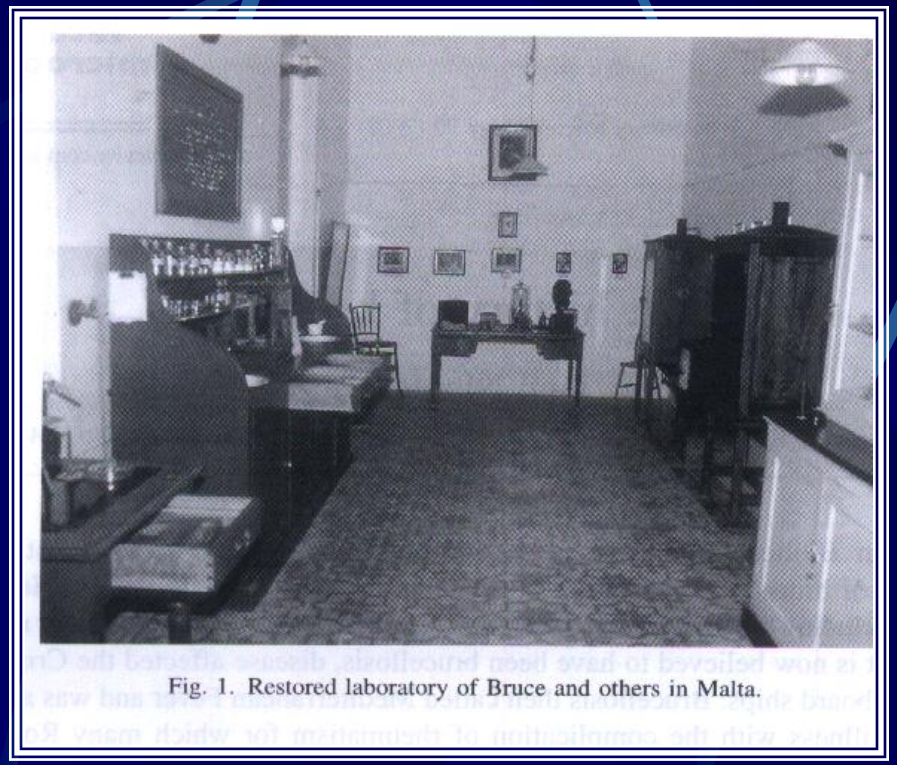
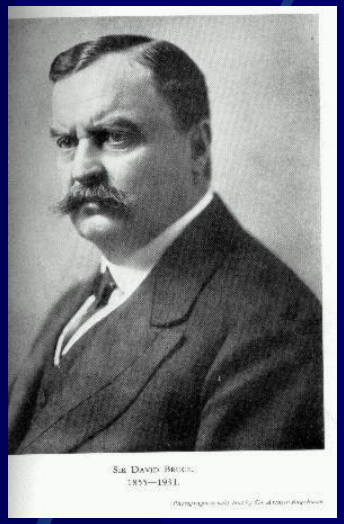
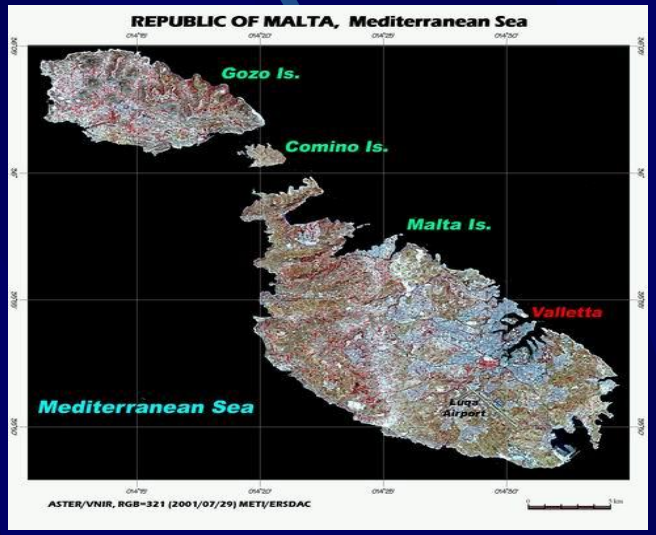


Fig. 1. Restored laboratory of Bruce and others in Malta.



