SCIENTIFIC COMMITTEE

STVM 2009 CONFERENCE ABSTRACT PROCEEDINGS EDITORIAL COMMITTEE

ACKNOWLEDMENTS AND CREDITS

Financial assistance was received from:

Applied Biosystems http://www3.appliedbiosystems.com/AB_Home/index.htm	**biolabproducts** http://www.biolabproducts.com/index.php
DFG http://www.dfg.de/	**DRG** http://www.drg-diagnostics.de/
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In Kind support was provided by:

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Welcome to the STVM Biennial Conference in Lübeck, Germany

The aim of the Society for Tropical Veterinary Medicine is to promote the international advancement of veterinary medicine, hygiene and related disciplines in tropical countries all around the world. The STVM moto is: **Working Together to Improve Animal Health Worldwide.**

The Society for Tropical Veterinary Medicine meets every two years in different locations, and after Hanoi, Vietnam in 2005 and Merida, Mexico in 2007 we will meet this year in Lübeck, Germany for our 10th Biennial Conference from June 28th to July 3rd, 2009. On behalf of the Organizing committee and the Scientific Board, I have the great pleasure to welcome you all coming from many countries around the world. The main scientific theme this year is: "**One Health, One Medicine: Building Bridges to Face the Challenge of Emerging and Zoonotic Diseases**".

The scientific program, prepared by our President-Elect Julio Figueroa, whom I would like to thank here, is highly interesting and exciting. This five-day conference will cover scientific topics from global zoonotic diseases to molecular research achievements from all over the World. This year representatives from the European Union, the OIE and European research networks will be interacting with scientists working on broad veterinary medicine topics. This conference should allow professionals to integrate and discuss research findings, policy requirements and educational needs for the benefit of a broad human and veterinary community and other stakeholders such as farmers, NGOs and the general public.

I also would like to thank our local organizers Ahmed Jabbar and Ulrike Seitzer who have been working hard with our President-Elect to ensure that the conference logistic and planning are so efficiently organised.

As provided during previous STVM conferences, participants presenting a talk or a poster would be able to publish their findings in *Transboundary and Emerging Diseases.*

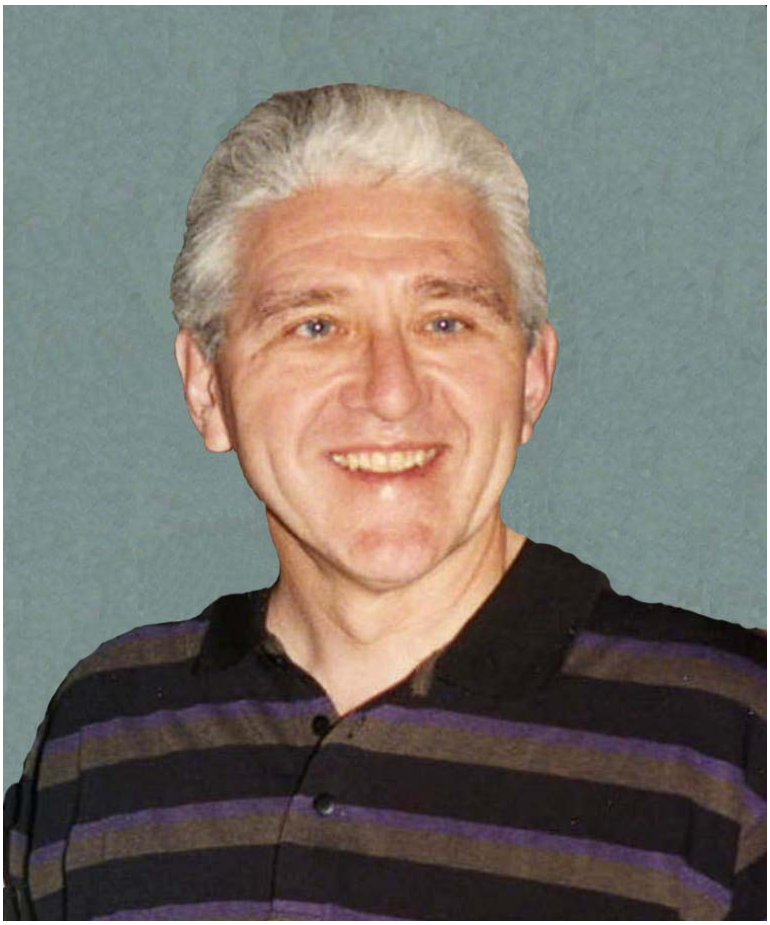
As usual with our STVM conferences and in addition to an exciting scientific program, the organizing committee has prepared many social events so participants can enjoy both highly professional presentations and some networking activities.

