Brucellosis 2003 International Research Conference

including the 56th Brucellosis Research Conference

September 15-17, 2003 - University of Navarra, Pamplona (Spain)

CONTENTS

Welcome

As Professor Paul Nicoletti reminds us, the "The first *Brucellosis Research Conference* was held at the Morrison Hotel in Chicago (USA) in November 1948 and was organized by B. T. Simms of the USDA-Bureau of Animal Industry. ... The reputation of this Conference is well-known as an excellent medium for presentation of results of experiments and exchange of research ideas." (*A brief history of the Brucellosis Research Conference*, 50th Anniversary Meeting, Chicago, November 8-9, 1998). The general format of the Conference has remained unchanged since then, with the only exception of the 53rd Conference which was held in Nimes (France) on 7-9 September 2000, and was excellently organized by J. J. Letesson from Laboratoire d'Immunologie et de Microbiologie, URBM-FUNDP, Namur (Belgique). During those days, it was suggested that the 56th Conference (year 2003) could be held again in Europe and the "brucellosis group" in Pamplona took over the challenge. Now, on behalf of the Organising Committee it is a great pleasure for me to welcome all of you again in Europe, in Spain, in Pamplona, and in the University of Navarra.

One of our main objectives was to encourage international participation. Thanks to the contribution of the FAO of the United Nations and the OIE of the World Organization for Animal Health, and with the special collaboration of the International Centre for Advanced Mediterranean Agronomic Studies through the Mediterranean Agronomic Institute of Zaragoza (CIHEAM/IAMZ) and the european COST Action 845 it has been possible to welcome more than 250 participants from 40 different countries (from Malaysia to the USA, from Finland to Nigeria). We want also to thank the Spanish Ministry of Science and Technology and the local Government of Navarra for their support. The sponsorship of several companies is also acknowledged.

Brucellosis is extremely widespread around the world and, but for a few developed countries, occurs almost everywhere. The disease has a truly international nature both in terms of its epidemiology, human health risks and impact on trade. Due to its complexity, research in brucellosis requires a multidisciplinary approach that can only be achieved by combined scientific expertise. The *Brucellosis 2003 International Research Conference* will be an efficient means of linking research teams across the world and facilitating the dissemination of information to the legislators and to the field.

We hope that the free expression and discussion of research results during these days in Pamplona will be one more step to win the battle against this zoonotic disease that cause heavy economical losses and human suffering around the world. Moreover, we are sure that all our efforts to control this infection represent an excellent opportunity to encourage the understanding among the countries and to work for the peace and developing of the human race.

Sincerely,

Ignacio López-Goñi, Chairman

Conference Commitees

Chair: Ignacio López-Goñi, Departamento de Microbiología, Universidad de Navarra, Pamplona (Spain)

Vice-Chair: J. P. Liautard, INSERM, Departement de Biologie Santé, Université de Montpellier II, Montepellier (France)

Vice-Chair Elect: Betsy J. Bricker, Bacterial Diseases of Livestock Unit, National Animal Disease Center, Agricultural Research Unit, USDA, Ames, Iowa (USA)

Past-Chair: Richard C. Essenberg, Department of Biochemistry and Molecular Biology, Oklahoma State University, Oklahoma (USA)

Secretary: Sue Hagius, Department of Veterinary Science, Louisiana State University, Louisiana (USA)

International Conference Secretary: Juan M. García-Lobo, Departamento de Biología Molecular, Facultad de Medicina, Universidad de Cantabria, Santander (Spain)

Scientific Program Committee

Javier Ariza Cardenal
Servicio de Enfermedades Infecciosas Hospital de Belvitge, Barcelona (Spain)

Bruno Garin-Bastuji
Unité des Zoonose, Laboratoire de Reference OIE pour la Brucellose, Paris (France)

R. Martin Roop II
Department of Microbiology and Immunology, East Carolina University School of Medicine, Greenville, North Carolina (USA)

José María Blasco
Servicio de Investigaciones Agrarias – DGA, Zaragoza (Spain)

Edgardo Moreno
Programa de Investigación en Enfermedades Tropicales, Escuela de Medicina Veterinaria, Universidad Nacional, Heredia (Costa Rica)

Jean-Jaques Letesson
Laboratoire d'Immunologie et de Microbiologie (Unite de Recherche en Biologie Moleculaire, Facultes Universitaires Notre-Dame de la Paix, Namur (Belgique)

Ottorino Cosivi
WHO, Animal and Food-Related Public Health Risks, Department of Communicable Disease, Surveillance and Response, Geneva (Switzerland)

Local Organising Committee

Ignacio López-Goñi, Depto. de Microbiología, Universidad de Navarra, Pamplona (Spain)
Ramón Díaz, Depto. de Microbiología, Universidad de Navarra, Pamplona (Spain)
Ignacio Moriyón, Depto. de Microbiología, Universidad de Navarra, Pamplona (Spain)
Carlos Gamazo, Depto. de Microbiología, Universidad de Navarra, Pamplona (Spain)
Juan M. García-Lobo, Depto. de Biología Molecular, Universidad de Cantabria, Santander (Spain)
Jesús Agüero, Depto. de Biología Molecular, Universidad de Cantabria, Santander (Spain)
Clara Marín, Unidad de Sanidad Animal, SIA-DGA, Zaragoza (Spain)
Antonio Orduña, Unidad de Investigación, Hospital Universitario de Valladolid (Spain)

KEYNOTE LECTURES AND SHORT ORAL PRESENTATIONS

Keynote Lectures will be 35 minutes in length plus 5 minutes for discussion, and Short Oral Communications will be 10 minutes in length.

Oral presentations can be made using slides, overheads or PowerPoint presentations (single projection only). For computer presentations it is recommended to send by e-mail (brucellosis2003@unav.es) your presentation before 10 September 2003. Alternatively, you may bring your PowerPoint presentations on a floppy disc or CD (**NOT ZIP DISC!**). You must deposit your CD or diskette in the Registration Desk at least three hours before your presentation. To avoid any confusion, please use "your name" in the title for the presentation when saving and/or sending. Computer, microphone and laser pointer will be provided by the organisation.

PERSONAL LAPTOP (COMPUTERS) WILL NOT BE ALLOWED

POSTER PRESENTATIONS

Two different Poster Sessions will be organised:

Poster Session I (poster number 1 to 60) will begin at 9:15 am Monday 15 and remain in place to 11:00 am Tuesday 16.

Poster Session II (poster number 61 to 118) will begin at 13:00 Tuesday 16 and remain in place to 18:30 Wednesday 17.

Poster Session will be located in the Science Building.

Posters are required to fit on a 90 cm (wide) x 120 cm (long) board. Adhesive material will be provided by the organisation.

Conference at a glance

Monday, September 15, 2003

8:15 - 9:15 (*Science Building Auditorium*) - **Registration**
9:15 - 9:30 (*Science Building Auditorium*) - **Welcome and Introductions**
9:30 - 10:45 (*Science Building Auditorium*) - **SESSION I - EPIDEMIOLOGY, CONTROL AND ERADICATION PROGRAMS (KEYNOTE LECTURES)**
- ***An overview of the epidemiology of brucellosis in selected countries.*** **William Amanfu.**
- ***Epidemiology of brucellosis: consequences in terms of control strategy*****. Bruno Garin-Bastuji.**

9:30 - 10:45 (*Lecture room 3A02*) - **SESSION II - HUMAN BRUCELLOSIS (KEYNOTE LECTURES)**
- ***Brucellosis: Facts and Fantasies*****. Edward J. Young.**
- ***Disease spectrum and laboratory diagnosis of human brucellosis*****. George F. Araj .**

10:45 - 11:15 - **Coffee break and Poster Viewing (Part I)**
11:15 - 13:00 (*Science Building Auditorium*)- **SESSION I – EPIDEMIOLOGY, CONTROL AND ERADICATION PROGRAMS (SHORT ORAL COMMUNICATIONS)**
11:15 - 13:00 (*Lecture room 3A02*) - **SESSION II – HUMAN BRUCELLOSIS (SHORT ORAL COMMUNICATIONS)**

13:00 - 15:00 - **Lunch and Poster Viewing (Part I)**

15:00 - 16:15 (*Science Building Auditorium*)- **SESSION III – DIAGNOSIS IN ANIMAL BRUCELLOSIS (KEYNOTE LECTURES)**
- ***Development and validation of diagnostic tools and typing methods for the certification of the absence of bovine brucellosis in Belgium*****. Jacques Godfroid.**
- ***Brucellosis in wildlife*****. Darla Ewalt.**

16:15 - 16:45 - **Coffee break and Poster Viewing (Part I)**
16:45 - 18:30 (*Science Building Auditorium*)- **SESSION III – DIAGNOSIS IN ANIMAL BRUCELLOSIS (SHORT ORAL COMMUNICATIONS)**

20:30 *(University Central Building)* - **Conference dinner**

Tuesday, September 16, 2003

9:00 - 10:30 (*Science Building Auditorium*)- **SESSION IV – IMMUNOLOGY, PATHOGENESIS AND HOST-PATHOGEN INTERACTION (KEYNOTE LECTURES)**
- ***Brucella LPS a key immunomodulator of immune responses in mice*****. Jean-Pierre Gorvel.**
- ***A stealthy but nasty intracellular bug named Brucella: aspects of interaction of the pathogen with the macrophage host cell*****. Stephan Köhler.**

10:30 - 11:00 - **Coffee break and Poster Viewing (Part I)**
11:00 - 13:00 (*Science Building Auditorium*) - **SESSION IV – IMMUNOLOGY, PATHOGENESIS AND HOST-PATHOGEN INTERACTION (SHORT ORAL COMMUNICATIONS - PART I)**

13:00 - 15:00 - **Lunch and Poster Viewing (Part II)**

15:00 - 16:15 (*Science Building Auditorium*)- **SESSION V – VACCINES (KEYNOTE LECTURES)**
- ***Classical and new generation vaccines against brucellosis in ungulates*****. Phil Elzer.**
- ***Alternative brucellosis vaccines: experiences with drug delivery systems*****. Carlos Gamazo.**

16:15 - 16:45 - **Coffee break and Poster Viewing (Part II)**
16:45 - 18:30 (*Science Building Auditorium*) - **SESSION V – VACCINES (SHORT ORAL COMMUNICATIONS)**
18:30 - 20:30 (*Lecture room 3A02*) - **COST-845 MC, WG1, WG2, WG3 and WG4 meeting**

Wednesday, September 17, 2003

9:00 - 10:15 (*Science Building Auditorium*) - **SESSION VI – TAXONOMY AND EVOLUTION (KEYNOTE LECTURES)**
- ***Phylogeny and evolution of alpha-proteobacterial genomes*****. Siv Andersson.**
- ***Comparison of the genomic sequences of Brucella melitensis, Brucella suis, and Brucella abortus biovars: structure and pseudogenes.*** **Shirley Halling.**

10:15 - 10:45 - **Coffee break and Poster Viewing (Part II)**
10:45 - 12:00 (*Science Building Auditorium*) - **SESSION IV – IMMUNOLOGY, PATHOGENESIS AND HOST-PATHOGEN INTERACTION (SHORT ORAL COMMUNICATIONS - PART II)**
12:00 - 13:00 (*Science Building Auditorium*) - ***Brucella*** **Taxonomy and Nomenclature Committee**

13:00 - 15:00 - **Lunch and Poster Viewing (Part II)**

15:00 - 16:15 (*Science Building Auditorium*) - **SESSION VII – GENOMICS AND PROTEOMICS (KEYNOTE LECTURES)**
- ***Proteomics and host-pathogen interaction: new paradigms*****. Michel Desjardins.**
- ***From genome sequences back to (systems) biology: using the C. elegans v1.1 ORFeome for functional proteomics*****. David E. Hill**.

16:15 - 16:45 - **Coffee break and Poster Viewing (Part II)**
16:15 - 16:45 (*Lecture room 3A02*) - ***International Society for Brucellosis*****business meeting**
16:45 - 18:30 (*Science Building Auditorium*) - **SESSION VI & SESSION VII – TAXONOMY AND EVOLUTION & GENOMICS AND PROTEOMICS (SHORT ORAL COMMUNICATIONS)**

18:30 (*Science Building Auditorium*) - **Closing Session**

Scientific Program

8:15 - 9:15 (*Science Building Auditorium*) - **Registration**

9:15 - 9:30 (*Science Building Auditorium*) - **Welcome and Introductions**

9:30 - 10:45 (*Science Building Auditorium*)

SESSION I - EPIDEMIOLOGY, CONTROL AND ERADICATION PROGRAMS (KEYNOTE LECTURES)
Chairman: **O. Cosivi**. WHO, Animal and Food-Related Public Health Risks, Department of Communicable Disease, Surveillance and Response, Geneva (Switzerland)

- ***An overview of the epidemiology of brucellosis in selected countries.***
  William Amanfu. FAO, Animal Health Service, Animal Production and Health Division, Roma (Italy)
- ***Epidemiology of brucellosis: consequences in terms of control strategy*.**
  Bruno Garin-Bastuji. National & OIE/FAO Animal Brucellosis Reference Lab., Bacterial Zoonoses Unit, Maisons-Alfort (France)

9:30 - 10:45 (*Lecture room 3A02*)

SESSION II - HUMAN BRUCELLOSIS (KEYNOTE LECTURES)
Chairman: **J. Ariza**. Servicio de Enfermedades Infecciosas. Hospital de Belvitge, Barcelona (Spain)

- ***Brucellosis: Facts and Fantasies*.**
  Edward J. Young. Medical Services, Veterans Affairs Medical Center, Houston (USA)
- ***Disease spectrum and laboratory diagnosis of human brucellosis*.**
  George F. Araj . Department Pathology and Laboratory Medicine, American University of Beirut Medical Center, Beirut (Lebanon)

10:45 - 11:15 - **Coffee break and Poster Viewing (Part I)**

11:15 - 13:00 (*Science Building Auditorium*)

SESSION I – EPIDEMIOLOGY, CONTROL AND ERADICATION PROGRAMS
SHORT ORAL COMMUNICATIONS

Chairman: **O. Cosivi**. WHO, Animal and Food-Related Public Health Risks, Department of Communicable Disease, Surveillance and Response, Geneva (Switzerland)

EO1- BRUCELLOSIS ERRADICATION PROGRAM IN URUGUAY.
Garín Alfredo. Jefe Programa de Errradicación de Brucelosis y Tuberculosis. Ministerio Agricultura y Pesca, División Sanidad Animal. Montevideo, Uruguay.

EO2- BOVINE BRUCELLOSIS CONTROL PROGRAM IN MÉXICO: PRESENT SITUATION AND FACTORS THAT LIMITS ITS ADVANCE.
R. Flores-Castro. Dirección de Investigación en Salud Animal y Salud Pública, DGIP/INIFAP. México.

EO3- BRUCELLOSIS IN NIGERIA.
R. A. Ocholi. Brucellosis Research Unit, Bacterial Research Department, National Veterinary Research Institute, Vom Plateau State, Nigeria.

EO4- BRUCELLOSIS - A SERIOUS THREAT TO CATTLE PRODUCTION IN MALAYSIA.
K. Palanisamy[1], R. Mahendran[2] and K. Idris[1]. (1) State Veterinary Department, Kubang Kerian, Kelantan, Malaysia, (2) Veterinary Research Institute, Jalan Sultan Azlan Shah, Ipoh, Perak, Malaysia.

EO5- TEMPORAL AND SPATIAL FEATURES OF BOVINE BRUCELLOSIS IN NORTHERN IRELAND: 1996 TO 2000.
D.A. Abernethy[1], D.U. Pfeiffer[2], R. Watt[1], S. McDowell[1,2]. (1) Veterinary Service, Department of Agriculture, Northern Ireland, U.K. (2) Royal Veterinary College, London, U.K.

EO6- BRUCELLOSIS RISK FACTORS IN SHEEP FLOCKS AT THE SOUTH OF BEIRA INTERIOR, PORTUGAL.

M. Martins[1], V. Almeida[2] e I. Neto[2]. (1) Escola Superior Agrária de Castelo Branco, Quinta da Srª de Mércules, 6001-909 Castelo Branco, Portugal. (2) Faculdade Medicina Veterinária, Polo Universitário do Alto da Ajuda, Rua Professor Cid dos Santos, 1300-477 Lisboa. Portugal.

EO7- HUMAN HEALTH BENEFITS FROM LIVESTOCK VACCINATION FOR BRUCELLOSIS: A CASE STUDY.

Felix Roth[1], Jakob Zinsstag[1], Dontor Orkhon[2], G. Chimed-Ochir[3], Guy Hutton[1], Ottorino Cosivi[4], Guy Carrin[4] and Joachim Otte[5]. (1) Swiss Tropical Institute, Swiss Centre for International Health and Department of Epidemiology and Public Health, PO Box, CH-4002 Basle, Switzerland. (2) Ministry of Public Health, Olympic Street 2, Ulaanbaatar 11, Mongolia. (3) Infectious Disease Research Centre, PO Box 48, Ulaanbaatar, Mongolia. (4) World Health Organisation, Avenue Appia 20, 1211 Geneva 27, Switzerland. (5) Food and Agriculture Organization of the United Nations (FAO), Viale delle Terme di Caracalla, 00100 Roma, Italy.

EO8- DIAGNOSTIC VALIDATION OF BOVINE BRUCELLOSIS SEROLOGICAL TESTS FOR EPIDEMIOSURVEILLANCE PURPOSES.

F. Boelaert[1], E. Venoo[1], D. Verloo[1], C. Saegerman[2], G. Maquet[3], M. Lomba[3], L. De Meulemeester[4], J. Wullepit[4], K. Walravens[1], K. Mintiens[1], J. Godfroid[1]. (1) Veterinary and Agrochemical Research Center, Brussels, Belgium. (2) Ministry of Health, Consumer's Protection and Environment, Brussels, Belgium. (3) Association Régionale de Santé et d'Identification Animales, Ciney, Belgium. (4) Diergezondheidszorg Vlaanderen, Leefdaal, Belgium.

EO9- DIFFERENCES IN SERUM ANTIBODY RESPONSES BETWEEN PIGS EXPERIMENTALLY INFECTED WITH *Brucella suis* BIOVAR 2 AND *Yersinia enterocolitica* SEROTYPE O:9.

G. Jungersen, V. Sørensen, S. Giese, and U. Riber. Danish Veterinary Institute, Copenhagen, Denmark.

EO10- NEW ANTIBRUCELLOSIS STRATEGY.

P. Ignatov[1], U. Immomaliev[2], I. Mamatkulov[2], A. Fedorov[1]. (1) IGN Corporation, USA. (2) Institute of Epidemiology and Microbiology of Uzbekistan.

11:15 - 13:00 (*Lecture room 3A02*)

SESSION II – HUMAN BRUCELLOSIS
SHORT ORAL COMMUNICATIONS

Chairman: **J. Ariza**. Servicio de Enfermedades Infecciosas. Hospital de Belvitge, Barcelona (Spain)

HO1- SIGNIFICANT REDUCED NUMBER OF *Brucella*-SPECIFIC IFN-g-PRODUCING CD3 T CELLS IN HUMAN CHRONIC BRUCELLOSIS.

Alireza Rafiei[1], Amina Kariminia[2], Sussan K. Ardestani[3], Abdolhosein Keyhani[1], Mino Mohraz[4], Ali Amirkhani[2]. (1) Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran. (2) Department of Immunology, Pasture Institute, Tehran, Iran. (3) Section of Immunology , Institute of Biochemistry and Biophysics, Tehran University, Tehran, Iran. (4) Department of Infectious Diseases, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.

HO2- *Brucella* IgM AND IgG FLOW ASSAYS FOR THE RAPID SERODIAGNOSIS OF HUMAN BRUCELLOSIS.

H. L. Smits[1], T. H. Abdoel[1], J. Solera[2], E. Clavijo[3], and R. Diaz[4]. (1) KIT Biomedical Research, Royal Tropical Institute / Koninklijk Instituut voor de Tropen (KIT), Amsterdam, The Netherlands. (2) Unit of Infectious Diseases, General Hospital of Albacete, Albacete, Spain. (3) Microbiology Unit, HCU Virgen de la Victoria, Malaga, Spain. (4) Servicio de Microbiología Clínica, Universidad de Navarra, Pamplona, Spain.

HO3- INTERFERENCE OF RHEUMATOID FACTOR IN THE IgM-BASED SEROLOGICAL DIAGNOSE OF HUMAN BRUCELLOSIS.

R. Díaz[1], H. Smits[2], T. H. Abdoel[2], M. Rubio[1], J. Ariza[3], A. Casanova[3], E. Clavijo[4], J. Solera[5], A. Orduña[6] and I. Dorronsoro[7]. (1) Departamento de Microbiología, Universidad de Navarra. Pamplona, Spain. (2) KIT Biomedical Research. Royal Tropiocal Institute. Amsterdam. The Netherlands. (3) Hospital de Bellvitge, Barcelona, Spain. (4) Hospital Virgen de la Victoria. Málaga. Spain. (5) Departamento de Medicina Interna. Facultad de Medicina. (6) Departamento de Microbiología. Universidad de Valladolid. Valladolid. Spain. (7) Hospital de Navarra, Pamplona, Spain.

HO4- RAPID DIAGNOSIS OF HUMAN BRUCELLOSIS BY SERUM QUANTITATIVE REAL-TIME PCR.

J.D. Colmenero[1], M.I. Queipo-Ortuño[2], María E. Pachón[3], M. Gonzalez[4], J.Mª. Reguera[1], G. Baeza[2], M.A. García-Ordoñez[1] and P. Morata[2]. (1) U. E. Infecciosas, Hospital Universitario Carlos Haya, Málaga, Spain. (2) Departamento de Bioquímica y Biología Molecular, Facultad de Medicina, Málaga, Spain. (3) Servicio E. Infecciosas, Hospital Universitario Virgen del Rocío, Sevilla, Spain. (4) U. E. Infecciosas, Hospital Universitario Virgen de la Victoria, Málaga, Spain.

HO5- CLINICAL MANIFESTATION AND LABORATORY TEST RESULTS IN 581 CASES OF BRUCELLOSIS IN BABOL, IRAN , 1997-2002.
M.R.Hasanjani Roushan[1], S. A. Asgharzadeh Ahmadi[1], M. J. Soleimani Amiri[1]. (1) Department of Infectious Diseases, Babol Medical University, Babol, Iran.

HO6- CHILDHOOD BRUCELLOSIS IN BABOL, NORTH OF IRAN.
M. R. Hasanjani Roushan[1], S. A. Asgharzadeh Ahmadi[1], Y. Zahed Pasha[2], M. J. Soleimani Amiri[1]. (1) Department of Infectious Diseases, Babol Medical University, Babol, Iran. (2) Amir Kola Children Hospital, Babol Medical University, Babol, Iran.

HO7- EFFICACY OF THREE DIFFERENT REGIMENS WITH TWO DIFFERENT DURATION OF THERAPY IN HUMAN BRUCELLOSIS.
M. R. Hasanjani Roushan[1], S. A. Asgharzadeh Ahmadi[1], M. G. Soleimani Amiri. (1) Department of Infectious Diseases, Babol Medical University, Babol, Iran.

HO8- ANIMAL AND HUMAN BRUCELLOSIS IN ITALY: OCCUPATIONAL, FOOD CHAIN EXPOSURE, OR BOTH?.
F. De Massis[1], A. Di Girolamo[2], A. Petrini[1], E. Pizzigallo[2], A. Giovannini[1]. (1) Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Teramo, Italy. (2) Clinica Malattie Infettive, Università degli Studi "G. d'Annunzio", Chieti, Italy.

HO9- BRUCELLAR SPONDILODISCITIS. A CASE REPORT IN A RELATIVELY LOW INCIDENCE AREA.
F. Vignale, P.G.Giuri, A. Di Girolamo, D. De Tullio, E. Pizzigallo, F. Ricci. Clinica Malattie Infettive, Università degli Studi "G. d'Annunzio", Chieti, Italy.

13:00 - 15:00 - **Lunch and Poster Viewing (Part I)**

15:00 - 16:15 (*Science Building Auditorium*)

SESSION III – DIAGNOSIS IN ANIMAL BRUCELLOSIS (KEYNOTE LECTURES)
Chairman: **B. Garin-Bastuji**. National & OIE/FAO Animal Brucellosis Reference Lab., Bacterial Zoonoses Unit, Maisons-Alfort (France)

- ***Development and validation of diagnostic tools and typing methods for the certification of the absence of bovine brucellosis in Belgium.***
  Jacques Godfroid. Veterinary and Agrochemical Research Center, Brussels (Belgium)
- ***Brucellosis in wildlife*.**
  Darla Ewalt. Diagnostic Bacteriology Laboratory, National Veterinary Services Laboratories, Ames (USA)

16:15 - 16:45 - **Coffee break and Poster Viewing (Part I)**

16:45 - 18:30 (*Science Building Auditorium*)

SESSION III – DIAGNOSIS IN ANIMAL BRUCELLOSIS
SHORT ORAL COMMUNICATIONS

Chairman: **B. Garin-Bastuji**. National & OIE/FAO Animal Brucellosis Reference Lab., Bacterial Zoonoses Unit, Maisons-Alfort (France)

DO1- SEROLOGICAL RESPONSE OF YOUNG AND ADULT SHEEP TO CONJUCTIVAL VACCINATION WITH REV-1 VACCINE ASSESSED WITH THE CLASSICAL AND RECENT TESTS.
A. Stournara[1], A. Minas[1], E. Bourtzi-Chatzopoulou[2], G. Koptopoulos[2], E. Petridou[2], K. Sarris[2]. (1) Veterinary Laboratory of Larissa, National Reference Laboratory of Brucellosis, 411 10 , Larissa, Greece. (2) Laboratory of Microbiology and Infectious Diseases , Faculty of Veterinary Medicine, Aristotelian University of Thessaloniki , 54 124, Thessaloniki. Greece.

DO2- EVALUATION OF THE COMPETITIVE ENZYME IMMUNOASSAY AND POLARIZED FLUORESCENCE ASSAY FOR BRUCELLOSIS DIAGNOSIS IN GOATS FROM NORTHERN MÉXICO.
C. Ramirez-Pfeiffer[1], A. C. Snydelaar-Hardwicke[2], F. Acosta-Martínez[2], J. Vázquez-Villanueva[2], E. Luna-Martínez[3], R. Gómez-Flores[4], K. Nielsen[5]. (1) Instituto Nacional de Investigaciones Forestales y Agropecuarias, CIRNE, C. E. Aldama, México. (2) Universidad Autónoma de Tamaulipas, Fac. Med. Veterinaria y Zoot., México. (3) Secretaría de Agricultura Ganadería, Desarrollo Rural, Pesca y Alimentación, SENASICA, México. (4) Universidad Autónoma de Nuevo León. Fac. Ciencias Biológicas, México. (5) Canadian Food Inspection Agency. Animal Disease Research Institute, Canada.

DO3- USE OF BP26 BASE ENZYME LINKED IMMUNOSORBENT ASSAY FOR DIFFERENTIATING RUMINANTS INFECTED WITH *Brucella abortus* OR *melitensis* FROM RUMINANTS INFECTED WITH *Yersinia enterocolitica*.
Kristine Klewer[1], Stéphane Gabriel[1], Laurence Guilloteau[2], Michel Zygmunt[2], Philippe Pourquier[1]. (1) Institut Pourquier, Montpellier, France. (2) INRA, Tours, France.

DO4- DIFFERENCES IN CELLULAR IMMUNE RESPONSES OF PIGS EXPERIMENTALLY INFECTED WITH *Brucella suis* AND *Yersinia enterocolitica* SEROTYPE O:9.
G.Jungersen and U.Riber. Danish Veterinary Institute, Copenhagen, Denmark.

DO5- BRUCELLOSIS IN THE NETHERLANDS: THE DEVELOPMENT OF *Brucella*-SPECIFIC SEROLOGICAL TESTS.
B. Kooi, L Mastebroek, P. Willemsen, D. Bakker and F. van Zijderveld. Central Institute for Animal Disease Control (CIDC) Lelystad, The Netherlands.

DO6- COMPARISON OF ROSE BENGAL IN ANALOGY OF 1:3 WITH SERUM AND ROSE BENGAL IN ANALOGY OF 1:1 WITH SERUM INTERPRETED IN SERIES WITH COMPLEMENT FIXATION TEST AS CONFIRMATORY TEST FOR THE DETECTION OF INFECTED SHEEP AND GOAT FLOCKS WITH *Brucella melitensis*. A FOUR-MONTH FIELD TRIAL DURING THE CYPRUS BRUCELLOSIS TEST AND SLAUGHTER ERADICATION PROGRAM.
M.V.Liapi[1], I.G.Ioannou[1], M.Papaprodromou[1]. (1) Veterinary Laboratories Department, Cyprus Veterinary Services, Nicosia, Cyprus.

DO7- DIAGNOSING OF OVINE AND CAPRINE BRUCELLOSIS TROUGH THE SERUM AND MILK ELISA - PRELIMINARY TEST VALIDATION.
Bosnakovski J., Mitrov D., Naletoski I. Veterinary Institute, Skopje, Republic of Macedonia.

DO8- THE USE OF FTA CARDS IN MOLECULAR DIAGNOSTIC TECHNIQUES FOR BRUCELLOSIS.
Betsy J. Bricker[1], Steven C. Olsen[1] and Darla R. Ewalt[2]. (1) USDA, Agricultural Research Service, National Animal Disease Center, Ames, IA, USA. (2) USDA, Animal and Plant Health and Inspection Service, Veterinary Services, National Veterinary Services Laboratories, Ames, IA, USA.

DO9- MARINE MAMMAL BRUCELLOSIS IN CANADA 1995-2003.
O. Nielsen[1], K. Nielsen[2], D. Ewalt[3], B. Dunn[1] and S. Raverty[4]. (1) Department of Fisheries and Oceans Canada, Winnipeg, Manitoba, Canada. (2) Canadian Food Inspection Agency, Animal Diseases Research Institute, Nepean, Ontario, Canada. (3) United States Department of Agriculture, National Veterinary Services Laboratories, Ames, Iowa, USA. (4) Animal Health Centre, British Columbia Ministry of Agriculture and Food, Abbotsford, British Columbia, Canada.

20:30 *(University Central Building)* - **Conference dinner**

9:00 - 10:30 (*Science Building Auditorium*)

SESSION IV – IMMUNOLOGY, PATHOGENESIS AND HOST-PATHOGEN INTERACTION (KEYNOTE LECTURES)
Chairman: **R. Martin Roop II**. Department of Microbiology and Immunology, East Carolina University School of Medicine, Greenville, North Carolina (USA)

- ***Brucella LPS a key immunomodulator of immune responses in mice*.**
  Jean-Pierre Gorvel. Centre d'Immunologie ISERM-CNRS de Marseille-Luminy, Marseille (France)
- ***A stealthy but nasty intracellular bug named Brucella: aspects of interaction of the pathogen with the macrophage host cell*.**
  Stephan Köhler. INSERM U-431, Universite Montpellier II, Montpellier (France)

10:30 - 11:00 - **Coffee break and Poster Viewing (Part I)**

11:00 - 13:00 (*Science Building Auditorium*)

SESSION IV – IMMUNOLOGY, PATHOGENESIS AND HOST-PATHOGEN INTERACTION SHORT ORAL COMMUNICATIONS - PART I

Chairman: **R. Martin Roop II**. Department of Microbiology and Immunology, East Carolina University School of Medicine, Greenville, North Carolina (USA)

PO1- HUMAN-*Brucella* INTERACTIONS: SPECIES SPECIFIC PERSPECTIVES.
A. Mathison, L. Eskra, G. Splitter. University of Wisconsin, Madison, USA.

PO2- SUBVERSION AND UTILIZATION OF THE HOST CELL CYCLIC ADENOSINE 5'-MONOPHOSPHATE/PROTEIN KINASE A PATHWAY BY *Brucella* DURING MACROPHAGE INFECTION.
Gross A., Liautard J-P and Dornand J. INSERM U431, Montpellier, France.

PO3- HUMAN NATURAL KILLER CELLS IMPAIR THE INTRAMACROPHAGIC DEVELOPMENT OF *Brucella suis* BY A CELL-TO-CELL CONTACT DEPENDENT MECHANISM.
J. P. Liautard , V. Lafont, J. Oliaro, A. Terraza, J. Dornand. INSERM U431, F-34095 Montpellier, France.

PO4- LPS FROM ENTEROBACTERIA AND *Brucella* SHOW DIFFERENT SIGNALING PROPERTIES ON TOLL-LIKE RECEPTORS.
A. Dueñas[1], A. Orduña[1], M. A. Bratos[1], A. Rodríguez Torres[1], M. Sánchez Crespo[2], C. García-Rodríguez[2]. (1) Hospital Clínico Universitario, Valladolid, Spain. (2) Instituto de Biología y Genética Molecular (Centro Mixto CSIC-Universidad de Valladolid), Valladolid, Spain.

PO5- *Brucella abortus* ACTIVATES DENDRITIC CELLS AND SPLENOCYTES TO SECRETE IL-12P40 AND TNF-a VIA DIFFERENT TOLL-LIKE RECEPTORS.
Li-Yun Huang, Julio Aliberti, Cynthia Leifer, and Basil Golding. CBER, FDA, and LPD NIAID, Bethesda, MD, 20892, USA.

PO6- THE ROLE OF HUMAN GAMMA 9 DELTA 2 T CELLS IN INNATE IMMUNITY AGAINST INTRACELLULAR *Brucella*.
Jane Oliaro, Sherri Dudal, Janny Liautard, Jean-Baptiste Andrault, Jean-Pierre Liautard and Virginie Lafont. Institut National de la Santé et de la Recherche Médicale Unité 431, Laboratoire de Microbiologie et Pathologie Cellulaire Infectieuse, Université de Montpellier II, Montpellier, France.

PO7- DO CD8 T CELLS HAVE A ROLE IN CONTROLLING *Brucella abortus* INFECTIONS?.
R. Goenka, M. Parent, and C. L. Baldwin. Department of Veterinary and Animal Sciences, University of Massachusetts, Amherst, MA 01003. USA.

PO8- CHARACTERIZATION OF QUORUM SENSING MOLECULAR ACTORS FROM *Brucella melitensis* 16M.
C. Deschamps, R-M Delrue, J. J. Letesson and X. De Bolle. Unité de Recherche en Biologie Moléculaire (URBM), Laboratoire d'Immunologie et de Microbiologie, Facultés Universitaires Notre-Dame de la Paix, Namur, Belgium.

PO9- VirB2 IS REQUIRED FOR THE FUNCTION OF THE *Brucella abortus* TYPE IV SECRETION SYSTEM.
Andreas B. den Hartigh, Hortensia G. Rolán and Renée M. Tsolis. Texas A&M University System Health Science Center, Departement of Medical Microbiology and Immunology, College Station, TX, USA.

13:00 - 15:00 - **Lunch and Poster Viewing (Part II)**

15:00 - 16:15 (*Science Building Auditorium*)

SESSION V – VACCINES (KEYNOTE LECTURES)
Chairman: **J. M. Blasco**. Servicio de Investigaciones Agrarias, DGA, Zaragoza (Spain)

- ***Classical and new generation vaccines against brucellosis in ungulates*.**
  Phil Elzer. Veterinary Science and Veterinary Immunology, LSU Department of Veterinary Science, Baton Rouge, LA (USA)
- ***Alternative brucellosis vaccines: experiences with drug delivery systems*.**
  Carlos Gamazo. Department of Microbiology, University of Navarra, Pamplona (Spain)

16:15 - 16:45 - **Coffee break and Poster Viewing (Part II)**

16:45 - 18:30 (*Science Building Auditorium*)

SESSION V – VACCINES
SHORT ORAL COMMUNICATIONS

Chairman: **J. M. Blasco**. Servicio de Investigaciones Agrarias, DGA, Zaragoza (Spain)

VO1- CALFHOOD RB51 VACCINATION FAILS TO PROTECT BISON AGAINST VIRULENT CHALLENGE.
D. S. Davis[1] and P. H. Elzer[2]. (1) Department of Pathobiology, College of Veterinary Medicine, Texas A & M University, College Station, TX 77843, USA. (2) Department of Veterinary Science, LSU Ag Center, Baton Rouge, LA 70803. USA.

VO2- INDUCTION OF IMMUNE RESPONSE IN GOATS WITH A EXPERIMENTAL DNA VACCINE ENCODING Omp31 OUTER MEMBRANE PROTEIN OF *Brucella melitensis* 16M.
V. K. Gupta, P.K. Rout, T. Chandrasekhar and V. S. Vihan. Central Institute for Research on Goats, Makhdoom, PO. Farah, Mathura, Uttar Pradesh 281 122, India.

VO3- IMMUNE RESPONSE IN CALVES VACCINATED WITH DNA VECTOR ENCODING Cu/Zn SOD PROTEIN OF *Brucella abortus*.
A. Oñate[1], I. Guzmán[1], A. González[1], S. Céspedes[1], C. Muñoz[1], R. Rivers[1], H. Folch[2], and E. Andrews[1]. (1) Laboratorio de Inmunología Molecular, Departamento de Microbiología, Facultad de Ciencias Biológicas. Universidad de Concepción. Concepción, Chile. (2) Instituto de Inmunología. Universidad Austral de Chile, Chile.

VO4- EVALUATION OF *Brucella abortus* S19 VACCINE STRAINS USED IN INDIA BY BACTERIOLOGICAL AND MOLECULAR TESTS AND VIRULENCE STUDIES IN BALB/c MICE.
Falguni Mukherjee[1], Jainendra Jain[1], María Jesús Grilló[2], José María Blasco[2], Mrinalini Nair[3]. (1) Research and Development, National Dairy Development Board, Anand 388 001, Gujarat, India. (2) Unidad de Sanidad Animal, Servicio de Investigación Agroalimentaria, Gobierno de Aragón, Apartado 727, Zaragoza, Spain. (3) Biotechnology Program, Department of Microbiology and Biotechnology Centre, MS University, Baroda 390 002, Gujarat, India.

VO5- COMPARISON OF SUBCUTANEOUS VERSUS INTRANASAL IMMUNIZATION OF MICE WITH *Brucella* SUBCELLULAR VACCINES.
A. Bhattacharjee[1], M. Izadjoo[2], W. Zollinger[1], and David L. Hoover[1]. (1) Walter Reed Army Institute of Research, USA. (2) Armed Forces Institute of Pathology, Washington, DC, USA.

VO6- PASSIVE TRANSFER OF PROTECTION AGAINST *Brucella melitensis* 16M INFECTION IN A MURINE MODEL.
Mina J. Izadjoo[1], Richard H. Borschall[2], Apurba K. Bhattacharjee[2], Mikeljon P. Nikolich[2], Ted L. Hadfield[1], and David L. Hoover[2]. (1) Armed Forces Institute of Pathology, Washington, D.C., USA. (2) Walter Reed Army Institute of Research, Forest Glen, Maryland, USA.

VO7- ORAL IMMUNIZATION WITH WR201, A LIVE, ATTENUATED PURINE AUXOTROPHIC STRAIN OF *B. melitensis*, PROTECTS MICE AND NONHUMAN PRIMATES AGAINST RESPIRATORY CHALLENGE WITH *B. melitensis* 16M.

D. Hoover[1], M. Izadjoo[2], R. Borschel[1], M. Mense[1], A. Bhattacharjee[1] and C. Paranavitana[1]. (1) Walter Reed Army Institute of Research, (2) Armed Forces Institute of Pathology, Washington, DC, USA.

VO8- DEVELOPMENT OF A GENETICALLY MODIFIED *Brucella melitensis* REV. 1 LIVE VACCINE ASSOCIATED TO A DIAGNOSTIC ASSAY ALLOWING DISCRIMINATION BETWEEN VACCINATED AND INFECTED SHEEP.

L. A. Guilloteau[1], A. Cloeckaert[1], I. Jacques[1], M. Grayon[1], K. Laroucau[1], S. Baucheron[1], F. Carreras[1], F. Cortade[1], V. Olivier-Bernardin[1], M. Olivier[1], M. S. Zygmunt[1], M. J. Grillo[2], C. M. Marin[2], M. Barberan[2], N. Vizcaino[3], A. Peix[3], L. Fernàndez-Lago[3], J. M.Blasco[2], J. M. Verger[1]. (1) Unité Pathologie Infectieuse et Immunologie, Institut National de la Recherche Agronomique, 37380 Nouzilly, France. (2) Servicio de Investigacion de Agroalimentaria de la Diputación General de Aragón, Zaragoza, Spain. (3) Universidad de Salamanca, Salamanca, Spain.

VO9- WANING *trans* COMPLEMENTATION OF ROUGHNESS IN A *Brucella melitensis wbo*A *pur*E DUAL MUTANT: A POTENTIAL FOR LIVE VACCINES AND HETEROLOGOUS ANTIGEN DELIVERY.

M. Nikolich, M. Izadjoo, C. Fernandez-Prada, E. Penn and D. Hoover. Walter Reed Army Institute of Research, Silver Spring, MD, USA.

VO10- DEVELOPMENT OF *Brucella abortus* STRAIN RB51 AS AN EXPRESSION VECTOR FOR HETEROLOGOUS EUKARYOTIC AND VIRAL PROTEINS AND AS A CARRIER FOR AIDS VACCINE.

Yakir Ophir[1], Gerhardt Schurig[2], Ramesh Vemulapalli[3], George N. Pavlakis[4], Barbara Felber[4], A.T.M. Shamsul Hoque[1], Weila Wang[1], Hana Golding[1] and Basil Golding[1]. (1) CBER, FDA, Bethesda, MD, USA. (2) VA-MD Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA, USA. (3) Purdue University, West Lafayette, IN, USA. (4) NCI, NIH, Fredrick, MD, USA.

VO11- PROTECTION AGAINST *Neospora caninum* IN A GERBIL MODEL USING *Brucella abortus* STRAIN RB51 EXPRESSING *N. caninum* PROTEINS.

S. Ramamoorthy, G. Schurig, D. Lindsay, R. Vemulapalli, S.M. Boyle, N. Sriranganathan. Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA 24061, USA.

18:30 - 20:30 (*Lecture room 3A02*)

COST-845 MC, WG1, WG2, WG3 and WG4 meeting

9:00 - 10:15 (*Science Building Auditorium*)

SESSION VI – TAXONOMY AND EVOLUTION (KEYNOTE LECTURES)
Chairman: **E. Moreno**. Programa de Investigación en Enfermedades Tropicales, Escuela de Medicina Veterinaria, Universidad Nacional, Heredia (Costa Rica)

- ***Phylogeny and evolution of alpha-proteobacterial genomes.***
  Siv Andersson. Department of Molecular Evolution, University of Uppsala, Uppsala (Sweden)
- ***Comparison of the genomic sequences of Brucella melitensis, Brucella suis, and Brucella abortus biovars: structure and pseudogenes.***
  Shirley Halling. NADC/ARS/USDA, Bacterial Diseases of Livestock Unit, Ames (USA)

10:15 - 10:45 - **Coffee break and Poster Viewing (Part II)**

10:45 - 12:00 (*Science Building Auditorium*)

SESSION IV – IMMUNOLOGY, PATHOGENESIS AND HOST-PATHOGEN INTERACTION
SHORT ORAL COMMUNICATIONS - PART II

Chairman: **Juan M. García-Lobo**. Departamento de Biología Molecular, Facultad de Medicina, Universidad de Cantabria, Santander, (Spain)

PO10- IivA, A PROTEIN INVOLVED IN THE *Brucella* spp. VIRULENCE.
S. L.Cravero, M. Carrica, E. Campos, A. Arese, J. Sabio y García, and O. L. Rossetti. Instituto de Biotecnología, Instituto Nacional de Tecnología Agropecuaria (INTA), Buenos Aires, Argentina.

PO11- THE *Brucella abortus xth*A2 GENE PRODUCT CONTRIBUTES TO RESISTANCE TO OXIDATIVE STRESS *in vitro* BUT IS NOT REQUIRED FOR WILD-TYPE VIRULENCE IN THE MOUSE MODEL.
Michael L. Hornback and R. Martin Roop II. The Brody School of Medicine of East Carolina University. USA.

PO12- ANALYSIS OF *Brucella* ANTIOXIDANT GENES REVEALS THE IMPORTANCE OF DEFENSE AGAINST STATIONARY PHASE ENDOGENOUS REACTIVE OXYGEN INTERMEDIATE ACCUMULATION *in vitro* AND *in vivo*.
M. Wright Valderas, J. M. Gee, R. B. Alcantara, M. E. Kovach, J. E. Baumgartner, and R. M. Roop II. Department of Microbiology and Immunology, East Carolina University School of Medicine, Greenville, NC 27834. USA.

PO13- THE GLUTAMATE DECARBOXYLASE SYSTEM IN *Brucella abortus* IS NOT REQUIRED FOR VIRULENCE IN THE MOUSE MODEL.
Tim Brown, Michelle Wright-Valderas, and R. Martin Roop II. East Carolina University, Brody School of Medicine, Greenville, NC 27834. USA.

PO14- IRON ACQUISITION STRATEGIES OF *Brucella abortus* 2308 DURING LIFE WITHIN THE MACROPHAGE.
James T. Paulley, R.M. Roop II. Department of Microbiology and Immunology, East Carolina University School Of Medicine, Greenville, NC 27834. USA.

PO15- LICENSE TO KILL: *Brucella abortus* ROUGH MUTANTS ARE CYTOPATHIC FOR MACROPHAGES IN CULTURE.
J. Pei and T. A. Ficht. Veterinary Pathobiology, Texas A&M University and Texas Agricultural Experiment Station, College Station, TX 77843-4467. USA.

PO16- MAP KINASE ACTIVATION IN ROUGH AND SMOOTH *Brucella*–INFECTED J774.A1 CELLS: RELATIONSHIP TO VIRULENCE.
M. P. Jiménez de Bagüés[1], A. Terraza[2], A. Gross[2], J. Dornand[2]. (1) Unidad de Sanidad Animal. Servicio de Investigación Agroalimentaria. Diputación General de Aragón. Ap. 727. 50080 Zaragoza, Spain. (2) INSERM U431. Université de Montpellier II. 34095-Montpellier. France.

12:00 - 13:00 (*Science Building Auditorium*)
***Brucella* Taxonomy and Nomenclature Committee** (public session)

13:00 - 15:00 - **Lunch and Poster Viewing (Part II)**

15:00 - 16:15 (*Science Building Auditorium*)

SESSION VII – GENOMICS AND PROTEOMICS (KEYNOTE LECTURES)
Chairman: **J. J. Letesson**. Laboratoire d'Immunologie et de Microbiologie, Unite de Recherche en Biologie Moleculaire, Facultes Universitaires Notre-Dame de la Paix, Namur (Belgique)

- ***Proteomics and host-pathogen interaction: new paradigms.***
  Michel Desjardins. Département de pathologie et biologie cellulaire, Université de Montréal, Québec (Canada)
- ***From genome sequences back to (systems) biology: using the C. elegans v1.1 ORFeome for functional proteomics.***
  David E. Hill. Dana-Farber Cancer Institute, Boston, MA (USA)

16:15 - 16:45 - **Coffee break and Poster Viewing (Part II)**

16:15 - 16:45 (*Lecture room 3A02*)
***International Society for Brucellosis* business meeting**

16:45 - 18:30 (*Science Building Auditorium*)

SESSION VI & SESSION VII – TAXONOMY AND EVOLUTION & GENOMICS AND PROTEOMICS SHORT ORAL COMMUNICATIONS

Chairman: **J. J. Letesson**. Laboratoire d'Immunologie et de Microbiologie, Unite de Recherche en Biologie Moleculaire, Facultes Universitaires Notre-Dame de la Paix, Namur (Belgique)

GO1- GENOME COMPARISONS OF THREE *Brucella* SPECIES: *B. suis*, *B. melitensis* AND *B. ovis*.
Rekha Seshadri and Ian Paulsen. The Institute for Genomic Research, 9712 Medical Center Drive, Rockville, MD 20850, USA.

GO2- THE *Brucella* ORFeome PROJECT.
De Bolle, X., Dricot, A. , Lambert, C. , Letesson, J.-J. URBM, University of Namur, 61 rue de Bruxelles, 5000 Namur, Belgium.

GO3- ANALYSIS OF THE UREASE GENE CLUSTERS IN *Brucella* spp.
F. J. Sangari and J. M. García-Lobo. Department of Molecular Biology, University of Cantabria, Santander, Spain.

GO4- SYSTEMATIC DISRUPTION OF GENES CODING FOR TRANSCRIPTIONAL REGULATORS IN *Brucella melitensis* 16M.
V. Haine , M. Dozot, A. Sinon, P. Lestrate, R-M. Delrue, A. Tibor, C. Lambert, J.J. Letesson and X. De Bolle. Unité de Recherche en Biologie Moléculaire (URBM), Laboratoire d'Immunologie et de Microbiologie, Facultés Universitaires Notre-Dame de la Paix, Namur, Belgique.

GO5- IDENTIFICATION OF A TRANSCRIPTIONAL REGULATOR INVOLVED IN *Brucella melitensis* FLAGELLAR GENE EXPRESSION.
S. Léonard, A. Tibor, J. Ferooz, D. Fretin, C. Nijskens and J.-J. Letesson. Unité de Recherche en Biologie Moléculaire (URBM), Laboratoire d'Immunologie et de Microbiologie, Facultés Universitaires Notre-Dame de la Paix, Namur, Belgique.

GO6- A MAJOR GENE CONTROLLING NATURAL RESISTANCE TO BOVINE BRUCELLOSIS.
J.W. Templeton[1], C. Schutta[1], L.G. Adams[1], E. Gormley[2], and D. Collins[2]. (1) Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M University, College Station, Texas, USA. (2) Department of Large Animal Clinical Studies, Faculty of Veterinary Medicine, University College Dublin, Ireland.

18:30 (*Science Building Auditorium*) - **Closing Session**

POSTER SESSION I
(from 9:15 am Monday 15 to 11:00 am Tuesday 16)

1- VALIDATION OF DIAGNOSTIC TESTS TO DETECT BRUCELLOSIS AND EPIDEMIOLOGICAL SURVEY IN THE ECUADORIAN ANDES.

J. Ron[1,4], J. Godfroid[2], C. Saegerman[3], K. Walravens[2], W. Benítez[1], J. Brandt[4], L. Utterhaegen[2], C. De Smedt[2], P. Michel[2] and D. Berkvens[4]. (1) Universidad Central del Ecuador, Centro Internacional de Zoonosis. Quito, Ecuador. (2) Veterinary and Agrochemical Research Center. Brussels, Belgium. (3) Ministry of Health, Consumer's protection and Environment. Brussels, Belgium. (4) "Prince Leopold" Institute of Tropical Medicine, Department of Animal Health, Antwerpen, Belgium.

2- SURVEILLANCE AND MONITORING OF BRUCELLOSIS IN SLOVENIA.

M. Štukelj. University of Ljubljana. Veterinary Faculty. Institute for pig diseases. Slovenia.

3- PREVALENCE OF BOVINE BRUCELLOSIS IN HERDS SUPPLYING MILK TO LOCAL MARKETS IN GUINEA, THE GAMBIA AND SENEGAL AND ASSOCIATED PUBLIC HEALTH RISK.