- ***Sir David Bruce* (1887 - Malta)**



GENUS *Brucella*

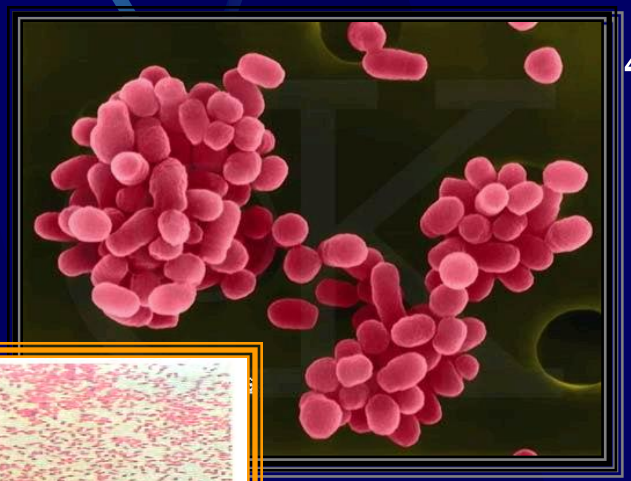
(α-2 subdivision of the class Proteobacteria)

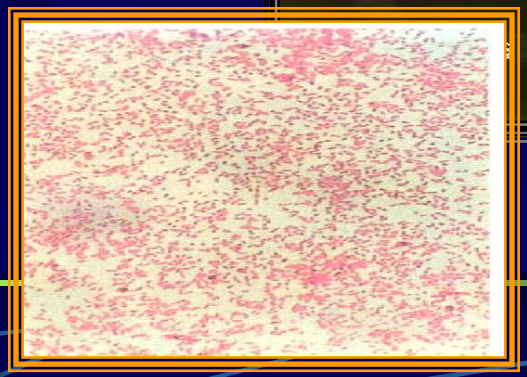
1. ***Brucella melitensis***   (3 bio-types: 1, **2**, **3**)

2. ***Brucella abortus***  (8 bio-types: 1, 2, 3, 4, 5, 6, 7 , 9)

3. ***Brucella suis*** 

4. ***Brucella canis*** 





5. ***Brucella ovis***

6. ***Brucella neotomae***

Human Brucellosis

Incubation : 1 week to 2-3 months (appr. 3weeks)

Spectrum of clinical manifestations:

"Undulant fever", night sweats, chills, malaise, often accompanied by severe headache, myalgias, arthralgias. lymphadenopathy, splenomegaly, hepatomegaly

Complications:

- **Meningoencephalitis, cerebellar abscess,**
- **Granulomatous hepatitis, hepatic and splenic abscesses, cholecystitis, arthritis, spondylitis, osteomyelitis**
- **Endocarditis,**
- **Granulomas in kidneys, orchiepididimitis etc.**

Mortality rate: very low

Brucellosis in animals

- Abortion (female)
- Infertility
- Orchitis&epididimitis (male)

Brucella spp. As a biological agent

- BSL-3
- Aerosol infection
- No human vaccine

Hypotetical bio-warfare attack:

(50 kg of agent by aircraft along a 2 km line

- **Upwind of a population center of 500.000**

Agent reach downwind 10 km,

- **500 dead, 125.000 incapacitated**

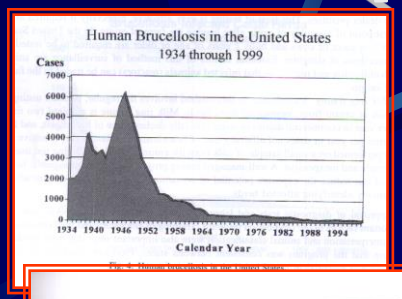
(Biological Weapons FAQ v. 0.43)