I wish you all a fruitful and enjoyable meeting.

Dr. Olivier Sparagano

STVM President

Dedication



The 10th Biennial Conference of the Society for Tropical Veterinary Medicine is dedicated to

Dr. Thomas E. Walton, Jr.

For his many contributions to the STVM and tropical veterinary medicine

In keeping with the tradition of the Society for Tropical Veterinary Medicine (STVM) Biennial Conferences, we are pleased to dedicate the 10th Biennial Conference of STVM to Dr. Thomas E. Walton, Jr. for his many important contributions to the field of tropical veterinary medicine and his many years of service to the STVM.

Dr. Walton received a DVM in 1964 from Purdue University and a Ph.D. from Cornell University in 1968. He was awarded an honorary Sc.D. from Purdue University in 1999 and Certificates from the Federal Executive Institute and Senior Executive Service in 1993 and 1995, respectively. He became a Diplomat of the American College of Veterinary

Microbiologists in 1970 and a Fellow of STVM in 2007. Dr. Walton has a long history of service with the United States Department, Agricultural Research Service, National Program Staff which began in 1972. From 1973 to 1985, Dr. Walton served as Research Leader and Supervising Veterinary Medical Officer at the USDA, ARS Arthropod-borne Animal Diseases Research Laboratory (ABADRL), Denver, Colorado, and in Laramie, Wyoming from 1985 to 1992. In 1994, he became the Acting Associate Director of the USDA, ARS, North Atlantic Areas and was stationed in Philadelphia, Pennsylvania. From 1992 to 1995, Dr. Walton served as the National Program Leader for Animal Health, USDA, ARS, National Program Staff, first at Beltsville, Maryland, and then as Director of the USDA, ARS, National Animal Disease Center (NADC) in Ames, Iowa. From 1997 to 2000, Dr. Walton was the Associate Deputy Administrator, Veterinary Services (VS), SUDA, APHIH in Washington, D.C. His last position before retiring in 2006 was Director, Centers for Epidemiology and Animal Health (CEAH), USDA, APHIS Veterinary Services in Fort Collins, CO.

Dr. Walton's numerous publications focus on many veterinary and tropical veterinary tropical diseases including feline viruses, Venezuelan encephalomyelitis, bluetongue virus, vesicular stomatitis virus, the impact of importation of animals and animal products and the threat of bioterrorism to the food supply.

Since retiring, Dr. Walton has served as consultant on many aspects of veterinary medicine, bio-containment and the plans to replace the current Plum Island Animal Disease Center of the Department of Homeland Security. He also was a consultant to the USDA, Foreign Agriculture Service and the Millennium Challenge Corporation on the control and eradication of foot and mouth disease and contagious bovine pleuropneumonia in Namibia.

Dr. Walton has received numerous recognitions and awards during his career including the Director's Award for Accomplishments in Program Management in 1981 and the Distinguished Alumnus Award from the College of Veterinary Medicine, Purdue University in 1996. In 1998 Dr. Walton received the Food and Drug Administration Commissioner's Special Citation and Harvey W. Wiley Medal, and he was awarded a Doctor of Science degree for career achievements from Purdue University in 1999.