F. Unger[1], A. Goumou[2], B. Diallo[2], M. Konte[4], M. Hempen[1], S. Münstermann[1] and K. H. Zessin[3]. (1) International Trypanotolerance Centre, Banjul, The Gambia. (2) Departement National d´ Èlevage, Conakry, Guinea. (3) Freie Universität Berlin, Department for International Animal Health, Berlin, Germany. (4) Institut Senegalais Recherche Agricole, Dakar, Senegal.

4- SEROLOGICAL SURVEY ON BRUCELLOSIS IN HUMAN, SHEEP AND GOATS IN CENTRAL PART OF IRAN.

T. Zahraei – Salehi[1] and A. Haseli Abarghoei[2]. (1) Department of Microbiology & Immunology, Faculty of Veterinary Medicine, Tehran University, Tehran – Iran. (2) Graduated from the Faculty of Veterinary Medicine, Tehran University, Tehran – Iran.

5- DESCRIPTION OF *Brucella melitensis* BODY DISTRIBUTION PATTERN IN DIFFERENT EPIDEMIOLOGICAL SITUATION: MICROBIOLOGICAL SAMPLING IN ERADICATION PROGRAMS.

Alvarez Del Castillo, L.[1], Latre Cequiel, M.V.[2], Yustes C.[3], Vendrell, J.[3], Bosch, A.[1], Gómez, B.[4], Garrido, F.[4], Duran-Ferrer, M.[4] (1) Food engineering and Biotechnology, UPC Barcelona, Spain. (2) Microbiology Department, Universidad de Zaragoza, Spain. (3) Paseo Sunyer , 51, Reus, Spain. (4) Central Veterinary Laboratory, Ministry of Agricultural Fisheries and Food, Santa Fe, Granada, Spain.

6- SEROLOGICAL INCIDENCE OF *Brucella*. ANTIBODY IN DOMESTIC ANIMALS AND MAN IN IRAN.

Tadjbakhsh, H. Department of Microbiology & Immunology, Faculty of Veterinary Medicine, Tehran University, P.O.Box: 14155-6453, Tehran – Iran.

7- IMPLEMENTATION OF A VACCINATION PROGRAMME AGAINST ANIMAL BRUCELLOSIS AND THE EFFECT ON THE INCIDENCE OF HUMAN BRUCELLOSIS IN WESTERN GREECE.

Ch. Bikas[1], Ch. Petropoulos[2] and E. Jelastopulu[3]. (1) Department of Internal Medicine, University Hospital of Patras, Greece. (2) Health Centre Erymanthia, Greece. (3) Laboratory of Public Health, Medical School, University of Patras, Greece.

8- EPIDEMIOLOGICAL STUDY ON 581 CASES OF BRUCELLOSIS IN BABOL IRAN 1997-2002.

M. R. Hasanjani Roushan[1], S. A. Asgharzadeh Ahmadi[1], M. J. Soleimani Amiri[1]. (1) Department of Infectious Diseases, Babol Medical University, Babol, Iran.

9- COUNTER-EPIDEMIC MEASURES TAKEN IN THE SITUATION OF BRUCELLOSIS EPIZOOTIC WIDELY SPREAD AMONG LIVESTOCK IN SOUTH KAZAKHSTAN.

K. Ospanov[1], T. Grushina[2], S. Kazakov[1]. (1) Kazakh Republican Sanitary - epidemiology station, Kazakhstan. (2) M. Aikimbayev's Kazakh Scientific Center for Quarantine and Zoonotic Diseases, Kazakhstan.

10- ANALYSIS OF SERUM BY ROSE-BENGAL TEST AND STANDARD TUBE AGGLUTINATION TEST FROM 20,663 PATIENTS IN SOUTHEAST TURKEY SUSPECTED OF HAVING BRUCELLOSIS.

T. Özekinci[1], S. Atmaca[1], N. Akpolat[1], S. Batun[2], S. Elçi[3] (1) Department of Microbiology, (2) Department of Hematology, Medical Faculty, (3) Education Faculty, Dicle University, Diyarbakir, Turkey.

11- *Brucella* SURVEILLANCE AND CLINICAL SAMPLING AMONG ANIMALS IN SWEDEN.

K.E Bergström[1], S. Boqvist[2]. (1) Dept. of Bacteriology, (2) Dept. of Disease Control and Biosecurity, SVA, SE – 751 89 Uppsala, Sweden.

12- SHEEP AND GOATS BRUCELLOSIS IN SICILY (1999-2002).

C. Di Bella[1], M. Bagnato[2], V. Bonomo[2], G. Vesco[1], G. Tumino[1], F. Prato[1], S. Vullo[1], F. Geraci[1], S. Agnello[1], F. Campo[1], R. Giunta[1], S. Caracappa[1]. (1) Istituto Zooprofilattico Sperimentale in Sicily "A. Mirri", Sicily, Italy. (2) Regional Ministry of Health, Sicily, Italy.

13- BOVINE BRUCELLOSIS IN SICILY (1999-2002).

C. Di Bella[1], M. Bagnato[2], V. Bonomo[2], G. Vesco[1], G. Tumino[1], F. Prato[1], S. Vullo[1], F. Geraci[1], S. Agnello[1], V. Ferrantelli[1], G. Cascone[1], A. Calabrò[1], S. Caracappa[1]. (1) Istituto Zooprofilattico Sperimentale (Experimental Zooprofilactic Institute) in Sicily "A. Mirri", Sicily, Italy. (2) Regional Ministry of Health, Sicily, Italy.

14- HIGH PREVALENCE OF SEROVAR 3 OF *Brucella abortus* IN THE REGION OF CANTABRIA (SPAIN).

J. Agüero[1], A. Ocampo[1], C. Marín[2], G. Gradillas[3] and J.M. García-Lobo[1]. (1) Departamento de Biología Molecular. Universidad de Cantabria, Spain. (2) Servicio de Sanidad Animal. Diputación General de Aragón, Spain. (3) Servicio de Laboratorio y Control. Dirección General de Ganadería. Gobierno de Cantabria. Spain.

15- ANTIMICROBIAL SUSCEPTIBILITY OF *Brucella canis* ISOLATED IN KOREA.

Jongwan Kim[1], Jongsam An[1], Jaehak Kim[1], Youngju Lee[1], Jongman Kim[1], Yiseok Joo[1], Ryunbin Tak[2]. (1) National Veterinary Research and Quarantine Service, (2) College of Veterinary Medicine, Kyungpook National University, Republic of Korea.

16- *Brucella cetaceae* - WHY DOES INFECTION OF DIFFERENT HOST SPECIES EXHIBIT DIFFERENT PATHOLOGIES?.

G. Foster. SAC Veterinary Science Division, Inverness. U.K.

17- SURVEILLANCE OF BRUCELLOSIS IN WILD BOAR IN SWITZERLAND.

Leuenberger R[1], Rüfenacht J.[1], Boujon P.[2], Miserez, R. [3], & Stärk K.D.C.[1] (1) Swiss Federal Veterinary Office, Bern, Switzerland. (2) Institute Galli-Valerio, Lausanne, Switzerland. (3) Institute of Veterinary Bacteriology, University of Bern, Switzerland.

18- SEROLOGICAL SURVEY FOR REINDEER (*Rangifer tarandus tarandus*) BRUCELLOSIS IN FINLAND.

E. Seuna[1] and V. Hirvelä-Koski[2]. Department of Bacteriology, Helsinki (1), Oulu Regional Unit (2), National Veterinary and Food Research Institute EELA, Finland.

19- PREVALENCE OF BRUCELLOSIS IN WILD SWINE (*Sus scrofa*) IN THE REPUBLIC OF CROATIA.

Z.Cvetnic[1], J. Toncic [2], S. Spicic [1], M. Lojkic [1], S. Terzic [1], L. Jemersic [1], A. Humski [1], M. Ocepek [3] and B.Krt [3] (1) Croatian Veterinary Institute Zagreb, Croatia. (2) Institute of Forestly, Jastrebarsko, Croatia. (3) Veterinary Faculty, Ljubljana, Slovenia.

20- HUMAN BRUCELLOSIS IN GEORGIA.

Ts. Zakaraia[1], N. Tarkhashvili[2], I. Antadze[3], O. Zenaishvili[1], G. Chubabria[1], N. Iashvili[1]. (1) Institute of Medical Parasitology and Tropical Medicine, (2) National Center for Disease Control, (3) Institute "Bacteriophage", Georgia.

21- CURRENT STATUS OF HUMAN BRUCELLOSIS IN KOREA.

Park, M. S., Y. S. Choi, J. S. Lee, S. K. Shim, H. K. Lee, J. H. Kim and M. Y. Park. Department of Bacteriology, National Institute of Health, Seoul, Korea.

22- *Brucella melitensis* BIOVARS FOUND IN THE REPUBLIC OF KAZAKHSTAN.

K. Ospanov[1], T. Grushina[2], A. Esmagambetova[1]. (1) Kazakh Republican Sanitary - epidemiology station, Kazakhstan. (2) M. Aikimbayev's Kazakh Scientific Center Quarantine and Zoonotic Diseases, Almaty, Kazakhstan.

23- IDENTIFICATION OF *Brucella* SPECIES ISOLATED FROM PROVEN PATIENTS IN IZMIR, TURKEY.

S. Kose[1], S. Kilic[2], G. Akkoclu[1], A. Kula[1], H. Ozinli[1], , S. Karacan[1], Y. Ozbel[3]. (1) Social Security Education Hospital, Yenisehir, Izmir, Turkey. (2) Ege University Medical School Department of Parasitology, Bornova, Izmir, Turkey. (3) Refik Saydam Hygiene Center, Microbiology and Clinical Microbiology, Ankara, Turkey.

24- THE SEROLOGIC SURVEY OF BRUCELLOSIS IN ABORTED WOMEN.

Dr. M. Rabbani Khorasgani, Dr. S. Sadeghi ,Dr. Ghasi Zadeh, Dr. S. Bokaee. The University of Tehran. Iran.

25- PRESENTATION OF ACUTE BRUCELLOSIS. A REVIEW OF 144 CASES.

Masouridou S., Andriopoulos P., Fokas S, Kalkani M., Dinosopoulou M., Asimakopoulos G., Tsironi M. Sparta General Hospital, Sparta, Lakonia, Greece.

26- CLINICAL AND IMMUNOLOGICAL FEATURES OF A HUMAN INFECTION BY THE M-STRAIN OF *Brucella canis*.

J. C. Wallach[1], G. H. Giambartolomei[2,3], P. C. Baldi[2], and C. A. Fossati[2]. (1) Servicio de Brucelosis, Hospital F. J. Muñiz, Argentina. (2) IDEHU, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina. (3) Laboratorio de Inmunogenética, Hospital de Clínicas José de San Martín, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina.

27- IMPORTED CASE OF BRUCELLOSIS COMPLICATED BY LIVER ABSCESS.

C. Mutini, M. Di Carlo, L. Coppolaro, A. Di Girolamo, E. Pizzigallo. Clinica Malattie Infettive, Università degli Studi "G. d'Annunzio", Chieti, Italy.

28- HUMAN *Brucella* ENDOCARDITIS: REPORT OF TWO CASES.

E. Vasileiadou, P. Tsekoura, E. Sidopoulos, D. Chrysagis, E. Papamihalis, D. Kolokotroni. Department of ?nternal ?edicine, Hospital of Infectious Diseases, Thessaloniki, Greece.

29- THE SIGNIFICANCE OF SEROLOGICAL TESTS IN DIAGNOSIS AND FOLLOWING UP OF NEUROBRUCELLOSIS: CASE REPORT.

Djordjevic M[1]., Lako B.[2], Ristanovic E[2]. Clinic for Infectious Diseases, Clinical Centre, Nis (1). Military Academy Belgrade (2).

30- CHRONIC COMPLICATIONS OF BRUCELLOSIS: CASE REPORT.

Djordjevic M[1]., Krstic M.[1],Lako B, [2]B.Djukic. Clinic for Infectious Diseases, Clinical Center ,Niš (1), Military Academy Belgrade (2).

31- BRUCELLOSIS AND CRIOGLOBULINEMIA.

Hermida I., Sáez L., Solera J. Servicio de Medicina Interna. Hospital General Universitario de Albacete. Facultad de Medicina, Universidad de Castilla La Mancha. Spain.

32- A CASE OF HUMAN BRUCELLOSIS GIVING NEGATIVE RESULTS IN THE SERUM AGGLUTINATION TEST (SAT)?: HOW TO AVOID THE PROZONE AND BLOCKING PHENOMENA.

J. L. del Pozo, T. Marrodán, M. F. Rubio, R. Díaz. Servicio de Microbiología Clínica. Clínica Universitaria. Universidad de Navarra. Pamplona, Spain.

33- IDENTIFICATION OF SMOOTH AND ROUGH FORMS IN CULTURES OF *Brucella melitensis* STRAINS BY FLOW CYTOMETRY.

C. M. Fernandez-Prada, E. B. Zelazowska, A. K. Bhattacharjee, M. P. Nikolich, and D. L. Hoover. Walter Reed Army Institute of Research (WRAIR), Silver Spring, MD 20910. USA.

34- EVALUATION OF A RAPID LATERAL FLOW TEST FOR DETECTION OF IgM AND IgG IN HUMAN BRUCELLOSIS.

J. Douglas[1], L. Okuhara[1], T. Abdoel[2], and H. Smits[2]. (1) Department of Microbiology, University of Hawaii, Honolulu, Hawaii, USA. (2) KIT Biomedical Research, Royal Tropical Institute, Amsterdam, The Netherlands.

35- DIAGNOSTICS OF HUMAN BRUCELLOSIS CAUSED BY *Brucella melitensis*.

T. Grushina[1], L. Tabatabai[2], L. Tserelson[3], M. Syzdykov[3], M. Rementsova[3], S. Daulbayeva[3], B. Beketov[1], K. Ospanov[4], A. Kouznetsov[3], S. Amireyev[3]. (1) M. Aikimbayev's Kazakh Scientific Center for Quarantine and Zoonotic Diseases, Kazakhstan. (2) National Animal Disease Center, Ames, Iowa, USA. (3) Hygiene and Epidemiology Research Center, (4) Kazakh Republican Sanitary - epidemiology station, Kazakhstan.

36- DETECTION OF *Brucella melitensis* BY VITAL SYSTEM.

St. Fokas, Sp. Fokas, M. Kalkani, M. Tsironi, S. Koutsoumpou, M. Dionysopoulou. Clinical Microbiology Department, General Hospital of Sparta, Lakonia, Greece.

37- EVALUATION OF AN AUTOMATED COMPLEMENT FIXATION TEST SYSTEM FOR THE DIAGNOSIS OF ACTIVE BRUCELLOSIS.

M. Tognini[1], P. Ardenghi[1], E. Clavijo[2], A. Orduna[3], S. Jimenez[4] and R. Diaz[5]. (1) DIESSE Diagnostica Senese SpA, Monteriggioni, Italy. (2) Hospital Virgen de la Victoria, Málaga, Spain. (3) Departamento de Microbiología, Facultad de Medicina, Valladolid, Spain. (4) Consejería de Salud, La Rioja, Spain. (5) Departamento de Microbiologia, Universidad de Navarra, Pamplona, Spain.

38- A COMPARISON OF ANTI-LPS IgG AS MEASURED BY THE 2ME TEST OR BY ELISA IN HUMAN BRUCELLOSIS.

J.C. Wallach[1,2], C. A Fossati[2], M.V. Delpino[2] and P.C. Baldi[2]. (1) Servicio de Brucelosis, Hospital F.J. Muñiz, Buenos Aires, Argentina. (2) IDEHU, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina.

39- DIAGNOSTIC USEFULNESS OF Omp31 FROM *B. melitensis* IN HUMAN AND ANIMAL BRUCELLOSIS.
J. Cassataro[1], K. Pasquevich, L. Bruno[1], J.C. Wallach[2,3], C. A Fossati[2], and P.C. Baldi[2]. (1) Laboratorio de Inmunogenética, Hospital de Clínicas, Universidad de Buenos Aires, Argentina. (2) Servicio de Brucelosis, Hospital F.J. Muñiz, Buenos Aires, Argentina. (3) IDEHU, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina.

40- LACTOBACILLI ANTAGONISM TO *Brucella*.
N. Gavrilova[1], T. Grushina[2], I. Ratnikova[1]. (1) Institute of Microbiology and Virology under the Ministry of Education and Science of the Republic of Kazakhstan. (2) M. Aikimbayev's Kazakh Scientific Center for Quarantine and Zoonotic Diseases, Kazakhstan.

41- RAPID DETECTION OF *Brucella* sp. DNA FROM HUMAN BLOOD SAMPLES USING REAL TIME PCR TECHNOLOGY.
C. Debeaumont, I. Pelloux, C. Recule, J. Croizé, M. Maurin. Laboratoire de Bactériologie, Cenre Hospitalier Universitaire de Grenoble, Grenoble, France.

42- DEVELOPMENT OF A RAPID AND SPECIFIC REAL TIME PCR ASSAY AND ITS VALIDATION FOR THE DETECTION OF HUMAN BRUCELLOSIS.
M.I. Queipo-Ortuño[1], G. Baeza[1], J.D. Colmenero[1], and P. Morata[2]. (1) Hospital Regional Universitario Carlos Haya, Málaga, Spain. (2) Departamento de Bioquímica y Biología Molecular, Universidad de Málaga, Málaga, Spain.

43- REAL-TIME PCR ASSAY FOR FIELD DIAGNOSIS OF *Brucella abortus* IN WILDLIFE POPULATIONS IN YELLOWSTONE NATIONAL PARK.
D.T. Newby and F.F. Roberto. Biotechnology Department, Idaho National Engineering and Environmental Laboratory, Idaho Falls, Idaho, USA 83415-2203.

44- DEVELOPMENT AND VALIDATION OF GENUS-SPECIFIC PCR FOR DIAGNOSIS OF *Brucella* INFECTION IN ANIMALS.
Jafar A. Qasem, Sabah Al-Momin, Salwa Al-Mouqati. Kuwait Institute for Scientific Research, Food Resources Division, Biotechnology Department. P.O.Box 24885, SAFAT, Kuwait 13109.

45- CLONING AND SEQUENCING OF 1.3 Kb RAPD FRAGMENT FOR THE DEVELOPMENT OF *Brucella* SPECIFIC PRIMERS.
Jafar Qasem, Sabah Al-Momin and Salwa Al-Mouqati. Kuwait Institute for Scientific Research, P. O. Box 24885 SAFAT, Shwayik City, Kuwait.

46- THE USE OF A COMBINATION OF RESTRICTION ENDONUCLEASES IN IS711-FINGERPRINTING FOR RESOLVING DIFFERENCES BETWEEN *Brucella* SPECIES.
Emma Young, John McGiven, Judy Stack and Alistair MacMillan. Department of Statutory and Exotic Bacteria, Veterinary Laboratories Agency, Addlestone, Surrey, UK.

47- MOLECULAR TYPING OF *Brucella* STRAINS BY RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD).
Marius Necsulescu, Virgilia Popa, Monica Vanghele, Daniela Botus. Pasteur Institute, Bucharest, Romania.

48- BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF *Brucella canis* ISOLATED IN KOREA.
Jongsam An[1], Jongwan Kim[1], Jaehak Kim[1], Youngju Lee[1], Jongman Kim[1], Yiseok Joo[1], Ryunbin Tak[2]. (1) National Veterinary Research and Quarantine Service, Republic of Korea. (2) College of Veterinary Medicine, Kyungpook National University, Republic of Korea.

49- MOLECULAR CHARACTERISATION OF FIELD STRAINS OF *Brucella* sp. ISOLATED IN ITALY IN THE YEARS 2001-2003.
M. Ancora[1], P. De Santis[1], E. Di Giannatale[1], T. Persiani[1], A.P. MacMillan[2] and V. Caporale[1]. (1) Istituto Zooprofilattico Sperimentale dell,Abruzzo e del Molise "G. Caporale", Italy. (2) Central Veterinary Laboratory, Weybridge, United Kingdom.

50- THE PRODUCTION AND CHARACTERISATION OF TWO RECOMBINANT PROTEINS, p18 AND bp26, FOR USE IN THE DEVELOPMENT OF AN IMMUNOASSAY FOR THE DETECTION OF BOVINE BRUCELLOSIS IN SERUM SAMPLES.
L. Dunne[1] and R. O'Kennedy[1]. (1) School of Biotechnology, Dublin City University, Glasnevin, Dublin 9, Ireland.

51- EVALUATION OF DIFFERENT CULTURE MEDIA FOR THE ISOLATION OF *Brucella suis*: COMPARISON OF DIFFERENT BASAL MEDIA AND SELECTIVE MEDIA.
A.C. Ferreira[1], R. Cardoso[1], D. Silva[1], M. Silva Pereira[2] and M.I. Corrêa de Sá[1]. (1) Laboratório Nacional de Investigação Veterinária, Lisboa, Portugal. (2) Estação Zootécnica Nacional -INIAP, Santarérm, Portugal.

52- COMPARATIVE EVALUATION OF BRUCELLOSIS SEROLOGY AND BACTERIOLOGY IN SLAUGHTERED ANIMALS IN PORTUGAL.
S. Rodeia, R. Cardoso and M. Dias. Serviço de Apoio aos Planos de Erradicação, Departamento de Bacteriologia, Laboratório Nacional de Investigação Veterinária (L.N.I.V.), Lisboa, Portugal.

53- ELISA AS AN ALTERNATIVE METHOD FOR THE DIAGNOSIS OF BRUCELLOSIS IN SHEEP.
Jalali, A.[1] , Hemmatzadeh, F.[2], Momtaz, H.[2] and Nilsson, E.[1]. (1) Svanova Biotech AB, Sweden. (2) Faculty of Veterinary Medicine, Tehran University, Iran.

54- EVALUATION OF THE ELISA IN DIAGNOSIS OF BRUCELLOSIS IN PIGS.
K. Szulowski, W. Iwaniak, J. Pilaszek. Department of Microbiology, National Veterinary Research Institute in Pulawy, Poland.

55- FLOURESCENCE POLARIZATION ASSAY PERFORMANCE IN THE DIAGNOSIS OF CAPRINE BRUCELLOSIS.
S. Conde[1], K. Nielsen[2], E.Piazza[1], L.Samartino[1]. (1) INTA-CCVyA-Castelar, Inst. Patobiología, Bacteriología. Buenos Aires, Argentina. (2) Canadian Food Inspection Agency, Animal Disease Research Institute, Ontario, Canada.

56- EVALUATION OF TWO SEROLOGICAL TESTS IN CANIN BRUCELLOSIS.
R. A. Radulescu, G. Petriceanu, V.E. Alexandru, A. Ragalie. Institute for Diagnosis and Animal Health, Bucharest, Romania.

57- SEROLOGICAL SURVEYS OF DOGS FOR *Brucella canis* INFECTIONS IN POLAND.
W. Iwaniak, K. Szulowski, J. Pilaszek. Department of Microbiology, National Veterinary Research Institute in Pulawy, Poland.

58- BRUCELLOSIS IN PINNIPEDS FROM ARGENTINA.
F. Capellino[1], S. Conde[1], M. Uhart[2], D. Albareda[3], L. Samartino[1]. (1) INTA CCVyA Castelar, Inst. Patobiología, Bacteriología, Bs. As., Argentina, (2) Wildlife Conservation Society, (3) Acuario de Buenos Aires, Argentina.

59- EFFICACY OF DIFFERENT ANTIGENS AND TESTS FOR THE SEROLOGICAL DIAGNOSIS OF BRUCELLOSIS IN CATTLE IN A CONTEXT OF FALSE POSITIVE REACTIONS DUE TO *Yersinia enterocolitica* O:9.
P. M. Muñoz[1], C. M. Marín[1], R. Díaz[2], I. Moriyón[2], B. Garin-Bastuji[3] and J. M. Blasco[1]. (1) Unidad de Sanidad Animal. SIA/DGA, Zaragoza, Spain. (2) Departamento de Microbiología, Universidad de Navarra, Pamplona, Spain. (3) Brucellosis Reference Laboratory, AFSSA, Maisons Alfort, France.

60- DIAGNOSIS OF HUMAN AND ANIMAL BRUCELLOSIS USING EXTRACTS OF BACTERIA PHYLOGENETICALLY RELATED TO *Brucella*.
M. V. Delpino[1], J. C. Wallach[1,2], C. A. Fossati[1] and P. C. Baldi[1]. (1) Instituto de Estudios de la Inmunidad Humoral, Facultad de Farmacia y Bioquímica, CONICET, UBA, Argentina. (2) Servicio de Brucelosis, Hospital F.J. Muñiz, Buenos Aires, Argentina.

POSTER SESSION II
(from 13:00 Tuesday 16 to 18:30 Wednesday 17)

61- HIGH PREVALENCE OF *Brucella pinnipediae* IN TISSUES FROM APPARENTLY HEALTHY GREENLAND SEA HOODED SEALS (*Cystophora cristata*).

M. Tryland[1,2], K. K. Sørensen[3], and J. Godfroid[4]. (1) Department of Arctic Veterinary Medicine, The Norwegian School of Veterinary Science, Tromsø, Norway. (2) Department of Microbiology and Virology, University of Tromsø, Norway. (3) National Veterinary Institute, Regional Laboratory, Tromsø, Norway. (4) Veterinary and Agrochemical Research Centre, Brussels, Belgium.

62- *Brucella melitensis* PERSISTENCE AND KINETICS OF THE IMMUNE RESPONSE IN EXPERIMENTALLY INFECTED EWES. PART I.

Tittarelli M, Di Ventura M., Scacchia M., Petrini A. Conte A., Caporale V. National Reference Centre Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo, Italy.

63- *Brucella melitensis* PERSISTENCE AND KINETICS OF THE IMMUNE RESPONSE IN EXPERIMENTALLY INFECTED EWES. PART II.

Tittarelli M., Di Ventura M., Scacchia M., De Massis F. Giovannini A., Caporale V. National Reference Centre Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo, Italy.

64- VIRULENCE OF *Brucella suis* BIOVAR 2 IN EXPERIMENTAL MODELS OF INFECTION.

David Fretin[1], S. Kohler[2], P. Michel[1], D. Desqueper[1], I. Danese[1], J. Godfroid[1]. (1) Veterinary and Agrochemical Research Center, Brussels, Belgium. (2) INSERM U-431, Université Montpellier 2, Montpellier, France.

65- PHAGOCYTOSIS AND INTRACELLULAR SURVIVAL OF *Brucella* IN MOCL3 OVINE MACROPHAGES.

M. P. Jiménez de Bagüés[1], M. Olivier[2], L. Guilloteau[2]. (1) Unidad de Sanidad Animal. Servicio de Investigación Agroalimentaria. Diputación General de Aragón. Ap 727. 50080 Zaragoza, Spain. (2) Laboratoire de Pathologie Infectieuse et Immunologie. INRA. 37380 Nouzilly. France.

66- ANALYSIS OF LONG TERM SURVIVAL OF *Brucella melitensis* IN OVINE CELLS MOCL3 AND EFFECT OF OPSONIZATION ON INFECTIVITY.

Paolo Pasquali[1], Rosanna Adone[1], Michel Olivier[2], Cinzia Marianelli[1], Claudia Pistoia[1], Paola Petrucci[1], Giacomo Marcon[1], Franco Ciuchini[1]. (1) Dipartimento di Sanità Alimentare ed Animale, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161, Rome, Italy. (2) Laboratoire de Pathologie Infectieuse et Immunologie. INRA. 37380 Nouzilly. France.

67- INTERACTION OF *Brucella abortus* WITH APOPTOTIC MECHANISMS.

Norman Rojas[1], Hazel Rojas[1], Monica Thelestam[2] and Edgardo Moreno[3]. (1) CIET, Facultad de Microbiología, Universidad de Costa Rica. (2) Microbiology and Tumor Biology Center, Karolinska Institute, Stockholm, Sweden. (3) PIET, Escuela de Medicina Veterinaria, Universidad Nacional, Costa Rica.

68- DOES OXYGEN TENSION MODULATE GENE EXPRESSION DURING GROWTH OF *Brucella* INSIDE ITS REPLICATIVE NICHE?.

S. Loisel, S. Köhler, J.-P. Liautard and V. Jubier-Maurin. INSERM U-431, Université Montpellier II, 34095 Montpellier, France.

69- INDUCTION OF ENHANCED CYTOTOXIC LYMPHOCYTE ACTIVITY BY *Brucella abortus* RB51 OVEREXPRESSING CU/ZN SUPEROXIDE DISMUTASE (SOD) AND LEAKING SOD.

Y. Hea[1], R. Vemulapalli[2], N. Sriranganathan[3], S. Boyle[3] and G. G. Schurig[3]. (1) Virginia Bioinformatics Institute, Virginia Tech, Virginia, USA. (2) Department of Veterinary Pathobiology, School of Veterinary Medicine, Purdue University, Indiana, USA. (3) Center for Molecular Medicine and Infectious Diseases, VA-MD Regional College of Veterinary Medicine, Virginia Tech, Virginia, USA.

70- DOES *Brucella* REPLICATE INSIDE THE AMOEBA *Acanthamoeba castellani*?.

C. Nlijskens, A. Tibor, RM. Delrue and J.J. Letesson. Unité de Recherche en Biologie Moléculaire (URBM), Laboratoire d'Immunologie et de Microbiologie, Facultés Universitaires Notre-Dame de la Paix, Namur, Belgique.

71- FLAGELLAR GENE HOMOLOGUES EXPRESSED IN *Brucella melitensis* 16M.

Brenda L. Soto and G. Splitter. University of Wisconsin, Madison, USA.

72- IMPLICATION OF FLAGELLAR GENES IN *Brucella* - HOST CELL INTERACTION.

C. Nlijskens, RM. Delrue, A. Tibor, S. Léonard, D. Fretin and J.J. Letesson. Unité de Recherche en Biologie Moléculaire (URBM), Laboratoire d'Immunologie et de Microbiologie, Facultés Universitaires Notre-Dame de la Paix, Namur, Belgique.

73- PURIFICATION, CHARACTERIZATION AND CLONING OF AN IMMUNOGENIC AMINOPEPTIDASE OF *Brucella melitensis*.

A. Contreras-Rodríguez[1], B. Ramírez-Zavala[1], A. López-Merino[1], A. Contreras[2], M. Seleem[2], N. Sriranganathan[2], and G. G. Schurig[2]. (1) Escuela Nacional de Ciencias Biológicas, I.P.N. MEXICO. (2) Center for Molecular Medicine and Infectious Diseases, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, Virginia 24061-0342, U.S.A.

74- IS A NEW POLYSACCHARIDE PRODUCED BY *Brucella melitensis* 16M?.

Delrue, R.M., Vanzenbergh, F. and J.J. Letesson. Unité de Recherche en Biologie Moléculaire (URBM), Laboratoire d'Immunologie et de Microbiologie, Facultés Universitaires Notre-Dame de la Paix, Namur, Belgique.

75- RHIZOPINE METABOLISM GENES ARE INVOLVED IN *Brucella* PATHOGENESIS.

P. Lestrate, A. Dricot, R-M. Delrue, V. Haine, A. Tibor, P. Michel, J. Godfroid, D. Billen, A. Krief, P. Murphy, X. De Bolle and J-J Letesson. Unité de Recherche en Biologie Moléculaire (URBM), Laboratoire d'Immunologie et de Microbiologie, Facultés Universitaires Notre-Dame de la Paix, Namur, Belgique.

76- THE AQUAPORIN GEN *aqp*X OF *Brucella abortus* IS INDUCED IN HYPEROSMOTIC CONDITIONS.

A. Seoane, M. C. Rodríguez, R. Hernández-Castro and J. M. García-Lobo. Departamento de Biología Molecular, Universidad de Cantabria. Spain.

77- DELETION OF THE *bac* A GENE FROM *Brucella abortus* INCREASES OR DECREASES VIRULENCE OF THE ORGANISM DEPENDING UPON THE GENETIC BACKGROUND OF THE HOST.

Parent, M.[1], R. Goenka[1], K. Levier[2], N. Carreiro[1], R.M. Roop II[3], G. Walker[2] and C.L. Baldwin[1]. (1) Department of Veterinary and Animal Sciences, University of Massachusetts, Amherst, MA, USA. (2) Department of Biology, Massachusetts Institute of Technology, Cambridge, MA, USA. (3) Department of Microbiology and Immunology, East Carolina State University School of Medicine, Greenville, NC. USA.

78- DhbR, A PUTATIVE AraC-LIKE TRANSCRIPTIONAL ACTIVATOR, REGULATES THE PRODUCTION OF THE SIDEROPHORE 2,3-DIHYDROXYBENZOIC ACID (2,3-DHBA) IN *Brucella abortus*.

E. S. Anderson[1], B. H. Bellaire[2] and R. M. Roop II[1]. (1) Department of Microbiology and Immunology, East Carolina University School of Medicine, Greenville, NC 27858, USA. (2) Department of Microbiology and Immunology, Louisiana State University Health Sciences Center, Shreveport, LA 71130. USA.

79- THE *rsh* GENE OF *Brucella melitensis* 16M IS ESSENTIAL FOR FULL VIRULENCE AND CONTROLS VirB EXPRESSION *in vitro*.

M. Dozot, R-M. Delrue,R. Hallez, J.J. Letesson and X. De Bolle. Unité de Recherche en Biologie Moléculaire (URBM), Laboratoire d'Immunologie et de Microbiologie, Facultés Universitaires Notre-Dame de la Paix, Namur, Belgique.

80- LIPOPOLYSACCHARIDE MODIFICATIONS IN *Brucella abortus* MUTATED IN THE TWO-COMPONENT REGULATORY SYSTEM BvrR/BvrS.

L. Manterola[1], I. Moriyón[1], E. Moreno[2], K. Brandenburg[3], and I. López-Goñi[1]. (1) Departamento de Microbiología, Universidad de Navarra, Pamplona, Spain. (2) PIET, Escuela de Medicina Veterinaria, Universidad Nacional, Costa Rica. (3) Forschungszentrum Borstel, Borstel, Germany.

81- EXPRESSION OF *Brucella abortus omp3a* AND *omp3b* UNDER DIFFERENT GROWTH CONDITIONS AND EFFECT OF *omp3a* OR *omp3b* DELETION ON OUTER MEMBRANE PROPERTIES AND VIRULENCE.

L. Manterola[1], C. Guzmán-Verrrr[2], M. J. Grilló[3], M. J. de Miguel[3], I. Moriyón[1], E. Chaves-Olarte[2], E. Moreno[2], and I. López-Goñi[1]. (1) Departamento de Microbiología, Universidad de Navarra, Pamplona, Spain. (2) PIET, Escuela de Medicina Veterinaria, Universidad Nacional, Costa Rica. (3) Unidad de Sanidad Animal, Servicio de Investigacion Agroalimentaria, Gobierno de Aragón, Zaragoza, Spain.

82- CLONING, EXPRESSION AND PURIFICATION OF *B. suis* OUTER MEMBRANE PROTEINS.

X. Ding[1], C. Paranavitana[1], M. Izadjoo[2] and D. Hoover[1]. (1) Department of Bacterial Diseases, Walter Read Army Institute of Reseach, Silver Spring, MD, USA. (2) Armed Forces Institute of Pathology, Washington, DC, USA.

83- HUMORAL AND CELLULAR IMMUNE RESPONSE TO *Brucella abortus* STRAINS RB51 AND S19 IN HEREFORD HEIFERS IN PATAGONIA REGION, ARGENTINA.

Robles, C.A.[1], Abalos, P.[2], Cabrera, R.[1], Petray, S.[1], Ibarra, L.[2]. (1) National Institute for Agricultural Technology – CC: 277 (8400) Bariloche, Argentina. (2) Facultad de Ciencias Veterinarias y Pecuarias. Universidad de Chile - Casilla 2, Correo 15, Santiago. Chile.

84- EFFICACY OF S-RB51 VACCINE IN ADULT CATTLE.

L. Samartino[1], M. Fort[2], S. Conde[1], E. Salustio[1]. (1) INTA. Centro de Ciencias Veterinarias, Buenos Aires, Argentina. (2) Estación Experimental Anguil, La Pampa, Argentina.

85- ATYPICAL POST-VACCINATION SEROLOGICAL RESPONSE IN COWS REVACCINATED WITH *Brucella abortus* RB51 IN ENDEMIC BRUCELLOSIS AREAS OF MÉXICO.

E. Díaz-Aparicio[1], B. Arellano-Reynoso[1], E. Herrera[1], M. Leal[2], and F. Suárez-Güemes[2]. (1) Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. México-Toluca Km. 15.5, Cuajimalpa, México, DF. 05110. México. (2) Facultad de Medicina Veterinaria y Zootecnia Universidad Nacional Autónoma de México. México.

86- PERFORMANCE OF THE *Brucella abortus* RB51 VACCINE IN HIGH PREVALENCE HERDS IN MÉXICO.

E. Herrera[1], E. Díaz-Aparicio[2], L.Hernández-Andrade[2], R. Pérez [2], F.Suárez-Güemes[1]. (1) Departamento de Microbiología e Inmunología, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, México D.F. 04510. México. (2) CENID Microbiología Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, México.

87- PROTECTION OF MICE AGAINST BRUCELLOSIS BY SIMULTANEOUS VACCINATION WITH *Brucella abortus wbkA* AND *bvrS* MUTANTS.

M. J. Grilló[1], L. Manterola[2], C. Marin[1], M. J. de Miguel[1], P. Muñoz[1], I. Moriyón[2], J. M. Blasco[1], and I. López-Goñi[2]. (1) Unidad de Sanidad Animal, Servicio de Investigación Agroalimentaria, Gobierno de Aragón, Zaragoza, Spain. (2) Departamento de Microbiología, Universidad de Navarra, Pamplona, Spain.

88- VIRULENCE AND VACCINE EFFICACY IN MICE OF LIPOPOLYSACCHARIDE MUTANTS OBTAINED FROM *Brucella melitensis* STRAINS 16M AND H38.

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89- COMPARISON OF *Brucella melitensis* R MUTANTS WITH INTACT OR DEFECTIVE LIPOPOLYSACCHARIDE CORE AS VACCINES IN BALB/c MICE.

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90- IMMUNE RESPONSE INDUCTION BY A NONVIABLE RECOMBINANT *B. abortus* RB51 STRAIN EXPOSED TO A MINIMUM DOSE OF GAMMA-IRRADIATION.

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91- DEVELOPMENT OF *Brucella melitensis* AS A VACCINE AGAINST BIOTERRORISM AGENTS.

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92- EVALUATION OF *B. abortus* M1luc AND I2 AS VACCINES AGAINST BOVINE BRUCELOSIS.

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93- EVALUATION OF NOVEL *Brucella abortus* AND *Brucella melitensis* DELETION MUTANTS AS POTENTIAL VACCINE CANDIDATES IN THE MOUSE AND GOAT MODELS OF BRUCELLOSIS.

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94- NEW SYSTEM TO ENCAPSULATE ANTIGENIC EXTRACTS FROM *Brucella ovis* FOR VACCINAL PURPOSES.

M. Estevan[1], C. Gamazo[1], M. J. Grilló[2], J. M. Blasco[2], C. M. Marín[2], G. García del Barrio[3] and J. M. Irache[3]. (1) Departamento de Microbiología, Universidad de Navarra, Pamplona, Spain. (2) Unidad de Sanidad Animal, SIA-DGA, Zaragoza, Spain. (3) Centro Galénico, Universidad de Navarra, Pamplona, Spain.

95- THE CONSTRUCTION AND *in vitro* EVALUATION OF DNA VACCINES AND CANDIDATE ANTIGENS FOR PROTECTION AGAINST BRUCELLOSIS.
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96- THE DEVELOPMENT AND ASSESSMENT OF 5 NOVEL DNA BASED VACCINES FOR PROTECTION AGAINST *Brucella* spp.
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97- IMMUNOGENICITY AND PROTECTIVE EFFICACY OF 5 NOVEL DNA VACCINES IN THE BALB/c MOUSE.
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98- THE SELECTION OF POTENTIAL PROTECTIVE ANTIGENS FOR DEVELOPMENT OF SUB-UNIT VACCINES AGAINST BRUCELLOSIS.
Nicky Commander, Pauline Groussaud, James Tucker. Veterinary Laboratories Agency, Woodham Lane, New Haw, Addlestone, Surrey, KT15 3NB, UK.

99- THE EFFECTS OF VARIOUS FACTORS ON THE VIABILITY OF *Brucella abortus* STRAIN 19 VACCINE.
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100- VARIATIONS IN THE LYSIS OF *Brucella melitensis* Rev 1 BY BACTERIOPHAGES.
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101- THE INOCULATION OF MICE WITH VIRULENT OR ATTENUATED *Brucellae* OR SMOOTH LIPOPOLYSACCHARIDE DOES NOT MODIFY THE COURSE OF SPLENIC INFECTIONS BY HOMOLOGOUS OR HETEROLOGOUS *Brucella* spp.
Grilló M. J.[1], M. J. de Miguel[1], P. M. Muñoz[1], C. M. Marín[1], M. Barberán[2], J. M. Blasco[1]. (1) Unidad de Sanidad Animal, Servicio de Investigación Agroalimentaria, Gobierno de Aragón, Zaragoza, Spain. (2) Departamento de Patología Animal, Universidad de Zaragoza, Zaragoza, Spain.

102- TAXONOMY AND NOMENCLATURE OF THE MEMBERS OF GENUS *Brucella*; A PROPOSED RE-EVALUATION OF THE TAXONOMIC RANK OF RESPECTIVE MEMBERS.
B. S. Osterman. Dept Clinical Microbiology, Karolinska Hospital, Stockholm, Sweden.

103- CONTRIBUTION TO THE CLASSIFICATION OF *Brucella* SPECIES BASED ON TANDEM REPEAT TYPING.
I. Jacques[1,2], M. Grayon[1], P. Le Flèche[3,4], G. Vergnaud[3,4], and L. Guilloteau[1]. (1) Unité de Pathologie Infectieuse et Immunologie, Institut National de la Recherche Agronomique, 37380 Nouzilly, France. (2) Institut Universitaire de Technologie, 29 rue du Pont Volant, 37083 Tours Cedex, France. (3) Centre d'Etudes du Bouchet, BP3, 91710 Vert le Petit, France. (4) Génomes, Polymorphisme et Minisatellites, Institut de Génétique et Microbiologie, Bat 400, Université Paris XI, 91405 Orsay Cedex, France.

104- IDENTIFICATION OF SPECIES OF *Brucella* USING FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR).
M. A. Miguel Gómez[1], M. A. Bratos Pérez[1], F. J. Martín Gil[2], A. Dueñas Díez[1], J. F. Martín Rodríguez[3], P. Gutiérrez Rodríguez[1], A. Orduña Domingo[1], A. Rodríguez Torres[1]. (1) Departamento de Microbiología, Hospital Clínico Universitario, Facultad de Medicina. Valladolid, Spain. (2) Servicio de Análisis Clínicos. Hospital Universitario Río Hortega, Spain. (3) Departamento de Estadística, Facultad de Medicina, Valladolid, Spain

105- AFLP: A TOOL FOR THE IDENTIFICATION AND TYPING OF *Brucella* ISOLATES?.
Adrian M. Whatmore, Terry Murphy, Sally J. Cutler, and Alastair P. Macmillan. Department of Statutory and Exotic Bacterial Diseases, Veterinary Laboratories Agency, Addlestone, Surrey, KT15 3NB, United Kingdom.

106- OXIDATIVE METABOLIC PROFILES OF *Brucella* STRAINS ISOLATED FROM MARINE MAMMALS. I. Jacques[1,2], M. Grayon[1], J.-M. Verger[1], and L. Guilloteau[1]. (1) Unité de Pathologie Infectieuse et Immunologie, Institut National de la Recherche Agronomique, 37380 Nouzilly, France. (2) Institut Universitaire de Technologie, 29 rue du Pont Volant, 37083 Tours cedex, France.

107- MAPPING OF THE PROTEINS OF *Brucella abortus* AND CROSS-REACTING BACTERIA USING TWO-DIMENSIONAL (2-D) ELECTROPHORESIS AND SELDI (SURFACE-ENHANCED LASER DESORPTION/IONIZATION) PROTEIN CHIP TECHNOLOGY .

Al Dahouk S[1], Tomaso H.[1], Bogumil R.[2], Bartling C.[1], Neubauer H.[1] (1) Institute of Microbiology, Federal Armed Forces, Munich, Germany. (2) Ciphergen Biosystems GmbH, Göttingen, Germany.

108- IDENTIFICATION OF IMMUNOGENIC PROTEINS IN THE PROTEOME OF *Brucella melitensis* AND *Brucella abortus* USING TWO-DIMENSIONAL ELECTROPHORESIS AND IMMUNOBLOTTING.

Al Dahouk S.[1], Tomaso H.[1], Bartling C.[1], Neubauer H.[1] (1) Institute of Microbiology, Federal Armed Forces, Munich, Germany.

109- COMPARATIVE PROTEOMIC ANALYSIS OF *Brucella suis* BIOVARS 1 AND 2 USING TWO-DIMENSIONAL GEL ELECTROPHORESIS AND MASS SPECTROMETRY.

M.S. Zygmunt , C. Boursier and F. Carreras. Unité de Pathologie Infectieuse et Immunologie, Institut National de la Recherche Agronomique, F-37380, Nouzilly, France.

110- IN SILICO ANALYSIS OF THE CtrA REGULON IN ALPHA PROTEOBACTERIA.

R.HALLEZ, A. F. BELLEFONTAINE, C. LAMBERT, J-J. LETESSON, J. VANDENHAUTE AND X. DE BOLLE. Unité de Recherche en Biologie Moléculaire (URBM), Laboratoire de Microbiologie et de Génétique Moléculaire, Facultés Universitaires Notre-Dame de la Paix, B-5000 Namur, Belgium.

111- ROLE OF THE ALTERNATIVE SIGMA FACTORS IN *Brucella melitensis* 16M VIRULENCE.

M. Delory, V. Haine, R-M. Delrue, T. Laurent, A. Tibor, C. Lambert, J.J. Letesson and X. De Bolle. Unité de Recherche en Biologie Moléculaire (U.R.B.M.), laboratoire d'Immunologie et de Microbiologie, Facultés Universitaires Notre-Dame de la Paix, Namur, Belgique.

112- IDENTIFICATION AND CHARACTERIZATION OF THE BRUCEBACTIN TRANSPORT SYSTEM.

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113- CHARACTERISATION OF *rpoB* MUTATIONS ASSOCIATED WITH THE RIFAMPIN-RESISTANT PHENOTYPE IN *Brucella* spp.

Cinzia Marianelli, Paolo Pasquali, Franco Ciuchini, Massimiliano Francia, Geraldina Riccardi and Rosanna Adone. Dipartimento di Sanità Alimentare ed Animale, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161, Rome, Italy.

114- EVIDENCE OF IS*711* TRANSPOSITION IN *B. ovis* AND *B. maris*.

Alain Ocampo Sosa and Juan M. García-Lobo. Laboratorio de Microbiología. Departamento de Biología Molecular. Universidad de Cantabria. Spain.

115- PHYLOGENETIC ANALYSIS OF THE GENUS *Brucella* WITH PARTICULAR REFERENCE TO MARINE MAMMAL ISOLATES.

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116- THE *Brucella melitensis* FLAGELLAR GENES ARE NOT CRYPTIC.

D. Fretin, S. Leonard, P. Lestrate, R.-M. Delrue, J.-J. Letesson and A. Tibor. URBM, Facultés Universitaires Notre-Dame de la Paix, rue de Bruxelles 61, B-5000 Namur, Belgium.

117- *Brucella* LIPIDS: CHARACTERIZATION OF *pmtA*, A GENE ENCODING A PHOSPHATIDYL-ETAHNOLAMINE-N-METHYLTRANSFERASE INVOLVED IN PHOSPHATIDYL-CHOLINE SYNTHESIS.

R. Conde-Álvarez , I. Moriyón and M. Iriarte. Departamento de Microbiología, Universidad de Navarra, Pamplona, Spain.

118- EXPRESSION OF THE ExoS PROTEIN FROM *Sinorhizobium meliloti* COMPLEMENTS BIOLOGICAL PROPERTIES OF A *Brucella abortus* MUTANT LACKING THE BvrS PROTEIN.

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Abstracts

AN OVERVIEW OF THE EPIDEMIOLOGY OF BRUCELLOSIS IN SELECTED COUNTRIES.

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Brucellosis is still a major disease problem in the Mediterranean region, western Asia, parts of Africa and Latin America. The Animal Production and Health Division of the Food and Agriculture Organization (FAO) of the United Nations, (UN) has primary responsibility for providing science based support to member countries to promote animal health and productivity. The Division recognizes the need for integration of human and animal health activities where zoonotic diseases affect human health and also livestock productivity and economics. Of particular importance is brucellosis, the zoonotic significance of which requires intersectoral collaboration in its control and subsequent eradication where feasible. FAO recognizes that for effective control of brucellosis to be achieved, this must be based on firm understanding of the epidemiological factors at play in these countries. To achieve this implies the effective use of disease intelligence and surveillance, support for institutional strengthening of veterinary services, international co-ordination and collaboration, the development of sound strategies and policies for animal disease management and facilitating the sustainable and productive use of animal and other natural resources in an integrated manner. FAO over the years has provided or facilitated the provision of technical advice and in some cases, logistic support to the control of brucellosis in particular regions/countries of the world which have recognized brucellosis as a disease of major economic and zoonotic significance and have therefore sought the Organization's support in its control. This presentation will discuss brucellosis particularly its epidemiological factors, socio-cultural determinants and livestock management systems encountered in participating countries of the Regional Animal Disease Surveillance and Control Network for North Africa, the Middle East and the Arab Peninsula (RADISCON) project, in Kosovo (Balkans), Tajikistan and Mongolia (Eurasia), and selected countries of sub-Saharan Africa.

EPIDEMIOLOGY OF BRUCELLOSIS: CONSEQUENCES IN TERMS OF CONTROL STRATEGY.

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Brucellosis is an important zoonotic disease widely distributed in both humans and animals throughout the world except for the growing number of countries where eradication in has been achieved (*W. Amanfu, this symposium*). The infection has been identified in most domestic animal species, primarily ruminants, camelids and swine but also horses and carnivores, and in several wild animal species (*D. Ewalt, this Symposium*). The disease is responsible for economic losses due to abortions, infertility and drop in milk production. A host specificity is commonly accepted (*B. abortus* in large ruminants, *B. melitensis* in small ruminants, *B. suis* in swine) but there are a lot of exceptions, *B. melitensis* in cattle being the most common. *B. melitensis* and the biovars 1 and 3 of *B. suis* are the more pathogenic for humans

while *B. ovis* and *B. suis* biovar 2 are generally considered as not. The major route of infection is through the mucous membranes of oropharynx, respiratory and digestive tracts, conjunctiva and genitalia. Vaginal excretion is the most important mean of *Brucella* spreading in the environment and the main source of infection of animals and professionally exposed humans. Shedding is also common in udder secretions and semen, which also contribute to the dissemination of the disease through oral or genital routes. *Brucella* ability to resist outside its mammalian host is relatively high and, under suitable conditions, they may retain infectivity during long periods in many materials, potential vehicles of infection.