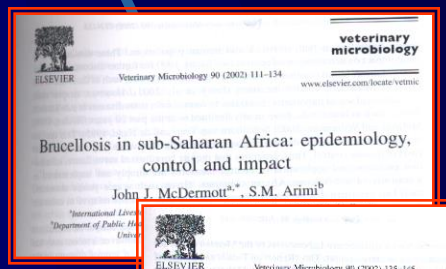
Brucella spp.	Human virulence	No of organisms
B. melitensis	HIGH	1-10
B. suis	High-Moderate	1.000-10.000
B. abortus	Moderate	100.000
B. canis	Low/immunosuppresed	>1.000.000

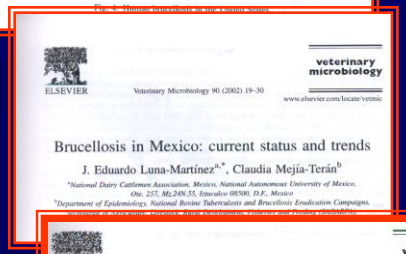
Costs (billions $):

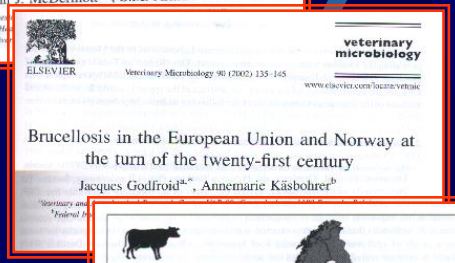
- livestock industry – production, abortions, lowered milk production, eradication costs, unrealized export, animal vaccination,
- absenteeism and treatment of patients.

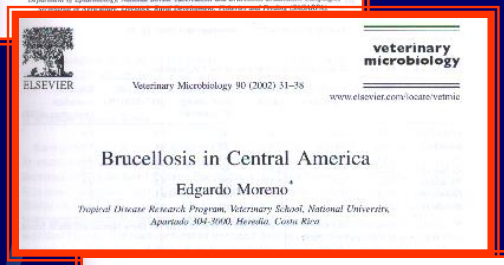
Epidemiology:

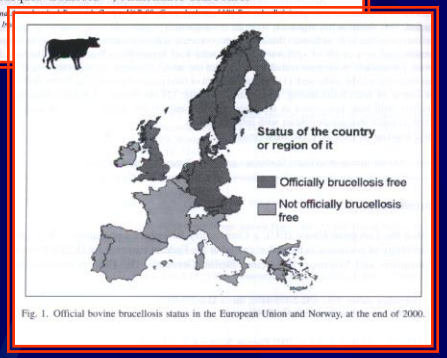






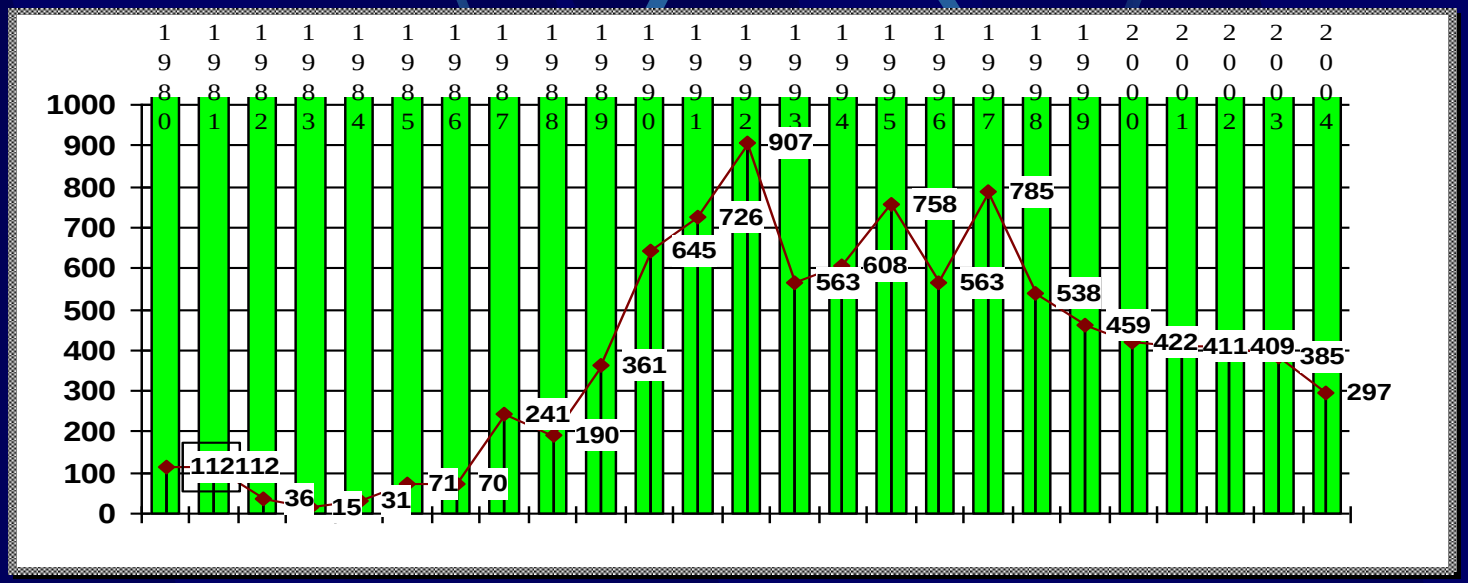






WHO: (Per year)
World - 500 000
Europe - 10-20 000

Macedonia:
Area: ~25.000 km²
Population: ~2.200.000



Human *brucellosis* (1980-2004) **9720 (total)****
2005 up to date 105 (1 soldier*)

- **Reservoirs:** goats, sheep,

- **Route of transmision:**
  - Ingestion of contaminated, unpasterized milk or diary products (21,7%, Sokolovski et al. 1997, Nikolovski, 2003)
  - Direct contact, inhalacion (39 %)
  - Combination (42,3%)

Diagnosis of Human *Brucellosis*

- ✓ Epidemiological data
- ✓ Clinical manifestations
- ✓ Laboratory tests

Laboratory tests:

- Culture
- Serology
- PCR

Culture
(BSL-3)

- **Specimens:**
  - **blood**
  - **sternum**
  - **lymph nodes**
  - **liquor**
  - **urine**
  - **abscess**
  - sputum, placenta, milk, vaginal - seminal secretion.

CVL-1996

AFIP-2000

✓ Isolation:

- **Blood culture systems:**
  - **API 20 NE**
  - **Bact/Aert™**
  - **Bactec**
  - **VITAL-bioMerieux.**

10-90%

Eissa et al. ,1990, (n= 87)
Sensitivity 75%.

Shehabi et al. ,1990, (n=106)
Sensitivity: blood 44,4%
bone marrow 27,7%.

Moreno et al. , 1992 (n= 119)
Sensitivity 70%

Diferrentiation

Диференцијација на видовите од родот *Brucella* [a,b]

Карактеристики	B. abortus	B. canis	B. melitensis	B. neotomae	B. Ovis	B. suis biotipovi 1	2	3	4
Лиза од fag RTD[v]									
Td	L	NL	NL	PL	NL	NL	NL	NL	NL
Wb	L	NL	NL	L	NL	L	L	L	L
Fi	L	NL	NL	L	NL	PL	PL	PL	L
Bk2	L	NL	L	L	NL	L	L	L	L
R/O	PL	NL	NL	NL	L	NL	NL	NL	NL
R/C	NL	L	NL	NL	L	NL	NL	NL	NL
Оксидација на субстрати:									
l- Alanine	+	g	+	g	g	g	_	g	_
l- Aspargine	+	_	+	+	+	_	g	_	_
l- Glutamic acig	+	+	+	+	+	_	g	g	g
l- Arabinose	+	g	_	+	_	+	+	_	_
g- Galactose	+	g	_	+	_	g	g	_	_
g- Ribose	+	+	_	g	_	+	+	+	+
g- Glucose	+	+	+	+	_	+	+	+	+
g- Xylose	g	_	_	_	_	+	+	+	+
l- Arginine, gl- Citruline dl- Ornithine	_	+	_	_	_	+	+	+	+
l- Lysine	_	+	_	_	_	+	_	+	
meso - Erythrol	+	g	+	+	_	+	+	+	+