Group photo taken at the STVM-93 Conference in Guadeloupe, FWI

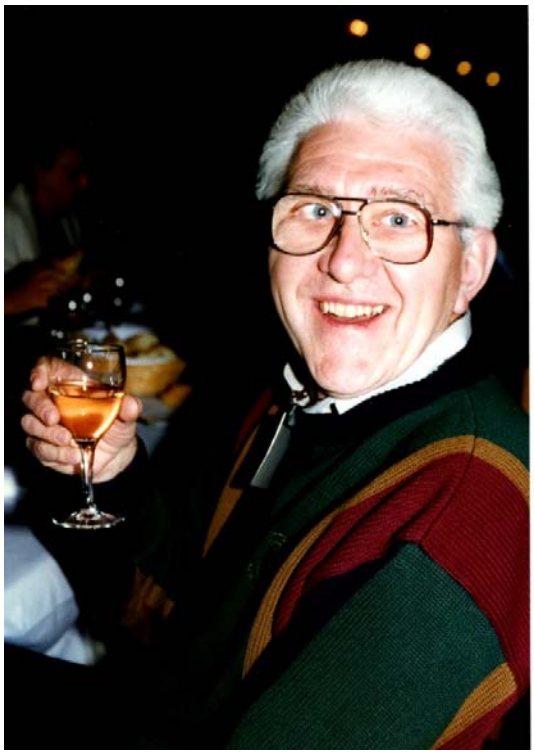
Dr. Walton was one of several participants at the 1st Biennial Meeting of STVM held in Puerto Rico in 1991 along with Drs. Nell Ahl, Ed Blouin, Bob Bokma, Emmanuel Camus, Julio Figueroa, Paul Gibbs, Carol House, Jim House and Kathy Kocan, who joined forces to form the foundation of STVM. Dr. Walton was elected Treasurer at this meeting and continued to serve as Treasurer and then Secretary-Treasurer until 2007. His wife, Mary Lou Walton, partnered with him to maintain the treasury and membership. Dr. Walton established the STVM as a non-profit organization and worked to establish the legal and governmental requirements needed to maintain the Society in compliance with regulatory agencies. He has maintained the financial stability of the STVM as a priority during the organization of each Biennial Conference and has served on the organizing committee of all previous conferences. It has been this dedication to maintaining the stability and infrastructure of the society that has allowed the STVM Biennial Conferences to remain successful.

Mary Lou Walton at the STVM-97 Conference in Montpellier, France

Mary Lou Walton and Dr. Jim House (Former STVM President)

Dr. Walton is recognized at STVM 2009 for building a solid treasury for STVM and for providing continual leadership which served as the ground-work and stabilizing factor that has bridged the biennial transitions among STVM Executive Committees over the years.

From all your friends and colleagues over your many years of service to STVM, we are proud to name Dr. Walton our 2009 Dedicatee. We congratulate and thank you for your many important contributions to the field of tropical veterinary medicine, your leadership in the USDA Agricultural Research Service and, most importantly, for your consistence excellent service and dedication to the continuance and growth of STVM.



Dr. Tom Walton at the STVM-97 Conference in Montpellier, France

STVM-09
Lübeck, Germany
June 28- July 3, 2009

Katherine M. Kocan and Edmour F. Blouin
Department of Veterinary Pathobiology
Center for Veterinary Health Sciences
Oklahoma State University
Stillwater, Oklahoma

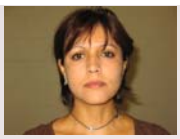
STVM NORVAL-YOUNG AWARD WINNER

Ruth Cecilia Galindo Ordoñez
Instituto de Investigación en Recursos Cinegéticos IREC
CSIC-UCLM-JCCM
Ronda de Toledo s/n
Ciudad Real
13005 Spain

Presenting: Thursday July 2nd, 2009, 13:45 h

O50: DIFFERENTIAL EXPRESSION OF INFLAMMATORY AND IMMUNE RESPONSE GENES IN SHEEP INFECTED WITH BACTERIA THAT TARGET IMMUNE CELLS.