The risk that a susceptible animal will be exposed to infection depends largely on the husbandry practices. Factors contributing to this risk include, for inter-herd transmission, primarily the movements of animals (replacement, transhumance) and the proximity to infected herds; for intra-herd transmission, the vaccination level, the herd size, the population density and the method of housing. Contamination from a wildlife source should be also considered (*B. suis* 2 in European pigs, *B. abortus* due to wild ruminants in North America). All these aspects should be taken in account for designing the most appropriate strategy of control and/or eradication in a specific area, finding the adequate diagnostic and vaccination strategy being frequently the minor constraint.

BRUCELLOSIS: FACT AND FANTASIES.

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"No disease, excepting syphilis and tuberculosis, is more protean in its manifestations"

MW Simpson, 1940

Brucellosis continues to fascinate scientists in disciplines from taxonomy to molecular genetics. It also challenges veterinarians and physicians faced with its myriad clinical presentations. A number of myths have grown up around the disease, some based on previously held beliefs that go unchallenged, others based on an incomplete understanding of its pathogenesis. Among them is the idea that brucellosis always becomes chronic, and, as a result is untreatable. Another is the postulate that chronic brucellosis is caused by immunosuppression and the only effective treatment must include immune stimulation. We will examine these concepts, review the relevant data, and compare some aspects of brucellosis with tuberculosis and syphilis.

DISEASE SPECTRUM AND LABORATORY DIAGNOSIS OF HUMAN BRUCELLOSIS.

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The diagnosis of human brucellosis remains a clinical challenge especially to the unaware since its presentation can affect any body organ or system. The protean clinical manifestations and multisystem involvement, mimicking other infectious and non-infectious diseases, merited labelling brucellosis as the “disease of mistakes”.

Routine biochemical and haematological laboratory tests show variable and overlapping results with many other diseases. The *Brucella*-specific tests are the mainstay in laboratory diagnosis of brucellosis. These include culture, slide or tube agglutination, indirect Coombs', ELISA, IFA, and molecular techniques e.g. PCR. Different types of antigen preparations have been utilized in detecting Ig classes and subclasses seeking reliable diagnostic and prognostic assays and markers. ELISA proved to be the test of choice especially in complicated and chronic cases. So far, the key approach in determining prognosis and relapse is a sequential follow-up of patients using serological tests. Discrimination between active and inactive stages can be very difficult, however, the use of certain antigens e.g anti *Brucella* cytoplasmic or membranous protein antibodies in ELISA seems useful. Molecular tests were shown to have a promising role in diagnosis, however, their potential value in patient follow-up and in distinguishing among different disease forms, remains to be determined. Knowledge about the advantages, disadvantages and limitations of these tests are essential for the proper interpretation of results, in relation to the history and clinical presentation of patients under investigation.

DEVELOPMENT AND VALIDATION OF DIAGNOSTIC TOOLS AND TYPING METHODS FOR THE CERTIFICATION OF THE ABSENCE OF BOVINE BRUCELLOSIS IN BELGIUM.

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In the beginning of the nineties, the prevalence rate of bovine brucellosis was very low in Belgium, although bovine brucellosis was not eradicated. This epidemiological situation allowed us to describe a new phenomenon: the emergence of "False Positive Serological Reactions" (FPSR) in bovine brucellosis screening tests. The most likely hypothesis to explain this phenomenon (but maybe not the only one) is a *Yersinia enterocolitica* O:9 infection. In parallel, we described for the first time, a *Brucella suis* biotype 2 enzootic brucellosis in wild boars (*Sus scrofa*) in Western Europe.

Therefore, new diagnostic tools had to be developed in order to differentiate beyond any doubt cattle infected by *Brucella abortus*, *Brucella suis* or *Yersinia enterocolitica* O:9. Our work allowed us to validate, under experimental conditions, the brucellosis skin test and the IFN-γ assay as bovine brucellosis diagnostic tools. A testing strategy integrating these new tools has been successfully implemented in the field in Belgium. This work has led to the incorporation of the brucellosis skin test in the new Directive 432/64/CEE that deals with living animals movements within the European Union.

Recently, our knowledge of the ecological niche of *Brucella* has been extended to marine mammals. It has been shown that these *Brucella* represent a new potential source of infection for both domestic animals and humans. Studies on the molecular typing of marine mammal *Brucella* reinforce the proposal of adding two new species in the genus *Brucella*: *Brucella cetaceae* (infection in cetacean) and *Brucella pinnipediae* (infection in pinnipeds).

BRUCELLOSIS IN WILDLIFE.

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The study of brucellosis in wildlife has become more important as the prevalence of the disease decreases in domestic animals. Numerous serological studies and bacteriological studies to detect *Brucella* antibodies and infection in a variety of species have been reported.

A major project involving the American bison located in the Greater Yellowstone National Park Area has provided valuable information concerning the pathogenesis and epidemiology of brucellosis in this population. Another problem area in the western United States is the elk that are fed at the National Elk Refuge in Wyoming. These animals are vaccinated with *B. abortus* strain 19 and the prevalence in the herd is monitored serologically from hunter killed animals.

Researchers in Alaska, Canada, and Siberia have been studying brucellosis in caribou and reindeer. These two animal species are infected with *B. suis* biovar 4 and pose a human health problem to the people raising and hunting them.

Feral swine remain a major source of infection. In the United States, the feral swine are located in 26 states and exceed 2 million animals. Several instances of the spread of *B. suis* from feral swine to domestic swine, cattle, and dogs have been documented. Numerous bacteriological and serological studies have been conducted to evaluate brucellosis in feral swine.

Since 1994, the prevalence of brucellosis in marine mammals has been studied. Several serological and bacteriological studies have been conducted. Current research is focused on studying the characteristics of the *Brucella* organism, identifying marine mammal species that are infected with *Brucella*, and determining the serological prevalence of the disease. *Brucella* has been isolated from a wide variety of animals including seals, whales, dolphins and sea otters.

Brucella LPS A KEY IMMUNOMODULATOR OF IMMUNE RESPONSES IN MICE.

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The properties of lipopolysaccharides (LPS) from intracellular Proteobacteria such as *Brucella, Chlamydia*, *Legionella*, *Rickettsia*, and even from plant endosymbionts such as *Rhizobium* reveal distinctive features that depart from enterobacterial LPSs. Remarkably, low endotoxicity, deficient induction of host cell activation and resistance to macrophage degradation of these LPSs are seen as a virulence mechanism, which is particularly useful for an intracellular parasitic life style. We have previously demonstrated that the low endotoxic *B. abortus* LPS captured by macrophages was recycled back to the plasma membrane where it was found associated with macrodomains. Here we show that LPS macrodomains behave as lipid mega rafts, segregating several raft components, LPS-binding proteins and the bulk of MHC class II molecules. These LPS macrodomains remain for several months in macrophages, are resistant to the disruptive effects of β-cyclodextrin on lipid rafts and hamper the presentation of peptides to specific T cells. In addition, the LPS from *Brucella abortus* triggers $CD4^+$ $CD25^+$ T lymphocytes with a phenotype compatible with activated/memory T cells recognizing MHC-II-LPS macrodomains on the surface of antigen presenting cells (APCs). LPS-specific T cell

recognition depends on the O-polysaccharide moiety and on MHC-II but not MHC-I molecules as demonstrated by the use of inhibitory antibodies as well as MHC-II and MHC-I deficient mice. LPS T cell recognition is restricted to the same MHC-II as the APCs. The low frequency of LPS-reactive T cells as compared to mitogen reactive T cells is consistent with a specific LPS T cell recognition. This important finding highlights a new role of low endotoxic LPS as immunomodulator of the immune response. Therefore, LPS-cell membrane complexes may be important entities involved in the pathogenesis and in the control of immune response during infection.

A STEALTHY INTRACELLULAR BUG NAMED *Brucella*: ASPECTS OF INTERACTION OF THE PATHOGEN WITH THE MACROPHAGE HOST CELL.

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The macrophage is the major host cell of the intracellular pathogen *Brucella*. Intramacrophagic multiplication depends on a certain number of factors that are essential, various classes of mutants therefore yield typical growth curves. Under nonopsonic conditions, brucellae enter the macrophage via lipid rafts located in the cytoplasmic membrane. This mode of entry is limited to smooth bacteria possessing an intact LPS O side chain. The two-component system BvrR/BvrS, affecting the outer membrane composition, is also involved in phagocytosis. In the following step, the rapid acidification of the early phagocytic vacuole by a vacuolar ATPase is crucial for survival of *Brucella*, as pH neutralization results in complete eradication of the bacteria. In this obviously harsh environment, *Brucella* proteins involved in stress response such as DnaK, and later Lon and HF-1, are essential. Another major characteristic event in macrophage infection by brucellae is the inhibition of phagosome-lysosome fusion, due to the impairment of recognition between the two compartments. The LPS O antigen is involved in this phenomenon, and only phagosomes containing rough *Brucella* fuse rapidly with lysosomes. The inhibition of phagosome-lysosome fusion enables the bacteria to activate specific virulence genes such as *virB*, triggered by the acidic environment and nutrient limitation. The type IV secretion system VirB participates in the establishment of the final replicative niche, as the corresponding mutants are rapidly eliminated from the infected macrophages. Once in its final niche, *Brucella* has to adapt to the environment encountered in this compartment, and a certain number of bacterial genes specifically induced within the macrophage have been evidenced. In parallel, several approaches by signature-tagged transposon mutagenesis and, more recently, by large-scale Tn5 mutagenesis yielded a more profound insight into the conditions the intracellular pathogen has to face: a nutrient-poor compartment devoid of amino acids and nucleic acid bases, characterized by an acidic pH in the early phase of infection, and by low oxygen tension. The set of genes that are essential for intracellular multiplication of brucellae has been termed "virulome". Furthermore, in the course of infection, intracellularly multiplying brucellae prevent apoptosis of macrophages by interference with the IFN-gamma apoptotic pathway. In addition to specific adaptation to the intracellular environment by formation of the replicative niche we termed "brucellosome", this behavior may be part of a general strategy of *Brucella* favoring its development within the macrophage host cell.

CLASSICAL AND NEW GENERATION VACCINES AGAINST BRUCELLOSIS IN UNGULATES.

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Worldwide there are five *Brucella* vaccines for use in animal populations: *B. abortus* 45/20, *B. melitensis* Rev-1, *B. suis* strain 2, *B. abortus* strains 19, and RB51. The killed vaccine strain 45/20 did not consistently protect against virulent challenge and also caused some abortions. *B. suis* strain 2 has not be evaluated extensively and can cause vaccinal titers. Rev-1 provides protection in sheep and goats against brucellosis; however, it can cause abortions and titers. Strain 19 protects cattle against brucellosis but may also cause abortions and titers; and it is not effective in wildlife species, primarily bison and elk.

Currently *B. abortus* strain RB51 is the only vaccine that does not cause vaccinal titers. RB51 appears to be safe in the majority of ungulates and non-target species tested in that it does not cause abortion. RB51 does cause abortions in pregnant reindeer, and it is not efficacious against virulent challenge in elk and bison. With no vaccines to protect wildlife, primarily bison, elk and feral swine, it is imperative that new vaccine candidates be evaluated.

New vaccine candidates for use in wildlife and domestic animals are being evaluated. OMP25 gene deletion mutants in *B. abortus*, *B. melitensis*, and *B. ovis* have been tested in cattle, goats and sheep and did not demonstrate any detrimental effects. Vaccinal titers can be distinguished from field strain titers using these vaccine strains. The *B. melitensis* mutant does not cause abortions and protects goats against virulent challenge. VTRS-1 is a rough derivative of virulent *B. suis*, and vaccination of swine with this strain does not cause vaccinal titers or abortion and provides sows protection against virulent challenge.

ALTERNATIVE BRUCELLOSIS VACCINES: EXPERIENCES WITH DRUG DELIVERY SYSTEMS.

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New generation avirulent vaccines are likely to be less immunogenic than traditional attenuated vaccines. Therefore, they require appropriate adjuvants. Vaccine delivery systems, such as emulsions, microparticles, ISCOMs and liposomes, are a new class of adjuvants that mainly function to target associated antigens into antigen presenting cells. Biodegradable microspheres are spherical polymeric particles which contain a drug dispersed throughout the matrix. The most widely used polymers for biodegradable microspheres are aliphatic polyesters made of lactide (PLA) and glycolide (PLGA). Biodegradable microspheres are useful to prolong and control the release of certain drugs and to target drugs to specific infection sites. PEC Microparticles – Hydrophobicity - *In vitro* stability - Do not acidify - Excellent tissue compatibility - Low cost. This review will focus on recent developments in vaccine delivery systems for immunoprophylaxis of brucellosis.

Currently in use vaccines against *Brucella* spp. display some problems related with its residual virulence. Therefore, development of fully avirulent vaccines is justified with the subcellular vaccines representing one of the most interesting approaches. An antigenic extract of *Brucella ovis* (HS), enriched in lipopolysaccharide, phospholipids and outer membrane proteins, was encapsulated

in poly-ε-caprolactone microparticles (HS-PEC) by the solvent evaporation method, as a vaccine delivery system for brucellosis. The resulting microparticles displayed sub-5 μm sizes. SDS-PAGE and immunoblotting of the extracted antigenic complex confirmed that the apparent molecular weight and antigenicity remained unaltered after the encapsulation procedure. The *in vitro* release profile of HS from HS-PEC microparticles appeared to be pulsatil. These microparticles were injected subcutaneously in BALB/c mice in order to observe the protection conferred against experimental infection with the virulent strains *B. melitensis* H38, *B. abortus* 2308 or *B. ovis* PA. The results showed that administration of HS-PEC microparticles gave high amounts of IFN-γ and IL-2 but low quantities of IL-4, and protected mice against any of the challenge strains used. Such protection was similar to that provided by the reference living attenuated *B. melitensis* Rev 1 vaccine. In a recent experiment, a single dose of HS-PEC (equivalent to 0.8 mg of HS) was able to protect rams challenged with *B. ovis*, the statistical level of significancy was not different to Rev1. Additional research must be performed with higher doses to establish the protective value of this innocuous rough subcellular vaccine.

PHYLOGENY AND EVOLUTION OF ALPHA-PROTEOBACTERIAL GENOMES.

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Members of the alpha-proteobacteria include pathogens of domestic animals like *Brucella*, while others are pathogens of humans causing diseases such as typhus, trench fever and cat scratch disease. Yet other species have evolved elaborate interactions with plants. The recent sequencing of a dozen alpha-proteobacterial genomes, including our own completed genomes of *Rickettsia prowazekii*, *Bartonella quintana*, the agent of trench fever and *Bartonella henselae*, the agent of cat-scratch disease, enables a global genomic comparison of human, animal and plant-associated bacteria. Here, we present the phylogenetic relationships of the alpha-proteobacteria for which complete genome sequence data is available and discuss genomic features that are shared between human, animal and plant pathogens. We identify differences in gene numbers, genomic contents and architectures that correlate with major lifestyle changes. We show that extreme genome size expansions of a few thousand genes have accompanied the evolution of the plant-associated bacteria. In contrast, eliminations of a few thousand genes are characteristic of shifts to intracellular animal environments and vector-mediated transmission pathways. We conclude that lifestyle characteristics and exposure or lack of phage attacks have influenced the genomic evolution of bacteria that have developed close interactions with plants and animals.

COMPARISON OF THE GENOMIC SEQUENCES OF *Brucella melitensis*, *Brucella suis*, AND *Brucella abortus* BIOVARS: STRUCTURE AND PSEUDOGENES.

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The whole genomic sequences of three classical species of Brucellae, *Brucella melitensis*, *Brucella suis* and *Brucella abortus* have been determined, and the sequence of *Brucella ovis* is nearly finished. These species preferentially infect goats, swine, bovine, and sheep, respectively. Comparative genomics may aid in answering questions related to species specificity, virulence, pathogenicity, as well as phenotypic differences observed amongst the Brucellae. Genomic sequences of *B. melitensis*, *B. suis* and *B. abortus*, excluding insertion/deletions (indels), are more than 99% similar to each other. There are only 7,301 SNPs between *B. suis* and *B. melitensis* among 3.2 Mbp. The SNPs are more or less spread randomly through out the chromosome but some clustering was noted. Close taxonomic relationship of Brucellae to the plant symbiont *Sinorhizobium meliloti* is supported by the extensive gene synteny between their genomic sequences, especially the large chromosome of Brucellae.

Determination of the genomic sequences of Brucellae confirmed the presence of two circular chromosomes in *B melitensis, B suis*, and *B. abortus*. One chromosome is about twice the size of the other. The largest chromosome is 2.1 Mbp and has a classical bacterial type origin of replication. The smaller chromosome is 1.2 Mbp and has a plasmid based origin of replication. The pulsed field map of the small chromosome of *B. abortus* 544 biovar 1 has a large inversion relative to others. This inversion was found in the *B. abortus* 9-941 genomic sequence and, by PCR, in *B. abortus* biovars, 2 and 4 but not *B. abortus* biovars 3, 5, 6, and 9.

There are two large regions encoding phage-associated proteins found among the Brucellae genomic sequences. One is in *B. suis* and the other in *B. melitensis.* The region in *B. melitensis* was also found in *B. abortus*. All three sequences have the large putative transposable element, Tn*1953*, encoding a large number of sugar and amino acid transport proteins. Some of these are polymorphic among the classical species. The genomic sequence of *B. abortus* is more similar to that of *B. melitensis* than *B suis*. However, the genomic sequence of *B. abortus* has several large insertion/deletions (indels) that are in the genomic sequence of *B. suis* but not *B. melitensis*.

Differences in genomic sequences of Brucellae were exploited to develop PCR-based diagnostic tests long before any of the genomes were sequenced. Currently used tests have varying ability to distinguish the classical species and biovars. Last year at the Brucellosis Research Conference, Bricker described variable nucleotide tandem repeats (VNTR) in Brucellae and established their potential as a strain typing epidemiological tool for source trace back in outbreaks of infection. A comparison of genomic sequences of the Brucellae lead to the identification of other loci that have the potential to be used for epidemiological trace back and biovar identification.

Pseudogenes were identified among the Brucellae, but any one of them was rarely found in all three Brucellae genomic sequences. Pseudogenes were often the result of premature stops caused by SNPs or frame shifts in regions where there were strings of identical bases or small deletions.

PROTEOMICS AND HOST-PATHOGEN INTERACTION: NEW PARADIGMS

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Phagocytosis, the process by which cells internalize large particles, plays key roles in several biological functions including embryogenesis, tissue remodeling and our innate ability to fight infection by intracellular pathogens. The aim of phagocytosis is to kill and degrade, within phagosomes, the internalized materials for further use of their molecular constituents and, in the case of our immune system, for processing and presentation of exogenous antigens at the cell surface. Surprising features of phagocytosis and phagolysosome biogenesis, the transformation of phagosomes into functional degradative compartments, have been revealed by the systematic analysis of these organelles using a proteomics approach. The phagosome, which was believed to be a simple organelle often molecularly described by using a handful of well known protein markers, turned out to be a rather complex entity made of hundreds, if not thousands, of proteins. Its representation, as a compartment made of an homogenous lipidic membrane with randomly distributed proteins, was modified to include specialized membrane microdomains displaying specific sets of lipids and proteins. The fundamental concept that phagocytosis proceeded by invagination of the plasma membrane had to be reconsidered to propose a model according to which the endoplasmic reticulum is recruited to the cell surface, where it fuses with the plasma membrane and contributes to phagosome formation. The association of ER proteins with phagosomes confers new functional properties, among which the ability to process exogenous antigens for presentation by both MHC class I and II molecules. Indeed, phagosomes were shown to be able to translocate peptides from their lumen to the cytoplasmic side of the organelles where the degradative ubiquitin/proteasome machinery associates. Upon processing and translocation back to the phagosome lumen, exogenous peptides are loaded onto MHC class I molecules to form immune complexes which are presented at the cell surface and able to trigger a $CD8^+$ T cell response. These results indicate that the ER and endocytic mixed features of phagosomes enable to link the natural ability of macrophages to kill microorganisms with the elicitation of an efficient adaptive immune response.

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FROM GENOME SEQUENCES BACK TO (SYSTEMS) BIOLOGY: USING THE *C. elegans* V1.1 ORFEOME FOR FUNCTIONAL PROTEOMICS.

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The availability of complete genome sequences suggests new approaches for biological research to complement conventional genetics and biochemistry. In this context, our goals are to functionally characterize a proteome. To achieve that goal, we are generating comprehensive protein-protein interaction, or interactome, mapping strategies for *C. elegans* and developing new concepts to integrate the resulting interactome data with other functional maps such as expression profiles (transcriptome), global phenotypic analysis (phenome), localization of expression

projects (localizome), etc. Such integrated maps should be valuable for a systems biology approach to development. *C. elegans* is a particularly useful model organism because of the current availability of a complete genome sequence coupled with the fact that the major signalling pathways (i.e., Ras, Akt, Rb, p53, and DNA damage response) are conserved in the worm. More importantly, novel interactions identified among worm proteins can be validated through forward and reverse genetics approaches to suggest function for novel, and in many cases, previously unknown genes. The starting point for our work is the creation and use of the *C. elegans* ORFeome, a physical collection of all expressed, protein-encoding genes cloned as full length open reading frames (ORFs). An immediate consequence of the ORFeome is that we are able to both verify the genome annotation and create a resource to functionally characterize the proteome. Approximately ~12,000 ORFs have been successfully cloned (ORFeome 1.1), of which ~4,000 correspond to genes that have remained untouched by any cDNA or expressed sequence tag (EST). Unexpectedly, more than 50% of predicted genes needed corrections in their intron-exon structures. Importantly, ~11,000 *C. elegans* proteins can now be expressed under many conditions and characterized using various high-throughput strategies, including large-scale interactome mapping. Coupled with other data, it appears that at least 85% of the ~21,000 predicted *C. elegans* protein-encoding genes are genuinely expressed. We suggest that similar ORFeome projects will be valuable for other organisms. A nearly complete set of full length ORFs in multiple expression vectors will facilitate whole-genome approaches to systems biology. Complementing the ORFeome effort, we have recently initiated the generation of a similar resource of all predicted promoters of *C. elegans*.

EO1- BRUCELLOSIS ERRADICATION PROGRAM IN URUGUAY.

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In 1926 bovine Brucellosis was diagnosed in tuhis country for the first time. Since then, different actions have been taken in order to diminish the prevalence of this disease. We went throug voluntary stages and obligatory ones, in wiche different tool available were used. In 1998 after a control stage that lasted thirty four years there started an Erradicacition Program using different procedures, depending on the type of cattle (milk producers or beef). With this Program development and surveillance increase, some problems have been detected. Today, the Official Veterinary Service, independents veterinarians and cattle breeders are devoted to solving these problems. This work develops the different stages of this Program dealing with what we consider achievements and errors, as wells. We believe this analysis to be important contribution to other countries that are also working on this desease.

EO2- BOVINE BRUCELLOSIS CONTROL PROGRAM IN MÉXICO: PRESENT SITUATION AND FACTORS THAT LIMITS ITS ADVANCE.

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Bovine Brucellosis control in Mexico has been performed since the second half of the 20th sentury, however it was until 1995 when the first official regulation was published, as a NORMA OFICIAL MEXICANA, for the control of the disease.The official proeradication program describes the authotized diagnostic tests, as well as the recommendations for the use of vaccines. During several years only strain 19 Vaccines were used, for calfs immunization. Later, the reduced dosis of this vaccine was included en the program and in the last 8 years RB 51 vaccines are officially used for the immunization of calf and cattle.

Despite the fact that we have all elements needed for a succesfull program, the advances are not as expected. From 1965 to 2001, the number of animals tested for brucellosis diagnostic were 14,351,061; from them 110,223 were identified as reactors (0.77%). In the year 2001, the number of tested animals was 2,372,609, with 13,975 reactors (0.59%). In contrast, the number of doses of vaccine used every year is only about one million. Last year, this number was 1,187,000 dosis, however, some animals are vaccinated two or more times. This means that less than a million animal are immnized every year, while the cattle population in this country is about 28 millions. Other important limitating factor is the fact that not all the reactors are eliminated or segregated, thus the bacteria persist in the farms.

EO3- BRUCELLOSIS IN NIGERIA.

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Brucellosis is recognised as an endemic disease in Nigeria, affecting large population of animals and posing big public health problems. Seroprevalence of brucellosis has been reported in cattle, sheep, goats pigs, dogs horses, chickens and

humans.Reports from various parts of the country indicates that the disease is widespread particularly in ranchaes, livestock breeding centres and dairy farms where prevalence is ranging between 3.7-48.8% and 0.4-8.1% among the traditional nomadic Fulani cattle herds. The most important causative agent encoutered is *Brucella abortus* biotype 1. Annual losses due to brucellosis in animals is estimated to be about $225 Million. The official programme for the prevention and control by mass vaccination of animals using Strain 19 started in 1944 was stopped in 1945. The production of the vaccine on a small scale for sale to farmers was started in 1995. At present there is no official programme for control in Nigeria. This aper reviews the current status of brucellosis in livestock and humans in Nigeria. Measures aimed at reducing the prevalence of the disease in animal and thus minimize the risk for human population are presented.

EO4- BRUCELLOSIS - A SERIOUS THREAT TO CATTLE PRODUCTION IN MALAYSIA.

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Malaysia embarked on a National Brucellosis Control and Eradication programme in 1978. Selective Strain 19 calf hood vaccination coupled with a test and slaughter policy was adopted. As a result of this strategy significant progress was achieved wherein the national prevalence declined from 8.7% in 1980 to 0.43% in 1993. However in 1998 the national prevalence rate jumped to 3.3% because cattle raised in oil palm plantations under an integrated system recorded an increase in the reactor rate. Cattle integration with oil palm has been a big success resulting in an increase in cattle population under plantation crop from 12,440 heads in 1994 to 130,000 heads in 2001. Brucellosis infection reached epidemic proportions in these schemes. The disease prevalence in 1995 was 0.8% which rapidly increased to 2.4% in 1997 and 6.9 % by 1998 with a herd infection rate of 27%. Though established brucellosis testing protocols were in place, a general lack of awareness among farmers resulted in purchasing of breeding cattle from various infected sources. Indiscriminate inter-herd movement under the integrated system hastened the rapid spread of the disease. The policy of vaccination using Strain 19 was discontinued in 1992. However the strategy was reviewed in 2000 with the introduction of the novel RB51 vaccine which was used on a trial basis in herds with high prevalence. The preliminary findings are encouraging and currently the national prevalence has declined to 1.69%. A more comprehensive control and eradication program is being implemented to control and eradicate brucellosis in the country.

EO5- TEMPORAL AND SPATIAL FEATURES OF BOVINE BRUCELLOSIS IN NORTHERN IRELAND: 1996 TO 2000.

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From 1996 to 2000 Northern Ireland experienced a rise in the incidence of bovine brucellosis due to *Brucella abortus*. Using data from the Department of

Agriculture's central database and veterinary investigation reports, the epidemiology of the disease is described, including both herd and animal-level parameters. 4 primary outbreaks in late 1996 and early 1997 resulted in 65 secondary outbreaks and, although infection was rapidly resolved in 2 foci in the north of the province, spread continued to occur within 2 areas in the south-west. The risk of infection was higher for beef-cow herds and most between-herd transmission was attributed to contiguous spread or contact at grazing. The role and value of detailed field investigation reports are discussed. Spatial features of the disease were explored using kernel smoothing techniques and space-time statistics, and the value of these methods over traditional choropleth and point-dot maps is also discussed.

EO6- BRUCELLOSIS RISK FACTORS IN SHEEP FLOCKS AT THE SOUTH OF BEIRA INTERIOR, PORTUGAL.

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An epidemiological study was carried out at the Castelo Branco district, south of the Portuguese Agricultural Region of Beira Interior during 1994-1999. The study objective was to identify the risk factors and to measure their degree of association with the occurrence of brucellosis in sheep flocks.

Study design: Unmatched case control. Two controls per case. α=95%; β=80%. Sample size: 150 sheep flocks. 50 infected (Sanitary classification $B_{2.1}$); 100 controls (B_3). Data sources: Brucellosis Eradication Programme (software PISA), local Animal Health Defence Association (OVIBEIRA) database and a personal interview. Methodology: A multivariate logistic regression model was developed to consider the individual and joint effects of the factors simultaneously. Pearson statistics was used to identify pairs of variables with good correlation. The model goodness-of-fit was described by the Hosmer and Lemeshow test: χ^2=1,750; p=0,972.

Results: Twenty-one potential risk factors were analysed but only four entered into the final model:

(1) Breeding female purchases (OR=10.43: χ^2=3,87; p<0.082; 0.779<OR<68.441).

(2) Lambing in March (OR=2.89: χ^2=9,12; p<0.056; 0.971<OR<10.001).

(3) Breeding female group isolation at lambing (OR=5.089: χ^2=5,43; p<0.029; 1.158<OR<16.333).

(4) Presence of dogs (OR=4.00: χ^2=14,61; p<0.038; 1.075<OR<13.241).

The model minimum probability of a brucellosis outbreak was estimated at 0.02 when only the risk factor - lambing in March - was present; the maximum probability of a brucellosis outbreak was estimated at 0.7 when the previous four risk factors were present simultaneously.

In conclusion, the present study reinforces the need for the improvement of the flock's biosecurity levels. Without it is accomplishment it will not be possible to increase and to maintain consistently a regional flock B_3 status.

EO7- HUMAN HEALTH BENEFITS FROM LIVESTOCK VACCINATION FOR BRUCELLOSIS: A CASE STUDY.

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This study estimates the economic benefit, cost-effectiveness and distribution of benefit of improving human health in Mongolia through the control of brucellosis by mass vaccination of livestock. Cost-effectiveness and economic benefit of mass vaccination against brucellosis, considering both human society and the agricultural sector is modelled. The intervention consists of a planned 10-year livestock mass vaccination campaign using Rev-1 livestock vaccine for small ruminants and S19 livestock vaccine for cattle. Cost-effectiveness, expressed as cost per DALY averted, was the primary outcome of this study. In a scenario of 52% reduction of brucellosis transmission between animals, conferred by mass vaccination, a total number of 49,027 DALYs could be averted. In the same scenario, estimated intervention costs are USD 8.3 millions and the overall benefit is USD 26.6 millions. This results in a Net Present Value of USD 18.3 millions and an average Benefit Cost Ratio for society of 3.2 (min. 2.27; max. 4.37). If the costs of the intervention are shared between the sectors proportionally to the benefit to each, the public health sector should contribute 11% to the intervention cost, yielding a cost-effectiveness of USD 19.1 per DALY averted (95% confidence intervals: 5.3 – 486.8). If private economic gain due to improved human health is included, the health sector should contribute 42% to the intervention costs and the cost effectiveness decreases to USD 71.4 per DALY averted. If costs of livestock brucellosis vaccination are allocated proportionally to all benefits, the intervention may become profitable and cost effective for both the agricultural and the health sectors.

EO8- DIAGNOSTIC VALIDATION OF BOVINE BRUCELLOSIS SEROLOGICAL TESTS FOR EPIDEMIOSURVEILLANCE PURPOSES.

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The last bovine brucellosis case in Belgium was identified in March 2000. In the context of the belgian epidemiosurveillance program, the objective of this study was to compare the diagnostic characteristics of, on one hand, different brucellosis serological tests performed on individual bovine sera (SAW-EDTA, RB, iELISA) or pooled bovine sera (pooled iELISA) and, on the other hand, a serial testing strategy: first testing the pooled sera followed by retesting the individual sera in case of positivity.

In order to evaluate the diagnostic specificity (Sp), sera were collected from animals over 2 years of age, between November 1st, 2001 and March 31, 2002. The

sample consited of 7185 individual sera. These sera and 719 pools, each consisting of 10 individual sera, were analyzed. In order to evaluate the diagnostic sensitivity (Sn), sera were analyzed, that were collected between 1990 and 1992, from recognized infected animals (animals from which *Brucella abortus* biovar 3 was isolated). 90 sera were analyzed individually and 90 pools were constituted by adding 9 ml of FCS to 1 ml of each of the 90 individual sera. The statistical analysis was performed using SAS®, version 8 and StatXact®, version 4. All tests were bilateral with a 0,05 level of significance.

Our results suggest that the Sp for individual and serial tests is very high (>99,4%) with rare false positive results (<1%). When pooled testing was solely applied, the Sp was unexpectedly lower with 3% false positive results. The Sn for the individual and serial tests were comparable and equal to 1, 95% confidence interval [0,9598-1]. A second analysis, using “Latent Class Models” via S-plus® and WinBugs® confirmed these results.

In conclusion, the hypothesis that Belgium is biologically free of bovine brucellosis (*B. abortus*) is statistically sustained (Prevalence: 0,00014, confidence interval 95% [0-0,0007].

EO9- DIFFERENCES IN SERUM ANTIBODY RESPONSES BETWEEN PIGS EXPERIMENTALLY INFECTED WITH *Brucella suis* BIOVAR 2 AND *Yersinia enterocolitica* SEROTYPE O:9.

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Infections in pigs and cattle with *Yersinia enterocolitica* serotype O:9 (YeO:9) may cause false positive reactions in serological tests for *Brucella* due to almost identical LPS O-antigens in the two bacteria. Denmark is free of brucellosis in cattle and sheep while few infections with *B. suis* biovar 2 have been observed in hares and free ranging pigs. YeO:9 was recently discovered in the porcine population and YeO:9 false positive reactions for *Brucella* is now a cause of problems in surveillance and export testing of pigs. The serological reactivity of pigs experimentally or naturally infected with YeO:9 or *B. suis* biovar 2 was compared in a number of *Brucella* assays and a novel serotype specific YeO:9 LPS ELISA.

Following experimental YeO:9 infection, cross-reactions in *Brucella* tests were limited to a 2-8 week period after inoculation while serum responses in the YeO:9 ELISA did not fade over time. In contrast, serum responses of pigs experimentally infected with a subclinical dose of *B. suis* biovar 2 were *Brucella* and YeO:9 test positive throughout the 20 week observation period.

In herds infected with YeO:9 and *B. suis* biovar 2, respectively, antibody responses among pigs in different age groups reflected these differences in *Brucella* and YeO:9 reactivity: In YeO:9 infected herds, *Brucella* reactors among YeO:9 ELISA positive pigs were predominant in young age groups while older pigs, with an affinity matured immune response to YeO:9, were positive in YeO:9 ELISA only. By contrast, in *B. suis* biovar 2 infected herds, a high number of YeO:9 reactive pigs were, irrespective of age, also test positive in tests for brucellosis. All samples with reactivity in any of the *Brucella* tests were also positive by the YeO:9 ELISA.

Conclusion: In case of serological reactions indicative of a *Brucella* infection in pigs, a combination of the highly sensitive YeO:9 ELISA and classical tests for

brucellosis can be used to support the evaluation of the suspicion of brucellosis in the originating herd.

EO10- NEW ANTIBRUCELLOSIS STRATEGY.

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We would like to present you new and very effective strategy that helps to fight brucellosis caused by *Br. melitensis*. It is possible to view brucellosis as parasitic system that includes sheeps, goats, and humans as its main hosts of *Brucella melitensis*. If we neutralize the source of infection therefore whole parasitic system can be destroyed. In order to do that we used new immunoactive medicine "IMNOMAC", that effectively protected animals from the Brucellosis. This unique medicine was given to animals together with food salt during 30 days before lambing period. Nurobad Region of Uzbekistan was the main region suffering from Brucellosis and was used as a study field in our research. Every year in that region had been officially registered 19-26 new cases of brucellosis of human and about 280 to 300 people asked for help in the hospitals from the recidivation of brucellosis. Also, among animals the number of brucellosis abortions reached approximately 20 - 25%. In the year 2002 all sheeps and goats in that region got medicine "IMNOMAC". As a result in the year 2002 no new cases of brucellosis of human in that region was registered and number of sick people with recidivation of brucellosis was reduced to 47. Abortions among sheeps and goats disappeared and were not registered. Before program was applied, 32.4% of all people in Nurobad region had positive reaction to agglutination test. After applying the program, there was less that 1% of people positively reacting to the test. This shows abrupt decrease in the activity of epidemic process. Obtained results allow us to hope for successful fight with this type of brucellosis and we invite everybody for cooperation.

HO1- SIGNIFICANT REDUCED NUMBER OF *Brucella*-SPECIFIC IFN-g-PRODUCING CD3 T CELLS IN HUMAN CHRONIC BRUCELLOSIS.

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Human brucellosis is a worldwide zoonotic infection caused by intracellular bacteria belong to the genus of *Brucella*. Based on the murine studies it has been shown that host resistance to *Brucella* depends on Th1 response, whereas Th2 response is involved in the severity of disease. Since the immune responses during human brucellosis have not been profoundly studied thus we tried to evaluate cytokines production in patients suffering from brucellosis. Two clinical forms, chronic and acute, were included in the study and sex and age-matched healthy individuals were also appraised as controls. Diluted whole blood samples were cultured in the presence of either mitogen; heat inactivated bacteria or medium alone. IL-12, IFN-γ and IL-10 were measured by specific sandwich ELISA. In addition, intracellular cytokine production was used in order to evaluate IL-13- and IFN-γ producing CD3 cells. It was found that not only IFN-γ production but also the number of IFN-γ producing CD3 cells were significantly decreased in response to antigen in the chronic group of patients. In contrast, IL-12 production in whole blood culture of chronic patients was higher than the acute patients. Although the percentage of IL-13-producing CD3 cells was dramatically high in the chronic group of patients no correlation was found between the number of IFN-γ producing and IL-13-producing CD3 cells. IL-10 production was also augmented in chronic patients but without any correlation to IFN-γ production. In conclusion, the correlation of Th2 cytokines production and progression of chronic human brucellosis was not demonstrated. Nevertheless, the number of IFN-γ-producing CD3 cells were found to be dramatically decreased in chronic group suggesting the induction of apoptosis to eliminate the Th1 cells which helps prolongation of brucellosis in chronic patients.

HO2- *Brucella* IgM AND IgG FLOW ASSAYS FOR THE RAPID SERODIAGNOSIS OF HUMAN BRUCELLOSIS.

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Laboratory testing is essential for the serodiagnosis of human brucellosis. Diagnostic testing for human brucellosis often is not available in remote areas that are endemic for brucellosis. As a consequence the illness may be overlooked or misdiagnosed. To fulfil in the need for a simple and rapid diagnostic test we have developed the *Brucella* IgM and IgG flow assays. The test can be performed and read without the need for formal training or special and expensive equipment. The assays are simply performed by the addition of a drop of serum and some running fluid to the sample well of a plastic assay device. The assay result is read after 10 to

15 minutes by visual inspection for staining of the antigen line in the test window of the assay device. A positive result is consistent with brucellosis. The relative expression of specific IgM and IgG antibodies depends on the stage of illness. Therefore the two flow assays should be used as complementary tests. The Rose Bengal test may be used as a screening test. The sensitivity as calculated for the initial serum sample collected at the time of first diagnosis from patients with confirmed brucellosis presented at different hospitals in Spain was 96%. Approximately 75% had specific IgM antibodies and 86% had specific IgG antibodies. The sensitivity for patients with acute or with persistent brucellosis was equally high. Testing of samples collected from patients with brucellosis from endemic areas have confirmed the high sensitivity and specificity. These results combined with the simplicity of the assay procedure make the tests ideal for use in medical facilities that lack appropriate diagnostic facilities or staff to perform the more complex standard diagnostic tests. The tests also are suitable for use in the field and may be performed on a drop of blood collected by fingerprick.

HO3- INTERFERENCE OF RHEUMATOID FACTOR IN THE IgM-BASED SEROLOGICAL DIAGNOSE OF HUMAN BRUCELLOSIS.

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Although occurrence of Rheumatoid Factors (RF) have often been linked to cases of rheumatic arthritis, some chronic bacterial infection also result in an induction of RF. In these instances, RF is frequently found to be of the IgM isotype.

Although several authors have reported the presence of RF in patients with brucellosis, the potential interference of RF in diagnostic tests based on the detection of IgM antibodies (Ab's) to *Brucella*, has not been evaluated. During the testing of a rapid flow immunochromatographic system (LF-IgM) for the detection of IgM Ab's in patients with brucellosis, we obtained a false positive result due to interference by RF. To further evaluate the potential relevance of this finding, we first screened sera (n=450) from brucellosis patients for RF using latex particles coated with human gammaglobulin. We then subjected the RF-positive samples (n=9) to serological assays for the diagnosis of human brucellosis including ELISA-IgM, LF-IgM and seroaglutinatión (SAT). All these tests were performed both subsequently and prior to absorption of the RF-positive sera with anti-RF Ab. Finally, the presence of other relevant Ab types (dithiothreitol-sensitive Ab's; non-agglutinating IgG Ab's, Native-Haptene-reactive Ab's and anti-cytosolic-proteins Ab's) was also investigated in the RF-positive samples.

Prior to their absorption with anti-RF-Ab, all 9 RF-positive sera gave also positive result as determined by ELISA-IgM, LF-IgM and LF-IgG. However, in five cases absorption with anti-RF Ab totally abolished reactivity detectable by ELISA-IgM, LF-IgM and SAT (the latter with the exception of a serum containing high titer of agglutinating IgA). Interestingly, these sera corresponded to patients with relapse (n=3) and patients with long-lasting brucellosis (n=2). Consistent with these findings,

the four remaining sera whose reactivity was unaffected by absorption with anti-RF Ab corresponded to acute cases and had high levels of IgM antibodies.

Although interference by RF potentially poses a serious problem to diagnostic tests based on IgM-detection, our results show that this phenomenon does not hinder achieving a correct serological diagnosis of human brucellosis as long as simultaneous performance of IgG tests are carried out. However, considering a potential interference by RF in patients with relapse and/or patients with long-lasting brucellosis is important because, as we show, specific test detecting IgM antibodies can yield false positive results due to the presence of RF.

HO4- RAPID DIAGNOSIS OF HUMAN BRUCELLOSIS BY SERUM QUANTITATIVE REAL-TIME PCR.

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In order to simplify molecular diagnosis of human brucellosis and make it more accessible to any clinical laboratory, we have now developed a Real-Time PCR assay and have evaluated its diagnostic yield in serum samples.

From April 2002 to May 2003, serum samples from 60 patients with active brucellosis were analyzed by quantitative Real-Time PCR. Two of the patients provided two samples each; one corresponding to the initial episode and the other corresponding to a relapse. Control samples were obtained from 55 subjects with febrile syndromes of other defined etiologies which had initially involved a differential diagnosis with brucellosis, asymptomatic patients with a history of brucellosis treated correctly during the previous 12 months, asymptomatic subjects professionally exposed to *Brucella* infection, with persistent high titers of antibrucella antibodies and healthy subjects.

A 223-base pair PCR target sequence present in the gene encoding a 31 kDa *Brucella abortus* antigen was selected for amplification. The LightCycler detection system and SYBR Green I Dye were used for amplification and online quantification. of PCR products.

Of the 60 patients included in the study, 36 (60%) had positive blood cultures and the other 24 (40%) were diagnosed based on clinical and serological criteria. Forty (66.6%) had fever with no apparent focus and 20 (33.3%) had one or more focal complications. Of the 62 samples from the patients with brucellosis, 57 (91.9%) were positive in the Real-Time PCR. The amplification threshold was 29.9+3.3 cycles (range 20.1-37.7 cycles). Two control samples (3.6%) had a false positive Real-Time PCR test. Thus, Real-Time PCR assay sensitivity, specificity and positive and negative predictive values were 91.9%, 96.4, 96.6, 91.4%, respectively. Positive and negative likelihood ratios were 25.3 and 0.08 respectively.

In conclusion, the high sensitivity and specificity of this Real-Time PCR assay, together with its speed, versatility, and risk reduction for laboratory personnel, makes this technique a very useful tool for the diagnosis of human brucellosis.

HO5- CLINICAL MANIFESTATION AND LABORATORY TEST RESULTS IN 581 CASES OF BRUCELLOSIS IN BABOL , IRAN , 1997-2002.

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This study was conducted for assessing of the clinical manifestations and laboratory test results on 581 cases of human brucellosis in department of infectious diseases of Babol Medical University from 1997-2002.

Of 581 cases, 329 (56.6%) were males and 252 (43.4%) females with the mean age of 31.9±17 years. 111 subjects were children younger than 15 years old . Artheralgia, sweating, fever, back pain and myalgia were the most clinical signs .The presentation of artheralgia, chills, fever, anorexia, nausea, vomiting, adenopathy were similar between children and adults, but sweating, back pain and myalgia were more common in adults than children ($P<0.05$). Splenomegally was seen in 19.6% of cases in children,but in 5.7% cases of adults ($P=0.0001$). Laboratory test results included: normal leucocyte count (89.4%), normal platelet count (95.8%), anemia (15.5%), CRP+ (34.5%) and RF+ (2.8%). Elevated erytherocyte sedimentation rate was seen in 54 cases, more in children than adults ($P=0.0001$). Complications were seen in 34.7% of cases. Peripheral arthritis, sacroileitis, spondylitis, and orchitis were common findings. This results show that the most clinical findings in human brucellosis were skeletal manifestations, but laboratory test results are non-specific, so in endemic area every patients with musculoskeletal signs and symptoms, brucellosis must be considered in differential diagnosis.

HO6- CHILDHOOD BRUCELLOSIS IN BABOL, NORTH OF IRAN.

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This prospective study was carried out on cases of childhood brucellosis in Babol, Iran, during a 6-year period from 1997-2002. 111 consecutive patients (61 males, 50 females) with the mean age 10 ± 3.6 years with active brucellosis were enrolled. 66 subjects (59.5%) were from rural regions. Brucellosis occurred in 80 cases (72%) at spring and summer seasons. In 10 cases, other family members also had active brucellosis. 96% of the cases had an acute and subacute presentation. Fever (74%), artheralgia (49.5%), splenomegaly (19.6%) were common findings. Peripheral arthritis was seen in 41 (36.9%) cases which usually presented as monoarticular arthritis involving the keens and hips, followed by sacroileitis 12 (16.7%) cases and genitourinary 9 (12.5%) of cases. Laboratory findings included anemia (16.8%), leucocytosis (11.7%), CRP+ in (67.6%), RF+ (8.5%), elevated ESR (33.3%) cases. 42 cases treated with Co-trimoxazole + Rifampine for 8 weeks, and relapse occured in 2 cases (4.7%) and 30 cases also treated by this regimen for 6 weeks and relapse rate was 3.3% ($P=0.125$). This study showed that in children with brucellosis, clinical manifestations are protoan and laboratory findings are not specific. Treatment with Co-trimoxazole + Rifampine for 8 weeks had equal efficacy with 6 weeks of therapy by this regimen.

HO7- EFFICACY OF THREE DIFFERENT REGIMENS WITH TWO DIFFERENT DURATION OF THERAPY IN HUMAN BRUCELLOSIS.

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The best regimen and duration of therapy for treatment of brucellosis is controversial. The aim of this study was to evaluate the efficacy of 3 different common regimens with duration of 6 and 8 weeks of therapy.

From April 1997 to April 2003, all patients attended to the department of infectious diseases of Babol Medical University were treated by one of the following regimens and all cases followed up for one year after cessation of therapy. 16 cases with the mean age of 35.4±14.6 years and 29 cases with the mean age of 33±18.6 years were treated by Doxycycline plus Rifampin for 6 and 8 weeks and relapses were occured in 8 (50%) and 10 (34.5%) of cases respectively (p=0.24). With Co-trimoxozol plus Doxycycline, 111 patients with the mean age of 36.52±16.5 years and 92 cases with the mean age of 34.5±15.42 years were treated for six and eight weeks, and the relapse rate was (7.9%) and (6.5%) respectively (p=0.386). 60 cases with the mean age of 32.7±18.17 years and 46 cases with the mean age of 33.19±15.9 years were treated by (Streptomycin plus Doxycycline and after 2 weeks, streptomycin replaced by Rifampin) for 6 and 8 weeks, but the relapse rate was 3.3% and 4.3% respectively (p=0.585).

The results of this study show that six weeks of therapy with regimen (streptomycin plus Doxycycline and after two weeks streptomycin replace with Rifampin) for six weeks is preferred regimen with other regimen of therapy longer duration of therapy is recommended.

HO8- ANIMAL AND HUMAN BRUCELLOSIS IN ITALY: OCCUPATIONAL, FOOD CHAIN EXPOSURE, OR BOTH?.

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Between 1970 and 2000 human brucellosis in Italy showed an incidence between 6.56/100 000 and 1.84/100 000. Between 1970 and 1990, *B. melitensis* was the most frequently isolated strain in human cases of brucellosis, reaching some 99% of total cases. Human data on *Brucella* isolates have not been updated since then, even though animal, mainly ovi-caprine, trends remained reasonably high in the years following 1990. A persistent correlation between human and animal infections has been demonstrated by regression analysis applied on both populations. Comparison studies for assessing the infection routes have not been conducted in our Country to date, but there are consistent data suggesting that foodborne infection could be the main diffusion route of the disease, due to the seasonal incidence peaks noted. Notifications of human brucellosis, that are mandatory in Italy, reach a peak between April and June. When we consider the standard incubation period of 2-4 weeks, and since lamb slaughter is traditionally concentrated at Easter time when lambs are around 60 days old, if an occupational exposure were suspected, we should expect a peak of human cases between March and May. Nevertheless, the peak of human cases is recorded between April and June. This could be related to

the production, and consumption, of fresh cheese, starting just after lamb slaughter. When the age of patients was considered, we did not observe a concentration in a particular age class, but a fairly uniform distribution among them. Moreover, when we analyzed incidence rates of human brucellosis between 1997 and 2000, the observed cases were similar to the expected, if we consider an occupational exposure risk of about 25%. Validation of the mathematical model was done on the basis of observations conducted on selected Italian regions, and considering a particular North/South gradient of human and animal brucellosis incidence.

HO9- BRUCELLAR SPONDILODISCITIS. A CASE REPORT IN A RELATIVELY LOW INCIDENCE AREA.

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Osteoarticular complications of brucellosis are quite common, and are reported in some 20-60% of cases worldwide. There is a wide spectrum of clinical manifestations, including arthritis, spondylodiscitis, osteomyelitis, synovitis and bursitis. In our settings, early diagnosis of such complications is somewhat difficult because of latency from acute infection, and due to the low incidence of brucellosis in Central Italy. Here we describe the case of a 52 years old female patient admitted to our Unit in December 2001 with complaint of diffuse back pain after a period of recurrent febrile episodes, max 38°C, occurred in the previous months, and resistant to non specific broad spectrum antibiotic treatment. Before admission, a NMR showed spondylitic alterations in the thoracic tract. Routine tests showed alteration of the inflammatory indices (ESR = 15mm/h, CRP = 1,41mg/dl), and a moderate neutrophilia. Wright serology was positive at high titer (1/1600), and both Rose Bengal test and complement fixation were positive for brucella antibodies. Blood culture and PCR were negative for brucella isolation. A further NMR of the dorsal column showed alteration of the D9 and D10 vertebral bodies, with intervertebral disc involvement. After exclusion of other causes of spondilodiscitis (i.e. tubercular or degenerative), a treatment with ciprofloxacin was initiated. After two months, because of symptoms and inflammation signs persistence, rifampin was associated, and doxycycline was then associated after discontinuation of ciprofloxacin. A progressive improvement of the inflammatory indices was noted, and a stable improvement of both clinical signs and symptoms was achieved. Wright serological test reverted to negative during treatment, and drugs were discontinued on August 2002. A follow-up NMR showed signs of healing D9-D10 spondylodiscitis, and the patient is in good clinical conditions to date. In our experience, better efficacy of doxycycline-rifampin association was noted, when compared to quinolones-rifampin, somewhat in contrast with other published reports. A prolonged treatment duration is frequently required for complicated brucellosis.