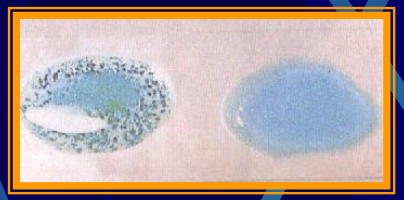
Average incubation time 13,6

Serologic tests

1. **Rose Bengal test , Brucelloslide test**
2. **Serum agglutination tube test - SAT- Wright**
3. **Coombs antihuman globulin test**
4. **Complement fixation test - CFT**
5. **2-Mercaptoethanol test**
6. **ELISA** (IgM, IgG, IgA)
7. **c-ELISA**
8. **Fluorescent Polarisation Assay (FPA)**

1. **Rose Bengal test , Brucelloslide test** (n=725, senz. 99,6%, spec. 95,4%, Taleski, 1996)

(n=1100, senz. 98% spec. 97%, Taleski,2000)



2. **Serum agglutination tube test - SAT- Wright** (n=725, snez. 84%, spec. 100%, 1996)

(n=1100, senz. 82% spec. 100%, Taleski,2000)

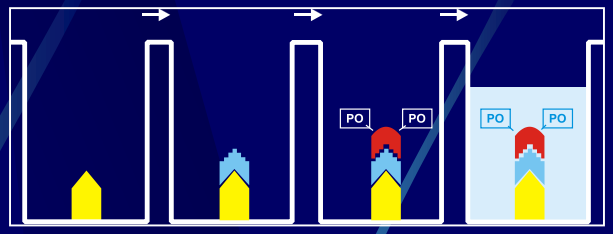
3. **Coombs antihuman globulin test** (n=725, snez. 89%, spec. 100%, 1996)

4. **Complement fixation test - CFT** (senz. 68,6%, Nikolovski, 1990)

(senz. 42% Taleski, 1996)

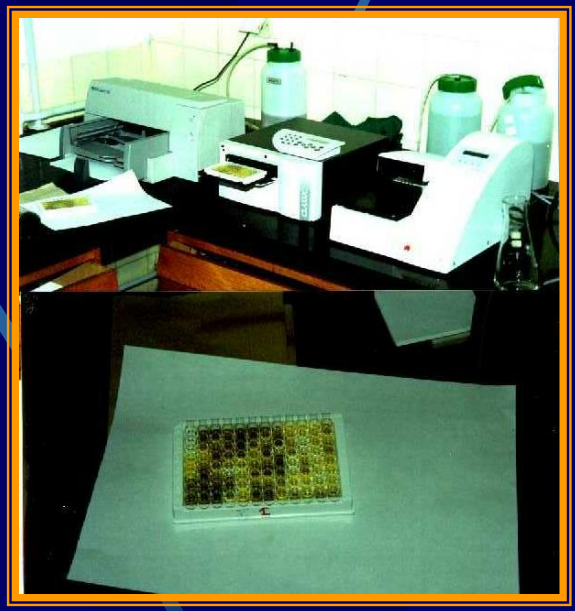
ELISA:

Ag-SLPS



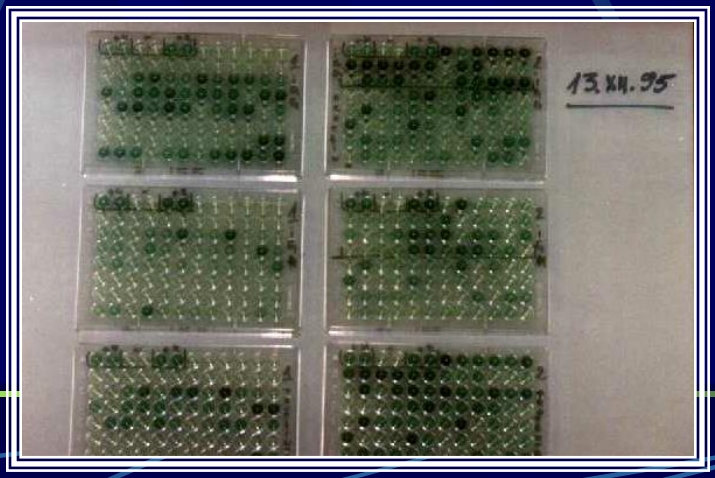
- Barbudhe at all. 1994 (n=80)
- Colmenero at all. 1994 (n=50)
- Araj at all. 1986, (n=173)
  1988, (n=573)