Ruth C. Galindo, Pilar M. Muñoz, María J. de Miguel, Clara M. Marin, José M. Blasco, Victoria Naranjo, Katherine M. Kocan, Christian Gortazar, José de la Fuente



I was born in Guatemala City, Guatemala, Central America on March 6th 1981. I attended elementary school in a little town where my father was a doctor; it was then when I realized that tropical diseases in animals have a big impact in the human population, domestic and wild animal population. When I started high school I moved to Guatemala City, in 1998 I began my studies as a veterinarian and I became aware of the important roll that simple and good animal health control programs play in humans health. When I finished college I worked for a nongovernmental organization from Spain that was introducing programs for undeveloped communities in the health care and well being of their animals, that is when I realized that I wanted to keep studying and decided to pursue my PhD. Unfortunately in under developed countries such as Guatemala it is difficult or next to impossible to be able pursue this kind of studies, so in 2005 I decided to move to Spain to start my working on my PhD. I hope that in the future my work can contribute to further discovery of vaccines and help to develop control diseases programs.

ALAIN PROVOST AWARD WINNER

Youmna M'ghirbi
Laboratory of Medical Entomology
Institute Pasteur Tunis
13 Place Pasteur – BP 74
1002 Tunis-Belvedere
Tunisia

Presenting: Thursday July 2nd, 2009, 14:05 h

O51: SEROLOGICAL AND MOLECULAR SURVEY OF *BABESIA* PARASITES IN CATTLE IN TUNISIA

Youmna M'ghirbi, Ali Bouattour

Curriculum Vitae

Date and place of birth: 11 may 1980 at Béni Khiar, Tunisia
Nationality: Tunisian

Degree courses

2005-2009: PhD in Laboratory of Medical Entomology, mention: honourable, Institute Pasteur Tunis, TUNISIA.
2002-2004: master of Ecology, July 2004; Laboratory of Medical Entomology, Institute Pasteur Tunis, TUNISIA.
2002-1998: masters, Sciences of life, option Biotechnology, May 2002; Faculty of Sciences, Tunis, TUNISIA.

Qualifications

Baccalaureate Sciences, Tunisia **(1998).**
Master's degree in Sciences of life, option biotechnology, Faculty of Sciences Tunis, Tunisia **(2002).**
Master of Ecology, Faculty of Sciences Tunis, Tunisia **(2004).**
Diploma course: « Arthropodes Vectors et Human Health», 18 April au 15 June **2006**, Institute Pasteur of Paris.
PhD, Faculty of Sciences Tunis, Tunisia **(2009).**

Professional Courses

- Teacher of immunology; **2007/2008**. Institute Superior of Biotechnology Sciences of Tunis (ISSBAT), TUNISIA.
- Post Doctoral student in Service of Medical Entomology; 2009.

Scientific Contributions

- 6 publications in peer reviewed journalsm, participation at 6 national and 9 international meetings

Contribution to Scientific Projects

1. Projet INCO-MED: Lyme borreliosis in North Africa. Risk assessment and implications for management and for control of human disease, EU ICAS-CT2000-30009.
2. Projet Actions Concertées Interpasteuriennes (ACIP): Les fièvres récurrentes au Maroc et en Tunisie. Etude clinique et épidémiologique.
3. Projet espagnol : les maladies transmises par les tiques.

Memberships

- Member of the association of Ancient student of Institute Pasteur of Paris.
- Member of the Tunisian association of Biologic Sciences (ATSB).
- Member of women and sciences association (FS).