DO1- SEROLOGICAL RESPONSE OF YOUNG AND ADULT SHEEP TO CONJUCTIVAL VACCINATION WITH REV-1 VACCINE ASSESSED WITH THE CLASSICAL AND RECENT TESTS.

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The serological response of young and adult sheep to conjuctival vaccination with REV-1 vaccine was assessed with the use of classical and new serological tests. One hundred and six (106) adult sheep and forty-nine (49) lambs 3-6 months old, reared in a brucellosis officially free flock, were used for this purpose. A normal dose ($1?10^9$ cfu) of REV-1 vaccine was administered conjunctivaly to all animals. Prior to vaccination the animals were bled and the sera collected were tested with the serological tests approved for the diagnosis of ovine and caprine brucellosis as Rose Bengal test (RBT) and Complement Fixation Test (CFT) (Directive 91/68 EEC) . The sera were tested also with the most sensitive modified Rose Bengal test (m-RBT) (Blasco, 1994 , OIE, 2002) and new tests as, indirect Elisa (i-ELISA) , (Jimenez de Bagues, 1991), competitive ELISA (c-ELISA) (Nielsen, 1995) and Flourescence Polarization Assay (FPA) (Nielsen, 1996). The results obtained by all tests performed were negative. The animals were bled at day 21, 42, 63, 91, 125, 159, 223 and 330 post vaccination and the sera collected were tested with the tests mentioned above.

The performed tests revealed that three weeks (21 days) post vaccination the seropositive adult animals to RBT, m-RBT, CFT, c-ELISA, i-ELISA and FPA represented the 100%, 100%, 98%, 88,6%, 87% and 83% of vaccinated adult animals respectively, while the seropositive lambs to these tests represented the 100%, 100%, 89,7%, 79,59%, 18,36 and 18,36% of vaccinated young animals respectively. Forty seven weeks (330 days) post vaccination the seropositive adult animals to RBT, m-RBT, CFT, c-ELISA, i-ELISA and FPA represented the 3,77%, 11,32%, 1,88%, 2,83%, 33% and 2,83% of vaccinated adult animals respectively . At the same time seropositive lambs that represented the 2,04% of vaccinated young animals were found with m-RBT, while the results obtained from all the other tests were negative.

DO2- EVALUATION OF THE COMPETITIVE ENZYME IMMUNOASSAY AND POLARIZED FLUORESCENCE ASSAY FOR BRUCELLOSIS DIAGNOSIS IN GOATS FROM NORTHERN MÉXICO.

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A preliminary study to determine the effectiveness of the fluorescence polarized assay (FPA) and competitive enzyme immunoassay (CELISA) in detecting negative serums was performed. Results were compared with those obtained with the card test using 3% of antigen concentration. Procedures and reagents used were

obtained from the Canadian Animal Disease Research Institute. For FPA, 40µl of serum were used. The card test was used in conformance with the Mexican Official Rules. Brucellosis negative goat serums were obtained from Canada which is officially Brucellosis free. CELISA showed to be the most efficient test for identifying as negatives all 284 negative serums tested. FPA identified correctly 94.8% of 477 serums tested with a cutting point at >95.4 mP and 99.4% at >105mP (Med Calc). ard test identified 98.3% of 526 negative serums. Additionally, 1,238 goat serums from northeast of Mexico, where are about of 40% of positive flocks and the goats are vaccinated, were tested with CELISA, FPA and the card test. Serums were classified as positive or negative according to the results obtained with CELISA and the results were compared with those obtained by FPA and the card test. According with the analysis FPA correctly identified 60.8% of the positive serums and 81.7% of the negative serums (>114.14mP). The card test identified 64.8% and 77.7% respectively. Results show that CELISA and FPA offer a viable alternative for goat brucellosis diagnosis in Northern Mexico although CELISA is more efficient in identifying negative goats. The card test usefulness is limited when dealing with animals from high prevalence regions and regions were vaccination is used. FPA has an advantage of being able to adjust the cutting point and can be used effectively as a screening procedure.

DO3- USE OF BP26 BASE ENZYME LINKED IMMUNOSORBENT ASSAY FOR DIFFERENTIATING RUMINANTS INFECTED WITH *Brucella abortus* OR *melietensis* FROM RUMINANTS INFECTED WITH *Yersinia enterocolitica*.

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Ruminant Brucellosis diagnosis was realised for years by serological techniques mainly based on LPS (ELISA, Rose Bengale, EAT, etc.). Since 1990, high rates of false positive serological reactions (FPSR) in bovine cattle have led to some difficulties in interpreting *Brucella* serologies (ref. 1).

Previously a *Brucella* protein named CP28, BP26, or Omp28 has been identified as an immunodominant antigen in infected cattle, sheep, goats, and humans (ref. 2). In this study, different ELISA systems using recombinant BP26, native BP26 and a panel of specific anti-BP26 monoclonal antibodies were evaluated in order to set up a specific BP26 ELISA test.

The cut-off of this test was settled to be in accordance with the European norms of detectability (ref. 3), to be possibly used furtherly within the framework of an European eradication program. At this cut-off level, different parameters were examined :

1. Sensitivity by comparison with the different results obtained by conventionnal methods, such as LPS base ELISA, Rose Bengale, Complement fixation and BP26 ELISA.
2. The specificity, which was studied in two contexts :
    a) In populations showing no or few FPSR
    b) In populations showing a strong FPSR prevalence

The first results indicate that this ELISA BP26 could be an interesting complementary tool in case of FSRP. Results will be presented and discussed.

Ref. 1 : B. Garin-Bastuji, R. Pouillot, C. Cau, P. Pourquier, L. Schalch, P. Very : Evaluation of two commercially available iELISA kits for the diagnosis of Bovine Brucellosis on pools of 10 sera. Comparison with the RB, CF and iELISA performed on individual sera.
Ref. 2 : Axel Cloeckaert, Sylvie Baucheron, Nieves Vizcaino, Michel s. Zygmunt : Use of Recombinant BP26 Protein in Serological Diagnosis of *Brucella melitensis* Infection in Sheep.
Ref. 3 : EEC Directive 64.432

DO4- DIFFERENCES IN CELLULAR IMMUNE RESPONSES OF PIGS EXPERIMENTALLY INFECTED WITH *Brucella suis* AND *Yersinia enterocolitica* SEROTYPE O:9.

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Due to almost identical LPS O-antigens, infections with *Yersinia enterocolitica* serotype O:9 (YeO:9) cause false positive reactions in serological tests for *Brucella*. As LPS are strong inducers of humoral immune responses, cellular immune responses to non-LPS antigens prepared from rough *Brucella melitensis* (Brucellergene®) was hypothesized to separate these two very different infections in pigs. Ten young pigs were experimentally inoculated in the eye sac with 2×10^7 cfu of *Brucella suis* biovar 2 and cellular immune responses were followed for 20 weeks. As evidenced by antibody responses in serum and cultivation of tissue samples at slaughter 7 pigs were successfully infected. Immune responses were compared with the responses in age matched pigs, experimentally infected per os with different doses of YeO:9.

Whole blood interferon-gamma (IFN-γ) test (stimulated with dialysed Brucellergene®, *Yersinia* secretory proteins (yop), positive and negative controls) revealed high IFN-γ levels, in response to Brucellergene® in *Brucella* infected pigs but not in YeO:9 infected or control pigs. In comparison, IFN-γ responses to yop and PBS were low in all groups of pigs irrespective of type of infection. The specificity of the cellular immune responses was confirmed by positive skin test in *Brucella*, but not *Yersinia*, infected pigs at different times after inoculation. Intracellular FACS staining for IFN-γ and BrdU incorporation showed $CD4^+$ and $CD8^{low}$ T cells were main producers of IFN-γ and the predominant proliferating cell subset following Brucellergene® stimulation of *Brucella* infected pigs. In conclusion, cellular mediated immune responses to non-LPS *Brucella* antigens were both specific and sensitive in discriminating subclinical experimental infections with *B. suis* from infections with YeO:9.

DO5- BRUCELLOSIS IN THE NETHERLANDS: THE DEVELOPMENT OF *Brucella*-SPECIFIC SEROLOGICAL TESTS.

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Since 1999 the Netherlands gained the status of an officially brucellosis free (OBF) member state on the basis of directive 64/432/EEC. Since then, 20% of the

herds have to be routinely tested for at least 5 years. Besides that, the EU prescribes notification of abortions and subsequent serological testing. False positive results of serological test are a major problem in countries free from brucellosis. Because the positive predictive value of a serological test in a population depends on the prevalence of the disease, this problem will increase when the prevalence decreases. By definition, in a free population, the positive predictive value will be zero, meaning that better tests with higher specificities need to be developed and validated. Since the early nineties, a significant increase of the number of FPR's have been reported, especially in Belgium and France. Serological cross reactivity has been described with *Yersinea enterocolitica* O:9 as well as other gram negative bacteria (Godfroid et al, 2002). To circumvent these cross reactivities, much effort have been put in the development of tests based on *Brucella* specific (cellular) antigens. The main problem thus far is the lack of sensitivity of these tests.

The aim of this project is to develop a new serological test which can be used as conformation test following positive results in the tests prescribed by the EU. Therefore, we constructed a genomic library of *Brucella* abortus strain 99. This library was screened with a highly positive *Brucella* serum (serum 2427) in order to identify cellular antigens reactive with this serum. In a first attempt, using the commercially available TripleX system (Clontech), we found a set of cellular antigens, most of which were previously described in the literature; i.e. BP26 (Zygmunt et al., 2002), HtrA (Roop et al., 1994) and dimethyl-ribityl-lumazine (DMRL; Hemmen et al., 1995). Because of this overlap, and due to some problems we and others encountered using the TripleX system, we decided to construct and screen a new library (ZapExpress; Stratagene), this time using two different *Brucella* field strains, a biotype 1 as well as a biotype 2 strain. In this screening we found, among the antigens described above, several new potential candidates which are currently being tested.

DO6- COMPARISON OF ROSE BENGAL IN ANALOGY OF 1:3 WITH SERUM AND ROSE BENGAL IN ANALOGY OF 1:1 WITH SERUM INTERPRETED IN SERIES WITH COMPLEMENT FIXATION TEST AS CONFIRMATORY TEST FOR THE DETECTION OF INFECTED SHEEP AND GOAT FLOCKS WITH *Brucella melitensis*. A FOUR-MONTH FIELD TRIAL DURING THE CYPRUS BRUCELLOSIS TEST AND SLAUGHTER ERADICATION PROGRAM.

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Rose Bengal antigen in analogy 1:3 (RB 1:3) with serum is recommended as more sensitive compared to the classic Rose Bengal (Rose Bengal antigen/serum=1:1, RB 1:1) for use in sheep and goats (Veterinary Record, 1994, 134, 415-420).

During the application of Cyprus Brucellosis eradication program, a four-month trial was performed to compare the RB 1:1 with the more sensitive RB 1:3 as flock screening tests. Blood samples from 50 animals per flock from two thousand two hundred fifteen (2215) flocks were collected and examined using RB 1:3. All RB 1:3 positive (showing any degree of agglutination) samples were examined using RB 1:1 and complement fixation test (CFT). Samples showing any degree of agglutination in

RB 1:1 were considered RB 1:1 positive and samples containing 20 ICFT units/ml were considered CFT confirmed positive. Three hundred fifteen flocks (315) showed one or more animals reacting with RB 1:3 including one hundred thirteen (113) flocks with one or more CFT confirmed positive animals. From the three hundred fifteen (315) RB 1:3 positive flocks, ninety-four (94) showed one or more animals reacting with RB 1:1 including forty eight (48) flocks with one or more animals confirmed positive with CFT. Among the two hundred twenty one (221) flocks that were negative to RB 1:1 and positive to RB 1:3, sixty five (65) flocks included one or more animals confirmed positive with CFT. In this trial, RB 1:3 and RB 1:1 detected from the population examined, in series with CFT, 5,1% and 2,16% positive flocks respectively while a proportion of 9,1% and 2,076% of the flocks reacting to RB 1:3 and RB 1:1 respectively, were not confirmed as positive. Also, the CFT confirmed as positive 35,8% of the flocks having animals with one or more reactions to RB 1:3. This proportions for RB 1:1 reached 51%.

DO7- DIAGNOSING OF OVINE AND CAPRINE BRUCELLOSIS TROUGH THE SERUM AND MILK ELISA - PRELIMINARY TEST VALIDATION.

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With ELISA methods it is possible to examine individual serum/milk samples (like confirmatory test) and pooled milk samples (like screening test). ELISA for pooled milk samples is useful for examination of flocks from 200 cows (*Brucella* bang), 100 sheep or 100 goats (*Br. melitensis*). The approach for standardization of tests is going trough the Bommeli ELISA-BESW (*Brucella* bang) standard and our institute (MKD) working standard (positive serum and milk, based on *Brucella melitensis* antigen). The specificity and sensitivity of the Chekit-Brucellotest ELISA and our institute (MKD) ELISA has been evaluated by the Department for Cattle Diseases at the Veterinary Institute, Skopje with routine samples serum/milk obtained parallel from brucellosis free herds and from infected herds, respectively. A total of 396 negative and 755 positive samples of both, sera and milk were included in the examination. The samples used in the preliminary validation were previously tested on RBT and CFT and were both negative in negative samples, and both positive in positive samples (combination of RBT and CFT used as a "golden standard").

The obtained specificity of the serum ELISA was 99.7 (at cut-off of 30%PP and 15% PP for Bommeli and MKD ELISA, respectively). The sensitivities of serum ELISAs at the same cut-off were 98.5% for Bommeli and 96.7% for the MKD test. Parallel milk samples from the same animals showed same specificity of 99.7% in both ELISA tests, but using different cut-offs of 30% for Bommeli test and 25% for the MKD test. The sensitivity of the milk ELISAs were 94.6% for the Bommeli test and 95.9% for the MKD test. Using ELISA technique it is possible to diagnose Brucellosis fast, safe exact and cheap.

DO8- THE USE OF FTA CARDS IN MOLECULAR DIAGNOSTIC TECHNIQUES FOR BRUCELLOSIS.

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There has recently been increased interest in the development of molecular-based diagnostic techniques for the identification and fingerprinting of *Brucella* isolates. One impediment for this technology is sample preparation, since most methods (especially PCR-based methods) are very sensitive to inhibitors from blood, milk, tissues, or foods. Culturing the bacteria is an effective way to eliminate inhibitors, but it is time-consuming. Alternatively, DNA extraction and purification from the inhibitors is also time-consuming and may lead to cross-contamination of samples. We describe the use of commercially available FTA cards as a rapid method for preparing *Brucella* DNA for PCR and other enzymatic reactions. These cards, commonly used for forensic analysis of blood and buccal samples, consist of filter paper impregnated with solubilizers and detergents. When a sample is spotted on the card the cells or bacteria are lysed and the DNA binds to the filter. Contaminants and inhibitors are easily removed by a few wash steps. We demonstrate that the treated samples are readily amplified by PCR without any significant loss of sensitivity.

DO9- MARINE MAMMAL BRUCELLOSIS IN CANADA 1995-2003.

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Since the discovery of marine mammal forms of *Brucella* in 1994 there have been numerous surveys to identify the strains involved, the hosts they infect, and their geographical distribution. Most of what is known about marine mammal *Brucellae* has been obtained from investigations of stranded or captive animals and a few serological surveys of wild animals. There has been some recent progress, as evidenced by reports that some cetacean stranding events are associated with meningoencephalitis caused by *Brucella*, accounts of reproductive failure in dolphins and baleen whales due to *Brucella* and that at least two human, community-acquired cases of neurobrucellosis resulted from *Brucella* infection that is believed to have arisen from marine mammal sources. A marine mammal sampling program of hunter-killed (and presumably healthy animals) and post mortem examination of stranded marine mammals has been on going in arctic and Pacific regions of Canada since the early 1980's and late 1990's respectively, and has provided some valuable insights regarding the epidemiology of marine mammal *Brucellae*. Specifically, serological testing using a *Brucella*-specific C-ELISA assay method has documented *Brucella* exposure in both phocid and cetacean species. Results from these investigations indicate that marine mammal brucellosis is widespread in Canadian waters affecting virtually all species tested. Prevalence is high and in some areas may approach 50%. Twelve isolations have been made from various species of

seals and beluga (*Delphinapterus leucas*) throughout Canada and all appear identical based upon their biochemical testing and characterization. The impact of brucellosis on endangered marine mammals is largely unknown but evidence is accumulating that it may be contributing to the decline of some fragile stocks. Canadian examples will be presented.

PO1- HUMAN-*Brucella* INTERACTIONS: SPECIES SPECIFIC PERSPECTIVES.

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A dynamic relationship exists between the human host and the bacterial pathogen during Brucellosis infection. Eukaryotic gene expression has an influential role in the clearance or persistence of various *Brucella* species within the host. An *in vitro* infection model was used to observe differential gene expression of the macrophage when infected with two different species of *Brucella*. *Brucella* species were chosen based on their dissimilar abilities to infect humans in nature. Human infections with *Brucella melitensis* 16M are severe in pathogenesis and human cases of infection have been widely reported; conversely, *Brucella ovis* has not been reported to cause human infection and pathogenesis is, at most, limited. Differentiated U937 macrophages infected over time with *B. melitensis* 16M and *B. ovis* exhibits divergent patterns of bacterial persistence and clearance, respectively. The bacterial clearance or persistence may, in part, be influenced by host macrophage genes that are differentially expressed when macrophages are combating *Brucella* infection. Observing the differentially expressed genes correlated to human persistence and clearance of *B. melitensis* and *B. ovis*, respectively, will provide insight into cellular mechanisms involved in the innate immune defense. These identified mechanisms could become molecular targets to improve host clearance of and protection against *Brucella* infection.

PO2- SUBVERSION AND UTILIZATION OF THE HOST CELL CYCLIC ADENOSINE 5'-MONOPHOSPHATE/PROTEIN KINASE A PATHWAY BY *Brucella* DURING MACROPHAGE INFECTION.

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Brucella spp are intramacrophage pathogens. Therefore, the macrophage response to infection has important consequences for both, the survival of phagocytozed bacteria and the further development of host immunity. Very little is known about the macrophage cell signaling pathways initiated upon infection and the virulence strategy that *Brucella* use to counteract these responses and secure their survival. In a previous study, we have shown that macrophages activated by SR141716A, a ligand of the cannabinoid receptor CB1, acquired the capacity to control *Brucella*. We observed that the CB1 receptor-triggering engages the microbicidal activity of phagocytes. As CB1 receptor belongs to the family of G-protein-linked receptors, we explored the cAMP signaling pathway. We showed that SR141716A activity implicate this pathway. Taking advantage of this result, we then demonstrated that *Brucella* infection elicited a stimulation of the cAMP pathway which resulted in prolonged activation of PKA and phosphorylation of the transcription factor CREB. Using specific inhibitor of the PKA pathway, we demonstrated that the activation of the cAMP/PKA pathway was crucial for the survival of *Brucella* within macrophages. Furthermore kinetics experiments which these inhibitors showed that the establishment of the bacteria at the early steps of infection required PKA activation. Preliminary results obtained with endosomal markers, suggested that the formation and nature of the phagosomal compartments in which *Brucella* resides are highly dependent of the activation of the cAMP/PKA pathway. We thus characterized, a primordial virulence strategy of *Brucella* involving

the host-signaling pathway, a novel point of immune intervention of this virulent pathogen.

PO3- HUMAN NATURAL KILLER CELLS IMPAIR THE INTRAMACROPHAGIC DEVELOPMENT OF *Brucella suis* BY A CELL-TO-CELL CONTACT DEPENDENT MECHANISM.

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The murine model is the most common model used to study *Brucella* infection. If mice are sensitive to *Brucella* spp, they develop diseases quite different from those observed in humans or domestic animals. These differences probably result from host immune response, suggesting deficiencies in the model. NK cells are implicated in early immune response to a variety of pathogens, they mediate innate protection and are capable of rapidly producing IFNgamma as well as lysing specific target cells. NK cells do not play a role in the control of *Brucella* infection in mice, a result rather surprising, since IFNgamma is a key cytokine which limits the development of *Brucella*. It is known for a long time that NK cell activity is impaired in patients developing brucellosis and human NK cells mediate the killing of various intramacrophagic bacteria. Therefore, NK cells could differently control infections in humans and mice. This prompted us to analyze the behavior of *B. suis* infecting human macrophages in the presence of syngenic NK cells. Our findings provide evidence that 1) NK cells impair the intramacrophagic development of *B. suis* , a phenomenon enhanced by NK cell activator, as IL-2; 2) NK cells cultured in the presence of infected monocytes are highly activated and secrete IFNgamma and TNF-alpha; 3) the impairment of bacterial multiplication inside infected cells is not (or poorly) associated with the production of cytokines which occurred during the early phase of infection of macrophages co-cultured with NK cells, 4) a direct cell-to-cell contact is required for NK cells to mediate the inhibition of *B. suis* development, this inhibition did not require the interaction of FasL and Fas. Works are in progress to determine mechanism(s) whereby human NK cells inhibit the intramacrophagic development of *B. suis.*

PO4- LPS FROM ENTEROBACTERIA AND *Brucella* SHOW DIFFERENT SIGNALING PROPERTIES ON TOLL-LIKE RECEPTORS.

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Toll-like receptors (TLR) are a family of microbe-detecting receptors involved in the innate immune response due to their ability to trigger an intracellular signaling cascade which leads to the activation of a number of proinflammatory genes regulated by the transcription factor NF-kB. TLR are currently considered as the cell receptors for LPS, but as the endotoxic effect of different LPS may differ, the question arises as to the structural features that allow productive binding of LPS on TLR. To address this issue, an ectopic expression system was developed in HEK

cells transfected with cDNAs encoding TLR-4, TLR-2, CD14 and MD-2, together with a reporter gene 5 x NF-kB coupled to the firefly luciferase gene. The assay of the kB-transcriptional activity elicited by LPS from *E. coli*, *Brucella melitensis*, *Brucella abortus*, *Yersinia enterocolítica*, and *Ochrobactrum anthropi* was addressed in this sytem. LPS from enterobacteriae such as *E. coli* and *Yersinia enterocolítica* produced a significant increase of kB-transcriptional activity on cells expressing TLR-4, CD14 and MD-2, whereas the remaining LPS failed to do so, even though the cells coexpressed TLR-2 as well. Attempts to address by ribonuclease protection assay some of the sets of genes, the expression of which could be influenced by LPS, showed a strong induction of chemokines (MIP-1a, MIP-1b, IL-8, and RANTES) by enterobacteria LPS, whereas a weaker expression was observed in response to *Brucella* spp and *Ochrobactrum anthropi* LPS. These data indicate that the different chemical structures of LPS might explain the induction of distinct proinflammatory effects through TLR or through other signalling pathway(s).

PO5- *Brucella abortus* ACTIVATES DENDRITIC CELLS AND SPLENOCYTES TO SECRETE IL-12P40 AND TNFa VIA DIFFERENT TOLL-LIKE RECEPTORS.

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Cattle and humans are susceptible to infection with the Gram-negative intracellular bacterium *Brucella abortus* (BA). Heat-killed BA (HKBA) is a strong Th-1 adjuvant and carrier. Previously, we have demonstrated that dendritic cells (DC) produce IL-12 in response to HKBA stimulation. In the present study, we use knockout mice and in vitro reconstitution assays to examine the contribution of signaling by Toll-like receptors (TLRs) and their immediate downstream signaling initiator, MyD88, in the activation of DCs following stimulation by HKBA. Our results show that HKBA-mediated induction of IL-12p40 and TNFa is dependent on the adapter molecule MyD88. To identify the TLR involved in HKBA recognition, we examined HKBA responses in TLR2 and TLR4 deficient animals. In serum, TNFa responses to HKBA were TLR4-independent; however, the response in TLR2 deficient mice was significantly delayed and reduced, although not completely abolished. Interestingly, the IL-12p40 response to HKBA, as well as the upregulation of CD40, CD80, and CD86 in DC, is both TLR2- and TLR4-independent, suggesting that other TLR may mediate HKBA recognition in these APC. Studies using reporter cell lines confirmed the results seen with knockout mice, namely TLR2, but not TLR4, can mediate cellular activation by HKBA. In addition, HEK293 cells, which do not respond to HKBA, were made responsive by transfecting TLR2, but not TLR1, TLR4, TLR6 or TLR9. Taken together, our data demonstrate that MyD88-dependent pathways are crucial for DC activation by HKBA and that TLR2 plays a role in TNFa, but not IL-12p40 pathways activated by this microbial product. Ongoing studies are aimed at identifying the possible molecular partners for DC recognition of HKBA.

PO6- THE ROLE OF HUMAN GAMMA 9 DELTA2 T CELLS IN INNATE IMMUNITY AGAINST INTRACELLULAR *Brucella*.

Jane Oliaro, Sherri Dudal, Janny Liautard, Jean-Baptiste Andrault, Jean-Pierre Liautard and Virginie Lafont. Institut National de la Santé et de la Recherche Médicale Unité 431, Laboratoire de Microbiologie et Pathologie Cellulaire Infectieuse, Université de Montpellier II, Montpellier, France.

Human gamma 9 delta2 T cells can contribute to immunity against intracellular pathogens through the release of soluble factors and direct cytotoxic activity against infected host cells. Here, we have used a co-culture model of human gamma 9 delta 2 T cells and *B. suis*-infected autologous macrophages to clarify the mechanisms used by gamma 9 delta 2 T cells. We found that: 1) gamma 9 delta 2 T cells, cultured in the presence of *B. suis*-infected macrophages, produce soluble factor(s) that have an effect on bacterial numbers; 2) Proteins of the granule exocytosis pathway are not responsible for this effect; 3) Fas-activated macrophage death is associated with a reduction in intracellular *Brucella*; 4) IFN-gamma and TNF-alpha, produced by gamma 9 delta 2 T cells, have no effect on bacterial numbers; 5) Pre-treatment of macrophages with gamma 9 delta 2 T cell supernatant prior to infection, results in a dramatic reduction in the number of intracellular bacteria; and 6) Soluble factors produced by gamma 9 delta 2 T cells can directly affect *Brucella* viability in the absence of host cells. Our results suggest that gamma 9 delta 2 T cells can orchestrate an immediate innate immune response that benefits the host by limiting the spread of intracellular pathogens.

PO7- DO CD8 T CELLS HAVE A ROLE IN CONTROLLING *Brucella abortus* INFECTIONS?.

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Acquired resistance to *Brucella abortus* clearly involves both antibodies and T cells initially following infection, however experimental evidence indicates that T cells are the component of the immune system involved in clearance. Moreover, the T cell cytokine interferon-g is crucial for survival of a brucella infection in the murine model. The relative contribution of CD4 and CD8 T cells to control and clearance of the infection remains unclear. Several studies evaluating control of the vaccine strain 19 injected intravenously suggest CD8 T cells are crucial for control of this attenuated strain. By contrast, one study shows a similar role for CD8 and CD4 T cells in control of the virulent field strain *B. abortus* 2308 injected intravenously while a study from our lab shows no role for CD8 T cells when 2308 is injected intraperitoneally. Here we sought to resolve whether the difference in control by different subpopulations of T cells reflected the virulence of the brucella strain or the route of infection. Studies compared the role of major histocompatiblity complex (MHC) class I restricted CD8 T cells with that of MHC class II restricted CD4 T cells following intravenous or intraperitioneal injection of strain 19 infections by using knock-out strains of mice lacking expression of class I or class II molecules. These studies are important for vaccine design since stimulation of CD4 and CD8 T cells have very different requirements due to differences in presentation of antigenic peptides on class I or class II MHC molecules.

PO8- CHARACTERIZATION OF QUORUM SENSING MOLECULAR ACTORS FROM *Brucella melitensis* 16M.

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The regulation of gene expression by Quorum Sensing is based on 3 actors: (i) a diffusible pheromone that increases in concentration as a function of cell density, (ii) an enzyme catalyzing pheromone production (synthase) and (iii) a transcriptional regulator which, when associated with pheromone, can regulate target genes. Quorum Sensing allows bacteria to communicate and coordinate gene expression and therefore controls the behaviour of an entire community.

In culture supernatant of *B. melitensis* 16M, we previously identified a N-acyl homoserine lactone (HSL) type pheromone suggesting that *Brucella* have a Quorum Sensing system. This pheromone negatively regulates the transcription of the *virB* operon encoding type IV secretion system involved in *Brucella* pathogenicity. Therefore study of this communication system might give insights into *Brucella* virulence.

Two coding sequences, *babR* and *vjbR* encoding Quorum Sensing regulators homologues have been identified by different screenings in *B. melitensis*. It has been shown that VjbR is an activator of the *virB* operon. The expression level of *babR*, *vjbR* and *virB* is analysed quantitatively during bacterial growth in wild type, BabR or VjbR mutant backgrounds with or without HSL. For this study, we used promoter of interest-*luxAB* reporter fusions on a replicative plasmid in *Brucella*. Until now, no HSL synthase has been identified in *Brucella*. Based on sequence similarities, an homologue of a novel HSL synthase, HdtS in *Pseudomonas fluorescens*, has been identified in the genome of *B. melitensis*. We investigate the production of HSL by this putative synthase in *Brucella*.

PO9- VirB2 IS REQUIRED FOR THE FUNCTION OF THE *Brucella abortus* TYPE IV SECRETION SYSTEM.

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The *Brucella* Type IV secretion system (T4SS) is encoded by the virB1 – virB12 genes. We found previously that transposon insertions in the virB1 3' region or in virB10 render *Brucella* unable to initiate persistent infection in mice. In *Agrobacterium* VirB2 has been shown to form a pilus with which VirB1 has been shown to associate. In order to determine whether these putative pilus-associated proteins are required for virulence and for function of the T4SS, we generated polar and non-polar mutants of virB1 and virB2, and tested them for virulence. Our preliminary results show that both polar and non-polar virB2 mutants are significantly attenuated for growth in macrophages and in mice. These findings suggest that VirB2 is essential for the function of the *B. abortus* T4SS.

PO10- IivA, A PROTEIN INVOLVED IN THE *Brucella* spp. VIRULENCE.

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According with the analysis of *Brucella melitensis* 16M genome, 22 % of the predicted ORFs have not an assigned function yet (Proc. Natl. Acad. Sci. USA 2002, 99:443). We report the characterization of one of this ORFs, that we named it *iiv*A (involved in virulence gene). This gene encodes for a protein of 11 kDa which presents a high content of alfa-helix, as deduced by prediction of the secondary structure using different algorithms and confirmed by circular dichroism of the recombinant protein. Northern blot analysis suggested that *iiv*A is monocystronic and BLAST homology searches indicated that IivA have uncharacterized homologues in *Sinorhizobium meliloti* and *Agrobacterium tumefaciens*.

Brucella abortus S2308 *iiv*A and *Brucella melitensis* 16M *iiv*A smooth strains shown a severe attenuation both in the BALB/c mice and guinea pig models of infection. When this strains were complemented in *trans* with a copy of the wild type *iiv*A gene, they recovered the full virulence. Using chemical crosslinking and a bacterial two-hybrid system we shown that IivA can assembly in homomultimers. The molecular bases of IivA function can contribute to understand the intimate mechanisms of the *Brucella* virulence.

PO11- THE *Brucella abortus xth*A2 GENE PRODUCT CONTRIBUTES TO RESISTANCE TO OXIDATIVE STRESS *in vitro* BUT IS NOT REQUIRED FOR WILD-TYPE VIRULENCE IN THE MOUSE MODEL.

Michael L. Hornback and R. Martin Roop II. The Brody School of Medicine of East Carolina University. USA.

Upon phagocytosis, *Brucella abortus* is able to reside within host macrophages and survive the decrease in external pH, low nutrient availability, and exposure to reactive oxygen intermediates (ROIs) encountered in the phagosomal compartment. The reactive oxygen intermediates generated by the oxidative burst of host macrophages are toxic to bacterial cells because these ROIs can react with proteins, lipids, and DNA and the accumulation of these damaged molecules results in death. In bacteria, there are two general defense mechanisms that are induced to provide resistance to oxidative killing. The primary mechanism of defense against ROIs involves enzymes that act directly to detoxify ROIs into harmless byproducts. The secondary mechanism of defense includes enzymes involved in repair of oxidatively damaged proteins, lipids, and DNA. Interestingly, studies involving *Salmonella* suggest that the secondary defense mechanisms, specifically DNA repair, may play a more important role in protecting bacteria from the oxidative burst of host macrophages than primary protectants such as catalase. In *Escherichia coli*, the base excision repair pathway is involved with repair of oxidatively damaged DNA. A major component of the base excision repair pathway is exonuclease III, which is encoded by the *xth*A gene. In *E. coli*, *xth*A mutants have been shown to be hypersensitive to exposure of hydrogen peroxide suggesting this DNA repair pathway is necessary for survival of this organism in response to ROIs.

Analysis of the recently sequenced genome of *Brucella melitensis* 16M has revealed the presence of two *xth*A homologs which has been designated *xth*A1 and *xth*A2. An isogenic *xth*A2 mutant constructed from *B. abortus* 2308 exhibits increased sensitivity to DNA damaging agents and hydrogen peroxide *in vitro*, but displays wild-type virulence in cultured murine macrophages and experimentally infected mice. These findings indicate that the *xth*A2 gene product contributes to the resistance of *B. abortus* 2308 to oxidative damage but is not required for wild-type virulence in the mouse model. The contributions of the *xth*A1 gene product to ROI resistance and virulence are presently under investigation.

PO12- ANALYSIS OF *Brucella* ANTIOXIDANT GENES REVEALS THE IMPORTANCE OF DEFENSE AGAINST STATIONARY PHASE ENDOGENOUS REACTIVE OXYGEN INTERMEDIATE ACCUMULATION *in vitro* AND *in vivo*.

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Brucellae are able to infect and replicate within host macrophages despite encountering the oxidative burst, acidic pH, and nutrient deprivation. Enhancing the amount of reactive oxygen intermediates (ROIs) produced during the macrophage burst either by bacterial IgG opsonization or by macrophage activation with interferon gamma fails to eliminate the intracellular brucellae in this cell type in culture. Mutational analysis of the *Brucella abortus* antioxidant genes encoding catalase (*kat*E), superoxide dismutase (*sod*C), and alkyl hydroperoxide reductase (*ahp*CD) has been undertaken, and the mutants assessed for ROI sensitivity *in vitro* and for their ability to resist killing by in cultured murine macrophages and persistence in the spleens of mice. While all three mutants were found to be sensitive to various ROI generating compounds in *in vitro* assays, only the *sod*C and *ahp*CD mutants demonstrated a defect in both their ability to replicate in cultured macrophages and to establish and maintain the typical chronic spleen infection in mice. The *sod*C and *ahp*CD genes are under the control of the stationary phase regulator Host Factor I (HF-1) and require this protein for optimal expression, while *kat*E is HF-1 independent. This link between antioxidant genes and stationary phase implies that defense against the macrophage oxidative burst may be less important to the bacterium's ability to effectively colonize the host cell than the capacity of the organism to detoxify endogenous ROIs which is key to its survival within its environmental niche.

PO13- THE GLUTAMATE DECARBOXYLASE SYSTEM IN *Brucella abortus* IS NOT REQUIRED FOR VIRULENCE IN THE MOUSE MODEL.

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Brucella abortus is an extremely successful intracellular pathogen of humans and domestic animals. This bacterium's ability to cause disease is a direct result of its ability to persist within host macrophages. It has been well documented that the brucellae must withstand exposure to a low pH environment within the phagosome in

order to establish and maintain the chronic infection state. Experimental evidence has shown that HF-I, the product of the *hfq* gene, is required for the efficient entry of *B. abortus* into stationary phase physiology. This physiologic transition is also essential for the maintenance of chronic infection in experimental and natural hosts. One of the consequences on entry into stationary phase for many bacteria is an enhanced resistance to acidic pH, and it is notable that the *B. abortus hfq* mutant Hfq3 displays a pronounced sensitivity to low pH *in vitro* that is only observed during stationary phase. In *Escherichia coli*, HF-I is an RNA binding protein that is required for translation of the mRNA encoding the stationary phase specific alternate s factor RpoS. Although the presence of a functional *B. abortus* RpoS homolog has yet to be experimentally established, 2D gel analysis has shown that > 40 *B. abortus* genes require HF-I for their optimal expression during stationary phase. One of these genes is *hde*A, which encodes a small chaperone that has been shown to be important in acid resistance in the enterics. Immediately upstream of *B. abortus hde*A is an operon consisting of three genes, *gad*B, *gad*C, and *gls*, encoding glutamate decarboxylase, a putative g-amino-butyric acid/glutamate antiporter, and glutaminase, respectively. The predicted products of these genes share significant amino acid identity with the *Escherichia coli* GadB (72.9% identity), GadC (29.2% identity), and glutaminase (36.5% identity). GadB catalyses the irreversible a-decarboxylation of glutamate to form g-amino-butyric acid (GABA), this reaction consumes a hydrogen ion and releases CO_2. The GABA produced in this reaction is then pumped out of the cell with the concomitant import of glutamate. In every organism in which the *gad*BC genes have been studied, these genes play an important role in both stationary phase and general resistance to low pH. In light of the homology shown with *E. coli* genes, and the known function of these genes in enteric organisms, it seems reasonable that the *B. abortus gad*BC*gls* operon products contribute to the survival of *Brucella abortus* in the acidic environment within the macrophage. To determine if *gad*B and *gad*C contribute acid resistance in *B. abortus* and virulence in the host, the polar isogenic *gad*B mutant HB1 and *gad*C mutant HB2 were constructed from virulent *B. abortus* 2308 by gene replacement. The *in vitro* and *in vivo* phenotypes of the *B. abortus gad*B and *gad*C mutants were compared to those of 2308, surprisingly, the gene products did not play a role in either *in vitro* acid resistance, or in long-term survival in the experimental mouse model.

PO14- IRON ACQUISITION STRATEGIES OF *Brucella abortus* 2308 DURING LIFE WITHIN THE MACROPHAGE.

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Almost all bacteria have an absolute requirement for iron, however, usable free iron is severely limited in the environment as well as in host tissues. To combat host iron restriction bacteria employ several mechanisms for iron acquisition. For pathogenic bacteria direct utilization of host iron containing or iron binding proteins is a commonly used strategy. Heme containing proteins and/or ferric dicitrate are often targets for bacterial iron acquisition machinery and can provide the bacteria with an

iron source during infection. Gram-negative intracellular pathogens commonly use specific receptors expressed on their surface to bind heme or ferric dicitrate and subsequently transport these compounds through the outer membrane and into the cell. *B. abortus* 2308 can efficiently utilize hemin or ferric dicitrate as iron sources in vitro, however the importance of these iron sources in the host remains to be determined.

Searches of the *Brucella melitensis* 16M genome sequence revealed the presence of two open reading frames with significant homologies to other genes encoding heme receptors in various pathogenic bacteria. These loci were targeted for mutagenesis in *Brucella abortus* 2308 to evaluate the role these genes play in heme acquisition and virulence in BALB/c mice. Mutations in these genes do not lead to altered growth phenotypes in rich media, however both mutants display dramatic decreases in viability during stationary phase in a low iron minimal medium. These decreases in viability can be relieved by the addition of $FeCl_3$. Both mutants exhibit defective survival and replication in cultured murine macrophages and one mutant, designated HR1703, is unable to maintain chronic spleen infection in experimentally infected BALB/c mice. These experimental findings suggest that heme represents an important iron source for the brucellae in the mammalian host.

PO15- LICENSE TO KILL: *Brucella abortus* ROUGH MUTANTS ARE CYTOPATHIC FOR MACROPHAGES IN CULTURE.

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Rough mutants of *Brucella* are attenuated for survival in animal models. However, conflicting evidence has been obtained concerning intracellular survival. Transposon-derived rough mutants isolated in our laboratory exhibited small, but significant reductions in intracellular survival using an *in vitro* assay. Several recent publications report that rough mutants exhibit increased macrophage uptake relative to their smooth parental strains, and a reduction in numbers at the end of the assay has been interpreted as intracellular killing. In an effort to explore the interaction of *B. abortus* mutants with macrophages, we have monitored uptake of rough mutants and survival *in vitro* using the murine macrophage cell line, J774.A1. The results confirm an increased uptake of rough mutants by 10-20 fold over the uptake of smooth organisms under standard conditions. Surprisingly, recovery of the rough mutants persisted up to 8 hours post infection, but at the point when intracellular replication of the smooth strains was observed the number of rough organisms recovered appeared to decline. When assays were performed in the absence of antibiotic, replication of the rough organisms was evident, and fluorescence microscopy confirmed the intracellular multiplication of smooth and rough organisms. Examination by phase contrast microscopy revealed lytic-death of macrophages infected with the rough mutants, which was confirmed by the release of lactate dehydrogenase (LDH) from the macrophages. Thus, the decline in number of rough organisms was the result of lysis of macrophages and not from intracellular killing. The cytopathic effect is characterized as a necrotic rather than apoptotic cell death based on early LDH release, Annexin V/propidium iodide staining, morphological changes of infected cells and nuclei, and glycine protection. These findings suggest a role for O-antigen during the early stages of host–agent interaction that is essential

in establishing an intracellular niche that maintains and supports persistent intracellular infection resulting in disease.

PO16- MAP KINASE ACTIVATION IN ROUGH AND SMOOTH *Brucella*– INFECTED J774.A1 CELLS: RELATIONSHIP TO VIRULENCE.

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J774.A1 cells challenged with rough *Brucella* expressed higher levels of cytokine mRNAs than smooth *Brucella*-infected cells. Furthermore, they released nitric oxide (NO), a deleterious molecule not synthesized in smooth *Brucella*-infected cells in the absence of gamma-IFN. In eucaryotic cells, one essential branch of cell signalling is the family of MAP kinases (MAPKs), among them p38 and ERK1/2 MAPKs that regulate the transcription of cytokine and inducible nitric oxide synthase (iNOS) genes. We investigated the activation of these kinases in J774.A1 macrophages infected with rough and smooth *Brucella*. Challenges with rough *Brucella* resulted in a powerful and sustained phosphorylation of the p38 and ERK1/2 MAPKs. This phosphorylation was markedly weaker or not observed in cells infected by smooth Brucella. In order to investigate whether MAPK activation was directly involved in NO production in rough *Brucella*-infected J774.A1 cells, iNOS expression was determined in assays performed in the presence or absence of specific inhibitors of p38 and ERK1/2 MAPKs: SB203580 and PD98059 respectively. These compounds inhibited the p38 and ERK1/2 pathways triggered by rough *Brucella* in a dose-dependent manner, these pathways being totally blocked by 25 microM of SB203580 or PD98059, respectively. At 25 microM, SB203580 consistently had no significant effect on the rough *Brucella*-induced expression of iNOS, but PD98059 dose-dependently repressed the expression of iNOS. This demonstrated an involvement of the ERK1/2 pathways in the control of iNOS expression. The findings showed that rough *Brucella* provided a cell signalling leading to one arm of the host antimicrobial defence, which is missing in smooth bacteria infection. This mechanism must be taken into account to explain the virulence associated to smooth and rough *Brucella*

VO1- CALFHOOD RB51 VACCINATION FAILS TO PROTECT BISON AGAINST VIRULENT CHALLENGE.

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The objective of this study was to evaluate the protection provided against exposure to virulent *B. abortus* in pregnant bison that had been vaccinated with RB51 vaccine as calves, either once or three times.

All bison were obtained from the State of South Dakota. The non-vaccinated controls and the one-time RB51 calfhood vaccinates were from a brucellosis-free certified herd. The three-time calfhood vaccinated animals (1st exposure at 6 months, 2nd at 12 months, 3rd at 18 months) were from a brucellosis-infected herd but were serologically negative. The vaccine was supplied by Colorado Serum Co., reconstituted and used according to manufacturer's instructions. RB51 was given subcutaneously at a dose of at least $1x10^{10}$ colony forming units (cfu) (Range 1-3.3 $x10^{10}$), which is the bovine calfhood dose. At approximately mid-gestation, all the vaccinated and control bison were challenged with virulent *B. abortus* strain 2308 (1 $x10^{7}$ cfu) via bilateral conjunctival exposure. Appropriate tissues and fluids were collected from the fetuses, calves or dams for culture.

Protection was defined as a statistical difference between the control and vaccinated groups. Decreases in abortion, decreases in fetal/calf colonization and decreases in maternal colonization were used to gauge protection. There were no differences in the number of live or dead calves between vaccinates and controls. There were no differences observed between the 1x vaccinates, 3x vaccinates or the controls with regards to fetal/maternal colonization. In this study, RB51 failed to provide protection against virulent challenge.

VO2- INDUCTION OF IMMUNE RESPONSE IN GOATS WITH A EXPERIMENTAL DNA VACCINE ENCODING Omp31 OUTER MEMBRANE PROTEIN OF *Brucella melitensis* 16M.

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In this study, we evaluated the ability of experimental DNA vaccine encoding the omp31 protein of *Brucella melitensis* 16M to induce cellular and humoral immune responses in goats. We constructed eukaryotic expression vectors called pTargeTomp31, encoding outer membrane protein (OMP 31) of *Brucella melitensis* 16M , and we verified that this protein was produced after transfection in to the goat muscle cells. pTargeTomp31 was injected intramuscularly three times, at 3-week intervals in a group of 10 goats of 6-12 months of age. pTargeTomp31 induced good antibody response in ELISA . pTargeTomp31 elicited a T-cell-proliferative response and also induced a strong gamma interferon and nitric oxide production upon restimulation with either the specific antigens or *Brucella melitensis* 16M extract. In this report, we also demonstrate that animals immunized with this plasmid elicited a strong and long-lived memory immune response persisted even after the 4 months after the third vaccination. Furthermore, pTargeTomp31 elicited a typical T-helper 1-

dominated immune response in goats, as determined by immunoglobulin G isotype analysis.

VO3- IMMUNE RESPONSE IN CALVES VACCINATED WITH DNA VECTOR ENCODING Cu/Zn SOD PROTEIN OF *Brucella abortus*.

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Brucella abortus is an intracellular facultative bacterium, able to proliferate in the host phagocytic cells, which allows it to avoid extra cellular defence's mechanisms and remain as a chronic form. In Chile bovine cattle is currently being vaccinated with *B. abortus* RB51 and it has a variable efficacy that does not assure a total protection. DNA vaccines are a powerful method of immunization, that has proved to elicit both humoral and cellular immune response. At the moment, new vaccination alternatives have been developed, the so-called DNA vaccines. This study was conducted to evaluate in calves the immunogenicity of a DNA vaccine encoding *Brucella abortus* Cu/Zn superoxide dismutase (SOD), a vector of known immunogenic capacity in mice (accepted Infection and Immunity 71: 2003). Cattle were immunized with expression plasmids in eukaryote cells (pcDNA3.1), either bearing or not bearing the insert for SOD protein gene (*sod*C). As a positive control, calves were vaccinated with *Brucella* RB51. Intramuscular (i.m.) immunization with pcDNA-SOD induced lymphocyte proliferation against SOD recombinant protein and bacterial in similar form to animals immunized with RB51. Also, prevalence of antigenic specific ?d T cells proliferation was observed with pcDNA/SOD vaccine. All experimental heifers groups (vaccinated with pcDNA/SOD and bacterial) developed low level of serum antibodies. Our results suggest that this newly made plasmid from pcDNA-SOD is effective in inducing an immune response against brucellosis in cattle.

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VO4- EVALUATION OF *Brucella abortus* S19 VACCINE STRAINS USED IN INDIA BY BACTERIOLOGICAL AND MOLECULAR TESTS AND VIRULENCE STUDIES IN BALB/c MICE.

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Two *Brucella abortus* S19 commercial vaccine strains used for vaccination campaigns against bovine brucellosis in India and two S19 strains used as a internal reference, were studied by both microbiologic and molecular assays, and tested for virulence in BALB/c mice. The four strains tested were identified as *B. abortus* biovar 1 and all had the characteristics of strain S19, according sensitivity to penicillin and *i* -

erythritol. However, when grew on medium containing *i*-erythritol, all strains tested originated spontaneous *i*-erythritol resistant colonies at mutation rates varying from 1.42×10^{-2} to 1.33×10^{-6}. The S19 characteristic 702 bp deletion in the erythrulose 1- phosphate dehydrogenase gene of the *ery* locus was present in the two S19 strains used as reference but not in the two commercial strains used for vaccination in India. Both commercial strains and one of the strains used as reference were showing reduced virulence in mice. The lack of the 702 bp deletion was unrelated with the mutation rates in the development of *i*-erythritol resistant colonies and with the virulence in mice.

VO5- COMPARISON OF SUBCUTANEOUS VERSUS INTRANASAL IMMUNIZATION OF MICE WITH *Brucella* SUBCELLULAR VACCINES.

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Intranasal immunization of mice with purified *Brucella melitensis* lipopolysaccharide (LPS) as a non-covalent complex with *Neissseria meningitidis* group B outer membrane protein (GBOMP) provided significant protection against disseminated infection of spleen and liver of immunized mice when challenged intranasally with virulent *Brucella melitensis* 16M (Infect. Immun. 2002;70:3324). In the present study we immunized mice either subcutaneously (s.c.) or intranasally (i.n.) with either purified *Brucella melitensis* LPS or with *Brucella melitensis* LPS-GBOMP non-covalent complex vaccine. Two doses of vaccine were given 4 weeks apart. Immunized mice and sham-immunized control mice were challenged intranasally with 10^4 CFU of virulent *B. melitensis* strain 16M, 4 weeks after the second dose of vaccine. The numbers of bacteria in lungs, livers, and spleens of mice were determined at 1, 8, and 12 weeks post challenge. Bacteria were found in the lungs of all mice at 1 week post challenge, but only 20-60% of spleens and livers were infected at that time. At 8 weeks post challenge spleens of 6/15 mice immunized s.c. with purified LPS were infected compared to 15/15 control mice ($p<.001$), 7/15 mice immunized i.n. with purified LPS were infected ($p=.002$), 5/15 mice immunized s.c. with LPS-GBOMP were infected ($p<.001$), and 6/15 mice immunized i.n. with LPS-GBOMP were infected ($p<.001$). The livers of 2/15 mice immunized s.c. with purified LPS were infected compared to 12/15 control mice ($p<.001$), 5/15 mice immunized i.n. with purified LPS were infected ($p=.025$), 2/15 mice immunized s.c. with LPS-GBOMP were infected ($p<.001$), and 6/15 mice immunized i.n. with LPS-GBOMP were infected ($p=.06$). There was no significant difference in the infection of lungs of immunized versus control mice. Similar protection from disseminated infection of spleen and liver of immunized mice were observed at 12 weeks post challenge. These results show that purified *B. melitensis* LPS vaccine, given subcutaneously or intranasally provides significant protection from disseminated infection of spleen and liver of mice, similar to the protection afforded by LPS-GBOMP non-covalent complex vaccine.