Sensitivity 89% - 100%
Specificity 77% - 100%



Our study (1999-2001):
(n=1100)

Sensitivity 98%
Specificity 100%



(Bosnjakovski, Taleski,1995)

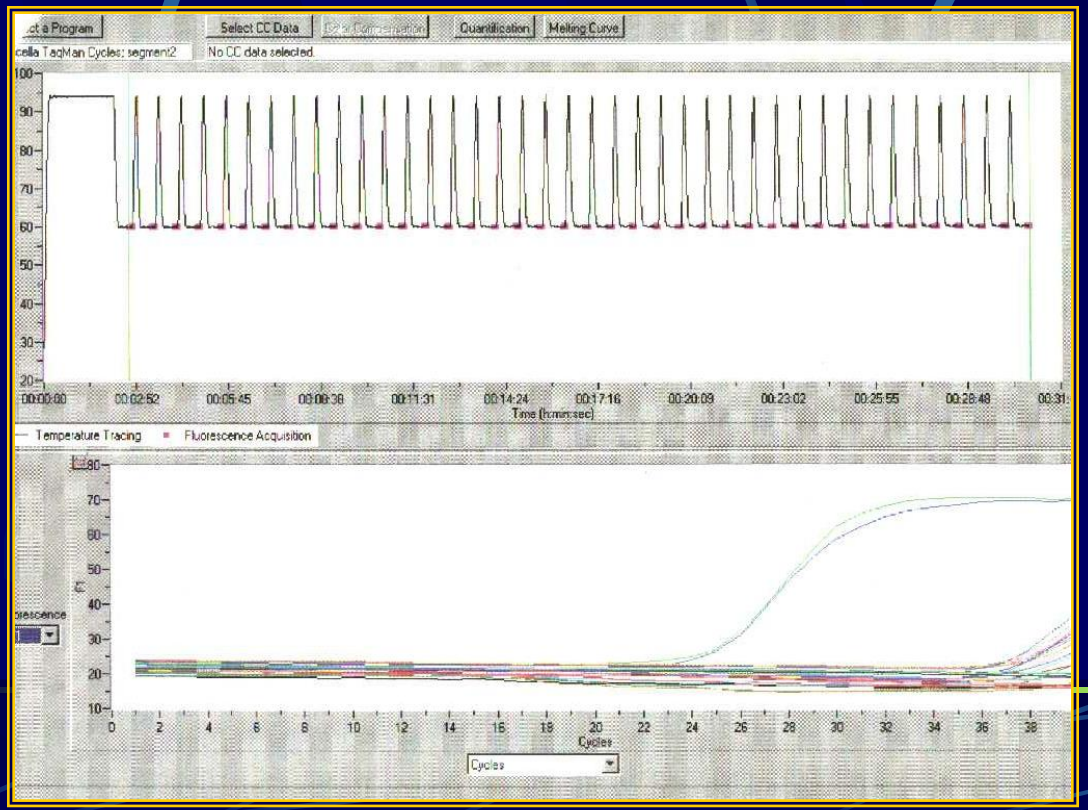
PCR (Polimerase Chain Reaction)

AFIP (Armed Forces Institute of Pathology)
Washington DC, US,2000

Conventional PCR

2-5^{h}

I. 1 cycle	94C^{0}	5’ (10’)
II. 30 cycles:	94C^{0}	1’
	60C^{0}	1’
	72C^{0}	1’ (1-3’)
III. 1 cycle	72C^{0}	3’ (10’)



R.A.P.I.D- PCR

6-30^{min}

I. 1 cycle	94C^{0}	2’
II. 40 cycles:	60C^{0}	20”
	94C^{0}	0’

Brucella Genes (GenBank-appr. 50 genes)