I N D E X

Scientific Program

SUNDAY JUNE 28TH

Participant arrival / Hotel transfers

Venue: Media Docks

14.00 Opening of Registration Desk

18.00-22.00 **Welcome Cocktail and Opening Reception**

MONDAY JUNE 29TH

8.00-8.30 Registration/Information

8.30-9.00 **Welcome Session**
(Chaired by Olivier Sparagano, STVM President, Ulrike Seitzer and Jabar Ahmed, STVM2009 Organizers, Julio V. Figueroa, STVM President-Elect and invited German Delegates)

9.00-10.00 **Session 1: "One Health**, **One Medicine**"
Chair: **Olivier Sparagano**

Keynote Address: Alain Dehove, World Fund Coordinator, OIE, Paris
O1. "One World - One Health"

10.00-10.30 Coffee Break

10.30-11.45 **Session 2: "Globalization/Zoonotic diseases"**
Chair: **Sathaporn Jittapalopong,** Co-Chair: **Klaus Lorenz**

10.30-10.45 **O2. Klaus Lorenz:** The Contribution of Tropical Veterinary Medicine to Veterinary Public Health in the Light of Globalisation

10.45-11.00 **O3. Maximilian P.O. Baumann:** The new Master Programme in Transboundary Animal Disease Management (MTADM) for Africa: an example for a North – South partnership to better cope with globalization

11.00-11.15 **O4. Wandee Kongkaew**: Trichinosis Foci in Thailand: Appraisal of Influential Factors from Local Behaviours

11.15-11.30 **O5. Tawin Inpankaew**: Serodiagnosis of *Toxoplasma gondii* infection in dairy cows in Thailand

11.30-11.45 **O7. Michael Furth**: Occurrence of *Taenia multiceps* Infection in Israel: New Zoonosis

11.45-13.00 Lunch

13.00-14.15 **Session 3: "Vaccines"**
Chair: **Jose de la Fuente,** Co-Chair: **Victoria Naranjo**

13.00-13.15 **O8. Sathaporn Jittapalapong:** Immune Response of Dairy Cows Immunized by Thai Anti-tick Vaccine (KU-VAC1) against *Rhipicephalus microplus*

13.15-13.30 **O9. Consuelo Almazan:** Vaccination with recombinant *Boophilus annulatus* Bm86 ortholog protein, Ba86, protects cattle against *B. annulatus* and *B. microplus* infestations

13.30-13.45 **O10. Srikanta Ghosh:** Efficacy of rHaa86, a Homologue of Bm86, Against Homologous Challenge Infestations of Hyalomma *anatolicum anatolicum*

13.45-14.00 **O11. Jose de la Fuente:** Understanding the function of the tick-protective antigen, subolesin, defines its role as a vaccine antigen for control of ticks and tick-borne pathogens

14.00-14.15 **O12. Olivier Sparagano:** Characterisation of the Immunological Response to *D. gallinae* Infestation in Domestic Fowl

14.15-14.45 Coffee break

14.45-16.15 **Session 4: "Ticks and Tick-borne diseases I"**
Chair: **Edmour Blouin** Co-Chair: **Gervasio Bechara**

14.45-15.00 **O14. Ivan G. Horak: T**icks at the Domestic Pets, Rodents/Human Interface in South Africa

15.00-15.15 **O15. Anne Marie Rhebergen:** Molecular Analysis of the Bm86 Orthologues from Argasid and Ixodid Ticks

15.15-15.30 **O16. Sathaporn Jittapalapong:** Immunization of Rabbits with SERPIN Recombinant Protein against cattle ticks (*Rhipicephalus (Boophilus) microplus*) infestation

15.30-15.45 **O17. Carlos R. Prudencio:** Combining the potential of antibody phage display and peptide phage display to select peptides mimicking epitopes of the tick *Boophilus microplus*

15.45-16.00 **O18. Alessandra Torina**: Prevalence of tick-borne pathogens in ticks in Sicily

16.00-16.15 **O19. Lygia M. F. Passos**: Dynamics of Natural *Ehrlichia canis* Infections in *Rhipicephalus sanguineus* Ticks in Brazil

Dinner at leisure

TUESDAY JUNE 30TH

8.00-8.30 Registration/Information

8.30-10.00 **Session 5: "Ticks and Tick-borne diseases II"**
Chair: **Kathy Kocan,** Co-Chair: **Consuelo Almazan**

8.30-8.45 **O20. Carlos R. Prudencio:** *Anaplasma marginale* epitope mapping based on random peptides libraries fused in filamentous bacteriophages

8.45-9.00 **O21. Annalisa Agnone:** Characterization of the Apical Membrane Antigen 1 in two Italian Strains of *Babesia bigemina*

9.00-9.15 **O22. J. Antonio Alvarez**: Immunization of *Bos taurus* steers with *Babesia bovis* recombinant antigens MSA-1, MSA-2c and 12D3

9.15-9.30 **O23. Dirk Geysen:** Recombination in buffalo-derived *T. parva* isolates trait to livestock of South Africa?