VO6- PASSIVE TRANSFER OF PROTECTION AGAINST *Brucella melitensis* 16M INFECTION IN A MURINE MODEL .

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Immunization with *Brucella melitensis* WR201, a live, attenuated purine auxotroph, protects mice against intranasal challenge with *B. melitensis* 16M. We have previously shown that passive transfer of antibody or adoptive transfer of T cells from mice immunized with WR201 protects Balb/c mice against intranasal challenge *with B. melitensis* 16M. To determine the relative role of antibody directed against surface O-polysaccharide (OPS) vs. antibody directed against non-OPS components, we immunized BALB/c mice with WR201 or WRR51, a rough, *wboA* mutant of 16M, collected serum 8 weeks later and purified IgG using a protein A/G column. IgG was transferred to naïve BALB/c mice, which were then challenged intranasally with 16M. IgG from mice immunized with WR201, but not from mice immunized with WRR51, protected mice. To further elucidate the protection against *Brucella melitensis* 16M infection in mice, we did passive transfer of immune nonhuman primate sera to mice and demonstrated protective effects. Data from these studies demonstrate that antibody is an important immune correlate of protection against infection with *Brucella* and demonstrate the utility of our mouse model to screen candidate vaccines in development.

VO7- ORAL IMMUNIZATION WITH WR201, A LIVE, ATTENUATED PURINE AUXOTROPHIC STRAIN OF *B. melitensis*, PROTECTS MICE AND NONHUMAN PRIMATES AGAINST RESPIRATORY CHALLENGE WITH *B. melitensis* 16M.

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Vaccination could provide protection for humans threatened by deliberate use of aerosolized *Brucella* as a bioweapon, which could infect via the respiratory tract and the conjunctiva. Successful vaccine development will require use of models of immunization and challenge by a relevant route, and finding the appropriate balance between safety and protective efficacy. To address these issues, we have orally immunized mice and Rhesus macaques with *B. melitensis* WR201, a purine auxotroph created by deletion of *pur*EK from *B. melitensis* 16M, and challenged them intranasally (mice) or via aerosol (monkeys). Mice given 10^{11} CFU of WR201 became systemically infected. Infection cleared from nearly all animals by 8 weeks post-immunization. Immunization led to dose-related increases in anti-LPS serum antibody and to production of IL-2 and IFN-γ by spleen cells cultured with *Brucella* antigens. Approximately 70% of mice were protected against disseminated infection after intranasal challenge; protection depended on immunizing dose and viability of immunizing organisms. Immunization also led to accelerated clearance of bacteria from the lungs of challenged animals. WR201 was also highly attenuated when given orally to Rhesus macaques, although low numbers of bacteria persisted in lymph nodes 8 weeks after immunization. Immunized monkeys developed serum anti-LPS

antibody and antigen-induced splenocyte production of IL-2 and IFN-γ. On challenge with 16M, 4 of 4 sham-immunized and 0 of 4 immunized animals lost weight and became febrile and bacteremic. At necropsy 8 weeks after challenge, 4/4 sham-immunized and 0/4 WR201-immunized had 16M in their tissues. One colony of WR201 was recovered from a lymph node and a testis of 1 immunized monkey at this time point. These data indicate that deletion of *pur*EK leads to a highly attenuated and immunogenic *Brucella* vaccine strain that is protective in a pathophysiologically meaningful challenge model in both mice and monkeys. The purine auxotrophy evidenced by this strain is a desirable attribute to limit intraphagosomal replication, but may require combination with additional attenuating mutations to enhance safety.

VO8- DEVELOPMENT OF A GENETICALLY MODIFIED *Brucella melitensis* REV. 1 LIVE VACCINE ASSOCIATED TO A DIAGNOSTIC ASSAY ALLOWING DISCRIMINATION BETWEEN VACCINATED AND INFECTED SHEEP.

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The live *Brucella melitensis* Rev. 1 strain is the best vaccine available for the prophylaxis of brucellosis in small ruminants. Vaccination by conjunctival route, associated to conventional serological tests, based on the use of LPS-S antigen, has resulted in a significant regression of the prevalence of the infection in small ruminants, particularly in France. We have focused our interest on the development of vaccine strains, at least as effective as the existing live attenuated vaccines, and allowing the discrimination of *Brucella* infected and vaccinated sheep. Vaccines strains were produced by deleting the chromosomal genes of Rev. 1 strain encoding BP26 and/or Omp31. These two proteins have a potential value for the diagnosis of *B. melitensis* and *B. ovis* infection respectively. No change in phenotypic and genetic characteristics was observed for any of the mutants. The residual virulence and immunogenicity of these strains in mouse and sheep were not different to those of the Rev. 1 strain. The CGV26 mutant, deleted in the *bp26* gene, and CGV2631 mutant, deleted in both the *bp26* and *omp31* genes, conferred significant protection, equivalent to the Rev. 1 strain, against *B. melitensis* and *B. ovis* infection in sheep. A diagnostic test, based on ELISA using the recombinant BP26 protein, was developed. Sheep vaccinated with the mutants did not induce an antibody response against BP26, whereas unvaccinated infected sheep developed a positive response. The combined use of the CGV26 vaccine and a BP26 ELISA in the field should allow the differentiation between vaccinated and infected animals, and therefore speed up the final eradication of sheep brucellosis.

VO9- WANING *trans* COMPLEMENTATION OF ROUGHNESS IN A *Brucella melitensis wboA purE* DUAL MUTANT: A POTENTIAL FOR LIVE VACCINES AND HETEROLOGOUS ANTIGEN DELIVERY.

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Brucella melitensis strain WRRP1 was constructed by deleting *wboA* and *purEK* loci in the *B. melitensis* strain 16M genome by allelic exchange. This rough purine auxotroph survived in significantly lower numbers in human monocyte-derived macrophages (MDMs) compared with either the Δ*wboA* parent strain WRR51 or with Δ*purEK* strain WR201. WRRP1 was also severely attenuated for virulence in BALB/c mice after intraperitoneal or oral introduction, where it persisted for less than one week. Provision of intact *wboA* on a pBBR1-derived plasmid in WRR51 restored both smoothness and a level of replication in MDMs indistinguishable from that of strain 16M. As expected, like *trans* complementation of *wboA* in WRRP1 restored smoothness and led to increased survival in MDMs in numbers not statistically different from those of Δ*purEK* strain WR201. However, a small subset of colonies recovered from MDMs infected with *wboA*-complemented WRRP1 lost the complementing plasmid and reverted to the rough phenotype. Plasmid loss was replicated in broth culture and preceded to completion in passage over two months. Loss of the complementing plasmid was also observed in colonies recovered from BALB/c mice orally infected with complemented WRRP1. Complemented WRRP1 persisted in the spleens of these mice for six weeks, compared with clearance by one week of the uncomplemented dual mutant. The duration of spleen persistence was not unlike that of Δ*purEK* strain WR201, which in most experiments was cleared before eight weeks. However, complemented WRRP1 persisted in spleens in significantly lower numbers at all timepoints than was seen with WR201. This reduction in bacterial load indicated loss of the complementing plasmid in a subpopulation of the bacteria within the host, resulting in uncomplemented WRRP1 attenuation and rapid clearance. The extended persistence of *wboA*-complemented WRRP1 in lower numbers in the host offers the potential for a safer live vaccine alternative to strain WR201 that may retain the protective qualities of the Δ*purEK* genotype. Loss of the complementing plasmid and reversion to roughness also provides a period of exposure of rough surface antigens within the host that may enhance immunogenicity. Additionally, *wboA*-complementation of WRRP1 clearly provided selective pressure for retention of the complementing plasmid; this selection was most pronounced in mice and MDMs, but was even observed in broth culture. The strong in vivo selection for the complementing plasmid can be exploited to maintain the expression within mammalian hosts of antigens from heterologous pathogens co-delivered on the plasmid in WRRP1, or a derivative live attenuated *Brucella* carrier. We demonstrated this potential of our system for foreign antigen delivery in vaccinees by expressing a heterologous gene encoding Green Fluorescent Protein from the *wboA*-complementing plasmid in BALB/c mice over a six-week period.

VO10- DEVELOPMENT OF *Brucella abortus* STRAIN RB51 AS AN EXPRESSION VECTOR FOR HETEROLOGOUS EUKARYOTIC AND VIRAL PROTEINS AND AS A CARRIER FOR AIDS VACCINE.

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HIV-1 infection is associated with a loss in T-helper cell responses prior to onset of AIDS. Therefore, therapeutic vaccines should be T-helper cell independent. Earlier, we demonstrated that heat-killed *Brucella abortus* conjugated to a V3-loop peptide from HIV-1 elicits neutralizing antibodies and CTL even in mice depleted of CD4+ T-cells. Currently we are attempting to express heterologous eukaryotic and viral genes in *B. abortus* RB51. It was previously reported that RB51 can express heterologous bacterial proteins and mice vaccinated with these recombinants developed Th1-like immunity against the expressed proteins. HIV-derived proteins (gag and pol) and ovalbumin (OVA) were selected for expression in strain RB51. These genes were cloned into three *B. abortus* expression vectors: pBBSODpro and pBBgroE under the *sod*C and *gro*E promoter sequences for constitutive expression; and pNOF100 under the tightly regulated inducible *tac* promoter. The expression of the cloned proteins was analyzed in whole cell extracts of *B. abortus* RB51 by Western blotting. It was possible to obtain low level expression of pol in pBBSODpro and improved expression in pNOF100 when grown in LB medium supplemented with glycerol, but not in TSB medium. OVA was only expressed in pNOF100 when grown in LB medium supplemented with glycerol. Optimal expression of eukaryotic genes in *Brucella* will most likely require selective codon optimization of the foreign genes, and possibly further modifications of expression vectors.

VO11- PROTECTION AGAINST *Neospora caninum* IN A GERBIL MODEL USING *Brucella abortus* STRAIN RB51 EXPRESSING *N. caninum* PROTEINS.

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N. caninum, the protozoan parasite, is of emerging importance as a cause of abortions in cattle. The attenuated *B. abortus* strain RB51 has been used as an effective vaccine for cattle brucellosis. Strain RB51 has also been used as a vector for heterologous protein expression. It was hypothesized that putative virulence factors of *N. caninum* could be expressed in strain RB51 to develop a combined vaccine for neosporosis and brucellosis. The GRA7, SRS2 and MIC3 genes of *N. caninum* were cloned separately into plasmid pBBR1MCS downstream of the *Brucella gro*E promoter and used to transform *B. abortus* strain RB51. The recombinant RB51vaccine strains were inoculated individually and in combination into groups of three gerbils. Each gerbil received intraperitoneally a primary dose of $6x10^8$ CFU/ml followed by a booster dose of $2x10^6$ CFU/ml administered four weeks later. Five weeks post immunization, the gerbils were challenged with $2x10^6$ *N. caninum* NC-1 tachyzoites. Four out of five gerbils survived in the groups administered GRA7 and SRS2 alone. Two out of three gerbils survived in the MIC3

group. Five out of five gerbils survived in the groups where all three antigens were combined and in the groups where GRA7 was combined with SRS2 and with MIC3. Brain, spleen, lung and liver tissues were fixed in formal saline and subjected to histopathological analysis. Tissues that had the largest number of lesions per field of examination were awarded the highest scores. The lowest score was found in the GRA7, MIC3 combination group followed by the SRS2, MIC3 group. These preliminary results indicate that this approach may be a practical method for prophylaxis against *N. caninum*.

GO1- GENOME COMPARISONS OF THREE *Brucella* SPECIES: *B. suis*, *B. melitensis* AND *B. ovis*.

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We report the complete genome sequence and analysis of a third *Brucella* species, *B. ovis*, an ovine isolate that is not demonstrated to be pathogenic in humans. The genus *Brucella* has been shown to be highly homogeneous with a > 95% nucleotide identity. Our comparisons of *B. suis* and *B. melitensis* revealed a 98-100% identity in over 90% genes with general synteny or conservation of gene order. Further comparisons reveals general regions of identity and a limited number of unique and other hyper-variable regions. A three-way genome comparison including *B. ovis* is likely to reveal factors accounting for differences in pathogenic nature of these organisms, and other insights into the evolution of their parasitic strategies in different hosts.

GO2- THE *Brucella* ORFeome PROJECT.

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The complete sequencing of several *Brucella* genomes opens the way to post-genomic studies, such as DNA microarrays, systematic disruption of predicted genes, proteomics, interactomics, etc.... Most of these studies are dependent on genomic ressources. A collection of predicted coding sequences (pCDS), also called ORFeome, may be one of these ressources. The ORFeome is constructed by the PCR amplification of pCDS, by adding *att*B1 or *att*B2 sequences allowing recombination using the integrase of lambda phage (Gateway system, Invitrogen). The PCR fragments are cloned in an entry vector having *att*P1 and *att*P2 sites using the recombination strategy. Pools of recombinant *E. coli* clones are obtained for each pCDS on the adequate selection medium. Each pool is sequenced to check that the pCDS inserted is the excpected one. The first steps towards the *Brucella* ORFeome have been made, first by a massive correction of the predicted translation start sites in the *B. melitensis* genome, and also by a pilot analysis using 100 pCDS. The initial results of this pilot analysis (J.-M. Delroisse et al., unpublished) suggests that the experiment is feasible a genomic level. Destination vectors such as those allowing complementation of mutants in *Brucella*, yeast two hybrid or tagging of proteins (Van Mullem et al., Yeast 2003, 20:739), are constructed. Beyond the ORFeome itself, the ressource will be particularly useful for interactomics, as suggested by recent analyses of two-components systems in *B. melitensis*, and for the low-cost generation of DNA microarrays.

GO3- ANALYSIS OF THE UREASE GENE CLUSTERS IN *Brucella spp*.

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Most members of the genus *Brucella* are characterised for possessing a strong urease activity. Analysis of the complete genome sequences revealed the

presence of two different urease clusters, conserved in the three main species. The sequences are highly conserved, except for a shorter *ureE* gene in *B. suis* cluster 1. We have isolated several mutants deficient in urease activity from a Tn*5* mutant library of the *Brucella abortus* strain 2308. All the mutants isolated carry a single insertion of Tn5, suggesting that only one enzyme is responsible for urease activity. To ascertain the contribution of each cluster to urease activity we have deleted by homologous recombination most of the gene codifying for the main structural subunit, *ureC*, in both clusters and in the three species, *B. abortus*, *B. suis* and *B. melitensis*. Measurement of urease activity in the parental strain, as well as the single and double mutants shows that in the three species tested, the only active cluster in laboratory conditions is cluster 1, as deduced by the initial mutagenesis. However, the activities differ greatly between strains. Given the similarities at the DNA level, we hypothesize the different activities depend on the presence or activity of regulatory proteins.

GO4- SYSTEMATIC DISRUPTION OF GENES CODING FOR TRANSCRIPTIONAL REGULATORS IN *Brucella melitensis* 16M.

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The genome sequence of three species of *Brucella* is now available (*B. melitensis*, *suis* and *abortus*). That information opens the way of the post genomic approach (transcriptome, proteome, interactome, etc …). We performed the systematic disruption of a category of genes coding for transcriptional regulators, the control of *Brucella* virulence genes being poorly documented. First, all *B. melitensis* 16M transcriptional regulators predicted were grouped into families based on sequence comparisons (essentially the helix-turn-helix signatures). We predicted 180 transcriptional regulators divided into 18 families. We selected 10 families that include regulators involved in virulence and/or symbiosis in other bacteria (AraC, ArsR, Crp, DeoR, GntR, IclR, LysR, MerR and TetR families). We constructed mutants for the ten families (94 regulators) by integrative disruption in *B. melitensis* 16M. The virulence of the mutants was tested in the BALB/c model of infection using a method based on STM (signature-tagged mutagenesis). We already identified 7 transcriptional regulators playing a role in *Brucella* virulence. This library of mutants is also very useful to study promoters of genes of interest in *Brucella*.

GO5- IDENTIFICATION OF A TRANSCRIPTIONAL REGULATOR INVOLVED IN *Brucella melitensis* FLAGELLAR GENE EXPRESSION.

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Even though brucellae are described as non motile, the *Brucella* genome contains three clusters of flagellar genes on the small chromosome. To identify an *in vitro* culture condition which was appropriate for stimulating expression of flagellar genes, we tested the effect of growth stage on flagellar gene expression by using a

*fli*F promoter-*lac*Z reporter fusion. FlgE (hook monomer) and FliC (flagellin) expression was also analysed by immunoblotting with specific polyserum.

The flagellar clusters contain a gene encoding a potential transcriptional regulator similar to response regulators of two component regulatory systems. A mutant of this coding sequence named 2C2 was constructed by integrative disruption. We studied the induction of the *fli*F promoter as well as expression of FlgE and FliC in this mutant and demonstrated that 2C2 is necessary for expression of these flagellar genes. The mutant was complemented in trans with a wild type copy of the ORF cloned downstream of its own promoter on a pMR10 replicative plasmid. The residual virulence of the 2C2 mutant was evaluated in BALB/c mice.

GO6- A MAJOR GENE CONTROLLING NATURAL RESISTANCE TO BOVINE BRUCELLOSIS.

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Twenty percent of three way cross cattle (Hereford, Jersey, & Brahman) were naturally resistant to a challenge of 10 million cfu's of Strain 2308 *Brucella abortus*. In searching for candidate genes that could be controlling this naturally resistant phenotype, bovine SLC11A1 (formerly NRAMP1) was shown to have a major effect on the natural resistance. In subsequent studies of SLC11A1, several characteristics of this gene have been identified: There are no consistent coding region differences between resistant and susceptible cattle. The gene product is highly conserved among mammalian species but the effect of the gene in each species is dramatically different. Even in closely related bovidaes (domestic cattle and American bison) the divergence of the gene is dramatic. We will present data supporting the proposal that differences in resistance and susceptible forms of the bovine SLC11A1 gene is in the regulatory gene sequences. These studies demonstrate the usefulness and limitations in comparative genetic studies and animal modeling in studying bovine brucellosis. In developing studies of the utility of using natural resistance to control brucellosis in Irish cattle, a preliminary study of nine Irish Holstein bulls at an AI stud, eight of the nine had a resistant form of bovine SLC11A1.

1- VALIDATION OF DIAGNOSTIC TESTS TO DETECT BRUCELLOSIS AND EPIDEMIOLOGICAL SURVEY IN THE ECUADORIAN ANDES.

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An epidemiological survey to determine the prevalence of brucellosis in cattle and farm workers was carried out in 23 farms in the Ecuadorian Andes between October 2002 and April 2003. Sera from dairy cattle (n = 516) and humans (n = 98) were tested with Rose Bengal (RB), Slow Agglutination of Wright (SAW), with EDTA, Complement Fixation test (CFT) and indirect Enzyme-Linked Immunosorbent Assay (iELISA). In addition, the brucellosis skin test (ST) using the purified allergen (Brucellergen, Synbiotics) was applied to the animals. Bacteriology was preformed on 103 (27 animals) milk samples collected in infected farms.

Bayesian analysis using the results of four tests (RB, SAW-EDTA, ELISA and ST) performed in animals guaranteed not vaccinated (n = 99) was carried out. Given a ST specificity of 100%, the animal prevalence rate was estimated to lie between 35% and 40%. Estimated test sensitivity and specificity were respectively 0.84 and 1 (ST), 0.47 and 0.87 (iELISA), 0.58 and 0.85 (SAW-EDTA), 0.40 and 0.90 (RB).

Brucella abortus biotype 4 was isolated in the milk of 4 animals. One of these samples originated from a B19 vaccinated cow, boosted with RB51. Six persons, living on the infected farms were seropositive for SAW-EDTA, iELISA and CFT. The official record of 183 human cases reported between 1960 and 2000, underlines the possible under reporting of human brucellosis in Ecuador.

Our results will help the "Centro Internacional de Zoonosis" CIZ to formulate recommendations to the "Ministerio de Agricultura y Ganaderia" and the "Ministerio de Salud Pública" with respect to diagnosis and prevention of brucellosis in Ecuador.

2- SURVEILLANCE AND MONITORING OF BRUCELLOSIS IN SLOVENIA.

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Brucellosis is an infectious disease that has been widespread world-wide. Brucellosis is usually caused by *Brucella abortus*, *B. melitensis* and *B. suis*. Brucellosis is manifested by abortion, retained placenta, orchitis, epididymitis (O.I.E. Manual of Standards for Diagnostic Tests and Vaccines. 2000).

Brucellosis in Slovenia is under systematic monitoring and control for years. The monitoring and control are part of annual Order of Ministry of Agriculture, forestry and food of Slovenia. In year 2002 we started with the programme to qualify Slovenia as country free from bovine brucellosis. According to Order 2002 for brucellosis were tested all cattle over 12 months of age except fattening animals, all bulls, all milk producing sheep and goats, all he-goats and rams, all boars and representative sample of breeding sows from selection farms.

In year 2002 were tested 275.611 cattle, 1062 goats, 196 he-goats, 3148 sheep, 938 rams and 2690 pigs in Rose Bengal test (RB). Used tests for serological diagnosis of brucellosis were RB, complement fixation test (CFT), c and Ab ELISA were used. All samples were tested in RB. In CFT were tested all positive sera in RB

and all male animals. Positive cattle in RB were tested also in c and Ab ELISA, other positive animals in RB were tested in cELISA. From all tested cattle in year 2002, 72 animals were positive in RB test. None of them were positive in CFT. In Ab ELISA were positive 3 samples. In cELISA 3 animals were positive but not the same as in Ab ELISA. Only 3 tested pigs were positive in RB and 1 he-goat which were negative in CFT and in cELISA. We shall satisfy the requirements to qualify Slovenia as country free from bovine brucellosis with testing of same number of cattle samples in 2003 as in 2002.

3- PREVALENCE OF BOVINE BRUCELLOSIS IN HERDS SUPPLYING MILK TO LOCAL MARKETS IN GUINEA, THE GAMBIA AND SENEGAL AND ASSOCIATED PUBLIC HEALTH RISK.

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Zoonoses are of particular public health importance in societies that live closely together with their livestock. Brucellosis, in particular, is easily transmitted via raw milk, the predominant consumption pattern in West Africa. In order to assess the importance of brucellosis, 2368 cattle in selected Districts supplying milk to the local markets in Guinea (Dubreka, 17 farms and Boke, 19 farms), The Gambia (Central River Division, 20 farms) and Senegal (Bassin Arachidier, 30 farms) were enrolled in a bovine brucellosis serological herd screening survey (May 2001 - October 2002). All serum samples were screened using Rose Bengal Plate Test (RBPT) and Complement Fixation Test (CFT) as confirmatory test for RBPT positive samples. Bulk milk samples were collected in Guinea and Senegal and subjected to Milk Ring Test (MRT) or ELISA test (BOMMELI®). Farmers were interviewed on their knowledge and awareness of brucellosis.

Results indicate different epidemiological situations for brucellosis in the three countries. Highest mean prevalences of all animals per area surveyed were found in Guinea with 12.7 % in Dubreka and 6.3 % in Boké. These values were considerably lower in The Gambia (1.1 %) and in Senegal (0.6 %). Highest herd prevalences were also demonstrated in Guinea with 16 of 17 herds (Dubreka) and 14 of 19 herds (Boké) testing at least one animal positive. In contrast, 2 of 20 herds were found positive in The Gambia and 3 of 30 in Senegal. Herds tested positive in their bulk milk samples (MRT or ELISA) were always amongst those serologically positive. The knowledge of farmers on the zoonotic character was generally poor. Milk was consumed nearly 100 % non-heated. High prevalences in cattle in Guinea prompted testing of volunteers (herdsmen and herd owners) in the same Districts with seven of 20 people diagnosed positive. This result underlines the high public health risk associated to bovine brucellosis in Guinea.

4- SEROLOGICAL SURVEY ON BRUCELLOSIS IN HUMAN, SHEEP AND GOATS IN CENTRAL PART OF IRAN.

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Brucellosis is one of the most important infectious and zoonotic diseases in Iran. Genus of *Brucella* has seven species including: *B. melitensis*, *B. abortus*, *B. suis*, *B. ovis*, *B. canis*, *B. neotomae* and *B. maris* which two of them (*B. melitensis*, *B. abortus*) are very dangerous in humans and animals in Iran. Brucellosis commonly is transmitted to human by animals and animals' products. The diagnosis of the disease is based on serological and bacteriological methods in Iran. The purpose of this study was to detect the incidence of brucellosis in Yazd province.

This study was carried out in the Abarkouh city of Yazd province of Iran. The populations of human, cattle and sheep of Abarkouh city were estimated about 50000, 8000 and 300000 respectively. Samples: One thousand and two hundred sera samples were taken from sheep (800 samples), goats (200 samples) and human (200 samples) in autumn and winter. Human sera samples were collected from central medical laboratory of Abarkouh city and sera samples of sheep and goats were collected from the farms around of the Abarkouh city. Tests: The samples were tested by using Slide (Rose Bengal) and tube (Wright) agglutination tests.

The results showed seropositive rate in goats, sheep and human were %2/5, %1 and %1/37 respectively. It is generally accepted that brucellosis induces both humoral and cellular immunity responses. The humoral response to brucella infection in human and animals has been subjects of many studies in many countries (1 and 2). Coming to conclusion, the results of our study have revealed the incidence rate of brucellosis in goat is more than human and sheep in central part of Iran.

References

1-Young, E.J. 1991: Serologic diagnosis of human brucellosis: analysis of 214 cases by agglutination tests and review of the literature. Rev. Infect. Dis. 13:359- 372

2-Alton, G.F., Jones, L.M., Angus, R.D. and Verger, J.M. 1988: Techniques for the brucellosis Laboratore. Institute National de la Recherché Agronomique, Paris.

5- DESCRIPTION OF *Brucella melitensis* BODY DISTRIBUTION PATTERN IN DIFFERENT EPIDEMIOLOGICAL SITUATION: MICROBIOLOGICAL SAMPLING IN ERADICATION PROGRAMS.

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112 animals belonging to sixteen sheep flocks suspicious (FCT positive) of *Brucella melitensis* infection were sacrificed and ten tissue samples taken from each one. Samples were analyzed by microbiology cultures in order to describe different microbial distribution pattern in sheep bodies and relationships with epidemiological and serological status of the flock. Special attention was made in very low (less than 1% serological positive reactors) and low (less than 5% positive reactors) prevalence flocks. The results suggest that mammary gland and lymph node biopsy will be the best options to confirm brucellosis infection in all of the epidemiological situations described and that very low positive flocks needs additional studies to asses their infection status.

6- SEROLOGICAL INCIDENCE OF *Brucella*. ANTIBODY IN DOMESTIC ANIMALS AND MAN IN IRAN.

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Brucellosis is one of the most important zoonosis in Iran. To find out the incidence of this disease we have done following study.

The occurrence of *Brucella* – antibody among 3647 sera of the different species of domestic animals and man has been studied in Iran. The percentage of infected animals are as fallow: sheep 4.24 p. 100 (female 4.3 p. 100 – male 2.6 p. 100), goats 2.18 p. 100, cattle 12 p .100 (calves 8.2 p. 100- male 4.7 p. 100- female 16.1 p. 100), horses 0.73 p. 100, swine 17.6 p.10, dogs 4.78 p. 100, buffaloes 5.5 p. 100, man 5.5 p. 100 (male 5. p. 100, fem. 6.8 p. 100). The results of two S.A.T. (Seroagglutination test) and C.F.T. (Complement fixation test) has been compared:

- 79.5 p. 100 of sera positive by S.A.T were also positive by C.F.T.
- 29.3 p. 100 of suspicious sera by S.A.T. were positive by C.F.T
- 9.9 p. 100 of negative sera by S.A.T. were positive by C.F.T.

Because of distribution of the disease in the different species of animals strict control procedures are essential.

7- IMPLEMENTATION OF A VACCINATION PROGRAMME AGAINST ANIMAL BRUCELLOSIS AND THE EFFECT ON THE INCIDENCE OF HUMAN BRUCELLOSIS IN WESTERN GREECE.

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Brucellosis still remains a major problem in the Mediterranean region, particularly in the agricultural and pastoral populations where the livestock-farming is the main source of income. The purpose of this study was to determine the incidence rate of brucellosis in a rural area of Greece before and after the implementation of a brucellosis control programme and to examine, if there is an effect on the incidence of human brucellosis.

The study was conducted in the Municipality of Tritaia in the Prefecture of Achaia in Western Greece and comprised all newly diagnosed human brucellosis cases between January 1997 and May 2003. In the time period from January 1999 till August 2002 a vaccination programme against animal brucellosis was realised by the Veterinary Service of the Ministry of Agriculture and included the gradually vaccination of all not pregnant female sheep aged over 3 months in the specific region. Descriptive methods were used to calculate age and gender specific incidence rates based on the census of 1991 and 2001 of the National Statistical Service of Greece (NSSG) before and after the implementation of the vaccination programme. The study revealed a statistically significant fall in the incidence of human brucellosis after the vaccination programme. In a 27-months period (1/1997-3/1999) there was a total of 140 cases, while only 30 cases were diagnosed in a 41-months period (1/2000-5/2003) in the same region. The study underlines the importance of control programmes of animal brucellosis in the prevention of human brucellosis.

8- EPIDEMIOLOGICAL STUDY ON 581 CASES OF BRUCELLOSIS IN BABOL IRAN 1997-2002.

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Brucellosis is more prevalent in developing countries especially in IRAN. Knowledge of the epidemiologic pattern of the disease may prevent the transmission of disease. From 1997 to 2002 all patients attended to Department of Infectious Diseases of Babol Medical University were entered in this study. Of 581 cases (329 males, 252 females) with the mean age of 31.9±17 years were evaluated. Among them, 111 cases were less than 15 years old. 222 cases (39.4%) was seen in urban regions, and the disease was seen in summer and autumn in 61% of cases. In 350 cases (60.3%), we have not found any risk factors for brucellosis. In 25 individuals, brucellosis was seen in = 2 cases in every family members. 541(93%) cases had acute and subacute presentation and only 384 cases (60%) were diagnosed as having brucellosis for less than one month after the onset of disease. This study shows that because of the lack of any risk factors in more than half of the patients and seasonal trends of this disease in our regions, it seems that consumption of common food, like meat and its products and also ice-cream and other dairy products may cause disease, which must be clarified.

9- COUNTER-EPIDEMIC MEASURES TAKEN IN THE SITUATION OF BRUCELLOSIS EPIZOOTIC WIDELY SPREAD AMONG LIVESTOCK IN SOUTH KAZAKHSTAN.

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Brucellosis morbidity rate in humans is 2,000 to 2,500 per year in the Republic of Kazakhstan. Highest morbidity (400-700 cases per year) has been registered in South Kazakhstan, where stock-raising is the main business. It can be explained by an ongoing (over 10 years) and wide-spread brucellosis epizootic among sheep owned by private farmers. There is an administrative unit in South Kazakhstan, where human brucellosis is found in everyone fourth of five villages. Livestock is continuously infected with brucellosis, as there are many small private farms (having 50 to 200 sheep), common grazing areas for a community of 20 to 30 farmers, and there is no control over barter deals involving sheep. It would be inefficient to take any anti-epidemic measures within one farm having *Brucella*-infected animals and brucellosis patient. Mass vaccination of animals for brucellosis undertaken over the last 5 years has not shown any effect on brucellosis morbidity in humans. Currently, it is not possible to early diagnose brucellosis because of big quantities of sheep in these areas and high costs of livestock preventive examination. It has been found that groups of people and families (5 persons and over) get sick with brucellosis in these administrative units. Local authorities announced quarantine in several villages. These measures were taken in five villages in the course of 1999-2001. No brucellosis cases were registered among the communities and livestock in the following three years. Therefore, the only method to combat brucellosis in the

situation of a long-lasting (chronic) epizootic among livestock is to impose strict quarantine and disinfection measures.

10- ANALYSIS OF SERUM BY ROSE-BENGAL TEST AND STANDARD TUBE AGGLUTINATION TEST FROM 20,663 PATIENTS IN SOUTHEAST TURKEY SUSPECTED OF HAVING BRUCELLOSIS.

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Brucellosis is a significant health issue in Mediterranean countries. The reasons for its endemic occurrence in this region are the excessive consumption of food made with uncooked milk due to traditional eating habits, and the failure to maintain standards of hygiene because of socioeconomic conditions.

Serum from 20663 patients attending our laboratory between August 2001 and December 2003 suspected of having brucellosis was examined by the Rose-Bengal (RB) test, and then positive sera underwent titration by the standard tube agglutination (STA) test. 463 (2.2%) of the 20663 sera tested positive by RB. On STA, 196 (0.9% of the total number) exhibited seropositivity below a titer of 1:160, and 267 (1.29% of the total number) exhibited seropositivity for a titer of 1:160 and higher. The results were assessed according to season based on a 12-month period, and it was determined that the highest assessment was during the summer months (32.3%) with 4688 positive serum samples, of which 73 tested positive on RB. 31 of these 73 were seropositive at a titer below 1:160, and 42 were seropositive at a titer above 1:160.

11- *Brucella* SURVEILLANCE AND CLINICAL SAMPLING AMONG ANIMALS IN SWEDEN.

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Sweden is declared officially brucellosis free (OBF) in cattle and fulfils the requirements on control measures in OBF EU member states. The last case of bovine brucellosis (*B. abortus*) was reported in 1957 and the disease has never been diagnosed in other native animal species.

Brucellosis is notifiable in all animals on the basis of clinical suspicion and suspected cases have to be confirmed serologically and/or by culture. Vaccination is not allowed and eradication measures would be implemented in case of brucellosis. In small ruminants, surveillance is based on serological surveys according to EU-legislation. Also, on a national initiative, serological surveys are regularly performed in cattle and pigs. The diagnostic tests used in dairy herds are tube agglutination, complement fixation (CFT) or milk ELISA. Whereas, in beef cattle, swine, sheep and goats the Rose Bengal plate test (RBT) or CFT is used. Surveillance is practically the same from year to year.

Result 2002: Bulk milk samples were analysed from 3000 (29%) of all dairy herds by use of an indirect ELISA (Svanova, Biotech, Uppsala) for *B. abortus*. Seven herds were found ELISA positive. Individual serum from all lactating cows (n=184) in

these seven herds were analysed by CFT and RBT with a negative result. 3000 individual blood samples from pigs were analysed for *B. suis*. Furthermore, 9305 samples from sheep at 281 holdings, and 695 samples from goats at 24 holdings were tested for *B. melitensis*. Also samples from breeding stations and clinical suspicions were collected, 925 cattle and 1865 pigs. Lastly, 104 samples from dogs (mostly import), 30 from reindeer and 58 from other animals were analysed. All tested negative.

12- SHEEP AND GOATS BRUCELLOSIS IN SICILY (1999-2002).

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Evaluation of the data from the regional eradication project, which covers the entire animal population, shows the development of the disease from 1991 to 2002, using the main epidemiological indicators. Analysis of the work carried out by the veterinary services confirms what we have already seen in cattle. It shows that the most critical point in the health process which has slowed down the eradication process of the disease to date, is the follow up on the flocks in the region. The major risk areas are individuated by using a measure of association, relative risk (RR), and some of the risk factors are also analysed.

The highest incidence of the disease recorded in 2002 compared to 2001, was in the province of Caltanissetta, with an increase of 43.25%. Analysis of the data shows that the increase in the number of new farms which proved positive, is mainly due to an increase in controls.

13- BOVINE BRUCELLOSIS IN SICILY (1999-2002).

C. Di Bella[1], M. Bagnato[2], V. Bonomo[2], G. Vesco[1], G. Tumino[1], F. Prato[1], S. Vullo[1], F. Geraci[1], S. Agnello[1], V. Ferrantelli[1], G. Cascone[1], A. Calabrò[1], S. Caracappa[1]. (1) Istituto Zooprofilattico Sperimentale (Experimental Zooprofilactic Institute) in Sicily "A. Mirri", Sicily, Italy. (2) Regional Ministry of Health, Sicily, Italy.

Evaluation of the data from the regional eradication project, which covers the entire animal population, shows the development of the disease from 1991 to 2002, using the main epidemiological indicators. Analysis of the work carried out by the veterinary services shows the most critical points in the health process which have slowed down the eradication process of the disease to date. In particular this refers to the follow up on the herds in the region. The major risk areas are individuated by using a measure of association, relative risk (RR), and some of the risk factors are also analysed.

The areas which fall under the Catania and Messina (Monti Nebrodi) AUSL (Local Health units) control have the highest prevalence; the infection percentage is 14.9 % of herds and 3.2% of the animals in Catania, while in Messina it is 20.6% of herds and 3% of animals.

14- HIGH PREVALENCE OF SEROVAR 3 OF *Brucella abortus* IN THE REGION OF CANTABRIA (SPAIN).

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Animal brucellosis is still highly prevalent in the region of Cantabria, which is located in the North of Spain. The main incidence is in cattle, where the current estimation of the presence of the disease is that of 0.85%.

We present herein our data resulting from the characterization of a wide number of field isolates of *Brucella* spp, mainly from cattle, collected over the last three years. The identification of species and biovars were determined both by phenotypic and genotypic methods. For the phenotypic characterization we performed the classical techniques addressed to this aim: dyes sensibility, CO_2 requeriment, SH_2 production, phage typing and agglutination with monoespecific sera. The genotypic studies were accomplished by PCR analysis, using oligonucleotides capable of distinguishing the species and or biovars isolated from the different samples. The strains were further characterized by RFLP analysis using the insertion sequence IS711 as genetic marker.

The majority of the strains analyzed proved to be belonging to the biovar 3 of *B. abortus*, and exhibited special genetic features which were not observed in the reference strain for this biovar. On the basis of these results, the possibility of the existence of an endemic clone of *B. abortus* in this region is discussed.

15- ANTIMICROBIAL SUSCEPTIBILITY OF *Brucella canis* ISOLATED IN KOREA.

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Antimicrobial treatment of canine brucellosis has been shown variable results, including relapse of brucellosis. The purpose of this study was to test the *in vitro* susceptibility of 15 antimicrobial agents against 3 and 52 isolates of *Brucella canis* isolated from dogs in Korea in 1994 and 2002, respectively. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were determined using conventional microdilution broth methods. Tetracyclines (minocycline, doxycline) and aminoglycosides (gentamicin, streptomycin) showed greatest suppressive activity against *Brucella canis* (MICs 0.06-0.5 μg/ml). Fluoroquinolones (norfloxacin, ciprofloxacine) and rifampin showed equivalent activity (MICs <1 μg/ml). Lincosamides (lincomycin, clindamycin), macrolides (erythromycin, spiramycin, tylosin), and sulfisoxazole showed least suppressive activity (MICs >32 μg/ml). Interestingly, recently isolated 52 strains showed 16-64 times higher macrolides (erythromycin, spiramycin, tylosin) MICs than 3 strains isolated in 1994. MBC analysis revealed aminoglycosides and fluoroquinolones showed greatest bactericidal activity (MBCs 0.12-1.0 μg/ml), while beta-lactam antibiotics (ampicillin,cephalothin), lincosamides, and sulfisoxazole showed least bactericidal activity (MBCs 16->32 μg/ml). Interestingly, tetracyclines showed highest MBC/MIC ratio (128X). In addition, macrolides showed broad range of MIC and MBC.

Our results showed that fluoroquinolones might be better antimicrobial agent for treatment of canine brucelllosis instead of aminoglycosides/tetracyclines complex, most commonly recommended antibiotics.

16- *Brucella cetaceae* - WHY DOES INFECTION OF DIFFERENT HOST SPECIES EXHIBIT DIFFERENT PATHOLOGIES?.

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A wide range of pathology has been reported for *Brucella cetaceae* infection in cetaceans. The nature and site of the pathology appears to be influenced to some extent by the individual host species involved. In particular sub-acute non-suppurative meningitis is the most frequent finding associated with *Brucella* in striped dolphins (*Stenella coeruleoalba*) but has not been observed in other cetaceae. In contrast harbour porpoise which is the species from which *Brucella* is most commonly found has had the organism recovered from brain on several occasions but the only associated neuropathology thus far has been a suppurative meningitis with numerous abscesses due to *Brucella* also seen in other tissues. Porpoise generally appear more able to cope with *Brucella* challenge, infection more often results in localised chronic infection or no associated pathology is evident. Whether these differences are due to strain variation or properties of the different host animals is unclear at present.

17- SURVEILLANCE OF BRUCELLOSIS IN WILD BOAR IN SWITZERLAND.

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Several economically important diseases, such as brucellosis or classical swine fever, are transmissible between wild boars and domestic animals. In Switzerland the wild boar population has been constantly increasing since the 1970ies and is still increasing. Consequently, diseases introduced into the wild boar population are likely to circulate among wild boars for increasing time intervals before eventually dying out naturally. In addition, wild boars in Switzerland are conquering geographic areas they did not inhabit previously. Further, keeping cattle and swine in housings with open front is becoming more and more popular. Therefore, the risk of transmission of diseases between wild boars and domestic animals is expected to increase.

A surveillance system may allow to identify newly introduced diseases at an early stage. Moreover, the impact of actions in order to prevent the further spread of such diseases can be assessed. Finally, a surveillance system allows documenting the freedom from disease. A surveillance system for diseases in wild boars in Switzerland has been initiated in the frame of a PhD-thesis (January 2001 – December 2003). Blood samples have been collected during two hunting seasons from wild boars shot. Antibodies against classical swine fever, Aujeszky's disease and brucellosis are being detected by ELISA. The results from the first hunting season (pilot study) did not indicate the occurrence of classical swine fever nor of Aujeszky's disease in the investigated regions. However, 0.8% (5/618) blood

samples tested positive for brucellosis (*B. suis*). In order to investigate brucellosis in further detail, the spleen and the reproductive organs (accessory sexual glands and the uterus respectively) have been collected in the second hunting season. Disease prevalence or freedom from disease respectively will be discussed for brucellosis among wild boars in Switzerland.

18- SEROLOGICAL SURVEY FOR REINDEER (*Rangifer tarandus tarandus*) BRUCELLOSIS IN FINLAND.

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Rangiferine brucellosis caused by *Brucella suis* biotype 4 has been reported in circumpolar areas, North America and Russia, but not in Scandinavia. No information is available of the occurrence of rangiferine brucellosis in Russian areas adjacent to Finland. There are 57 reindeer herding co-operative districts in the northern part of Finland. Serological surveys were conducted in 1997-1998 and 2001-2002 to study the occurrence of *Brucella* antibodies in semi-domesticated reindeer. The total number of reindeer in 1997-1998 was about 286 000, while in 2001-2002 the figure was 297 000. 2 307 serum samples from 14 districts and 1 676 samples from 39 of the 57 districts were collected at slaughter in 1997-1998 and 2001-2002, respectively. The samples were tested for *Brucella* antibodies using Rose Bengal Plate Agglutination Test in both surveys. No seropositive samples were detected. The negative results indicate that the prevalence of brucellosis was less than 0.13% in 1997-1998, and less than 0.18% in 2001-2002, with 95% confidence.

19- PREVALENCE OF BRUCELLOSIS IN WILD SWINE (*Sus scrofa*) IN THE REPUBLIC OF CROATIA.

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During 2001 and 2002 on seven localities in Croatia a survey on the prevalence of brucellosis in wild swine was carried out. The survey included 271 (52.7%) female and 243 (47.3%) male animals between 7 months and 4 years of age. On that occasion 514 blood samples of wild swine were serologically analysed. For serological analysis Rose Bengal test (RBT), slow agglutination test (SAT), complement fixation test (CFT) and indirect enzyme immunoassay (iELISA) were used. In all of the wild swine from all of the localites investigated positive reactions to brucellosis were established. Most of the positive reactions were established by iELISA (13.6%), then by RBT (11.5%), CFT (10.5%) and SAT (8.9%). The samples of 134 testes, 39 uteri and 5 fetuses of piglets were analysed bacteriologically. *Brucellae* spp. were isolated from 18 (10.1%9 samples that were collected from all of the localities investigated. The results of PCR using BRU-UP and BRU-LOW primers confirmed that all of the isolates were from *Brucella* spp. Genus, whereas, for the analysis of fragment IS 711 DNA multiplication using two specific primers (IS 711 and *B. suis* - specific primer) was carried out. In all the isolates investigated and in the standard cultures of *B. suis* biovar 1 (1330) and *B. suis* biovar 2 the product of

multiplicatio of approximately 285 bp was established. *B. suis* biovar 2 was identified from the isolates also by traditional bacteriological analysis. On the basis of our investigations it can be concluded that in Croatia wild swine are natural reservoirs of *Brucella suis* biovar 2, representing in this way a permanent and potential source of brucellosis for domestic and wild animals.

20- HUMAN BRUCELLOSIS IN GEORGIA.

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Brucellosis represents one of the major public health problems for Georgia. In the Institute of Medical Parasitology and Tropical Medicine brucellosis registration started since 1949. We reviewed medical charts and epidemiological data since 1993. Information from the charts was abstracted using a standardised questionnaire.

Based on these data we found out that a total of 1282 cases of brucellosis have been registered in our Institute during the last decade. 686 patients out of 1282 were hospitalized. B. primaria accounts 59% of cases, 31% - for B. latenta, 6 % - B recidiva, 4% - statust post brucellosis. Serologically brucellosis was diagnosed in every patient, but bacteriologically only in 17,2% of cases recovered causative agent – *B. melitensis*, biovar III. No other serotypes and biotypes have been detected during the last decade. Eastern Georgia accounts majority of cases (90%), were the source of infection is a sheep, while in the western Georgia source proved to be cows. Seasonal pattern is detected as well. Brucellosis picked in May-June accounting 29% cases. This pattern of seasonality is associated with deliveries of sheep. Most cases among humans were male (91%), aged 20-39 years old (49%). The most common source of infection was infected amniotic fluid of sheep. Clinical presentation of brucellosis tends to become "milder". Compared with previous years, prolonged fever and joint pain rarely are observed, mainly due to antimicrobial therapy. Severe cases commonly are attributed to contacts with goat rather than sheep.

Brucellosis incidence still remains high. Additional efforts are needed to prevent brucellosis among humans and animals in Georgia.

21- CURRENT STATUS OF HUMAN BRUCELLOSIS IN KOREA.

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Brucellosis may imitated as other infections and asymptomatic conditions and therefore, the diagnosis of the disease is like to be missed or delayed because of the wide spectrum of its clinical manifestations. Despite of multi-outbreak of bovine brucellosis during the last decade in Korea (a total 2,700 cows with brucellosis were slaughtered during last 3 years), human brucellosis has very few occurred unusually since the first identification of *Brucella abortus* in 1939. But from the middle of last year to this June, 9 cases of human brucellosis were found among 23 occupational exposurer in 2 different provincial areas where were spread bovine brucellosis at that

time. To confirm the cases, laboratory diagnosis and epidemiological surveillance were performed as well as clinical features were considered. Standard tube agglutination (STA), ELISA, PCR and blood culture were applied for the laboratory diagnosis. Of the 9 blood samples from patients with brucellosis, *B. abortus* were isolated from 3 patients. And the antibody titers to *B. abortus* in convalescent-phase sera tested by STA was distributed between 1:160 and 640. The antibody level had persisted for around 10 weeks but followed by a sharp reduction at two weeks after the end of antibiotic treatment. While in ELISA, even though the new rising of IgM and IgG antibody titers to *B. abortus* were not observed in all patients after the end of antibiotic therapy, we need to monitor the changes of the antibody levels over time because the IgM and IgG antibody remain as high titer up to now. In PCR assay using primer pair derived from the gene coding for a 31-kDa *Brucella* antigen, only 5 of them were PCR positive. Epidemiological background showed that all cases were directly related to occupational activities that seven patients of them were livestock workers and two were veterinarians. One of them had often ingested unpasteurized milk of cow. And the others had ever contacted directly without individual measures to vaginal discharges, aborted fetuses and placentas of infected beef cattles. The main clinical presentations were irregular fever of variable duration, fatigues, weakness, sweats, chills and weight loss. With appropriate antibiotic therapy (streptomycin, doxycyclin), symptomatic therapy the diseases had favourable outcome. These were the first case experience in our laboratory, however, studies for validation of the cases will be needed in relation to epidemiological background, diagnostic procedures and clinical features.

22- *Brucella melitensis* BIOVARS FOUND IN THE REPUBLIC OF KAZAKHSTAN.

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Brucellosis morbidity rate in humans is 2,000 to 2,500 per year in the Republic of Kazakhstan. Serological and bacteriological methods have been used to examine each patient. *Brucella* cultures have been isolated from 75% of blood samples; they are mainly *Brucella melitensis* biovars 1, 2, and 3. Given bacteriological laboratory tests, we monitor the dynamics of *Brucella melitensis* biovars isolated from humans residing in various parts of Kazakhstan. The 1999-2002 monitoring data show that *Brucella* structures (biovars) have been changing in several parts of Kazakhstan. We conducted an epidemiological analysis of such changes for 4 administrative units of Kazakhstan with highest brucellosis morbidity in humans. We noted that percent of *Brucella* biovar 1 went up from 5.7% to 83.6% in the 1st administrative unit; from 50.0% to 90.9%, in the 2nd one; from 46.1% to 61.6%, in the 3rd one; and from 30.4% to 36.7%, in the 4th one. At the same time, the three studied administrative units showed a decrease in *Brucella* biovar 3 from 85.7% to 13.2% in the 1st administrative unit; from 34.2% to 33.3%, in the 3rd one; and from 50.0% to 37.9%, in the 4th one. We have not been able to identify any reasons for such changes in *Brucella* biovars.

23- IDENTIFICATION OF *Brucella* SPECIES ISOLATED FROM PROVEN PATIENTS IN IZMIR, TURKEY.

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In many parts of the world, brucellosis has significantly decreased, but it is still a problem in some regions of Turkey. We studied 11 patients hospitalized in the SSK with a diagnosis of brucellosis. The diagnosis was made on the basis of a clinical picture compatable with brucellosis and one of two laboratory criteria: agglutination titers of =1:160 and isolation of *Brucella* sp. from blood. Serum dextrose agar and brucella agar were used for growing and identification of isolates. For detecting of variation in in-vitro colonies, the methods of stereoscopic microscopy and “crystal violat staining” were used. For species identification, (i) agglutination using monospecific antisera (A and M); (ii) CO_2 requirements of isolates; (iii) detection of urease activation; (iv) detection of H_2S production; and (v) stain inhibition test (fuchsin, thionin) were performed. Three reference strains (*Brucella abortus* 19, *B. melitensis* 16M, *B. suis* 1330) were used for all identification methods. After the identificaton, sensitivity of isolates against antibiotics were determined using MIC values. Ten and one out of 11 isolates were identified as *B. melitensis* biovar 3 and *B. melitensis* biovar 1 respectively. All isolates were found to be sensitive against to Doxycycline, Rifampin, Ciprofloxacin and Cephtriaxone. Only one of 11 isolates were found to be resistant against to Co-trimoxazole.

24- THE SEROLOGIC SURVEY OF BRUCELLOSIS IN ABORTED WOMEN.