Functional Group:	Gene/Protein Designation
Cell Envelope	*omp 1, 2a, 2b, 10, 19, 16A, 25, 28, bmp 18, bp 26, BA 41, CP 24, cds A, lpx D, fab Z, Ipx A, mepA*
Cellular processes	*htr A, htra-like, dnaD, dnaJ, groEL, groES,*
Energy Metabolism	*GLK, GLUp, ERY*
DNA RNA building blocks	*purE, purK, purA*
Respiratory Functions	*katE, sodC*
Replication	*recA, uvrA, adenine methyl transferase*
Translation	*16S dRNA, 23S dRNA*
Unknown	*BCSP31, p39, ORFP17*
Repeated DNAs	*IS711 *(IS6501), Bru-RS1, BRU-RS2*

***Brucella* genom appr. 58% GC**
2 Circular Chromosomes:
2100 kbp
1150 kbp

- Genus specific
  - BCSP31, 16S rRNA
- Species/biotypes
  - AMOS-PCR, IS711, omp2A,omp2B,

Brucella DNA detection

Sets of primers :

- **Specific primers for gene *BCSP 31* *(amplicon 134 bp)***

BCSP 31 **F-622** 5’-GCG TTG GGA GCG AGC TTT-3’, 18 nucleotides

BCSP 31 **R-681** 5’-GCC AGT GCC GAT ACG GAA-3’, 18 nucleotides

Taqman probe (640):

6FAM-CGG TTG CAC AGG CCC CGA CA-TAMRA, 20 nucleotides.

Literature data (PCR):

- **16S rRNA, Romero et al., 1995;**
- ***omp-2* gen, Klevezas et al., 1995; Rincon et al., 1997;**
- ***IS711* (*IS6501*) , Bricker et al., 1994; 1995;**
- ***BCSP31* , Matar et al., 1996; Quiepo-Ortuno et al., 1997; Morata et al., 1998.**

- **Quiepo-Ortuno et al.,1997, *BCSP31*, (n=47), convencional PCR ,**
- **Senz. 100%, spec. 98,3%.**
- **Navaro et al.,1999, (n=10 patient, 5 healthy)**
- **senz. 50% spec. 60 %.**
- **Romero et al. 1995, milk of 37 infected cows, convencional PCR ,**
- **senz. 87,5% and ELISA (for *brucella* antibodies) in 98,2%**

PCR (R.A.P.I.D.-PCR) Results:

(n=330 peripheral human blood samples)

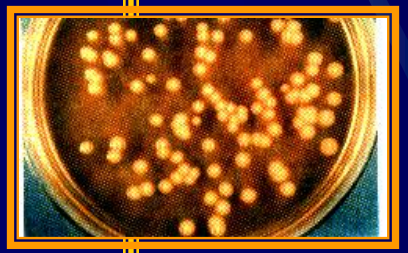
BCSP 31

- Sensitivity 56%
- Specificity 100%

Isolates (n=16)

BCSP 31

- **Sensitivity 100%**
- **Specificity 100%**



✓Results (n=330)

Sensitivity	ELISA %	PCR *BCSP31* %
A. Patients in acute phase(100)	100	56
B. With symptoms after treatment (100)	23	17
C. No symptoms after treatment (100)	9	2
D. Healthy persons (30)	0	0
Specificity	100	100

CONCLUSIONS:

- **Incidence of brucellosis caused by Brucella melitensis in sheep, goats and humans is still significant problem in Macedonia. Not problem for Army personal, but Army medical personal can help to control the problem.**

- **ELISA method (sens. 98% and spec. 100%) should be a reference method in diagnosis of human brucellosis.**

- **PCR (R.A.P.I.D) is a promising tool to overcome well known problems in bacterial isolation and identification of *Brucella spp*. allows diagnosis in a very short time and avoids the risk from intralaboratory infections.**

- **Effective PCR detection of *Brucella* DNA from peripheral blood in order to get a better correlation with serology will require :**
  - **-sampling in the beginning of the disease,**
  - **-concentration techniques or larger volumes of blood for processing.**