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Brucellosis is one of the most important zoonotic disease in Iran with economic and public health hazards. *Brucella* can locolized in different organs and may creat disturbances in many systems of body. Abortion, usually occurs in the animals but there are a little reports that *Brucella* causes abortion in women. So we researched on humoral responses to *Brucella* in aborted women by serological methods. We performed some serological tests including: RBPT, Wright, Coombs and, 2ME tests for detection of anti-*Brucella* antibodies in three hundred aborted women in some hospitals and three hundred women with natural delivery. The results of this study showed three samples of aborted women were positive by RBPT, Wright and Coombs tests but they were negative in 2ME test. All sera samples of women with natural delivery were negative by serological tests. The findings of three patients with positive reaction will be presented at main article. Brucellosis may be regarded as an agent of abortions with unknown etiology in women so in endemic area the serological tests for diagnosis of brucellosis may be useful for evaluation and interpretation of abortion in women.

References

1- Khan, M.Y., Mahm, W. M., and Memish. Z. A., 2001: Brucellosis in pregnant women. J. Clinical infection Disease, 15:32 1172-7.

2- Sharif, A., Reyes, Z. and Thomassen, P. 1990: Screening for brucellosis in pregnant women . J. Trop. Med. Hyg, 93: 1.

25- PRESENTATION OF ACUTE BRUCELLOSIS. A REVIEW OF 144 CASES.

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Human brucellosis is a disease presenting with a variety of symptoms, affecting multiple systems and causing different forms of localized infection. We describe and discuss the presentation of acute human brucellosis during the past 14 years (1990-2003) in an endemic area of Greece. 144 patients presenting with acute brucellosis and hospitalized in Sparta General Hospital - in internal medicine, urology and pediatrics - are described. Their clinical symptoms at presentation, the sites of localization, the laboratory evaluation and therapeutic regiments used are presented, together with a comment on the outcome.

All patients presented with fever, 56% had splenomegaly, 29,1% low back pain, 25,6% hepatomegaly, 24.3% arthritis of large joints (hip, knee, shoulder etsc.), 22% hematological involvement (20% anemia, 4% neutropenia, 2% thrombocytopenia), 6% elevated liver function tests. Two cases with cholecystitis, one with mammary abscess and one with acute abdomen were also treated. Diagnosis was based on history, clinical presentation, Wright agglutination test and blood cultures. 86% of the patients had titters higher than 1/160, and titters 1/160 and lower were associated with positive blood cultures. The overall percentage of positive blood cultures was 79%. The patients were treated with combined regiment of streptomycin - doxycyclin (91%), with alterations for pediatric patients. Occupational history accounted only for 19.8% of the patients.

Brucellosis remains a disease with unpredictable presentation. Proper evaluation of the patient' s history, presentation, laboratory, occupational and epidemiological data can lead to proper diagnosis and treatment.

26- CLINICAL AND IMMUNOLOGICAL FEATURES OF A HUMAN INFECTION BY THE M-STRAIN OF *Brucella canis*.

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Brucella canis is the causative agent of canine brucellosis, but can be transmitted to humans. Symptomatic human infections are rare, probably due to the low virulence of *B. canis*. Since suspensions of wild-type *B. canis* show autoagglutination, a less mucoid laboratory strain (termed M-) has been widely used for diagnosis. Innoculation of high doses of this strain to dogs does not produce the typical signs of brucellosis. The pathogenic potential of the M- strain in humans remains unknown. Here we report a human infection by the *B. canis* M- strain, which produced a disease similar to that produced by wild-type *B. canis*.

A 35-year-old man was referred with recurrent fever, headache, arthralgia, weakness and constipation. The patient worked in a laboratory that produced antigens for diagnostic use. Three weeks before symptoms began, he had been handling a culture of *B. canis* M- without personal protection. The clinical examination

disclosed cervical adenomegaly, and laboratory tests revealed neutropenia and a mild increase of hepatic enzymes. The patient denied close contact with dogs or other animals. Blood samples were drawn for culture, which was positive for *Brucella canis*. Conventional tests for antibodies to smooth brucellae yielded negative results, but slide agglutination for *B. canis* was positive at 1:10 dilution. The patient received oral doxycycline for 42 days and parenteral gentamicin for 10 days, and experienced clinical recovery. Blood cultures performed 2 weeks after the end of antibiotic therapy were negative for *B. canis*. Serological follow-up included slide agglutination and 3 ELISAs performed with a hot-saline extract of *B. canis* M- (HS), LPS-free cytoplasmic proteins of *B. abortus* (CP) or recombinant *Brucella* lumazine synthase (rBLS). Antibodies to all the 3 antigens were detected at the time of diagnosis. Antibodies to CP and BLS reached negativization 103 days after diagnosis and antibodies to HS (and slide agglutination) were negative at 200 days after diagnosis. Evidence of cellular immune response to BLS and CP was also obtained with peripheral blood mononuclear cells drawn at the time of diagnosis. The patient remained asymptomatic at the time of the last clinical control, 4 years after initial diagnosis.

27- IMPORTED CASE OF BRUCELLOSIS COMPLICATED BY LIVER ABSCESS.

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Hepatic involvement is a common feature of *Brucella* infection, but liver abscess is a less frequent complication. We report a case of brucellar hepatic abscess in a 42 year old man with a 4 week complaint of fever, abdominal pain, anorexia, and weight loss. Forty days earlier he was back from a 2 years staying in Albania. A detailed epidemiologic history showed that he consumed, about 2 week before the onset of symptoms, fresh unpasteurized cheese. At admission the patient was febrile (39°C), and tachycardic (100 bpm). Abdomen was diffusely painful, especially in the right upper quadrant. Liver was 2 cm below the costal margin. Blood tests showed mild neutrophilia (6 680 cells x mm^3), anaemia (haemoglobin 10.6 g/dl), and hypoalbuminemia (3 034 mg/dl), ESR 94 mm/h and C-reactive protein 15,1 mg/dl. Abdominal ultrasound scan showed an abscess within the VI hepatic segment of about 55 mm in diameter which was promptly drained. Blood cultures were positive for *Streptococcus anginosus*, but Wright test for *Brucella* was also positive at high titer (>1600). Clinical course was complicated by right sided pleuric effusion that needed evacuation. I.V. ciprofloxacin, doxycycline P.O., and metronidazole P.O. were initially administered. Antibiotic regimen was then switched to doxicicline P.O. and gentamicin I.V. for 6 weeks, accomplishing a progressive improvement of general conditions. *E. coli* and *Streptococcus anginosus* were isolated from the abscess drainage. Any attempt of isolating *Brucella* spp., including biomolecular approaches, failed. Wright serology was repeated at discharge, showing a decrement in titers. A review of published cases was done, finding a brucellar hepatic abscess incidence of some 1%. Since the isolated strains in this patient did not clearly indicate a brucellar etiology, some question could be raised on the real involvement of *Brucella* spp. in this case. The possibility are that normal intestinal flora could reach, by contiguity, the liver. Otherways, the initial brucellar colonization could have been replaced, during abscess evolution, by superinfecting strains that made the

specific diagnosis impossible. The latter mechanism could also explain the low incidence of confirmed brucellar liver abscesses in other series.

28- HUMAN *Brucella* ENDOCARDITIS: REPORT OF TWO CASES.

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Endocarditis is a rare complication of human brucellosis. Diagnosis is not easy, may be delayed, with increasing possibility of therapeutic failure. Two cases of human *Brucella* endocarditis are presented, both managed successfully with combined medical and surgical treatment.

Two male patients 67(A) and 73(B) year-old, cattle-breeders, had been treated for brucellosis for 40 days and 3 months respectively, but they did not improve. They had neither a history of heart disease nor any signs of heart involvement at the time of first diagnosis. Both of them had fever, sweets, weakness, prostration, dyspnoea. Their condition worsened the last days. A strong diastolic aortic valve murmur was appeared. Bacterial endocartitis vegetations and severe aortic valve deficiency were showed at echocardiogram. High *Brucella* serologic titers were found in both patients. Six blood cultures were negative (patient A), whereas peripheral blood PCR for *Brucella* was positive. Two blood cultures revealed *Brucella melitensis* (patient B). Affected valves were replaced surgically. Tissue cultures from vegetations were negative. Cardiac tissue PCR for *Brucella* was positive in patient A. Medical treatment included aminoglucosides, tetracyclines, co-trimoxazole and rifampicin before surgery, followed by tetracyclines, rifampicin and co-trimoxazole. Antibiotic therapy continues for 7 (A) and 6 months (B) after surgery. Patients' physical condition and serology have improved dramatically.

PCR is an alternative laboratory method for diagnosis of *Brucella* endocarditis, especially after prolonged antibiotic therapy, or any time when blood cultures are negative. The management of choice for this severe complication of brucellosis is the combination of valve replacement with a specific multiantibiotic therapy, which should last for a long time postoperatively.

29- THE SIGNIFICANCE OF SEROLOGICAL TESTS IN DIAGNOSIS AND FOLLOWING UP OF NEUROBRUCELLOSIS: CASE REPORT.

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The case report of severe form of Brucellosis named neurobrucellosis with clinical feature of meningoencefalitis and positive epidemiological data: cattle contact was reported. Rapid agglutination test – BAB, classic agglutination test – Wright and immunoenzymatic test – Elisa were used in serodiagnosis of the disease. An increasing antibody titer from 1:320 in the first month to 1:640 in the third month of the disease, regarding brucella antigen was obtained. One year latter, patient doesn't have any symptoms, clinical state and biohumoral status were all right. Serologically, BAB was positive, Wright was 1:20 and ELISA: IgM negative and IgM positive. In conclusion, in our circumstances ELISA represents the most sensitive method for determination the class of immunoglobulins as same as the stadium of

the disease activity. Peripheral-blood PCR assay would have been the highly sensitive and specific as well as rapid and easy to perform and therefore it would be considered the most useful tool for diagnosis of brucellosis.

Key words: neurobrucellosis, serodiagnosis, ELISA.

30- CHRONIC COMPLICATIONS OF BRUCELLOSIS: CASE REPORT.

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The case report of epidemiological, clinical and serological verified brucellosis with osteoarticular manifestations like lumbosacral spondilitis and chronic hepatitis was reported. 33 years old patient with fever of unknown origin and dull pain under the right costal arch.in duration of one month and a half with positive epidemiological data was represented. Epidemiological data showed that he consumed row cheese at the last three months in Kosovo. Diagnosis was confirmed by serological analyses: BAB +. Wright 1:500. Besides appropriate antibiotic (tetracyclines, rifampicin) and symptomatic therapy and the initial recovery, after two months of the discharge from the hospital he complained about back pain, paresthesia of left leg and headache. Electromyoneurographia and electrostimulation showed proximally lesion L5-S1, more manifested in radix L5 on the left. Radiography confirmed lumbosacral spondilitis in level L5-S1. Serological analyses showed BAB +, Wright -. After one year backpain persisted, hepatic ensimes were elevated, BAB persisted + and Wright -. After four years of the beginning of the disease, in February 2003, patient complained again about lumbosacral and neck back pain and dull pain under the right costal arch.There were elevated levels of hepatic enzymes, biopsy showed chronic hepatitis, serologicaly both HBs Ag and anti HCV negative. Ultrasound of liver showed hepatic enlargement and inhomogenous, hypoehogenous liver. Serological analyses showed BAB- , Wright 1:10 , Elisa: IgM – and IgG +. Conclusion: lumbosacral spondilitis and chronic hepatitis stayed as a chronic complications of the brucellosis. It is a reliable evidence that intracellular location of *Brucella* protects bacteria from the effects of antibiotics. Also, *Brucella* organism's display many mechanisms to evade the intracellular killing, wich appear to be the reason for the success of the bacterium in dwelling within macrophages.

Key words: brucellosis, chronic complications, lumbosacral spondilitis, chronic hepatitis.

31- BRUCELLOSIS AND CRIOGLOBULINEMIA.

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A 59-year-old male was admitted for evaluation of a 2-weeks history of progressive abdominal distension, edema and purpuric rash. He had a history of heavy smoking and alcohol abuse. His physical exam was significant for ascites, hepato-splenomegaly, edema and petechiae wich are most prominent on the legs. Laboratory tests showed an anemia with a haemoglobin level of 9.8 g/dl and thrombopenia with a platelet level of 51,000/mm^3, serum creatinine of 1.8 mg/dl, serum albumin of 2.3 g/dL, increase of GGT and FAL. Low levels of C3 and positive rheumatoid factor (184 UI/mL), cryoglobulins 1,4% (polyclonal IgG-IgA-IgM).

Urinalysis was notable for proteinuria 450 mg/24 h and hematuria. Ascitic fluid: WBC 600/mm^3, 95% monocytes and 5% neutrophils. Abdominal ultrasonography: hepatosplenomegaly, ascites and signs of portal hypertension. Skin biopsy: leucocitoclastic vasculitis. *Brucella* spp was isolated of blood cultures and ascitic fluid. Serologic test showed: positive Rose Bengal test, SAT 1:320 and Coombs anti-*Brucella* 1:1280. Treatment with doxycycline, levofloxacin and rifampin during 45 days was administered with resolution of rash, ascites and edema and normalization of renal function and platelet level. An abdominal ultrasonography after therapy showed resolution of ascites and portal hypertension.

Cryoglobulinemia has been described in infectious diseases but rarely reported in brucellosis. Cutaneous, renal and hepatic involvement could be related to vasculitis secondary to cryoglobulinemia by *Brucella* spp. Resolution of disease can be obtained with specific antimicrobial therapy similar to others cases of the cryoglobulinemia secondary to others infections.

32- A CASE OF HUMAN BRUCELLOSIS GIVING NEGATIVE RESULTS IN THE SERUM AGGLUTINATION TEST (SAT)?: HOW TO AVOID THE PROZONE AND BLOCKING PHENOMENA.

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Human brucellosis is an important but often neglected cause of morbidity in many regions of the world. The serum agglutination test (SAT) is the most commonly used serological assay for the diagnosis of human brucellosis but in some cases the correct interpretation of SAT results is hindered by the presence of blocking antibodies of the IgA type. Even in sera from culture-confirmed cases of human brucellosis, these antibodies have been shown both to cause the prozone phenomenon and to produce false-negative SAT results due to the so-called blocking phenomenon. We have demonstrated that the prozone phenomenon can be avoided by means of: 1, pre-absorption of the sera with anti-IgA antibodies; 2, the use of a buffer adjusted to pH 5.0 in the SAT; 3, the addition of dithiothreitol. Similarly, we have proven that the blocking phenomenon can be avoided by: 1, pre-absorbing the sera with anti IgA antibodies, although in this case, a positive SAT result appears only if there is a sufficient amount of agglutinating IgG antibodies; 2, using a buffer adjusted to pH 5.0 and, even better utilizing the antigen provided by the manufacturers of the Brucellacapt (Vircell, Granada, Spain) tests.

Conclusion: IgA antibodies are the responsible for both the prozone and the blocking effects. These two phenomena can be avoided by using a buffer adjusted to pH 5 in the SAT. Interestingly, Rose Bengal test is not affected by these two phenomena and we hypothesize that this fact is due to the acidity of its working solution (pH=3.6).

33- IDENTIFICATION OF SMOOTH AND ROUGH FORMS IN CULTURES OF *Brucella melitensis* STRAINS BY FLOW CYTOMETRY.

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We developed a flow cytometric method to determine the proportion of *B. melitensis* cells displaying surface O-polysaccharide (OPS) in liquid culture. OPS was detected using polyclonal antibodies from rabbits immunized with smooth (S) or rough (R) *Brucella* LPS. First, we evaluated the binding of these antibodies to 16M (S), WRR51 (R) and complemented WRR51 expressing the *wboA* gene (S) as well as to their corresponding GFP-expressing derivative strains 16M/GFP, WRR51/GFP and WRR51/GFP+*wboA*. The rough mutants did not react with anti-S-LPS nor did the smooth strains react with anti-R-LPS. Second, using different ratios of 16M/GFP and WRR51/GFP, we were able to detect the presence of 1% or fewer rough bacteria spiked into a sample of smooth organisms. Third, we evaluated the purity of cultures of *B. melitensis* strains grown in a fermenter. Finally, we sequentially examined the spontaneous loss of green fluorescence and surface OPS in stationary phase cultures of smooth *B. melitensis* strains 16M/GFP and WRRP1pGSG5. Plasmid pGSG5, which contains both *wboA* and GFP genes, complements *wboA* and restores a smooth phenotype to rough strain WRRP1 (Δ*purE*Δ*wboA B. melitensis*). We found that 16M/GFP lost the plasmid faster but displayed OPS longer than WRRP1pGSG5. After 40 days in culture, only 1% of the population of 16M/GFP was green. However, more than 70% of 16M/GFP bound anti-S-LPS antibody while 30% bound anti-R-LPS antibody. In contrast, 5-10% of WRRP1pGSG5 cultured for the same period of time were still green and bound anti-S-LPS antibody, while at least 80% bound anti-R-LPS antibody. These flow cytometric methods may be useful for quality control of process development for large-scale vaccine production.

34- EVALUATION OF A RAPID LATERAL FLOW TEST FOR DETECTION OF IgM AND IgG IN HUMAN BRUCELLOSIS.

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In 1999, we participated in evaluating a rapid IgM dip stick method for diagnosis of acute brucellosis and found this three hour test to be both highly sensitive and specific (J. Clin. Microbiol. 37:4179-4182). Here we describe our results based on lateral flow technology. Sera were examined from individuals infected with *Brucella suis*. These sera had been tested by the dipstick method for IgM. Thirty-four sera from slaughter house patients were tested. Of the 34 sera 20 were from acute cases. These sera were positive, ranging from 2+ to 4+ and the results were comparable with earlier IgM dipstick tests. We also tested 14 sera from treated patients. Here we found 10 of 14 sera ranged from weakly positive to 2+. One of two 2+ individuals was positive after 24 months since diagnosis, suggesting a relapse or treatment failure. The other was 2+ after one month of treatment. The four remaining sera from treated individuals were negative. The study included 12 sets of paired sera (acute and treated). All paired sera showed a decline in the IgM. The IgG lateral flow test gave varied results ranging from negative to 2+. Thirteen of the 20 acute

sera were positive with four giving a 2+ reaction and six were negative. Six sera from treated patients were negative and eight were weakly positive or 1+. Our IgM findings were consistent with our previously tested dipstick results. IgG was detected in some samples and showed declines with treatment. Although, we noted the declines in IgG, the test may be more useful for chronic brucellosis. One case with apparent treatment failure case, became IgG negative from an initial 1+ value. We concluded that the value of the test over previous methods was its 15-minute reaction time, simplicity and potential for use in remote areas.

35- DIAGNOSTICS OF HUMAN BRUCELLOSIS CAUSED BY *Brucella melitensis*.

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About 2,200 new cases of human brucellosis are recorded annually in Kazakhstan; the morbidity rate has a growing trend and is in the range from 14.8 (average for the country) to 178 per 100,000 people in some districts. Clinical, epidemiological and laboratory studies were undertaken covering 210 patients who were residents of two rural districts with the morbidity rate 3 times higher than the average for the country. We identified 114 patients (54.2%) having various clinical forms, such as latent brucellosis – 30 cases (26. 3%), acute brucellosis -13 (11.4%), chronic brucellosis -62 (54.3%), and residual effects – 9 (7.9%). Latent and chronic forms were prevailing. *Brucella melitensis*, biovar 1 (2 cases) and biovar 2 (4 cases) were isolated from the blood samples of 6 patients having acute (2), chronic (2) and latent (2) brucellosis. In 3 of the 6 cases, we identified sero-negative patients with chronic (1) and latent (2) brucellosis. In 3 patients with spondylitis, osteoarthritis *Brucella* were isolated 5, 8, and 9 months after the disease had begun. It was noted that 4 patients with *Brucella melitensis* ran a conventional livestock farming in Kazakhstan, with cows and sheep kept together. It suggests that *B. melitensis* pathogen could migrate onto cattle. The studies showed that human brucellosis remains a serious threat to the health of people living in rural districts. Brucellosis is difficult to diagnose, if possible at all, when using the routine techniques.

36- DETECTION OF *Brucella melitensis* BY VITAL SYSTEM.

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This study was conducted to evaluate Vital automated blood culture system performance in terms of time-to-detection (TTD) of bacteremia due to *B. melitensis* as well as to determine the need for blind subcultures after the incubation period. We studied retrospectively 31 separate cases of bacteremia caused by *B. melitensis* over a 2-years period (2001-2002) using the Vital system for the routine diagnosis of bacteremia. Two blood cultures (four aerobic bottles) were processed per patient and ten millilitres of blood were inoculated in each bottle. The Vital bottles were monitored for 11 days and then were transferred to a regular incubator for 10 additional days of

incubation. All the bottles detected positive by the instrument were subcultured; if not, blind subcultures were performed at days 11, 16 and 21. By using a 11 days incubation protocol and considering all bottles from each patient the Vital system detected 24 positive cultures (77%) and the other 7 were revealed by blind subcultures while the instrument showed a negative result (missed positive cultures 23%). Earlier detections were seen in 3 days and the latest ones at 11 days. The median time-to-detection of *B. melitensis* was 6.6 days and the majority of isolates (18 isolates, 79.2%) were detected within 7 days of incubation. Cumulative percentage rates were 4.2%, 12.5%, 29.2%, 58.3%, 79.2%, 87.5%, 96%, 100% for days 3,4,5,6,7,8,9 and 11, respectively. In our own experience the Vital system is useful for isolating *B. melitensis* in blood cultures and a monitoring period of 11 days can be used for practical reasons. In every case prolonged incubation and periodic blindly subculturing of negative bottles is required to maximize the recovery of *B. melitensis*.

37- EVALUATION OF AN AUTOMATED COMPLEMENT FIXATION TEST SYSTEM FOR THE DIAGNOSIS OF ACTIVE BRUCELLOSIS.

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The CFT is reliable only when carefully standardized throughout. All reagents involved must be used at optimal reactivity. Therefore it is imperative that all reagents be carefully prepared and standardized to insure a completely balanced system. These standardizations, which involve titrations of sheep red blood cells, haemolysin, complement, and antigens before the test can be properly performed, render the complement fixation test rather difficult and time consuming. Aim of this work is the evaluation of the Seramat System for the performance of the CFT for the serological diagnosis of brucellosis. The Seramat system is composed of the Seramat instrument, that automatically processes the samples and of diagnostic kits: haemolytic system set and antigens. The haemolytic system set kit contains pre-titrated and ready-to-use complement and haemolysin and stabilized (6-months shelf life) sheep red cells. The haemolysin, being monoclonal, assures a higher sensibility and a minor loss of activity and stability with time compared to the polyclonal ones. Up to 40 samples/hour can be processed as the reaction takes place at 37°C. The final results re calculated through the use of a dedicated software. The antigens used for this work are *B. abortus* S 19 and *B. melitensis* cytosolic soluble extract. Sera were from patients with current and past infection.

38- A COMPARISON OF ANTI-LPS IgG AS MEASURED BY THE 2ME TEST OR BY ELISA IN HUMAN BRUCELLOSIS.

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Among traditional serological tests for human brucellosis, the value of tube agglutination in the presence of 2-mercaptoethanol (2ME) has remained

controversial. While the concentration of 2-mercaptoethanol used in the test is assumed to destroy only IgM, the extent to which IgG is affected has never been addressed. We conducted a comparison between 2ME titers and levels of IgG to *Brucella* smooth lipopolysaccharide (LPS) measured by ELISA in 158 serum samples referred to our laboratory. These samples were from people with confirmed recent or past brucellosis (patients), individuals with positive slide agglutination in blood bank or pre-laboral tests, and persons with symptoms compatible with brucellosis that were referred for serological confirmation of the disease (suspected brucellosis). Anti-LPS IgG levels were expressed as a positivity index (PI), calculated as the optical density yielded by each sample divided by the cut-off of the assay. Anti-LPS IgG was considered negative when the PI was < 1, weakly positive for PI between 1 and 2, and strongly positive when the PI was higher than 2. Sixty-four samples were negative by 2ME, 20 were positive with a titer of 25, 28 yielded a titer of 50, and 46 had a titer of 100 or higher. Anti-LPS IgG was strongly positive for 52 (81%), 19 (95%), 26 (93%), and 46 (100%) of these samples, respectively. A separate analysis was performed for the samples that showed the most discordant results (negative or 25 by 2ME, strongly positive by ELISA). The standard tube agglutination (STA) had yielded significant titers (≥100) in 13 (25%) of the 52 samples with negative 2ME and in 8 (42%) of the 19 samples with a 2ME titer of 25. Among the 52 subjects with negative 2ME and high levels of IgG, 32 had documented recent or past brucellosis, including 25 (48%) with current clinical manifestation; other 18 individuals had compatible symptoms but lacked bacteriological confirmation of the disease. Among subjects with a 2ME titer of 25, 14 (74%) had documented brucellosis with current clinical manifestation. These results suggest that a significant proportion of patients with active brucellosis are missed by the 2ME test, but can be correctly diagnosed if anti-LPS IgG is measured by ELISA.

39- DIAGNOSTIC USEFULNESS OF Omp31 FROM *B. melitensis* IN HUMAN AND ANIMAL BRUCELLOSIS.

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The group 3 of outer membrane proteins (OMP) of *Brucella includes* OMP25 and OMP31, which share 34% identity. OMP25 is highly conserved in *Brucella* species, and OMP31 is present in all species, except *B. abortus*. Some antigenic differences have been described between OMP31 from *B. ovis* and that from *B. melitensis*. We obtained recombinant purified OMP31 (*B. melitensis*) and tested by indirect ELISA its recognition by sera from humans and animals suffering from brucellosis. Sera from 73 patients, 55 sheep and 31 dogs were analyzed. All patients had yielded hight titers of IgG against *Brucella* smooth LPS and all but one were also positive for IgG to total cytoplasmic proteins (both measured by ELISA). Blood cultures were available for 52 patients and were positive for *Brucella* in 29 cases (9 *B. suis*, 5 *B. abortus*, 9 *B. melitensis* and 6 *Brucella* spp.). Forty-one patients (56%) were positive for antibodies to OMP31, including 5 cases of *B. suis* infection, 1 of *B. abortus*, 9 of *B. melitensis* and 4 of *Brucella* spp. Among patients included in the study, 34 had yielded negative result for antibodies to a particular cytoplasmic protein

of *Brucella* (lumazine synthase -BLS-); 20 of these patients were positive for antibodies to OMP31. Among 38 sheep infected with *B. melitensis* (biovars 1 and 3), 24 (63%) were positive for antibodies to OMP31. Anti-OMP31 antibodies were also detected in 12 out of 17 rams (70%) in which *B. ovis* was isolated from semen. All the 31 dogs included in the study had *B. canis* isolated from blood and had yielded positive result by direct agglutination (*B. canis* M-) and by ELISA against cytoplasmic proteins from *Brucella*. Antibodies to OMP-31 were detected in 25 (80%) of these dogs.

These results suggest that an indirect ELISA using recombinant purified OMP31 from *B. melitensis* would have a limited usefulness for the diagnosis of human and animal brucellosis. Nevertheless, the potential usefulness of this antigen in combination with other recombinant proteins from *Brucella* should not be dismissed. Notably, 59% of the patients that were negative for anti-BLS antibodies had positive anti-OMP31 reactivity. Regarding *B. melitensis* infection in sheep, although the percentage of detection remained low, the results obtained by ELISA were largely superior to those obtained previously by other authors using Western blotting.

40- LACTOBACILLI ANTAGONISM TO *Brucella*.

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The antibiotics in the treatment of brucellosis can have harmful side effects, and disbacteriosis is formed. There is an opportunity to destroy *Brucella* by using specific biological agents, i.e. lactobacilli and their metabolites. These microorganisms are symbionts of the gastrointestinal tract and are harmless for people. First *in vitro* studies of lactobacilli antagonistic activity with respect to *Brucella* were conducted using 20 lactobacilli strains and reference strains having 5 *Brucella* species. Six *Lactobacillus* strains exhibited a pronounced activity with respect to all *Brucella* species. One *Lactobacillus* strain had a suppression factor not less than 1:10000 and retained its properties over a long storage period (up to 4 years). Antimicrobe activity of the strain was determined *in vivo* using white mice of no breed infected with *B. abortus*, and a comparison was made with gentamicin action. The mice were divided into 8 groups depending on the period of treatment with gentamicin and lactobacilli, as well as time when they were killed by cervical dislocation to perform bacteriological tests of their 8 internal organs. The studies showed that the *Lactobacillus* strain antagonistic activity with respect to *Brucella* was comparable with that of gentamicin. It was found that the mice that had got lactobacilli with their food before and after being infected and that were treated with gentamicin definitely exhibited lower infection indices and less intensive proliferation of the infection into their internal organs in comparison to the reference group of mice that were infected and not treated. It confirms that there is an opportunity to use *Lactobacillus* for brucellosis treatment and prevention.

41- RAPID DETECTION OF *Brucella* sp. DNA FROM HUMAN BLOOD SAMPLES USING REAL TIME PCR TECHNOLOGY.

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Brucella species, the agents of brucellosis, are class 2 potential bioterrorism agents as defined by the CDC (USA). Clinical presentation of brucellosis is unspecific, and diagnosis remains often based upon isolation of the pathogen from blood. However, culture is slow, poorly sensitive in sub-acute phase of the disease, and exposes personnel to laboratory-acquired brucellosis. Serological tests detect specific antibodies only 2 to 3 weeks following infection, and are poorly specific due to antibody cross reactions. PCR-based techniques have been used successfully to detect *Brucella* sp. DNA from animal or human samples. These techniques are both highly sensitive and specific, and allow earlier detection of *Brucella* sp. in infected patients. Real time PCR technology now offers further advantages such as low risk of false positive tests due to laboratory cross contamination, reduced turn around time, and the possibility to quantify DNA targets.

We elaborated two real time PCR assays to detect *Brucella* sp. DNA from clinical samples, either targeting *B. melitensis* omp31 gene encoding a 31 kDa outer membrane protein found in all *Brucella* species except in *B. abortus*; or targeting a 31 kDa *Brucella abortus* protein (BCSP31)-encoding gene, whose nucleotide sequence is highly conserved at the genus level. Both assays were first validated by amplification of DNA extracts from 15 *Brucella* strains (kindly provided by Dr. B. Garin-Bastuji, AFSSA, France) of the various nomenspecies. Specificity of the technique was determined by the lack of amplification of DNA from *Yersinia enterocolitica* serotype O:9, *Afipia clevelandensis*, *Escherichia coli* O:157, *Bartonella henselae*, and various other bacterial species. The tests were then applied to 10 sera/blood samples from patients with proven brucellosis (positive blood culture). Preliminary results indicate that sensitivity of real time PCR assays is about 1 colony forming unit (cfu) in 5µl of DNA extract.

42- DEVELOPMENT OF A RAPID AND SPECIFIC REAL TIME PCR ASSAY AND ITS VALIDATION FOR THE DETECTION OF HUMAN BRUCELLOSIS.

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Brucella organisms are intracellular pathogens that have the capacity to survey and multiply within the phagocytes of the host. Several PCR methods have been described for diagnosis of human brucellosis. We have developed a rapid and reproducible method for assessment of brucellosis in serum samples. The method combines SYBRGreen I technology and the LightCycler Real Time detection system. The primers selected have been reported before by us and amplify at 223 bp sequence of the gene encoding the 31 kDa *Brucella abortus* antigen, which is conserved in all *Brucella* species. The primer annealing temperature and Mg^{++} concentration was optimised so that there was no wanted non-specific amplification. The number of cycles was optimised so that as little as 10 fg (2 genomic equivalents) of purified bacterial DNA could be consistently detected. The sensitivity of this assay

was comparable to these previously described PCR protocols. The assay showed a high reproducibility of 96 % to 99 % and was lineal in a range between 101 and 108 fg of *Brucella* DNA. The coefficient of regression of the standard curve was on average 0.98. The intra-assay and the inter-assay coefficient of variation of the threshold cycle were 1.3% and 6.63% respectively. For accurate quantification of the number of copies of *Brucella* in samples containing unknown quantities, we have used serial dilutions of a vaccine *B. abortus* B-19. The fact that no-signal were obtained with closely related organisms argues for a high specificity of this newly developed method.

In conclusion, the high sensitivity, simplicity and reproducibility of the real-time Brucellosis DNA quantification method, makes it especially suitable for Brucellosis diagnosis, as capillaries do not have to be reopened for post-PCR analysis, the risks of carry-over contamination could be minimised.

43- REAL-TIME PCR ASSAY FOR FIELD DIAGNOSIS OF *Brucella abortus* IN WILDLIFE POPULATIONS IN YELLOWSTONE NATIONAL PARK.

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Brucellosis is estimated to be one of the top five zoonoses globally, with outbreaks remaining common in both wildlife and domesticated animals. Bovine brucellosis has not yet been eradicated in the U.S., and wildlife species, such as bison and elk, remain an important potential reservoir for the disease. We have developed a real-time PCR assay for *B. abortus* using a field-portable instrument for use in diagnosing infection in wildlife to facilitate improved wildlife management, and thus minimize the potential for transmission of the disease to cattle that range near Yellowstone National Park.

Blood, tissue, and amniotic fluid have been screened using this assay which targets a 156 bp amplicon encompassing portions of the *alk*B gene and the IS711 insertion element of *B. abortus*. Using genomic DNA as template, this assay is linear over 7 orders of magnitude (7.5 ng down to 7.5 fg). This lower detection limit corresponds to 2 genomic copies; in tissues the lower detection limit increases to 50 to 500 genomic copies. Concentrations of *B. abortus* DNA in cow blood samples ranged from less than 7.5 fg (estimated to be 2.1×10^4 cells/ml blood) to as high as 1.8 pg (estimated to be 5.0×10^6 cells/ml blood) in a sample from a spontaneous abortion. A similar infectious load was estimated in the amniotic fluid of an infected bison cow (6.8×10^6 cells/ml), while analysis of secondary sex organ tissue from an infected bison indicated a much lower load of 4.4×10^3 cells/ml. Preliminary real-time PCR results for blood, tissues, and bodily fluids are in agreement with culture results, suggesting that this approach may be very useful for field-testing of animals suspected to be infected with *B. abortus*.

44- DEVELOPMENT AND VALIDATION OF GENUS-SPECIFIC PCR FOR DIAGNOSIS OF *Brucella* INFECTION IN ANIMALS.

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Brucellosis is a common zoonotic disease of worldwide distribution, which infects mainly cattle, sheep, goats and swine, resulting in a decrease of reproduction efficiency and abortion. The disease is caused by different species of *Brucella* existing as Gram-negative coccobacillary rods.

This work was conducted to develop a diagnostic method using PCR. Two primer sets were developed from RAPD generated fragment that was specific for *Brucella* genus. The two sets of oligonucleotide primers KW1&KW2 and KW3&KW4 were optimized for direct PCR amplification. Primers KW3 and KW4 were used for field test on samples from infected animals; 6 liver tissue samples, 6 kidney tissue samples and 8 lymph node tissue samples were tested for *Brucella* by PCR. One liver (16.6%), 3 kidney (50%) and 7 lymph (87.5%) tested positive for *Brucella* by PCR. The PCR amplification was specific for *Brucella* genus, as these primers were not able to amplify any non-*Brucella* organism associated with farm animals. The results suggest that application of the established PCR techniques may be more useful than the conventional tests in the specific diagnosis of animal Brucellosis.

45- CLONING AND SEQUENCING OF 1.3 Kb RAPD FRAGMENT FOR THE DEVELOPMENT OF *Brucella* SPECIFIC PRIMERS.

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Brucellosis is a common zoonotic disease of worldwide distribution, which infects mainly cattle, sheep, goats and swine, resulting in a decrease of reproduction efficiency and abortion. The disease is caused by different species of *Brucella* existing as Gram-negative coccobailary rods. Transmission to human occurs by exposure to infected animals or by ingestion of contaminated milk or milk products. A *Brucella abortus* RAPD profile was generated by 10 mer primer OBP-01 5'-GTTCGCTCC-3'. In order to develop primers for direct-PCR amplification we cloned and sequenced the 1.3 kb fragment generated by OPB-01 random primer (Al-Momin et al. 1998 WJMB 14, 415-420). The fragment was cloned using the T/A cloning method relying on the terminal extendase activity of Ultima Taq polymerase into pCR2.1 vector and transformed in *E. coli* TIOP10f'. The DNA sequence was determined using the dideoxy ribonucleotide chain termination method. From the sequence data two primer sets were developed primer KW-1 and KW-2, which gave a 1050 bp amplification product. A second amplification product of 700 bp was generated when primers KW-3 and KW-4 were used. Both segments were amplified from *Brucella* genomic DNA. These generated PCR-fragments were specific for *Brucella* spp. As these were not able to amplify any non-*Brucella* organisms associated with farm animals. We hope to use these primer sets for species-specific detection of *Brucella* organisms and *Brucella* infection.

46- THE USE OF A COMBINATION OF RESTRICTION ENDONUCLEASES IN IS711-FINGERPRINTING FOR RESOLVING DIFFERENCES BETWEEN *Brucella* SPECIES.

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The use of IS711-fingerprinting has proved to be a useful molecular tool in the identification of *Brucella* species. Digestions with common restriction endonucleases, such as *Eco*R1, are unable to completely distinguish the individual species of *Brucella* and their biovars. Genomic DNA from the 19 *Brucella* reference strains and 3 vaccine strains were analysed by IS711-fingerprinting using a combination of two restriction endonucleases (*Eco*R1 + *Dde*I). Digested DNA was separated by agarose gel electrophoresis and transferred to a nylon membrane. The membrane was hybridised using a DIG-labelled IS711 probe and fingerprint patterns were obtained. The use of the two restriction enzymes combined produced fingerprint patterns that allowed greater differentiation of the 19 reference strains and 3 vaccine strains analysed compared to using *Eco*R1 restrictions alone. In particular, the combination of *Eco*R1 and *Dde*I allowed further resolution of the *B. abortus* and *B. suis* biovars. The use of the combination of these restriction endonucleases may provide a useful tool in epidemiological studies and molecular identification of *Brucella*.

47- MOLECULAR TYPING OF *Brucella* STRAINS BY RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD).

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The genetic diversity of 18 *Brucella* strains from our collection, isolated between 1954 – 1994, (7 *B.canis*, 4 *B.suis*, 3 *B. abortus*, 2 *B. melitensis* and 2 *B. ovis*), was evaluated by RAPD, with a commercial kit and by using the DNA templates obtained by thermal lysis of bacterial cells, aliquoted and stored at –20ºC. The software package Treecon for Windows 3.1 was used for cluster analysis of the RAPD fingerprints. The strains were grouped in 11 genetic types, ID: 0.89, and the distance-based methods UPGMA and Neighbour-joining gave a clear discrimination among *Brucella* species. *B. abortus* demonstrated a high genetic variability (each strain was grouped in an individual cluster), *B. melitensis* and *B. ovis* were genetically homogenous, while *B.canis* were discirminated in two cluster. RAPD can be an useful method to distinguish related bacterial strains and a simple, quick and sensitive technique for the epidemiological investigation of brucellosis.

48- BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF *Brucella canis* ISOLATED IN KOREA.

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Molecular characterization provides powerful tool to differentiate highly conserved *Brucella* species (*Brucella melitensis*, *Brucella suis*, *Brucella abortus*,

Brucella ovis, *Brucella canis*, *Brucella neotomae*) instead of time consuming biochemical test. The purpose of this study was to compare biochemical test and PCR-RFLP to investigate the prevalence of *Brucella canis* in dogs in the future. A total of 260 dogs were randomly selected from two different breed kennels, (1) one kennel that brucellosis has occurred (group 1, 126 dogs from 6 farms), and (2) random selected breed kennel (group 2, 134 dogs from 6 farms). Of 126 dogs in group 1, 47 dogs (37.3%) were bacterial culture positive, while 5 dogs (3.7%) were culture positive in group 2. *Brucella canis* was isolated from all 6 farms in group 1, while *Brucella canis* was isolated from 2 farms in group 2. To characterize and differentiate *Brucella canis* isolates from *Brucella melitensis, Brucella suis, Brucella abortus*, *omp*-31, *wbk*A, and *per* were amplified and treated with restriction enzyme. *Omp*-31 was amplified from all *Brucella* species, except *B. abortus*. PCR-RFLP analysis of *omp*-31 revealed that all *Brucella canis* showed species specific B type following digestion with *Bme*18I. However, all the *Brucella* strains showed the same pattern following treatment with *Sal*I. PCR-RFLP analysis of *wbk*A revealed that all the *Brucells* spp. showed the same pattern (A type) following digestion with *Hind*III. PCR-RFLP analysis of *per* revealed that *Brucella canis* and *Brucella suis* showed same pattern following treatment with *Hind*III. However, *B. abortus* 544 and *Brucella melitensis* 63/9 showed different pattern. Our results showed that PCR-RFLP of *omp*-31 can be applicable to investigate the prevalence of *Brucella canis*.

49- MOLECULAR CHARACTERISATION OF FIELD STRAINS OF *Brucella* sp. ISOLATED IN ITALY IN THE YEARS 2001-2003.

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Classically the genus *Brucella* is deemed to comprise six species: *B. melitensis, B. abortus, B. ovis, B. canis, B.suis* and *B. neotomae*, these subdivided further into biovars or biotypes. On the basis of the homology shown during DNA-DNA hybridisation studies, it has been suggested that *Brucella* is a monospecific genus represented by the single species *B. melitensis* comprising various infra-species considered as biovars. Some databases and culture collections (GenBank; National Culture Collection, UK) have accepted this scheme, but other authors, on the basis of host range and species-specific outer membrane protein gene markers, etc, prefer to retain the six *Brucella* species as biologically separate entities. In our study we applied molecular methods in the routine identification of field strains of *Brucella* isolated in Italy between 2001-2003. A PCR method, based on the insertion sequence IS711, is used to verify that a field isolate is a *Brucella*, and then a second, more refined, species-specific PCR method, based on published data, is used to identify an isolate to the species level. The different *Brucella* species were finally analysed by RFLP, using prior amplification, by PCR, of the *omp*2a, *omp*2b and *omp*25 genes, and subsequent restriction analysis of the amplicons with selective restriction enzymes to finally assign the *Brucella* species to the correct biovars. All 245 *Brucella* field isolates have been processed; 152 were shown to be *B. melitensis* biovar 3, 23 *B. abortus* biovar 1, 39 *B. abortus* biovar 6, 3 *B. ovis*, 30 *B. suis* biovar 1, and 1 *B. suis* biovar 2. For all the field isolates screened by the methods described, there was complete agreement with the identifications made by conventional

bacteriological methods to the species level. In most cases it was also possible to identify the biovars within the species.

50- THE PRODUCTION AND CHARACTERISATION OF TWO RECOMBINANT PROTEINS, p18 AND bp26, FOR USE IN THE DEVELOPMENT OF AN IMMUNOASSAY FOR THE DETECTION OF BOVINE BRUCELLOSIS IN SERUM SAMPLES.

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Brucellosis is a worldwide zoonosis caused by gram-negative, intracellular bacteria, from the genus *Brucella*. In animals the disease is characterised by foetal abortions, lowered fertility and reduced milk yields, and in humans symptoms include undulant fever, arthritis and osteomyelitis. The disease results in economic losses worldwide and limitations in the trade of animals and animal products internationally. Therefore, there is an increased need for the development of rapid, sensitive and specific methods of detection.

Currently research is focusing on the use of recombinant proteins in the development of a highly sensitive and specific immunoassay for the detection of bovine brucellosis. The genes encoding two recombinant proteins, an 18kDa cytoplasmic protein (p18) and a 26kDa periplasmic protein (bp26) have been cloned into the high level expression vector pQE60 and the proteins expressed in XL 10-Gold *E. coli*. The recombinant proteins have been purified using immobilised metal affinity chromatography (IMAC) and the suitability of the purified proteins for use in an indirect ELISA (iELISA) established. The recombinant proteins allowed the discrimination between Brucella-positive and negative serum samples. It is proposed to develop an indirect immunoassay incorporating both recombinant proteins, which will enable sensitive and specific detection of a *Brucella* infection in bovine serum samples.

51- EVALUATION OF DIFFERENT CULTURE MEDIA FOR THE ISOLATION OF *Brucella suis*: COMPARISON OF DIFFERENT BASAL MEDIA AND SELECTIVE MEDIA.

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The information about the performance of *Brucella suis* in culture media is scarce. These brucellae can grown in different commercial basal media but selective media are necessary for primary isolation due to the high number of overgrowing contaminants usually present in field samples. This study evaluates four comercial basal media namely, Tryptic Soya Agar (TSA), Blood Agar Base (BAB), Brucella Medium Base (BMB), GC Medium (GC) and the Plommet Medium (P) (M.Plommet, 1991). All media were used without supplements and with 5% horse serum, 1% haemoglobin and 0.1% yeast extract (except for Blood Agar Base and Plommet Medium). The eighteen media were tested using the viable counting techinque with twenty-two *B. suis* field strains isolated from domestic pigs. Ten strains from biovar 2

were isolated and typed in our laboratory by standard procedures and the remaining strains, eleven biovar 2 and one biovar 1, were kindly supplied and typed by C.M. Marín (Unidad de Sanidad Animal, Servicio de Investigacion Agrária, Zaragoza). Three reference strains, *B. suis* biovar 1/1330, biovar 2/Thomsen and biovar 3 (NCTC-PHLS, London) were used as controls.

The results were analised by classical analysis of variance using the procedure GLM of SAS System. This analysis showed a significant difference amongst strains ($p<0.0001$), media ($p<0.0001$) and in the interaction effect between strain and medium ($p<0.0001$). The mean multiple comparison test-LSD was used to compare the strains, the media and the interaction between strains and media. Best results were obtained with the TSA and TSA with 5% horse serum (TSA-5%S), BAB and GC supplemented with 1% haemoglobin. They showed no statistical difference. The GC media, simple and supplemented with horse serum or yeast extract, showed not to be suitable for *B. suis* growth. Additionally, once the TSA-5%S gave good results in *B. suis* growth, the efficacy of this basal media supplemented with two different antibiotics concentrations, chosen according to personal data and previous studies (C.M. Marín et al.,1996a), was studied with 375 field samples of 75 infected pigs belonging to one herd with 80% of abortions. The M1 medium (TSA-5%S with 5 mg/L nalidixic acid, 100 mg/L cycloheximide, 100 000 UI/L nystatine, 1 800 UI/L polymixine B sulphate and 20 mg/L vancomycine) and M2 medium (TSA-5%S with 4 000 UI/L bacitracin, 100 mg/L cicloheximide, 100 000 UI/L nystatin, 1 800UI/L polymixine B sulphate, 3 mg/L vancomycine and 2.5 mg/L amphotericine B) were compared with the Farrell's selective medium (Farrell, 1974) and the modified Thayer-Martin selective medium (C.M. Marín, 1996a). Results were evaluated by Cohen' Kappa concordance measurement and Cochran' Q test.

52- COMPARATIVE EVALUATION OF BRUCELLOSIS SEROLOGY AND BACTERIOLOGY IN SLAUGHTERED ANIMALS IN PORTUGAL.

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In the scope of the National Brucellosis Eradication Programme, 1100 animals (879 sheep, 142 goats and 79 cattle) mainly from the Beira Interior and Beira Litoral region in Portugal, were sampled (blood, lymph nodes, spleen, liver, uterus, udder and testes), from January 2001 to June 2003. Rose Bengal (RBT) and Complement Fixation (CFT) tests were carried out on each serum sample and culture for *Brucella* isolation was performed for all tissue samples. The aim of this study was to compare results from both serological tests (at pre and post slaughtering) and the bacteriology. Agreement between tests was assessed with Kappa statistics, which showed substantial agreement between CFT and RBT at post slaughtering. On the other hand, moderate to substantial agreement was observed between pre and post slaughtering CFT, whereas RBT results indicate slight agreement. RBT (ca. 98%) had higher values for sensitivity than CFT (ca. 90%). When comparing pre and post slaughtering results, a high percentage of sampled animals showed a decrease in CFT titres. *Brucella melitensis* (biotype 3) was isolated in 246 animals (22,4%), mainly from udder, lymph nodes and spleen.

53- ELISA AS AN ALTERNATIVE METHOD FOR THE DIAGNOSIS OF BRUCELLOSIS IN SHEEP.

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Brucellosis is an important zoonotic disease caused by the bacterium *Brucella*, affecting various animal species. Although many countries have succeeded to control this disease in cattle, brucellosis in small ruminants has been less favourable. However, due to the economical and health impacts of this issue, there is a need for easily standardized and performed tests for this purpose.

Two antibody ELISA's (SVANOVIR™), one indirect and one competitive were evaluated on sheep sera. In the first stage 150 negative (Sweden) and 120 positive (Iran and Israel) samples were tested on both assays. The samples were characterized by serology and/or cultivation. The results were as follows:

Assay	Sensitivity	Specificity	Cut off
Indirect ELISA	94,3	100	PP >= 15
Competitive ELISA	100	100	PI >= 30

To confirm validity of the chosen cut off, the trial was repeated on 1094 sheep samples from herds in Iran. All samples were tested with Rose Bengal (RBT), Wright and 2-ME according to standard procedures. Out of the 1094 sheep samples, 83 % were negative in all three conventional assays. Out of these only 5 % was positive in the C-ELISA whereas 41 % in the I-ELISA. Out of the 14 % tested positive in all three agglutination tests, 97 % was positive with the I-ELISA and 69% with the C-ELISA. Two percent of the total material showed discrepancies in the agglutination tests. However, in this group the specificity of the C-ELISA against 2-ME was 82%. The study shows that the ELISA tests could be suitable replacements for the conventional tests for screening as well as for confirmatory applications.

54- EVALUATION OF THE ELISA IN DIAGNOSIS OF BRUCELLOSIS IN PIGS.

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According to Manual of Standards for Diagnostic Tests and Vaccines the serological methods recommended in diagnosis of brucellosis in pigs are ELISA, BBAT and FPA. The aim of the studies was to evaluate the properties of the indirect ELISA (I-ELISA) used in diagnosis of swine brucellosis in Poland. In the test the microplates coated with the "smooth" lipopolysaccharide (S-LPS) obtained from the strain S19 of *Brucella abortus*, the conjugate of anti-swine immunoglobulins with horseradish peroxidase and ABTS with H_2O_2 as the substrate, were used. The controls of the ELISA kit consisted of: strong positive swine serum (S++), weak positive serum (S+) and negative serum (S-) prepared respectively on the base of sera from pigs coming from *Brucella* infected herds and pigs free of brucellosis. In the examination 105 sera from pigs from 3 infected herds, 92895 sera from healthy pigs monitored for brucellosis from territory of Poland and 3662 sera from imported pigs were used. Besides ELISA the traditional methods such as RBT, CFT and additionally for some samples, SAT and 2-ME were used. Of pigs from infected herds 102 sera reacted positively in the ELISA, whereas 95 sera were positive in the RBT and 98 in the CFT. In the screening surveys only 11 positive results were obtained in

the ELISA, whereas 71 in the RBT and 2 in the CFT. Of sera from imported pigs 44 were positive in the ELISA, 20 in the RBT and 37 in the CFT. Positive results in the ELISA, CFT and most of positive results in the RBT concerned pigs from herds, where positive results for brucellosis were considered as false positive (FPSR), probably caused by *Yersinia enterocolitica* O:9. The obtained results indicate that the ELISA is a sensitive and specific method when testing pigs from territory of Poland. The diagnostic problems relevant to false positive reactions may appear in testing swine sera from pigs coming from countries where problem of FPSR occur.

55- FLOURESCENCE POLARIZATION ASSAY PERFORMANCE IN THE DIAGNOSIS OF CAPRINE BRUCELLOSIS.

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Conventional tests may be used as herd tests to detect caprine brucellosis, however, such tests are less useful for detecting infection in individual animals. In this study, the fluorescence polarization assay (FPA) and some conventional tests were used for the serological diagnosis of brucellosis in individual goats. Two hundred samples from *Brucella* infected herds and 88 samples from brucellosis free herds were tested. None of the animals were vaccinated. The buffered antigen plate agglutination test (BPA), the tube agglutination test (SAT), the 2-merchaptoethanol modification of the SAT(2-ME), the complement fixation (CFT), indirect enzyme immunoassay (IELISA) and FPA were used. The tests were performed as described elsewhere (INRA. 1988; J. Immunol. Meth. 195:161-168, 1996).

The results obtained with samples from brucellosis free animals showed agreement between all tests. The samples from *Brucella* infected goats showed that 15 sera that were negative in the conventional tests or anticomplementary in the CFT were positive in the FPA. Eleven of these animals became positive to the conventional test 45 days later indicating that FPA may detect the infection in advance to other test. Forty-three animals positive in all tests gave values of 85 millipolarization units (mP) or higher in the FPA. ROC analysis suggested an FPA cut off of 84 mP for areas where REV 1 vaccine is not used. Both the IELISA and FPA performed well, however, for control programs, especially in areas of intense goat farming, a convenient, accurate, rapid and simple test is required. The data indicates that the FPA fulfills these criteria and can be a useful tool for the diagnosis of caprine brucellosis.

56- EVALUATION OF TWO SEROLOGICAL TESTS IN CANIN BRUCELLOSIS.

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Study was performed since January 1998 till January 2003, in Serology department of IDAH, on blood samples from companion dogs with clinical signs of brucellosis. The diagnosis used tests were: Rapid agglutination test (RBT) with *B. canis* strain colored with Rose Bengale and Complement Fixation Test (CFT) with *B. ovis* strain. In the studied period were examined 693 blood samples and we found

299 (43, 1%) with positive results in CFT and 488 (64, 6%) in RBT and with doubtful results: 88 (12, 6%) in CFT and 19 (2, 74%) in RBT. Though the positive results of both test shown to be comparable, however CFT it was proved to be more conclusive from a diagnostically point of view, because of the easy interpretation and by titration of specific antibody we may obtain a diagram of the antibody dynamic since evolution of the disease and also we could evaluate the efficiency of the prescribed treatment.

57- SEROLOGICAL SURVEYS OF DOGS FOR *Brucella canis* INFECTIONS IN POLAND.

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Brucellosis of dogs is infectious disease caused by *Brucella canis*. In serological diagnosis of brucellosis in dogs the following tests are used: RSAT, 2ME - RSAT, TAT, 2ME-TAT, AGID and ELISA. The aim of the research was evaluation of diagnostic properties of the tests used in recognizing of brucellosis in dogs in Poland. In investigations 765 sera originating from dogs were used – in this number 3 diagnostic sera, 42 sera from dogs naturally infected (obtained from USA) and 720 sera from dogs from Poland (17 of them originated from dogs with clinical symptoms, which could indicate brucellosis). Antigenic preparations were made in own range from "M-" (less mucoid variant) strain *B. canis*. The commercial antigens for RSAT and AGID – both made in USA, were also used in the research. When examining 42 sera from naturally infected dogs, 41 reacted positively in RSAT and 2ME-RSAT, 39 in TAT and 37 in 2ME-TAT. In AGID positively reacted 34 sera and 41 in ELISA. What concerns dogs` sera from Poland – 9 of them reacted positively in 2ME-RSAT, 5 in 2ME-TAT and AGID and 15 in ELISA. Of sera from dogs with symptoms which could indicate brucellosis, 1 serum reacted positively in 2ME-TAT and AGID whereas 3 in ELISA. The investigations showed a high correlation between the results obtained by the own and commercial antigens. The highest sensitivity showed RSAT and then in succession TAT, ELISA and AGID. The results of investigations confirmed a good quality of home made antigens. The tests utilizing these antigens can be used in serological diagnosis of brucellosis in dogs caused by *B. canis*. The examination enabled to ascertain the native reservoir of *B. canis*.

58- BRUCELLOSIS IN PINNIPEDS FROM ARGENTINA.

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Brucellosis in marine mammals has been recently described in several parts of the world (Handbook of Marine Mammal Medicine, 2001, 4:6). We studied the levels of antibody induced by *Brucella* sp in pinnipeds from Argentina's extensive coastline. We also evaluated the performance of conventional and non-conventional tests used for brucellosis detection. One hundred and sixty samples were analyzed. Twelve from fur seals (*Arctocephalus australis*), 4 from southern sea lions (*Otaria flavescens*) and 144 from southern elephant seals (*Mirounga leonina*) were tested. Seven different serological tests were evaluated: Buffered Plate Antigen (BPA), Wright

agglutination (SAT), 2-mercaptoethanol (2-ME), Complement fixation (FC), indirect and competitive enzyme immunoassay (ELISA) and fluorescence polarization assay (FPA) (J. Wild. Dis. 2001; 37:89 -100). Six of 160 animals were positive by at least 3 tests (BPA, CELISA and FPA). These 6 positive animals were elephant seals. All other animals were negative to all tests performed. We found 2 non-expected reactions, first CF showed high anticomplementary activity in most of the cases (149/160). Second 2-ME showed higher titers than Wright in 26 cases. Furthermore, the IELISA was negative for all these animals. We did not have enough positive samples to fully evaluate these tests, however it can be suggested that either FC, Wright, 2ME and IELISA are not suitable tests to evaluate brucellosis in pinnipeds. Our results show that elephant seals from the coast of Argentina have been exposed to antigen of *Brucella* sp. The good performance of FPA and CELISA indicate that these tests should be validated for the detection of brucellosis in pinnipeds.

59- EFFICACY OF DIFFERENT ANTIGENS AND TESTS FOR THE SEROLOGICAL DIAGNOSIS OF BRUCELLOSIS IN CATTLE IN A CONTEXT OF FALSE POSITIVE REACTIONS DUE TO *Yersinia enterocolitica* O:9.

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Serological tests using whole smooth cells or smooth lipopolysaccharide (S-LPS) as antigens are the most widely used for diagnosing Brucellosis in cattle, but are susceptible to false positive serological reactions (FPSR) when cattle are or have been recently infected by *Yersinia enterocolitica* O:9. The best strategy known to differentiate *Brucella* from *Y. enterocolitica* O:9 infections is based on the use of cytosolic *Brucella* proteins in a skin test. This test is cumbersome and very expensive and researching simple and cheaper diagnostic tests is advisable. This work compared the efficacy of several antigens and tests for the serological differentiation of *Brucella* from *Y. enterocolitica* O:9 infections in cattle. None of the *Brucella* homologous S-LPS, polysaccharide (native hapten, O-chain and poly B), proteins (cytosolic and BP26 recombinant protein) and rough extracts (*B. ovis* hot saline or *B. abortus* per A R-LPS) or heterologous antigens (*E. hermanni* S-LPS and *O. intermedium* cytosolic proteins) tested in an indirect ELISA (iELISA) was able to fully differentiate FPSR from responses due to brucellosis. An iELISA/S-LPS using 3M KSCN as chaotropic reagent improved the specificity of the iELISA/S-LPS alone for such differentiation. A competitive ELISA using Mab M84 of C/Y specificity as competing reagent was not useful for the same purpose. By contrast, gel precipitation tests with *Brucella* native hapten polysaccharide or cytosolic proteins were useful (about 90% sensitivity and 100% specificity) for identifying *Brucella* infected animals in a FPSR context. These tests could be used as confirmatory tests at the herd level in areas with no obvious risk of *Brucella* infection and where false positive reactions due to *Y. enterocolitica* O:9 are observed.

60- DIAGNOSIS OF HUMAN AND ANIMAL BRUCELLOSIS USING EXTRACTS OF BACTERIA PHYLOGENETICALLY RELATED TO *Brucella*.

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Members of the genus *Brucella* are gram negative alpha-proteobacteria that cause brucellosis, an infectious disease affecting livestock and humans. The production of antigens for the serological diagnosis of brucellosis implies the handling of live *Brucella*, which is a dangerous pathogen. We speculated that cytoplasmic proteins from other alpha-proteobacteria (*Agrobacterium* sp, *Sinorhizobium* sp and *Ochrobactrum* sp) would show cross-reactivity with *Brucella* antigens and could be used to diagnose brucellosis in humans and animals. Proteins from *A. tumefaciens*, *S. meliloti* and *O. anthropi* were obtained by French press disruption, followed by ultracentrifugation and DNAse and RNAse digestion. Indirect ELISAs were designed in which the plates were coated with the corresponding cytoplasmic proteins at 0.5 µg/well. Cut-off values were calculated as mean ± SD of specific optical densities ($sOD = OD_{\text{with antigen}} - OD_{\text{without antigen}}$) obtained with normal sera (humans, 20; sheep, 20; cows, 36; dogs, 34). These tests were used to assay sera from humans (n=32), sheep (n=75), cows (n=59), and dogs (n=61) infected with different *Brucella* species. These sera were all positive for antibodies against lipopolysaccharides and cytoplasmatic proteins of *Brucella sp* by previously described ELISA systems. Canine infection by *B. canis* was detected with high specificity (97,6% for *Agrobacterium*, 93,3% for *Sinorhizobium*, 100% for *Ochrobactrum*) and sensitivity (32% for *Agrobacterium*, 77% for *Sinorhizobium*, 100% for *Ochorbactrum*) by all the ELISAs tested, and it was possible to diagnose the disease shortly after the exposure to the pathogen (15 days). In contrast, normal sera from humans, sheep, and cattle yielded high sOD with all the antigens, which resulted in high cut-off values and, consequently, in low sensitivities. For human sera, cut-off values were 2.550 for *Agrobacterium*, 1.820 for *Sinorhizobium*, 1.500 for *Ochrobactrum*; for sheep, cut off values were 1.821, 1.255, and 0.856, respectively; for cattle, cut off values were 0.822, 0.930, and 0.665, respectively. These results show that antibodies to cytoplasmatic proteins from related proteobacteria have no diagnostic role in ovine, bovine and human brucellosis. In contrast, they allow the specific and sensitive diagnosis of canine brucellosis and the detection of the infection by *B. canis* shortly after the exposure to the pathogen.

61- HIGH PREVALENCE OF *Brucella pinnipediae* IN TISSUES FROM APPARENTLY HEALTHY GREENLAND SEA HOODED SEALS (*Cystophora cristata*).

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We have previously reported a high prevalence (35%) of anti-*Brucella* antibodies in sera from hooded seals. The aim of the present study was to look further into *Brucella*-infections in hooded seals by bacteriological and serological

investigations in apparently healthy animals caught in their natural habitat. The animals were caught during a scientific hunt between Spitsbergen (Svalbard) and Greenland in September 2002. Serum and samples (tonsils, lung, mediastinal lymphnodes, spleen, liver, kidney and testes) from 29 seals were obtained. The organ samples (n=174) showed no gross lesions. Tissue-homogenates were plated on blood agar and modified Farrell's medium (without nalidixic acid and bacitracin). Suspected colonies were identified as *Brucella* sp. based on morphological and biochemical characteristics and PCR on IS711/IS6501. Marine *Brucella* sp. were recovered from 37% (11/29) of the animals. The highest prevalence was in spleen (10/29) and mediastinal lymph node (10/24). PCR assays based on the IS711 element found downstream of the *bp26* gene that is specific for marine mammal isolates, gave consistently positive results. Three new insertion sequences were identified and named ISBm1, ISBm2, and ISBm3, in course of sequencing of the *wbk* locus of *B. melitensis* 16M. ISBm1 *Eco*RI RFLP allows the differentiation between pinniped and cetacean *Brucella* isolates. All isolates were classified as *Brucella pinnipediae*. These results were confirmed by the recently described specific PCR identification tests. The sera were tested for anti-*Brucella* antibodies by SAW (with and without EDTA), RB, CFT and a protein-A ELISA, and considered positive only if found sero-positive in all tests. Anti-*Brucella* antibodies were detected in the serum of 8/11 bacteriologically positive animals. Two additional animals also were serologically positive, giving a total sero-prevalence of 33% (10/29), confirming our previous results. The most striking findings were the high prevalence of infection and the high number of infected organs in apparently healthy animals.

62- *Brucella melitensis* PERSISTENCE AND KINETICS OF THE IMMUNE RESPONSE IN EXPERIMENTALLY INFECTED EWES. PART I.

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The authors studied the evolution of *Brucella melitensis* infection in order to assess infection persistence and kinetics of immune response in experimentally infected ewes and their lambs. Forty-six brucellosis free ewes were experimentally infected and monitored together with their lambs through three subsequent reproductive cycles.

Results of direct and indirect tests performed on ewes and their lambs during the first two reproductive cycles are analyzed in this study. During the first pregnancy, all ewes except one, aborted. At the end of the second reproductive cycle 39 lambs were born. Infection persistence was assessed by bacteriological tests performed on organs of dead sheep and lambs and on milk samples. Antibody kinetics was studied by serological assays (RBT, CFT and i-ELISA). During lactation, m-ELISA tests were also performed on milk samples. Finally, sensitivity of diagnostic methods was assessed.

63- *Brucella melitensis* PERSISTENCE AND KINETICS OF THE IMMUNE RESPONSE IN EXPERIMENTALLY INFECTED EWES. PART II.

Tittarelli M., Di Ventura M., Scacchia M., De Massis F. Giovannini A., Caporale V. National Reference Centre Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo, Italy.

The authors studied the evolution of *Brucella melitensis* infection in order to assess infection persistence and kinetics of immune response in experimentally infected ewes and their lambs. Forty-six brucellosis free ewes were experimentally infected and monitored together with their lambs through three subsequent reproductive cycles. This paper describes results obtained after the third synchronization and subsequent mating of 35 ewes. Results of direct and indirect tests performed on ewes and their lambs and on slaughtered animals at the end of the experimental study are analyzed. Antibody kinetics was assessed in ewes and in the 23 lambs born during the third cycle by RBT, CFT and i-ELISA. During lactation, m-ELISA test was also performed on milk samples. Bacteriological tests were carried out on milk, dead lambs and organs of all ewes involved in the research and slaughtered at the end of the study.

Overall results showed that animals infected during the first reproductive cycle resulted still infected through the two successive pregnancies. *B. melitensis* was isolated only in one of the 17 lambs which died during the study; the antibodies were detected at least for the first 5 months of age.

64- VIRULENCE OF *Brucella suis* BIOVAR 2 IN EXPERIMENTAL MODELS OF INFECTION.

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Brucella spp. infections are characterized by a marked preferred animal host speciation. However, in cattle, besides *B. abortus* infections, *B. melitensis* and *B. suis* infections have been reported. For the latter one, the infection is considered to be a “spill over” from a wildlife reservoir. In Europe, wild boars (*Sus scrofa*) and hares (*Lepus capensis*) are infected by *B. suis* biovar 2 (BS2) and represent a risk for cattle to be sensitized to *Brucella* antigen and hence to develop antibodies that will be detected in serological tests. BS2 is characterized by its very low pathogenicity for humans and cattle and presumably sheep and goats too.

The aim of this study is to assess the virulence of BS2 in comparison to the virulence of *B. suis* biovar 1 (BS1) in the mouse model and in different macrophages cell lines. Preliminary results show that in the mouse and in the murine macrophage cell line J774 models of infection, the virulence of BS2 is reduced as compared to the virulence of BS1. In the human macrophage cell line THP1, BS2 shows a remarkable reduction of virulence in comparison to BS1. These results may partly explain the very low pathogenicity of BS2 for humans.

65- PHAGOCYTOSIS AND INTRACELLULAR SURVIVAL OF *Brucella* IN MOCL3 OVINE MACROPHAGES.

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Brucella is a Gram negative pathogen which is able to penetrate and multiply within host cells. Up to now no small ruminant cell line was available for in vitro studies of pathogen-monocyte interaction of *Brucella*. An ovine blood monocyte derived cell line named MOCL3 has been established for this purpose. In order to determine if the cell line could be used as an in vitro model for the study of brucellose we analysed the phagocytosis and intracellular survival of different smooth (S) and rough (R) strains of *Brucella* in MOCL3 macrophages. The strains used were *B. ovis* Reo 198 (R), *B. melitensis* 16M (S), *B. melitensis* R5 (R), *B. melitensis* B3B2 (R) and Rev1 (vaccinal S strain), *B. abortus* 2308 (S), *B. abortus* RB51 (R), *B. abortus* 45/20 (R) and B19 (vaccinal S strain), *B. suis* 1330 (S) and *B. suis manB* (R). All strains except *B. ovis* were internalized at a different extents in our conditions. Smooth strains multiplied within the macrophages during the 48h period experiments while R strains did not or multiplied slightly between 24 and 48h. Vaccinal strains like Rev 1 and B19 were less virulent than their corresponding wild types *B. melitensis* 16 M and *B. abortus* 2308. Nitric Oxide was not detected when MOCL3 cells were stimulated with *E. coli* LPS or infected with different strains of *Brucella*. We conclude that the MOCL3 cell line could be a suitable tool for *in vitro* studying the pathogen-host interactions in brucellosis.

66- ANALYSIS OF LONG TERM SURVIVAL OF *Brucella melitensis* IN OVINE CELLS MOCL3 AND EFFECT OF OPSONIZATION ON INFECTIVITY.

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Brucellae are gram-negative facultative intracellular bacteria, able to infect macrophages, within they can multiply, evading the effector mechanisms of phagocytes. Most of the available knowledge arises from studies using murine phagocytes. On that account the availability of an *in vitro* model using ovine cells could represent a great advantage to intimately understand the interplay between bacteria and phagocyte from animals which are natural host of the infection. Ovine blood monocyte derived cell line, named MOCL3, were infected with *Brucella melitensis* 16M, *B. melitensis* Rev1 at a ratio of 10 bacteria to one cell or were kept as uninfected controls. At 24, 48, 96, 168 and 240 hours after infection, the viability of cells and the multiplication of bacteria were monitored. Uninfected MOCL3 showed a viability curve which reached the plateau phase after 96 hours. When macrophages were infected with *B. melitensis* Rev1 we observed a similar trend, with only a significant difference at 24h when macrophages infected resulted more viable than the uninfected controls. Macrophages infected with the virulent *B. melitensis*, on the contrary, showed a different pattern characterised by a lower viability until 48 hours with a rapid increase around 96 hours. This trend showed a positive correlation with

the number of bacteria present intracellularly, suggesting that *Brucellae* are able to modulate the viability of the cell line. In a separate set of experiments cells were infected with *B. melitensis* opsonised with positive and negative ovine sera or remained unopsonised. After 72 hours intracellular bacteria were counted. We found that bacteria opsonised with negative serum and bacteria unopsonised gave similar results, while bacteria opsonised with positive serum were significantly lower than the others. It could imply that bacteria opsonised with positive serum induced a cellular pathway which let to a better control of bacterial intracellular multiplication of *B. melitensis*.

67- INTERACTION OF *Brucella abortus* WITH APOPTOTIC MECHANISMS.

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One of the most intriguing features of *Brucella abortus* infection resides in its capacity to survive and multiply within professional and non-professional phagocytes. The intracellular survival is accompanied, at least in the first phases of bacterial multiplication, by a minimum damage to cellular integrity and inhibition of apoptotic mechanisms (Chaves-Olarte et al. 2002. Cell. Microbiol. 4:663; Gross et al. 2000. Infect. Immun. 66:342). Initially, the anti-apoptotic activity of *Brucella abortus* infection on murine RAW264.7 macrophage cell line was determined by protection assays against several drugs generating DNA fragmentation. Virulent smooth strain *B. abortus* 2308 significantly protected macrophages from apoptosis induced by cycloheximide, TNF-α, and actinomycin D, drugs acting at different targets. The protection was observed over a relatively wide range of concentrations and time of incubation with the drugs, suggesting a persistent anti-apoptotic stimuli soon after bacterial infection. Flow cytometry analysis confirmed the ELISA results, showing over three times more apoptosis in non-infected cells as compared to 2308-infected cells. To study the involvement of outer membrane antigens, such as smooth lipopolysaccharide (LPS), the rough mutant strain *B. abortus perA* was included in the protection assays. Smooth LPS seemed not to be involved in protection from apoptosis, since the *perA* mutant effectively protected murine macrophages from apoptosis at the initial stages, but the overgrowth and high cell attachment of these rough bacteria decreased cell survival after 24 h of infection. More detail analysis with different rough mutants is necessary before drawing definitive conclusions. Another virulence-related system investigated was the type IV secretion virB system, by using polar and non-polar *virB B. abortus* mutants. Polar mutant did not protected macrophages from apoptosis, but interestingly non-polar mutant showed significant levels of protection, similar to virulent *B. abortus* 2308. Fluorescent staining of brucellae, cells and nuclei confirmed the ELISA findings, suggesting that *virB* locus integrity is important for the inhibition of apoptosis and persistence of *Brucella* in eukaryotic cells.

68- DOES OXYGEN TENSION MODULATE GENE EXPRESSION DURING GROWTH OF *Brucella* INSIDE ITS REPLICATIVE NICHE?.

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Our previous study of the nik gene cluster of *Brucella suis* has shown that its intracellularly induced promoter is also activated under *in vitro* microaerobic conditions. On the other hand, a *cydB* mutant of *B. abortus* lacking the cytochrome bd oxidase of high affinity for oxygen, was found highly attenuated in the mouse model of infection. The complete genome sequence has revealed that *Brucella* possesses a locus potentially encoding another high oxygen affinity oxidase, very homologous to the *cbb3* –type terminal oxidase of *Rhizobium meliloti*. Moreover, all the necessary genes for a complete anaerobic respiratory system were discovered , which could allow *Brucella* to use nitrate, for instance, as terminal electron acceptor. More recently, our work identified among the whole set of mutants representing the virulome of *B. suis*, two attenuated strains showing miniTn5 insertion in *cydD*, part of the operon encoding the cytochrome bd oxidase, and in *caiB*, encoding one of the enzymes required for the carnitine metabolism during anaerobiosis. We conclude that this environmental condition was a characteristic of the brucellosome, necessitating bacteria adaptation. We decided to study how regulation by low oxygen tension can influence expression of genes implicated in a better adaptation of *B. suis* to the phagosomal environmental conditions. Plasmids were constructed in which *B. suis* promoters were cloned upstream the *gfp* reporter gene to analyze their regulation. Expression of the *fixK* gene, a putative oxygen sensor, and of its potential target *narK*, first gene of the Nar operon encoding the nitrate reductase, were examined in the wild type *B. suis* strain and in the *fixK* mutant under aerobic, microaerobic and anaerobic conditions. Results were compared to the level obtained in intracellular expression. Unexpected findings indicated that these two promoters were expressed under normal oxygenation. Activation of the *narK* gene was actually shown to be dependent of *fixK*.

69- INDUCTION OF ENHANCED CYTOTOXIC LYMPHOCYTE ACTIVITY BY *Brucella abortus* RB51 OVEREXPRESSING CU/ZN SUPEROXIDE DISMUTASE (SOD) AND LEAKING SOD.

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Brucella abortus is a Gram negative, facultative intracellular pathogen of several mammals, including humans. Cell-mediated immunity (CMI) is critical for protection against brucellosis. *B. abortus* strain RB51 is currently being used as the official live vaccine against bovine brucellosis in the US and several other countries. We have previously reported that overexpression of *Brucella* Cu/Zn superoxide dismutase (SOD) in a recombinant strain RB51 (strain RB51SOD) significantly increased its vaccine efficacy against virulent *B. abortus* challenge in a mouse model. Here, we describe that strain RB51SOD and another recombinant of strain RB51 which overexpresses homologous SOD and simultaneously expresses

mycobacterial antigen 85A (RB51SOD/85A) induce significantly higher cytotoxic T lymphocyte (CTL) cytolytic activity against virulent *B. abortus* infected target cells. We further demonstrate that the overexpressed SOD was "secreted out" of RB51SOD and RB51SOD/85A bacteria. Overexpressed SOD is the first reported protein to be "secreted out" of *B. abortus*; the secretion may be necessary for the induction of stronger CTL cytolytic activity and enhanced protection.

70- DOES *Brucella* REPLICATE INSIDE THE AMOEBA *Acanthamoeba castellani*?.

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To study the interactions between *Brucella* and the host cell with more easiness, we tried to infect a fresh water amoeba, *Acanthamoeba castellanii*, with *Brucella melitensis*. This amoeba is naturally infected by *Legionella pneumophila*. In this study, different conditions were tested for infection: the contact time between *Brucella melitensis* and *A. castellanii*, the multiplicity of infection (number of bacteria per amoeba), the presence or absence of the antibiotic gentamycin during the infection and the culture medium of *A. castellanii*.

Our results indicate that *Brucella melitensis* is unable to replicate in *A. castellanii*. This amoeba seems to efficiently digest *Brucella melitensis*.

71- FLAGELLAR GENE HOMOLOGUES EXPRESSED IN *Brucella melitensis* 16M.

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Brucella melitensis is an intracellular facultative Gram-negative, nonmotile bacterium that causes Brucellosis. The purpose of this work is to investigate the expression of flagellar-like genes recently identified in *Brucella melitensis. B. melitensis* flagellar gene homologues may encode proteins that form a flagellar type and/or type III secretion system which may contributes to host colonization and intracellular survival of *Brucella* spp. In this study we investigate: 1) the role of the flagellar gene homologues in *B. melitensis* 16M pathogenesis and 2) the kinetics of expression of these genes under different environmental stimuli, the results of these investigation will be presented. The current study is expected to provide information on potential pathogenic mechanisms of *B. melitensis* and related organisms and on potential target specific therapies (e.g. vaccines).

72- IMPLICATION OF FLAGELLAR GENES IN *Brucella* - HOST CELL INTERACTION.

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Brucella is described as a non-motile coccobacille, however this pathogen possesses all the genes necessary to build a flagellum. Nevertheless, the chemotactism genes are absent. Expression of *fliF* (encoding the MS ring), hook monomer and flagellin have been demonstrated *in vitro*. In addition, different flagellar mutants of *B. melitensis* are attenuated in BALB/c mice. To understand the role of the flagellar regulon in *B. melitensis* pathogenesis, we evaluated the ability of different flagellar mutants to replicate inside macrophages and HeLa cells. By comparing internalisation of the wild type (WT) and *fliF* mutant, we determined if flagellar genes are involved in invasion of host cells. We also studied the induction of the *fliF* promoter during infection of macrophages by fluorescence microscopy with a *pfliF*-GFP fusion.

73- PURIFICATION, CHARACTERIZATION AND CLONING OF AN IMMUNOGENIC AMINOPEPTIDASE OF *Brucella melitensis*.

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An immunogenic aminopeptidase was purified from *Brucella melitensis* strain VTRM1. The purification procedure consisted of ammonium sulfate fractionation and three chromatographic steps. This procedure resulted in a yield of 29 % and a 144-fold increase in specific activity. The aminopeptidase appeared to be a monomeric enzyme with a molecular mass of 96 kDa and an isoelectric point of 4.8. It's activity was optimal at pH 7.0 at 40°C. The enzyme was strongly inhibited by EDTA, 1,10-phenathroline, and divalent cations (Zn^{+2} and Hg^{+2}), suggesting that this protein was a metallo-aminopeptidase. The enzyme showed preference for alanine at the N termini of aminoacyl derivatives. The Km values for Ala-pNA and Lys-pNA were 0.35 mM and 0.18 mM, respectively. The N-terminal sequence of aminopeptidase was used to search homologous in the genomes of *B. melitensis* 16M and *B. suis* 1330. The analysis revealed an exact match of the probe sequence (36 bp) with an open reading frame (ORF) of 2,652 bp, encoding a protein predicted to be alanyl aminopeptidase (aminopeptidase N). Collectively, this data suggests designation of the *B. melitensis* enzyme as an aminopeptidase N. The aminopeptidase was recognized by sera from patients with acute and chronic brucellosis, suggesting that the enzyme may have important diagnostic implications. To detect the aminopeptidase gene, a polymerase chain reaction (PCR) with a set of designed primers was performed. The expected product of 2,652 bp was obtained *in B. melitensis, B. abortus, B. suis, B. canis, B. neotomae* and *B. maris*. We cloned and expressed the aminopeptidase N protein in *Escherichia coli*.

74- IS A NEW POLYSACCHARIDE PRODUCED BY *Brucella melitensis* 16M?.

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The genus *Brucella* is described as constituted by six species: *Brucella melitensis, B. abortus, B. suis, B. ovis, B. canis* and *B. neotomae*, each preferenttially infecting an animal host. Recently, a 25-Kilobase DNA fragment was detected in each reference's *Brucella* species excepted in *B. abortus*. This DNA fragment contains a number of genes that might be involved in polysaccharide biosynthesis, including glycosyl transferases, an acetyltransferase, enzymes involved in sugar interconversions and transporters, along with possible regulatory proteins (Vizcaino et al., 2001). However, except for the *omp31*, no functionnal characterization of these genes have been performed. In the present study, we evaluated, for several genes contained in this region, *in vitro* expression in rich medium and the residual virulence of their respective mutants in cellular and mice models. The results will be presented.

75- RHIZOPINE METABOLISM GENES ARE INVOLVED IN *Brucella* PATHOGENESIS.

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In some Rhizobiale species, mos and moc genes are involved in rhizopine synthesis and degradation respectively. The rhizopine (L-3-O-methyl-scyllo-inosamine (3-O-MSI)), is an inositol derivative and the enzymes involved in its synthesis pathway are encoded by the *mos* operon, formed of four genes (*orf1*, *mosA*, *mosB* and *mosC*) and located on the symbiotic plasmid. Rhizopine is a specific growth substrate for strains of *Rhizobia* bearing the *moc* operon. This operon, located in the vicinity of the *mos* operon, is composed of four coding sequences (*mocABC* and *mocR*) involved in rhizopine catabolism. Recently, a DFI screen showed that in *B. abortus*, an homologue of *mocC* is specifically induced in macrophages, it has also been shown that in *B. melitensis*, a *mosA* mutant is attenuated. Analysis of the *B. suis* and *B. melitensis* genome allowed us to find close homologues of *mocA*, *mocC* and *mosC*. Mutants for *mosC, mocC* and *rpiR* genes were constructed, RpiR being homologuous to a transcritptional regulator located in the vicinity of *mocC* and *mocA*. All these mutants were found to be attenuated in mice but not in cellular models of infection. Transcriptional fusions between upstream sequences of *mosA, mosC, mocC* and *rpiR* with green fluorescent protein (GFP) were constructed and GFP expression was followed by FACS analysis in the wild-type and *rpiR* mutant backgrounds, with and without exogenous rhizopine. The results suggest that RpiR acts as a repressor controlling both its own expression as well as the expression of *mocC*. The rhizopine was also found to be an inducer of *rpiR* expression and to have an inhibitory effect on *mosA* transcription. Rhizopine was also shown to promote *Brucella* growth. Therefore the result presented here suggest that rhizopine might have a role in *Brucella* pathogenesis.

76- THE AQUAPORIN GEN *aqp*X OF *Brucella abortus* IS INDUCED IN HYPEROSMOTIC CONDITIONS.

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Aquaporins belong to a widespread protein family involved in water homeostasis. They are essential in eukaryotic organisms for rapid transport of water across the cytoplasmatic membrane. However, aquaporins appear only sporadically in bacteria and its physiological role in osmoregulation is still not well known. In a previous work, we have identified in *Brucella abortus* an aquaporin gene whose deduced gene product, AqpX, showed 70% aminoacid sequence identity to the *E. coli* water channel AqpZ, which serves as a model for bacterial water channels. We have also shown that AqpX mediates rapid and large water fluxes in both directions in response to sudden up-or downshifts in osmolality. In order to study the expresión of the *aqpX* gene of *B. abortus*, we have disrupted this gene by allelic exchange using an *aqpX*::*lacZ*-Km gene fusion. The *aqpX* null mutant did not show any significant difference in growth rate compared to the wild-type strain either in rich or minimal media. This result demostrated that disruption of the *aqpX* gene was not letal for *B. abortus*. The role of the AqpX water channel was investigated by exposing the cells to hypo-and hyperosmolar conditions, through a reporter gene fusion (beta-galactosidase assays), RT-PCR and primer extensión analysis. We found that the expresión of *aqpX* gene was osmotically controlled with a maxim level of transcription under hyperosmotic growth conditions. Moreover, *B. abortus aqpX* gene expresión levels were enhanced during the mid-logarithmic phase of growth. These results indicated that the expresión of *aqpX* was regulated along the growth curve and was induced in hyperosmotic conditions, suggesting that the presence of aquaporins could be important for adaptation to osmotic shock/stress conditions.

77- DELETION OF THE *bac* A GENE FROM *Brucella abortus* INCREASES OR DECREASES VIRULENCE OF THE ORGANISM DEPENDING UPON THE GENETIC BACKGROUND OF THE HOST.

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Deletion of the *bac* A gene from *Brucella abortus* strain 2308 has been shown to result in a decreased recovery of colony forming units from the spleens of normal immune-intact BALB/c mice following infection. However in interferon-γ-gene disrupted C57BL/6 mice the deletion of the *bac* A gene results in more pathology than does infection of the same mice with the parent strain 2308. This is despite the fact that the number of colony forming units recovered is less from mice infected with the *bac* A deletion mutant. Recent studies have suggested that the *bac* A gene may affect the lipopolysaccharide (LPS) structure. Here studies are reported that evaluated the sensitivity of the *bac* A mutant to complement-mediated lysis, a trait correlated with LPS structure, as well as the ability of the *bac* A mutant to stimulate macrophage release of pro-inflammatory cytokines. Differences in LPS structure

have been hypothesized to result in differences in interactions with CD14/toll receptors on macrophages and subsequent release of cytokines. It was speculated that differences in cytokine production might be responsible for the difference in pathology associated with infection with the *bac* A mutant.

78- DhbR, A PUTATIVE AraC-LIKE TRANSCRIPTIONAL ACTIVATOR, REGULATES THE PRODUCTION OF THE SIDEROPHORE 2,3-DIHYDROXYBENZOIC ACID (2,3-DHBA) IN *Brucella abortus*.

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Iron is essential to the survival of most bacteria, but the mammalian host represents an extremely iron-restricted environment. To persist, a pathogen must circumvent this restriction. This is accomplished, in part, through the secretion of iron binding compounds called siderophores, but due to the toxicity of excess iron, bacteria must tightly regulate this iron uptake. The majority of bacterial genes encoding components of iron acquisition are regulated either by the ferric iron uptake regulator (Fur), or a transcriptional repressor with Fur-like activities.

Brucella abortus reportedly produces two catechol-type siderophores, both produced through the enzymatic activities of the products of the *dhb* operon. Expression of the *dhb* operon is tightly regulated in response to environmental iron levels. Two consensus Fur boxes are located within the *dhb* promoter, but an isogenic *fur* mutant constructed from *B. abortus* 2308 displays wild-type repression of *dhb* expression in response to iron-replete growth conditions. A homolog of AlcR, the AraC-like transcriptional activator that controls expression of the siderophore alcaligin in *Bordetella*, has recently been identified in *B. abortus*. The *B. abortus alcR* mutant, BEA5, shows decreased expression of the *dhb*CEBA operon under iron-deplete conditions, when compared to the parental 2308 strain, indicating that the product of this gene, termed DhbR (dihydroxybenzioc acid regulator), serves as a transcriptional activator for the 2,3-DHBA biosynthesis genes. The nature of the complex interplay between Fur, DhbR and other iron-responsive transcriptional regulators in controlling expression of the *dhb* operon is currently under investigation.

79- THE *rsh* GENE OF *Brucella melitensis* 16M IS ESSENTIAL FOR FULL VIRULENCE AND CONTROLS VirB EXPRESSION *in vitro*.

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Several studies suggest that *Brucella* has to cope with nutritional deprivation during its trafficking inside professional and non professional phagocytes. Moreover, data from Kohler et al. (2002) and Kim et al. (2003) suggest that *rsh* (for *relA spoT* homolog) is important for the virulence of *Brucella*. This gene encodes the main factor of starvation response (also called stringent response) in bacteria. In *E. coli*, the RelA and SpoT proteins synthesize the nucleotides (p)ppGpp which mediate the

adaptation to starvation. In pathogenic or symbiotic bacteria like *Legionella* and *Rhizobium*, a single protein called Rsh is involved in the adaptation to the intracellular environement.

In order to understand the role of *rsh* in the virulence of *Brucella*, we created a strain of *B. melitensis* 16M deleted for *rsh* using a non polar cassette called aphA4. It is expected that *rsh* deletion will have pleiotropic effects, as demonstrated in *E. coli*. In agreement with this, we observed that the *rsh* mutant presents a particular morphology: after growth in a rich medium, the *rsh* mutant is two to three-fold bigger than the wild-type strain when observed with electron microscopy. This phenotype was very similar to the one described for the double *relA spoT* mutant of *E. coli*. The non polar *rsh* mutant fail to replicate in ovine macrophages and HeLa cells. The virulence of the *rsh* mutant in a murine model is currently under investigation. Western blot analysis revealed that some virB proteins are less abundant in the *rsh* mutant compared to the wild-type strain when they are grown until mid-exponential phase in rich medium. These data suggest that Rsh may indirectly regulate the expression of the virB operon.

KOHLER, S., FOULONGNE, V., OUAHRANI-BETTACHE, S., BOURG, G., TEYSSIER, J., RAMUZ, M., AND LIAUTARD, J-P. (2002). Proc Natl Acad Sci USA 99(24):15711-6.

KIM, S., WATARAI, M., KONDO, Y., ERDENEBAATAR, J., MAKINO, S, AND SHIRAHATA, T. (2003). Infect Immun 71(6): 3020-3027.

80- LIPOPOLYSACCHARIDE MODIFICATIONS IN *Brucella abortus* MUTATED IN THE TWO-COMPONENT REGULATORY SYSTEM BvrR/BvrS.

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The BvrR/BvrS two-component sensory-regulatory system is essential for penetration and intracellular survival of *B. abortus*. BvrR/BvrS mutants display increased surface hydrophobicity, sensitivity to polycationic bactericidal peptides and adherence to epithelial cells and macrophages. This set of properties is suggestive of envelope changes and, in fact, we have shown that these mutants do not express Omp3a (Omp25) and Omp3b (Omp22) (Guzmán-Verri et al. Proc. Natl. Acad. Sci. U. S. A. 99:12375-12380). To test whether other envelope molecules are changed in BvrR/BvrS mutants, several envelope fractions were analyzed. No quantitative differences were found in periplasmic cyclic 1,2-β-glucans, lipopolysaccharide (LPS) or native hapten polysaccharide content, or in free lipid profiles. However, chimeric *bvrS* mutant cells bearing the parental LPS showed increased polymyxin resistance and, conversely, chimeric parental cells bearing the LPS of the *bvrS* mutant displayed increased sensitivity and, by electron microscopy, the envelope morphological alterations associated with the peptide action. Increased binding by the LPS of *bvrR* and *bvrS* mutants was observed by fluorimetry with dansylated polymyxin, and polymyxin had a stronger acyl-chain fluidifying effect on the LPS of the mutants. No differences in the degree polymerization or ^{13}C-NMR spectra of the O-polysaccharides were observed, an increase of underacylated forms in the lipid A of the *bvrR* and *bvrS* mutants was observed by high-performance thin layer chromatography. Although further research is necessary to ascertain whether this lipid A change represents a true regulation by BvrR/BvrS or simply results from a

pleiotropic effect caused by the absence of Omp3a, and Omp3b, lipid A alterations could contribute to explain the lack of virulence of the BvrS/BvrR mutants.

81- EXPRESSION OF *Brucella abortus omp3a* AND *omp3b* UNDER DIFFERENT GROWTH CONDITIONS AND EFFECT OF *omp3a* OR *omp3b* DELETION ON OUTER MEMBRANE PROPERTIES AND VIRULENCE.

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The two-component regulatory system BvrR/BvrS of *Brucella abortus* is essential for intracellular penetration and survival within mammalian cells. We have previously shown that this system controls the expression of *B. abortus* Omp3a (Omp25) and Omp3b (Omp22) (Guzmán-Verri et al. Proc. Natl. Acad. Sci. U. S. A. 99:12375-12380). Thus, we explored the kinetics of Omp3a and Omp3b expression and their role in *B. abortus* virulence. The level of expression of *omp3a* and *omp3b* promoters in response to different growth conditions was measured by the β-galactosidase assay in chromosomal *omp3a::lacZ* and *omp3b::lacZ* transcriptional fusions. The level of *omp3a* transcription increased when cells growth in rich medium (TSB or BHI) were incubated for 6 h. in minimum medium. On the contrary, the level of *omp3b* transcription increased when cells growth in minimum medium were incubated in rich medium.

In contrast to BvrS/BvrR mutants, the sensitivity of these Omps mutants to sodium dodecyl sulphate, Sarkosyl and polymyxin B were not significantly different from that exhibit by the virulent strain. Similarly, the adherence, internalization and replication of both Omp mutants in non-professional phagocytes HeLa cells and RAW 264.7 macrophages, assayed by double immunofluorescence and direct bacterial counts, were not significantly different from that of the virulent parental strain. Whereas a large number of *bvrS* mutant bacteria were found extracellularly in HeLa cell cultures, *omp3a* and *omp3b* mutants were seldom found extracellularly. Finally, the survival of both Omp mutants in BALB/c mice was similar to that of the wild type strain. These results demonstrate that the previously described attenuation of BvrR/BvrS mutants is not due only to down-regulation of Omp3a and Omp3b and indicate that additional factors are involved in the attenuation.

82- CLONING, EXPRESSION AND PURIFICATION OF *B. suis* OUTER MEMBRANE PROTEINS.

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Brucella, an aerobic, nonsporeforming, nonmotile Gram-negative coccobacillus, is a NIH/CDC category B bioterror threat agent that causes incapacitating human illness. Medical defense against the bioterror threat posed by *Brucella* would be strengthened by development of a human vaccine and improved diagnostic tests. Central to advancement of these goals is discovery of bacterial

constituents that are immunogenic or antigenic for humans. Outer membrane proteins are particlularly attractive for this purpose. In the present study, we cloned, expressed and purified five predicted outer membrane proteins of *B. suis*, including two large ones of 88 kD and 68 kD molecular weight. The recombinant proteins were designed with 6-his and V5 epitope tags at their C termini to facilitate detection and purification. The *B. suis* genes were PCR synthesized based on their ORF sequences and directly cloned into an entry vector. The recombinant entry constructs were propagated in TOPO 10 cells, recombined into a destination vector, pET-DEST42, then transformed into *E. coli* BL21 cells for IPTG-induced protein expression. The expressed recombinant proteins were confirmed with western blot analysis using anti-6-his antibody conjugated with alkaline phosphatase. These *B. suis* outer membrane proteins were purified by immuno-capture using a specific antibody against the V5 epitope. Spleen cells from mice previously immunized with the purine auxotrophic live, attenuated vaccine strain *B. melitensis* WR201 were cultured with recombinant proteins. After 24 hours, culture supernatant fluids were assayed for IFNγ and interlukin 2 content. Recombinant *B. suis* proteins were readily expressed and purified by this approach and stimulated cytokine production from *B. melitensis*-immune mouse spleen cells.

83- HUMORAL AND CELLULAR IMMUNE RESPONSE TO *Brucella abortus* STRAINS RB51 AND S19 IN HEREFORD HEIFERS IN PATAGONIA REGION, ARGENTINA.

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A controlled field study was carried out using two homogeneous groups of 25 nine-month-old Hereford heifers. On day cero all the animals were bled and subcutaneously vaccinated with commercially S19 or RB51 vaccines at recommended doses. Both groups were bled at days 30, 90, 210 and 360 post vaccination. On each opportunity blood with anticoagulant was taken and cultivated in sterile plates by duplicates. One was incubated adding a cytosolic antigen of *Brucella abortus* RB51 while the other with PBS as control. After 18 hours of incubation at 37ºC with an atmosphere of 5% of CO_2, plasma was obtained. Also blood samples without any anticoagulant were taken for sera collection. The sera samples were processed with BPAT and with two standard I-ELISA, using S-LPS (for S19 antibodies) or R-LPS (for RB51 antibodies) respectively. The plasma samples were processed with a capture ELISA (Bovigamtm, CSL), for the detection of bovine γ-interferon (INFγ). The humoral immune response generated by RB51 measured with an I-ELISA using a R-LPS was similar to the one generated by S19, measured with an I-ELISA using a S-SPLS. In both cases, on day 30, similar antibody picks were produced with the following figures: pp 90.4 ± 26.6 for RB51 vs. pp 91.8 ± 21 for S19. RB51 vaccine showed a cellular immune response, measured through the production of INFγ, similar to the one produced by S19 (pp 22.1 ± 11.6 for RB51 vs. pp 20.5 ± 14.7 for S19). *Brucella abortus* RB51 generated, in comparison to S19, a similar humoral and cellular immune response without any interference with diagnosis. It could be a good alternative for adult animal vaccination in areas were it

is difficult to obtain a good vaccine coverage in young animals under 8 month old, as Argentinean regulation states.

84- EFFICACY OF S-RB51 VACCINE IN ADULT CATTLE.

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Strain *B. abortus* RB51 (S-RB51), was developed to protect cattle for brucellosis and the level of protection was comparable to S-19 (Am. J. Vet. Res. 1996: 57, Am. J. Vet. Res. 1998: 59). Besides, the S-RB51 do not induce the production of antibodies even if it is applied multiple times (Prev. Vet. Med. 2000: 45). In this experiment we study the efficacy of S-RB51 applied for adult cattle which were calf vaccinated either with S-RB51 or S-19. Four groups of cattle were established: *A*:23 cows were vaccinated with S-19 and revaccinated with S-RB51. *B*: 21 cows were vaccinated with S-RB51 and revaccinated with S- RB51 *C*: 28 cows were vaccinated with S-19 and revaccinated twice with S-RB51 *D*: 12 cows were not vaccinated. All animals were challenged in the middle of pregnancy with $2.1x10^7$ *B. abortus* strain 2308 intraconjuntivally Serological studies were done using BPA, 2 ME, CFand ELISA tests. Bacteriological studies were performed from aborted foetuses, vaginal swabs and milk. All cows vaccinated and revaccinated with S-19 became seropositive but they remained negatives when were vaccinated and revaccinated with S-RB51. The number of abortions were 4, 3, 3 and 6 in groups A, B, C and D. Strain 2308 was recovery from 6, 5, 8 and 10 cows for the same groups and CF titres (1/40) were detected in 6, 6, 10 and 12 cows respectively 6 months post-challenged. These animals were considered infected. According to these results we did not see any differences in the efficacy among these combination of vaccines, however protection given by the vaccines was much better in groups A, B and C than the control group.

85- ATYPICAL POST-VACCINATION SEROLOGICAL RESPONSE IN COWS REVACCINATED WITH *Brucella abortus* RB51 IN ENDEMIC BRUCELLOSIS AREAS OF MÉXICO.

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It is know that RB51 vaccination does not induce antibodies, that interfere with diagnosis, due to this fact, any positive reaction is considered as infection, however, we observed in cattle revaccinated with RB51, animals without infection, that were serologically positive. The aim of this work was to elucidate which antibodies were present in these animals with an “atypical” response. Sera samples were collected from an infected dairy herd, ten sera from each group were used, RB51 vaccinated groups: 1 card (CT) and rivanol (RT) negative; 2 CT positive, RT negative; 3 CT and RT positive (1:50 or 1:100); 4 CT and RT positive (1:400). RB51 revaccinated groups: 5 CT positive, RT negative; 6 CT and RT positive (1:50 or 1:100). Positive group with *B. abortus* isolation, and negative non-vaccinated group. All vaccinated

and revaccinated animals and negative group were bacteriological negatives. The tests used were: Radial Immunodiffusion (RID) and I-ELISA with *B. abortus* 2308 and RB51 LPS as capture antigens, and polyclonal antibodies peroxidase conjugates for anti bovine IgG_1, IgG_2, IgM and IgA. For RID, sera from group 4 and infected group were positives. I-ELISA S-LPS, in vaccinated cows groups 1, 2, 3, the response to all isotypes was similar to the negative group. In revaccinated cows groups 5 and 6, IgG_1 response was high. For groups: 4 and infected, positives to RT 1:400 and RID tests, had a high levels of IgG_2 different with respect to other vaccinated and revaccinated groups. For I-ELISA R-LPS all groups, included infected cows did not presented difference in all isotypes with negative cows. We concluded that infected cows had high IgG_2 levels indicative of a Th1 response. An atypical response in Brucellosis endemic zones is present after vaccination with RB51. The RID, I-ELISA S-LPS with IgG_2 and rivanol tests are good possibilities for differentiate vaccinated or infected cows.

86- PERFORMANCE OF THE *Brucella abortus* RB51 VACCINE IN HIGH PREVALENCE HERDS IN MÉXICO.

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The *Brucella abortus* RB51 vaccine was introduced in Mexico in 1998, the absence of the O antigen do not induce antibodies formation detected by the standard diagnostic tests. In Mexico the use of this vaccine is recommended in two different doses, normal dose for calves and reduce dose for pregnant catlle. We have been monitoring the behavior of the vaccine in areas with high prevalence of Brucellosis. It was found 94% of protection against natural challenge, the vaccine strain was isolated from milk and vaginal swabs it was also found a serological response in the card test but not in Rivanol test. In a prospective study, we follow 1150 vaccinated cows for 660 days, the animals were from two different herds one with low (<10%) prevalence (1) and the other with high (> 10%) prevalence (2). In herd 1 all positive animals were eliminated as well as all the new cases, the incidence of the disease after one year was 1% by the 2nd year it was 0%. In herd 2 they eliminated the positive animals by the time of vaccination, but the new cases were not eliminated, the incidence was higher each month during the 1st year, time when they decided to eliminate positive animals and new cases, by the day 540 the incidence dropped to 1%. Several animals presented a positive reaction to the card test but were negative again after 2 or 3 months, these animals were negative to rivanol test. It is important to keep in mind that the positive reaction might be due to a secondary respond after the animal is exposed to the field strain.

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87- PROTECTION OF MICE AGAINST BRUCELLOSIS BY SIMULTANEOUS VACCINATION WITH *Brucella abortus wbkA* AND *bvrS* MUTANTS.

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A rough *B. abortus wbk*A mutant bearing an intact lipopolysaccharide (LPS) core induces protection against *B. abortus* in mice lower than that conferred by vaccine S19, and controls with smooth LPS (S-LPS) suggest that anti-O-polysaccharide antibodies account for the difference (Monreal et al. 2003. Infect. Immun. 71:3261-3271). It was thus hypothesized that the vaccine efficacy of *wbk*A could be improved by the simultaneous inoculation of a smooth strain showing an attenuation such that, while priming the development anti-O polysaccharide antibodies, it would not to evoke a strong anti-S-LPS antibody response. To test this, we used a *B. abortus bvrS* mutant unable to replicate intracellularly and avirulent in mice but carrying S-LPS. BALB/c mice were vaccinated with: (1), mutants *wbkA* and *bvrS* (10^8 CFU intraperitoneally); (2), *wbkA* plus formalin-killed *bvrS* cells (10^8 CFU or its equivalent intraperitoneally); and (3), *wbkA* plus S-LPS (10^8 CFU and 100 μg intraperitoneally). Additional controls were mice vaccinated with either mutant alone, formalin-killed *bvrS,* LPS or S19 (10^5 CFU, subcutaneously). The protection conferred by *wbkA* plus *bvrS* was better than that obtained with either mutant alone and better ($P<0.005$) than that conferred by S19. The protection afforded by *wbkA* plus *bvrS*, dead *bvrS* cells, or S-LPS was better to that afforded by the single administration of *wbkA* ($P<0.0001$, $P=0.01$, and $P=0.001$, respectively), thus showing the effect of S-LPS. However, the *wbkA* plus live *bvrS* was the best of all the combinations tested. Splenic growth curves evidenced that mutant *bvrS* was more attenuated than mutant *wbkA* and proved that their simultaneous inoculation increased *wbkA* spleen persistence.

88- VIRULENCE AND VACCINE EFFICACY IN MICE OF LIPOPOLYSACCHARIDE MUTANTS OBTAINED FROM *Brucella melitensis* STRAINS 16M AND H38.

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Rough (R) *Brucella* vaccines deficient in the O-polysaccharide of the lipopolysaccharide represent an approach to circumvent the interference created by smooth vaccines in standard serological tests. To examine whether the virulence and immunizing properties of R mutants is affected by variations in the genetic background of parental strains, 16M-*per*-R and H38-*per*-R were obtained from *Brucella melitensis* 16M and H38 by transposon disruption of *per* (a gene involved in perosamine synthesis, the only O-polysaccharide sugar in *Brucella*), and analyzed in the BALB/c mouse model. Splenic growth curves evidenced the attenuation of both *per*-R mutants. However, differences in virulence between the mutants were observed during the acute phase of infection. When administered at 10^6 CFU/mouse, H38-*per*-R reached splenic CFU numbers similar to those of either parental strain 2

weeks after infection whereas 16M-*per*-R was always in lower numbers. Although less markedly, the difference between H38-*per*-R and 16M-*per*-R was also observed at 10^7 and 10^8 CFU/mouse doses. At later times, the splenic CFU numbers decreased gradually for both mutants, faster in the mice inoculated with the higher doses (10^7 or 10^8 CFU) of the more virulent mutant (H38-*per*-R mutant), suggesting that initial splenic levels and persistence were conversely related. When tested as vaccines in the mouse model against *B. melitensis*, both mutants conferred protection similar to that afforded by smooth Rev 1. At 10^7 CFU/mouse, H38-*per*-R improved (P=0.027) the protection conferred by 16M-*per*-R, suggesting that H38-*per*-R and 16M-*per*-R differ in vaccine properties.

89- COMPARISON OF *Brucella melitensis* R MUTANTS WITH INTACT OR DEFECTIVE LIPOPOLYSACCHARIDE CORE AS VACCINES IN BALB/*c* MICE.

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The attenuation and vaccine properties of *B. abortus* rough mutants in mice depend on the extent of their lipopolysaccharide (LPS) core defects (Monreal et al. 2003. Infect. Immun. 71:3261-3271). To test whether this also occurs in *B. melitensis*, mutants in the O-polysaccharide *wbkD* and core $manB_{core}$ genes were obtained from the virulent smooth strain H38. Consistent with the predicted gene role, the *wbkD* and $manB_{core}$ mutants bore an intact and a markedly deficient LPS core, respectively. Studies in mice proved that: i), both mutants were attenuated as compared to H38; ii), inoculated at 10^8 to 10^5 CFU, mutant $manB_{core}$ did not multiply in spleens and was cleared by week 6; iii), at 10^6 or 10^5 CFU, mutant *wbkD* multiplied to reach a plateau between weeks 2 and 3 and splenic numbers close to those of strain H38; iv), no splenic multiplication of mutant *wbkD* could be observed when inoculated at 10^7 or 10^8 CFU, and the numbers declined faster than at 10^6 or 10^5 doses; and v), mutant *wbkD* persisted in spleens for more than 6 weeks. When tested as vaccines against *B. melitensis* in mice under the conditions established for rough and smooth vaccines (10^8 CFU intraperitoneally and 10^5 CFU subcutaneously, respectively), mutant $manB_{core}$ failed, and mutant *wbkD* and the smooth vaccine Rev 1 protected ($P<0.05$) at a similar level. These results confirm and extend our previous observations on the importance of the LPS core in the virulence and vaccine properties of rough *Brucella* mutants in mice.

90- IMMUNE RESPONSE INDUCTION BY A NONVIABLE RECOMBINANT *B. abortus* RB51 STRAIN EXPOSED TO A MINIMUM DOSE OF GAMMA-IRRADIATION.

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Brucella abortus strain RB51 is an attenuated, stable rough mutant that is being used as live vaccine in several countries to control bovine brucellosis. Using a

plasmid-based expression system, we have previously demonstrated that strain RB51 can serve as a vector for the delivery of heterologous proteins to induce a preferential, specific Th1 type of immune response. Because safety concerns may preclude the field use of strain RB51-based recombinant live vaccines, we are exploring strategies to inactivate recombinant RB51 strains without interfering with the induction of Th1 type immune responses. In this study, we compared the ability of heat-killed and gamma-irradiated recombinant RB51 strains to induce heterologous antigen-specific immune responses in BALB/c mice. Our studies revealed that exposure of strain RB51LacZ (a recombinant RB51 expressing β-galactosidase of *E. coli*) to a minimum of 300 krad of gamma-radiation rendered the bacteria nonviable. These bacteria, however, remained metabolically active as shown by their active electron transport chain. A single intraperitoneal inoculation of mice with 10^9 CFU-equivalent gamma-irradiated RB51LacZ, but not heat-killed RB51LacZ, induced a β-galactosidase-specific Th1 type immune response. On day 3 post-inoculation, mice inoculated with gamma-irradiated, but not heat-killed, RB51LacZ had enlarged spleens with detectable levels of nonviable strain RB51LacZ. Preliminary experiments with dendritic cells indicated that both gamma-irradiated and heat-killed RB51LacZ were equally efficient in activating dendritic cell maturation. However, dendritic cells exposed to gamma-irradiated bacteria secreted more IL-12. These results suggest that recombinant RB51 strains exposed to a low dose of gamma-radiation become nonviable, but remain metabolically active, and retain their ability to stimulate a strong Th1 type immune response specific to the expressed heterologous antigens.

91- DEVELOPMENT OF *Brucella melitensis* AS A VACCINE AGAINST BIOTERRORISM AGENTS.

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The goal of this research is to develop a live, attenuated, single vaccine against anthrax and brucellosis in humans. The 2.3-kb pag gene encoding the *Bacillus anthracis* protective antigen (PA) was fused to *Brucella* GroE promoter in broad host range plasmid pBBR4MCS to produce pBB4PA. A *purEK* deletion *Brucella melitensis* strain WR201 was electroporated with plasmid pBB4PA to produce strain WR201PA. The 2.1-kb *Brucella wboA* gene encoding a mannosyltransferase involved in O-side chain synthesis, was cloned into pBB4PA to produce pBB4PA/WboA. A *purEK, wboA* mutant B. melitensis strain WRRP1 was electroporated with pBB4PA/WboA to produce strain WRSPA. Immunoblot analyses using rabbit anti-PA polyclonal serum was performed to determine the expression of PA by recombinant strains. *Escherichia coli* carrying pBB4PA and *B. melitensis* strains WR201PA and WRSPA each produced an approximately 63-kDa protein equivalent to the full length PA and a series of proteins between 4 to 30-kDa. Immunoblot analyses using monoclonal rat antiserum to *Brucella* O-side chain revealed that O-side chain synthesis was complemented in strain WRSPA. However, crystal violet staining indicated that strain WRSPA exhibited a rough phenotype. One week after inoculation, strain WRRP1 completely cleared from BALB/c mouse spleens, whereas strain WRSPA cleared by 2.3 to 3.5 $\log_{10}$ compared to *B.*

melitensis wild type virulent strain 16M. Four weeks after inoculation, strain WRSPA completely cleared from spleens. These results indicate that strain WRSPA is not as virulent as strain 16M and not as attenuated as strain WRRP1. The recombinant strains WR201PA and WRSPA have potential as vaccine candidates against anthrax and brucellosis in humans.

92- EVALUATION OF *B. abortus* M1luc AND I2 AS VACCINES AGAINST BOVINE BRUCELOSIS.

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B. abortus M1luc is a mutant strain derived from S19 in which most of *bp26* sequence has been replaced by the *luc* gene. I2 is a double mutant in which, apart from *bp26* mutation, most of *omp19* has been deleted. In BALB/c mice, M1luc behaved as S19. I2 was more attenuated than S19 but conferred the same level of protection against challenge with S2308. The objective was to obtain strains that could be easily distinguished from field strains (by use of the luc marker) and that had an associated diagnostic test (using BP26 antigen). In I2, the objective was to generate a strain that would also have less residual virulence than S19, retaining its protection capacity. For evaluation in the natural host, groups of 5-month old heifers were either not vaccinated or vaccinated with $2x10^{10}$ CFU of S19, M1luc or I2 (N=15, each). All vaccines generated equal humoral immune response against LPS. Animals were inseminated and then challenged, in the third trimester of pregnancy, with $3x10^{7}$ CFU of S2308. The protection levels against abortion were 78.6% for S19, 81.8% for M1luc and 45.5% for I2. From the non-vaccinated group, 25% did not abort. These results indicate that Omp19 is necessary for S19 full protection capacity. The BP26-i ELISA was highly specific for animals vaccinated with M1luc or I2. S19 vaccinated animals developed anti-BP26 antibodies after 6 months of vaccination. The control group did not present antibodies against BP26 at any time. However, sensitivity after the experimental challenge was much lower than the one obtained for naturally infected cattle.

93- EVALUATION OF NOVEL *Brucella abortus* AND *Brucella melitensis* DELETION MUTANTS AS POTENTIAL VACCINE CANDIDATES IN THE MOUSE AND GOAT MODELS OF BRUCELLOSIS.

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Using in vivo screening of signature tagged mutant banks, we have identified several *B. abortus* genes that are attenuated in virulence due to a reduced ability to either establish or maintain a successful infection in the mouse model. Based on the ability of selected mutants to stimulate a protective immune response in the mouse, we hypothesized that one or more deletion mutants may prove to be superior to currently available vaccines in large animals. The creation of unmarked deletion

mutants serves two purposes. First, mutants may be used without concern for the spread of antibiotic resistance. Second, unmarked, non-polar deletions will confirm that the previously observed attenuation was due to the interrupted gene and not to polar effects resulting from transposon insertion. Knockouts were constructed via overlapping extension PCR and subsequent SacB counter-selection. The mouse model was used to compare survival of marked (kanamycin resistant-Km^R) to unmarked deletions (Km^S) and wild-type. Both marked and unmarked deletions exhibited the reduction in virulence relative to the parental wild-type while no noticeable difference in attenuation was observed between marked and unmarked mutants. In addition, mutants made in either *B. abortus* or *B. melitensis* revealed no phenotypic differences in the mouse model resulting from differences in genetic background. Several of the mutants have been evaluated in the pregnant goat model, in order to compare safety. One mutant, BMV2, caused neither seroconversion nor abortion in the goats, which was predicted by rapid clearance in the mouse model. A non-rapid clearing mutant, BM8, colonized the dams but was unable to colonize the fetuses. Future plans include evaluating the efficacy of these mutants including several correlates of protective immunity.

94- NEW SYSTEM TO ENCAPSULATE ANTIGENIC EXTRACTS FROM *Brucella ovis* FOR VACCINAL PURPOSES.

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Vaccination against *Brucella* infections in animals is usually performed by administration of live attenuated smooth *Brucella* strains. However, live vaccines present some inconvenient, such as the residual virulence or the interference with serological diagnosis. Therefore, it was previously developed an innocuous subcellular vaccine based on microparticles (Mp) as transport vehicles (adjuvants) for the control release of antigenic extract of *Brucella ovis* (HS) [Murillo et al., Vaccine 19 (2001): 4099-4106]. Founded on that approach, we have now designed a procedure to prepare microparticles by a w/o/w emulsion solvent evaporation method carried out by TROMS (Total Recirculation One-Machine System). This method is a new procedure based on the formation of a multiple emulsion by the injection of the phases under a turbulent regime, avoiding the use of aggressive homogenisation techniques. The method is easily reproducible and applicable on a semi-industrial scale, permits complete control of all production parameters and can be performed under sterile conditions [García de Barrio et al., J. Contr. Rel. 86 (2003) 123-130]. Mp characterization was performed according to the following parameters: size (2.09 ± 0.85 μm), zeta potential (-20.05 ± 0.90 mV), HS content (15.2 ± 1.9 μg/mg), encapsulation efficiency (73.5 ± 13.7 %), distribution of HS on/in microparticles (surface 8.4%, strongly binding to surface 11.1 %, inside of particle 80.5 %). Different parameters may affect on the characteristics of resulting Mp (such as pharmaceutical auxiliaries and cyclodextrins). Thus, around 32 % of the loaded HS was released from the formulations without β-cyclodextrin (β-CD) and Pluronic® F-68 (F 68). In contrast, the release of HS from formulations without with both components was only 4.2 %, and 15.5 % when F-68 was omitted. Chemical and serological analysis

indicated that HS encapsulated conserved its antigenicity. Therefore, a study of protection against an experimental challenge with *B. melitensis* (Bm) H 38 in BALB/c mice was performed. The animals were vaccinated subcuteanously with both free and encapsulated HS. Immunized and control mice were challenged 8 weeks later with Bm (ip). HS-MP conferred significant protection in comparison with free HS immunized mice or non-immunized control group. Moreover, this protection was similar to that conferred by the Rev 1 reference vaccine.

95- THE CONSTRUCTION AND *in vitro* EVALUATION OF DNA VACCINES AND CANDIDATE ANTIGENS FOR PROTECTION AGAINST BRUCELLOSIS.

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Five novel candidate antigens were selected for development into DNA vaccines. Specific primers were designed for each of the candidates to include kozac sequences and unique restriction sites to facilitate cloning. Commercially available eukaryotic expression constructs were used as the backbone for the DNA vaccine. Prokaryotic expression constructs designed to generate specific recombinant fusion proteins were also produced. All plasmid identities were verified by sequencing. Expression from prokaryotic constructs was determined through SDS PAGE and western blotting studies. Specific proteins were purified through various affinity chromatography techniques. Expression from DNA vaccines was verified through transient transfection of Cos7 cells, and detection of specific gene transcription through RT-PCR or detection of expressed protein through indirect immunofluoresence assay. This poster describes the generation and quality control aspects of DNA vaccine production that are essential to the success of the study.

96- THE DEVELOPMENT AND ASSESSMENT OF 5 NOVEL DNA BASED VACCINES FOR PROTECTION AGAINST *Brucella* spp.

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Brucellosis is a zoonotic disease of considerable socio-economic importance. Vaccination is considered to be an important component of control and eradication strategies. Currently available vaccines for animal brucellosis are based upon live attenuated strains of the organism. Although effective, these vaccines are impractical due to their persistence in the host and associated residual virulence, pathogenicity to humans, and induction of an immune response that cannot be distinguished from a natural infection. Effective defined non-living or sub-unit vaccines have the potential to overcome these problems. Here we describe the rational selection, construction and evaluation of 5 novel DNA based vaccines. Candidate antigens were selected from genome data and post genomic analysis. DNA vaccines based upon *omp25*, *acvB*, FrpB, invasion protein B, and FliC have been assessed for immunogenicity and protective efficacy in the Balb/c mouse model. Each vaccine was capable of generating a *Brucella* specific Th1 biased immune response, as determined by serology and antigen specific cytokine responses. Two of the vaccine candidates, *omp25* and invasion protein B, were able to provide a significant level of protection to

challenged Balb/c mice. Vaccines based upon *omp25* were shown to be at least as protective as Rev1 in challenge studies with *B. melitensis* 16M.

97- IMMUNOGENICITY AND PROTECTIVE EFFICACY OF 5 NOVEL DNA VACCINES IN THE BALB/c MOUSE.

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The development and *in-vitro* assessment of DNA vaccines encoding the genes *omp25*, FliC, FrpB, AcvB, and invasion protein B is described in accompanying posters 1 and 2. The immunogenicity and protective efficacy of these candidates was assessed in the Balb/c mouse. Each candidate was shown to elicit a *Brucella* specific immune response. Serological assessment indicated a bias from all candidates toward IgG_{2a} isotype antibody production, suggesting a Th1 mediated immune response. Specific cytokine production was measured through RT-PCR and ELISA of cell lysates or supernatants from specific antigen stimulated splenocytes. IFN-γ and IL-4 were detected up to 12 weeks post vaccination. In protection studies, vaccinated mice were challenged with *B. melitensis* 16M. Mice receiving the DNA vaccines based upon invasion protein B and *omp25* were able to control infection to a similar level to those receiving vaccination with the live attenuated vaccine Rev1. Here we describe the immunological findings and their relation to the protection results.

98- THE SELECTION OF POTENTIAL PROTECTIVE ANTIGENS FOR DEVELOPMENT OF SUB-UNIT VACCINES AGAINST BRUCELLOSIS.

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Identification of protective antigens is the first step in rational vaccine design. The recent completion of the *Brucella* spp. genomes and the availability of numerous techniques for in depth in-silico analysis, has enabled us to identify a selection of potentially important antigens for investigation as vaccine candidates. Homologies to known protective antigens, structural information and putative T and B cell epitopes were assessed through bioinformatics approaches. RT-PCR techniques were then used to determine the expression of these candidate genes from *B. melitensis* 16M, cultured under various conditions. This poster describes the first step toward the development of rationally designed DNA vaccines through this selection and elimination of putative vaccine candidates.

99- THE EFFECTS OF VARIOUS FACTORS ON THE VIABILITY OF *Brucella abortus* STRAIN 19 VACCINE.

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This study aimed to produce *Brucella abortus* strain S.19 vaccine in a safe and effective form that meets the recognized international laboratories standards. And in a lyophilized form that eases its distribution. This study also endeavored the use of bioreactor in the mass production of S.19 vaccine in the Sudan. In this research the factors that affect the viability of *Brucella abortus* S.19 vaccine were studied. The temperatures 25ºC and were found of be optimum for the stability of the vaccine. This indicates that vaccination under Sudan conditions where the temperature ranges between 25-40ºC does not affect the viability of the vaccine up to 40 minutes, which is the time advised for vaccination from this finding. All the cells were destroyed when the vaccine was heated to 60ºC for 5 minutes. This concludes that pasteurization and boiling of milk completely eliminate the hazard of the sheded brucella S.19 cells in milk post vaccination. S.19 vaccine was highly susceptible to the direct sun light and it lost about 53.1% of its viability within two hours of exposure to direct diffuse day light. Where as using dark non-transparent bottle restored its viability after the same time of exposure. This illustrates the importance of containing the vaccine in dark protected bottles during vaccination in shiny hot season to ensure the recommended viable cells per dose. The phosphate buffered saline and the distilled water were found sufficient to restore the viability of the vaccine when they were used in suspending the harvested cells where as viability of the cells was adversely affected when normal saline was used for the same period of time. The effect of four disinfectants on the viability of S.19 vaccine was studied. Viz.: formalin, 70-75% alcohol, 0.5% phenol and chlorophenol. Out of these formalin was found to be the best with 100% lethality of the cells during the first ten minutes. Seventy percent and 75% ethyl alcohol were also able to inactivate the cells, but to a lesser degree compared to formalin. When 0.5% phenol solution and absolute chlorophenol were used, only partial inactivation was observed. The vaccine was freeze-dried, and the best cell-surviving rate was 99.4% after 11 months of storage in –20ºC when the lyophilizing stabilizing stabilizer was used. And 98.7% when the serum-lactose solution was used under the same conditions. When the PBS was used during lyophilization, it did not protect the cells against the lyophilization effect. Also during this study, the *Brucella abortus* S.19 vaccine was produced by bath method using Göttingen bioreactor. The estimated number of doses per a single batch (two liters) was 29,000 full dose. When the OIE reference lab checked the quality of the vaccine, it met the international standard.

100- VARIATIONS IN THE LYSIS OF *Brucella melitensis* Rev 1 BY BACTERIOPHAGES.

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Brucella melitensis Rev 1 confers a high level of protection against virulent *B. melitensis* in goats and ewes. The purpose of this work, was to report the variations of strains from eight different production batches of *B. melitensis* Rev 1 vaccine. Three *B. melitensis* and one *B*. *abortus* reference strains were used as controls. The colonial phase of *B. melitensis* Rev 1 cultures were determined using the acriflavine test and crystal violet stain. Phage sensitivity testing was done with phages Tbilisi (Tb), Weybridge (Wb), Izatnager (Iz) and R/C, at routine test dilution 10_4 (RTD), they were incubated at 37ºC in atmosphere with 10% CO_2 and later lysis was observed.

The vaccine strains differed from the recognized pattern in one or more characteristics. These strains were atypical regarding their growing on fuchsin and thionin at recommended concentrations, the other strains gave suitable results for the *Brucella* genus. In the phage sensitivity Rev 1 strains were found to be lysed for all phages for the other hand reference strains of *B. melitensis* were lysed only by Iz phage.

101- THE INOCULATION OF MICE WITH VIRULENT OR ATTENUATED *Brucellae* OR SMOOTH LIPOPOLYSACCHARIDE DOES NOT MODIFY THE COURSE OF SPLENIC INFECTIONS BY HOMOLOGOUS OR HETEROLOGOUS *Brucella* SPP.

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It has been suggested that vaccination and immunotherapy have a therapeutic effect in brucellosis because these treatments reestimulate macrophages to resolve the infection, thus minimizing the clinical impact of disease. However, it has been reported that treatment with a subcellular fraction of S19 does not modify the course of *B. abortus* 544 chronically infected mice (Plommet et al 1982. Ann Rech Vet, 13 : 127-132.), and that the infection caused by smooth attenuated strains *B. abortus* S19 (Plommet and Plommet 1988. Ann Rech Vét, 19: 245-251) *or B. melitensis* Rev 1 (N. Bosseray, personal communication) can be reactivated after inoculating homologous virulent strains. To study whether such a therapeutic effect could be observed depending on the virulence of the strains involved, BALB/C mice infected with virulent *B. abortus* 2308 were inoculated with virulent *B. abortus* 544, attenuated *B. abortus* S19 or smooth lipopolysaccharide at day 4 (acute phase) or week 4 (chronic phase) post infection. None of these treatments modified the splenic 2308 CFU levels during the acute or the chronic phases of the infection. Similarly, the time course of infections by rough virulent *B. ovis* PA, virulent *B. melitensis* H38 or attenuated *B. melitensis* Rev 1 were not modified by inoculation with virulent *B. abortus* 544. It is concluded that no matter the virulence and surface characteristics of the strain inducing the primary infection, the course of brucellosis in BALB/c mice is not modified by a second infection with homologous, heterologous, virulent or attenuated brucellae or by inoculation with smooth lipopolysaccharide.

102- TAXONOMY AND NOMENCLATURE OF THE MEMBERS OF GENUS *Brucella*; A PROPOSED RE-EVALUATION OF THE TAXONOMIC RANK OF RESPECTIVE MEMBERS.

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Since the discoveries of the six nomenspecies of genus *Brucella* respectively, from the discovery by David Bruce of the ”Micrococcus of Malta fever” in 1887 and the creation of the *Brucella* genus by Meyer and Shaw in 1920, there has been a continuous debate on the relevance of looking upon this genus as being multispecific or monospecific. At the meeting of the Subcommittee on Taxonomy of *Brucella* in Manchester in 1986, a decision on genus *Brucella* as a monospecific genus was taken, based on results of genetic analysis methods and related criteria for species

definition. Reviewing the evolution during this passed century of *Brucella* discussions, with a focus on the post-decision time period, about the consequently questioned concept of a multispecific genus *Brucella*, I will present relevant facts for a re-evaluation of the mentioned Manchester decision. Thus, bringing a pragmatic approach to the taxonomy and nomenclature of genus *Brucella*, based on the *Brucella* Subcommittee discussions and on the updated scientific dealing with this topic in published articles, including reference work, and incorporating the results of emerging genetic analysis methods, I find there are reasons enough today to re-evaluate genus *Brucella* as comprising earlier validated five nomenspecies, ie the three major nomenspecies *Brucella melitensis* (ATCC 23456), *Brucella abortus* (ATCC 23448), *Brucella suis* (ATCC 23444), and their acknowledged biovars, and the two minor nomenspecies *Brucella ovis* (ATCC 25840), and *Brucella neotomae* (ATCC 23459); and also to reclassify *Brucella canis* (ATCC 23365) as the sixth biovar of *Brucella suis*.

103- CONTRIBUTION TO THE CLASSIFICATION OF *Brucella* SPECIES BASED ON TANDEM REPEAT TYPING.

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The genus *Brucella* is known to be very homogenous, making difficult the discrimination between strains, whatever the methods used, including phenotypical typing associated with molecular tests. The recent reports about isolates of *Brucella* from marine mammals reinforce the need for adapted molecular markers, in order to study the epidemiology of all *Brucella* strains. Sequences of whole genomes of *B. melitensis* 16M and *B. suis* 1330 are now available. It thus makes possible a systematic search for tandem repeats, which have been shown to be an interesting source of markers for the typing of some pathogenic bacteria. One-third of the tandem repeats, with at least 4 repeat units, are made of 8 base-pairs repeat units as shown by searching the tandem repeat database (http://minisatellites.u-psud.fr). We have explored on *Brucella* the polymorphism associated with this family of tandem repeats. For this purpose, 21 *Brucella* strains including 18 classical reference strains from the different *Brucella* biovars and 3 marine strains have been typed for 17 octameric repeats loci. One-half of the loci detect 8 alleles or more in the limited strain collection used here. Nei's polymorphism index varies from 0.5 to 0.9. All strains can be clearly differentiated with this set of markers, some of which are extremely variable. The clustering analysis which can be done on the resulting data aggregates the *B. abortus* strains well apart from the others.

This provides a very powerful tool for strain identification. The phylogenetic value of the approach will now need to be validated by the typing of much larger strain collections.

104- IDENTIFICATION OF SPECIES OF *Brucella* USING FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR).

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Fourier Transform Infrared Spectroscopy is a technique that has been used over the years in chemical analysis for the identification of substances and is one that may be applied to the characterisation of microorganisms. Since 80s it has successfully been applied to the differentiation of a wide range of microorganisms. Bacteria belonging to the *Brucella* genus are classified into six recognised species *B. melitensis*, *B. abortus*, *B. suis*, *B. canis*, *B. ovis* and *B. neotomae,* within some of which different biovars have been recorded. The marked tendency towards variation in the smooth rough phase, together with the laboriousness and risk involved in the methods used in their identification make their classification difficult. We studied the type strains of the different species and biovars of *Brucella* and 11 isolates of human origin of *B. melitensis*, six corresponding to biovar 1, one to biovar 2 and five to biovar 3. The results of lineal discriminant analysis performed using the data provide an above 95% likelihood of correct classification, over half of which are in fact above 99% for the vast majority of *Brucella* strains. Only one case of *B. melitensis* biovar 1 has been incorrectly classified. The rest of the microorganisms studied have been classified correctly in all cases to a likelihood of over 80%. In the graphic representation of the analysis a grouping of these can be seen in clusters, which include the different species. One of these comprises *B. melitensis*, another *B. abortus,* and another wider one is made up of *B. suis*. The *B canis, B. ovis* and *B. neotomae* strains appear separate from the previously described groups.

105- AFLP: A TOOL FOR THE IDENTIFICATION AND TYPING OF *Brucella* ISOLATES?.

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Brucellosis is a zoonotic disease of major public health, animal welfare and economic significance worldwide. Although the UK has achieved Officially Brucellosis-Free (OBF) status the constant threat of reintroduction highlights the need for improved diagnostic and epidemiological tools. Such tools enhance the speed of management of outbreaks by facilitating rapid identification of the infecting organism, its source and means of spread. In light of the complexity of classical *Brucella* typing the application of molecular techniques provides new approaches that will speed up the identification and typing process and hence reduce the risk of spread and the threat to human and animal welfare. Here we describe the development of AFLP procedures applicable to *Brucella*. Some fifty distinct combinations of restriction enzymes and/or selective primers were tested. Two, which gave the most promising profiles in terms of band size range, band spread and band number and showed differences between reference strains of distinct *Brucella* species, were selected for further study. These combinations (*Eco*RI+0/*Mse*I+TC and

*Apa*I+0/*Mse*I+G) were applied to AFLP studies of around 250 isolates of *Brucella* representing all type strains and known species of *Brucella*, including the recently identified marine mammal isolates, and a wide range of clinical isolates from animals and humans.

106- OXIDATIVE METABOLIC PROFILES OF *Brucella* STRAINS ISOLATED FROM MARINE MAMMALS.

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In spite of their genomic homogeneity, members of the genus *Brucella* still remain classified into six conventional species –*i.e.*, *B. melitensis*, *B. abortus*, *B. suis*, *B. ovis*, *B. canis* and *B. neotomae*- mainly on the basis of differences in pathogenicity and host preference. Since the 1990's, many bacterial strains were worldwide isolated from marine mammals. They were shown to have phenotypic characteristics of *Brucella*, without however being identified to existing species. These marine isolates were later confirmed to belong to the genus *Brucella* on the basis of DNA/DNA hybridization studies. Molecular studies, particularly on the polymorphism of the *omp2* locus, allowed the differentiation between terrestrial and marine *Brucella* and also between the marine isolates themselves. In this study, thirty-one *Brucella* strains isolated from various marine mammals (seals, porpoises, dolphins…) were examined for their oxidative metabolic pattern on a sample of 12 conventional amino-acid and carbohydrate substrates. The oxygen uptake for each substrate was measured on a Warburg apparatus. QO_2(N) values were then graphically presented, and compared with the oxidative profiles of the reference strains for the six known *Brucella* species. Three main oxidative profiles were identified in the marine sample, which were different from those of the *Brucella* reference strains. Interestingly, the subdivision of the marine *Brucella* by the oxidative metabolism was quite identical to that previously obtained with the *omp2* polymorphism studies on the same isolates. Thus, on the basis of both oxidative and *omp2* studies, at least two species, *B. pinnipediae* and *B. cetaceae*, could be proposed to classify the marine isolates within the genus *Brucella*.

107- MAPPING OF THE PROTEINS OF *Brucella abortus* AND CROSS-REACTING BACTERIA USING TWO-DIMENSIONAL (2-D) ELECTROPHORESIS AND SELDI (SURFACE-ENHANCED LASER DESORPTION/IONIZATION) PROTEIN CHIP TECHNOLOGY .

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Brucellosis is a re-emerging zoonosis resulting in a severe multiorgan disease in humans. Clinical signs and symptoms of brucellosis can be misleading as they are unspecific and may mimic many other febrile illnesses. Serological tests usually detect antibodies to LPS of smooth *Brucella* strains causing various cross-reactions. The antibody response to a preparation of cytoplasmatic proteins depleted of LPS has already proved to be more reliable and specific. A comprehensive proteomic

study of *B. abortus* and cross-reacting bacteria was conducted to identify proteins, which may help to distinguish *Brucella* spp. from *V. cholerae*, *F. tularensis*, *X. maltophilia*, etc. *B. abortus* and several cross-reacting bacteria were lysed. Total proteins were separated by isoelectric focusing in the first dimension using immobilized pH gradient strips, followed by SDS-PAGE in the second dimension. Proteins were visualized by silver staining and all gels were analyzed using 2D-Elite ImageMaster Software. For Surface-enhanced Laser Desorption/Ionization Mass Spectrometry (SELDI-MS) proteins were prepurified by using affinity ligands on ProteinChips. Retained proteins were eluted by laser desorption and ionization and their mass was determined by Time-of-Flight Mass Spectrometry.

Clear differences between *B. abortus* and cross-reacting bacteria were detected both in 2D-gels and in SELDI spectra. Potential biomarkers may be isolated this way. Although various differences in the proteome of the bacteria could be identified, the immunological role of these proteins has to be examined in future studies. Thus, proteomics may lead to medical advances in brucellosis, e.g. more significant diagnostic tools and possible vaccine candidates.

108- IDENTIFICATION OF IMMUNOGENIC PROTEINS IN THE PROTEOME OF *Brucella melitensis* AND *Brucella abortus* USING TWO-DIMENSIONAL ELECTROPHORESIS AND IMMUNOBLOTTING.

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Human brucellosis is predominantly caused by three species of the genus *Brucella* – *B. melitensis, B. abortus*, and *B. suis*. Clinical signs and symptoms of brucellosis are unspecific, antibiotic therapy often fails and effective human vaccines are still not available. Because of these characteristics *Brucella* was among the first biological agents weaponized in the 1950ies. An effective human vaccine at least for personnel with high occupational risks of infection would be desirable. The aim of our study was to identify specific immunogenic proteins in the proteome of *Brucella melitensis* and *Brucella abortus*. *B. melitensis* and *B. abortus* were lysed and total proteins were separated by isoelectric focusing in the first dimension using immobilized pH gradient strips, followed by SDS-PAGE in the second dimension. Proteins were visualized by silver staining and all gels were analyzed using 2D-Elite ImageMaster Software. A second gel was blotted on a nitrocellulose membrane and the immunogenic proteins were identified using *B. abortus* or *B. melitensis* hyperimmune serum.

Live-attenuated *Brucella* strains are used in veterinary medicine for vaccination. As accidental inoculation may cause a severe human disease, antigenic fractions of *Brucella* have been discussed for human uses, e.g. *Brucella* protective antigen and phenol-insoluble fraction. However, neither the efficacy nor the duration of protection could be clearly established. Using two-dimensional gel electrophoresis we were able to demonstrate the whole proteomes of human pathogenic *Brucella* spp. Immunoblotting revealed various immunogenic proteins in *B. melitensis* and *B. abortus*. A single specific protein or a defined cocktail of several immunogenic proteins may be the key for an adaequate human vaccine.

109- COMPARATIVE PROTEOMIC ANALYSIS OF *Brucella suis* BIOVARS 1 AND 2 USING TWO-DIMENSIONAL GEL ELECTROPHORESIS AND MASS SPECTROMETRY.

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Brucella suis is a Gram-negative, facultative, intracellular pathogen that causes brucellosis in both domestic and feral pigs. Porcine brucellosis is characterized by abortion, low production, infertility, and arthritis in infected animals. *B. suis* can also cause a serious infection in humans–characterized by a systemic, febrile illness. Although *B. suis* (biovars 1, 2 and 3) is still widely distributed in the world, prevalence in domestic pigs is low excepted in South-East Asia and South America. Since approximately 10 years, *B. suis* biovar 2 is emerging in outdoor breedings of domestic pigs in France. The population of wild boars in this country are largely contaminated by *B. suis* biovar 2 with up to 40% of serological prevalence. It is well known that transmission of *B. suis* biovar 2 between wild boars and domestic pigs is possible. However, no definitive explanation has been provided for the lower pathogenicity of this biovar for the wild boars compared to domestic pigs. Moreover, in contrast to *B. suis* biovar 1, human infections with *B. suis* biovar 2 have not yet been reported . In the present study, we compared the proteome of *B. suis* biovar 1, for which the genome sequence has recently been released and published, to that of strains of *B. suis* biovar 2, by using two-dimensional gel electrophoresis and matrix-assisted laser desorption/ionization (MALDI)-mass spectrometry (MS), to elucidate differences between the protein expression pattern of the two biovars and to identify potential molecular markers related to host preferentialism. Differentially expressed proteins involved in sugar transport, amino acid binding, oxydative pathways were identified. Expression of an immunogenic 31-kDa protein was altered in *B. suis* biovar 1.

110- IN SILICO ANALYSIS OF THE CtrA REGULON IN ALPHA PROTEOBACTERIA.

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CtrA is a transcriptional regulator found in all alpha proteobacteria analysed to date. In this group, we find notably the free living bacterium *Caulobacter crescentus*, the plant's symbionts *Sinorhizobium meliloti* and *Mesorhizobium loti*, the plant's pathogen *Agrobacterium tumefaciens*, or yet the facultative or obligate animal's pathogens *Brucella* sp. or *Rickettsia prowazekii*, respectively. In *C. crescentus*, CtrA is controlled by a complex multicomponent signal transduction system that regulates and synchronises multiple cell cycle events (Quon et al., 1996). All the promoters directly regulated by CtrA contain a characteristic DNA motif (TTAA-N7-TTAAC or TTAACCAT), defined as the CtrA binding sites since CtrA is able to bind it *in vitro* and *in vivo* (Laub et al, 2002). We analysed the CtrA regulon in other alpha proteobacteria to get a better understanding of the CtrA role in these bacteria. The release of the alpha proteobacterial genomes has permitted us to characterize in silico both the upstream and downstream pathways of CtrA regulons. Our results indicate first that the upstream regulating pathway is partially conserved between

these bacterial species. Indeed, in all genomes except for *R. prowazekii*, we find not only one homolog for each factor potentially involved in the control of a CtrA activity in *C. crescentus*, but also other potential CtrA activating factor(s) in some species. For identification of CtrA targets, we have searched all promoter regions containing the CtrA binding site consensus in the seven genomes. Our observations suggest that similar processes are regulated by CtrA through the control of distinct target genes. In conclusion, our results indicate that the alpha proteobacteria are adapted to their lifestyles while keeping an essential transcriptional regulator with a view to controlling generic cellular processes.

Laub, M. T., Chen, S. L., Shapiro, L. and McAdams, H. H. (2002) Proc. Natl. Acad. Sci. 99 : 4632-4637.

Quon, K. C., Marczynski, G. T., and Shapiro, L. (1996) Cell 84 : 83-93.

111- ROLE OF THE ALTERNATIVE SIGMA FACTORS IN *Brucella melitensis* 16M VIRULENCE.

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One of the most important step in regulation of bacterial virulence genes is the initiation of transcription. For recognition of promoter sequences, the RNA polymerase recruits an additional protein known as a sigma factor. Based on sequence similarity, six Sigma factors were identified in *Brucella melitensis* 16M : Sigma 70 (the housekeeping Sigma factor), two Sigma 32 homologs, two ECF sigma factors and Sigma 54. The Sigma 32 and Sigma ECF proteins are alternatives sigma factors. Some of these Sigma factors might play a role in the regulation of virulence-related factors in *Brucella*, as observed in other bacterial pathogens such as pilin in *Pseudomonas aeruginosa* [Totten P. et al. 1990, J. Bacteriol. 172, 389] and HtrA, a stress-induced serine protease, in *Salmonella typhimurium* [Humphreys, S., et al., 1999 Infect. Immun. 67, 1560].

We constructed mutants for the predicted alternative Sigma factors and Sigma 54 by gene replacement in *B. melitensis* 16M. The five mutants were characterized for their ability to replicate in bovine macrophages and in HeLa cells. One Sigma ECF mutant was identified as defective for its intracellular survival in HeLa cells. Mutants were also tested in a murine infection model (BALB/c mice). Two mutants, including the Sigma ECF mutant unable to replicate in HeLa cells, were attenuated in the mouse at 4 weeks post-infection.

112- IDENTIFICATION AND CHARACTERIZATION OF THE BRUCEBACTIN TRANSPORT SYSTEM.

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Brucella abortus produces Brucebactin, a strong catecholic siderophore different from 2,3-DHBA. The genes involved in the biosynthesis of this siderophore showed high homology to the enterobactin biosynthesis region in *E. coli*. Brucebactin is probably a complex siderophore which needs a transport system similar to

enterobactin. At the present time nothing is known about how iron is transported into brucellae, unlike other gram–negative bacteria *B. abortus* did not show induction of membrane proteins when grown in iron deprived medium (Infect. Immunity 60:4496-4503). A BLAST search performed on the sequence data of *B. melitensis* 16M and *B. suis* 1330, revealed the presence of two clusters encoding components of two putative receptors for such a siderophore. One of the systems shares homology to the anguibactin receptor from *Vibrio anguillarum*, while the other shows homology to Vitamine B12 and siderophore receptors from different organisms. We have constructed nonpolar deletion mutants at the two loci in the *B. abortus* 2308 strain. The mutant in the anguibactin system, 2308 DBMEII0607 was unable to grow in minimal medium in presence of 2,2´dipyridyl, however it produces brucebactin, as showed by CAS and Arnow assays and TLC. Catecholic extracts from *B. abortus* 2308 and 2308 DBMEII0607 culture supernatants, both positive in CAS assay, complemented strain B. abortus BAM41, that is unable to grow in iron deprived medium due to its unability to synthesize brucebactin (Microbiology 148:353-360), in crossfeeding assays in minimal medium with 2,2`dipyridyl. This result suggests that 2308 DBMEII0607 produces brucebactin but is unable to assimilate iron, probably due to unability to transport ferri-brucebactin. BMEII0607 thus seems to constitute an integral component of the brucebactin transport system. The mutants with deletions in the locus homologous to Vitamim B12 and siderophore receptors seems to be unaffected in iron assimilation.

113- CHARACTERISATION OF *rpo*B MUTATIONS ASSOCIATED WITH THE RIFAMPIN-RESISTANT PHENOTYPE IN *Brucella* spp.

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Brucella abortus strain RB51, currently used in United States as vaccine in cattle, is a rough rifampin-resistant mutant derived by repeated passages of *B. abortus* virulent strain 2308 on media supplemented with rifampin. Rifampin was added to the medium to induce the rough phenotype and because previous studies indicated that rifampin-resistant (Rifr) organisms were less virulent than rifampin-susceptible (Rifs) strains. The genetic bases for rifampin resistance in RB51 were not investigated: currently, no data about mutations associated with rifampin phenotype are available in *Brucella* spp. The rifampin antibiotic target in prokaryotes is the b-subunit of the DNA-dependent RNA polymerase encoded by *rpoB* gene. Several mutations were mapped to the *rpoB* gene and associated with rifampin resistance in *Escherichia coli* and *Mycobacteria*. In this report we analysed the nucleotide sequence of the *rpoB* gene (4134 bp) in 20 Rifs *Brucella* strains, 20 Rifr colonies derived from two clinical isolates of *B. melitensis* rough mutants and the well-known rifampin-resistant RB51.The Rifr colonies were obtained by culturing *B. melitensis* rough strains on *Brucella* Agar supplemented with rifampin from 40 to 400 μg/ml. After several passages on unsupplemented medium in order to stabilise putative mutations, the sequence of *rpoB* gene of Rifr colonies was compared with that of Rifs strains of *B. melitensis* biovars 1, 2 and 3, *B. abortus* biovars 1, 3, 4,5 and 6, *B. ovis*, *B. suis* biovars 1, 2, 3 and 4. The sequence of *rpoB* gene of *B. melitensis* 16M was considered as reference. No mutations were observed in Rifs strains. On the

contrary, all Rifr strains carried one or two missense mutations within the *rpoB* gene resulting in eight different genotypes. The most frequently *rpoB* mutations affected either Asp-536 or Val-154 and Met-1249. Our results represent the first study to detect specific *rpoB* mutations and evaluate their correlation to resistance phenotype in *Brucella* spp.

114- EVIDENCE OF IS*711* TRANSPOSITION IN *B. ovis* AND *B. maris*.

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Brucella genome was shown to harbour an IS element denominated IS711 or IS6501. IS711 is specific of *Brucella* and it is found in the genomes of all the different species in a variable copy number ranging from 7 copies in *B. abortus, B. melitensis* and *B. suis* to about 30 in *B. ovis* and those species isolated from marine mammals (named *B. maris*) There was no evidence of transposition of IS711 so far, but the fact of that it has recently been reported a copy of IS711 interrupting the *wboA* in *B. abortus* strain RB51, not present in the rest of species and biovars, and the large copy number of this element in *B. ovis* and *B. maris* isolates, lead to think that IS711 can still transpose.

In this study we report an evidence of IS711 transposition from *B. ovis* and *B. maris* by using a “transposon trap” plasmid pGBG1 for isolating mobile DNA elements. The selection module that contains the mutagenesis target is composed of a silent *tetA* gene controlled by the pR promoter of bacteriophage l, which is repressed by the l CI repressor in which mutations produce a tetracycline resistance phenotype. *Brucella melitensis* 16M, *B. abortus* RB51, *B. ovis* BOC22 (field strain) and *B. maris* strains (B14/94, B1/94,B2/94) containing pGBG1 were grown in specific culture media with tetracycline (Tc) till the appearance of Tc resistant mutants (TcR). Then the TcR colonies were analysed by PCR with specific primers from the target sequence to detect the presence of transpositional mutations. TcR mutants due to IS711 transposition events were only detected in *B. ovis* and *B. maris* strains, for the rest of *Brucella* strains tested were only found TcR mutants due to point mutations and nucleotide deletions Five different copies of IS711 were found to transpose to the repressor.

115- PHYLOGENETIC ANALYSIS OF THE GENUS *Brucella* WITH PARTICULAR REFERENCE TO MARINE MAMMAL ISOLATES.

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Brucella strains have been isolated from marine mammal species since the mid 1990’s and have subsequently been typed by classical and molecular methods. Typing approaches indicate that marine strains are clearly different from the terrestrial strains and PCR-RFLP of the *omp2* loci (Microbiol. 1998, 144, 3267-3273) IS711 fingerprinting (J. Clin. Microbiol. 2000, 38, 1258-1262), and AFLP analysis also indicate that there are differences between the marine strains. To resolve these

apparent differences a phylogenetic analysis was undertaken on twenty-two reference and thirty-five marine mammal *Brucella* strains to derive an hypothesis of the evolution of the genus and form the basis of an appropriate taxonomic classification of marine *Brucella* species. Strains compared in the phylogenetic analysis included isolates from pinniped and cetacean species gathered from European and North American coastal locations. Results of the phylogenetic reconstruction suggest that marine isolates are a diverse group forming distinct clades that closely follow their host specificity and their geographic distribution. European strains isolated from pinnipediae and cetaceae appear to be host specific i.e. a monophyletic clade of pinniped strains and a monophyletic clade of cetacean strains. North American strains isolated from pinnipediae species form a separate monophyletic clade to the European strains. The nestedness of the phylogenetic reconstruction indicate that although pinniped and cetacean strains form distinct groups these results indicate that each species is further differentiated by their geographic location. The results of this study should be considered when making decisions on the taxonomic classification of *Brucella* strains isolated from marine mammal species.

116- THE *Brucella melitensis* FLAGELLAR GENES ARE NOT CRYPTIC.

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Even though brucellae are described as non motile, the *Brucella* genome contains three clusters of flagellar genes on the small chromosome. We identified all the strutural genes for building a flagellum as well as for its rotation however no gene for chemotactic receptors or transducers were detected. The major part of the 3 clusters showed a clear syntheny with other *Rhizobiaceae*. Several coding sequences typical of α-Proteobacteria flagellar systems are also present.

The following results demonstrate that *Brucella* flagellar genes are not cryptic. An STM screen of *B. melitensis* mutants in mice identified a mutant in the *fliF* gene encoding the flagellar MS ring homologue. The *fliF* promoter is specifically induced intracellularly. The genes *fliF*, *flgE* and *fliC* are expressed *in vitro*. The presence of *fliF* is necessary for FlgE and FliC expression indicating the existence of a flagellar regulon in *Brucella*. Mutants in coding sequences typical of flagellar systems (*motB*, *flgI*, *flgE* and fliC*)* are attenuated in BALB/c mice.

117- *Brucella* LIPIDS: CHARACTERIZATION OF *pmt*A, A GENE ENCODING A PHOSPHATIDYL-ETAHNOLAMINE-N-METHYLTRANSFERASE INVOLVED IN PHOSPHATIDYL-CHOLINE SYNTHESIS.

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Brucella cell envelopes are rich in phosphatidyl-choline, a phospholipid unusual in gram-negative pathogens. A search in the complete genomic sequence of *Brucella melitensis* 16M revealed a ORF (BMEI2000) putatively coding for a phosphatidyl-ethanolamine-N-methyltransferase (PmtA) with homologous in other members of the α-*Proteobacteria*. Analysis of the DNA region showed that

BMEI2000 is flanked by: (1), a putative transcription terminator for the gene situated immediately upstream coding for the (putative) chaperon protein DnaJ; and (2) a ORF situated about 150 bp downstream with homology to genes involved in pyrimidine metabolism, suggesting that BMEI2000 may be an independent transcriptional unit. Two possible translation starting codons were identified in the sequence but only the second is preceded by a putative ribosome binding site. The corresponding protein contained an amino acid stretch (VLEFGPGTGV) which corresponds to the consensus amino acid sequence described in methyltransferases. To ascertain its function, BMEI2000 was cloned in the expression vector pET21a and introduced in *E. coli* BL21(DE3). Cells induced with IPTG synthesized a new protein of the expected molecular weight. Research is in progress to ascertain whether expression of *Brucella* PmtA in *E. coli* results in a change in its phospholipid profile, as a first step to determine the role of phosphatidyl-choline in the unusual properties of *Brucella* cell envelopes.

118- EXPRESSION OF THE ExoS PROTEIN FROM *Sinorhizobium meliloti* COMPLEMENTS BIOLOGICAL PROPERTIES OF A *Brucella abortus* MUTANT LACKING THE BvrS PROTEIN.

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Brucella abortus is an intracellular pathogen that relies upon unconventional virulence factors to infect hosts. Of these factors, a two component regulatory system has proven to be essential for virulence since mutants in either the sensor (BvrS) or the regulator (BvrR) have lost the ability to penetrate to and survive within epithelial and phagocytic cell lines. In this work we have evaluated the degree of complementation provided by the sensor (ExoS) of an analogous two component system in the related plant pathogen *Sinorhizobium meliloti*. A *Brucella abortus* strain lacking BvrS (*B. abortus* 2.13) was transformed with a cosmid containing ExoS and a tetracycline resistance cassette which allows selection. Isolation of the cosmid from resistant strains confirmed successfull transformation. The complemented strain (2.13-ExoS) was tested for its ability to penetrate and survive within phagocytic and non-phagocytic cell lines. 2.13-ExoS recuperated the ability to invade non-phagocytic HeLa cells as determined by Colony Forming Units counting and Immunofluorescence. Mirroring the wildtype strain (2308), 2.13-ExoS was able to reach the endoplasmic reticulum avoiding lisosomes and replicated successfully within this compartment. In the same manner, 2.13-ExoS invaded the phagocytic cell line RAW 264.7 and followed a similar replication kinetic than strain 2308. Altogether these results imply that the BvrR-BvrS system from *Brucella abortus* is functionally interchangeable with the ExoS system from *Sinorhizobium meliloti.* The implications of these observations for the pathophysiology of brucellosis is discussed.

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