9.30-9.45 **O24. Abdalla A. Latif:** Corridor Disease (*Theileria parva* buffalo-associated infection in cattle) in South Africa: The Carrier-state in Cattle

9.45-10.00 **O25. Mohammed A. Bakheit:** Species Identification of *Theileria equi* and its Differentiation from *Babesia caballi* by High Resolution Melting (HRM) Analysis

10.00-10.30 Coffee break

10.30-12.00 **Session 6: "Host-vector-pathogen interactions"**
Chair: **Jabbar Ahmed** Co-Chair: **Julio V. Figueroa**

10.30-10.45 **O27. Katherine M. Kocan:** Silencing of genes involved in *Anaplasma marginale*-tick interactions affects the pathogen developmental cycle in *Dermacentor variabilis*

10.45-11.00 **O28. Edmour F. Blouin:** *Anaplasma phagocytophilum* infection in *Ixodes scapularis* (ISE6) cultured cells modifies tick gene expression

11.15-11.30 **O29. Zorica Zivkovic:** Differential expression of genes in salivary glands of male *Rhipicephalus* (*Boophilus*) *microplus* in response to infection with *Anaplasma marginale*

11.30-11.45 **O30. Agustín Estrada-Peña:** Vector-pathogen interactions: the impact of tick ecology on the evolution of *Anaplasma marginale*

11.45-12.45 Lunch

12.45-14.15 **Session 7: "Genetics**, **Genomics, Transcriptomics"**
Chair: **Ulrike Seitzer**, Co-Chair: **Nathalie Vachiery**

12.45-13.00 **O32. Victoria Naranjo:** Characterization of the chromosomal locus for the *Ixodes scapularis* tick protective antigen, subolesin

13.00-13.15 **O33. Nathalie Vachiéry:** Selective capture of transcribed sequences method of *Ehrlichia ruminantium* for transcriptomic study

13.15-13.30 **O34. Julio V. Figueroa:** *Babesia bigemina*: Expressed Sequence Tag Analysis of the intraerythrocytic stage

13.30-13.45 **O35. Stefano Reale:** Microsatellite Multilocus Polymorphism Analysis to Characterize *Leishmania infantum* Strains Isolated in Sicily

13.45-14.00 **O36. David Berthier**: Quantification of mRNA cytokine using specific qRT-PCR during an experimental trypanosome infection on African trypanotolerant and trypanosusceptible bovine

14.00-14.15 **O37. David Berthier**: Use of qRT-PCR Method to Validate SAGE Expression Data in Experimental *Trypanosoma congolense* Infection

14.15-14.30 Coffee break

14.15-16.00 **Session 8: "Poster session I"**

16.00-18.00 **ICTTD-3 Satellite meeting**

16.00-16.15 **Frans Jongejan**: Overview and progress of the ICTTD Project

16.15-16.45 **Jabbar Ahmed**: Asian Component of ICTTD-3

16.45-17.00 **Ivan Horak**: "A world list of valid ixodid tick species names supported by ICTTD"

17.00-17.15 **Abdalla Latif**: "Summary of activities of the Taxonomy group"

17.15-17.30 **José de La Fuente**: "Allopatric speciation in ticks: genetic and reproductive divergence between geographic strains of *Rhipicephalus* (*Boophilus*) *microplus*"

17.30-17.45 **Agustin Estrada Pena**: "ICTTD database development and predictive mapping"

17.45-18.00 **Umberto Vesco**: "Zoonoses database development"

Dinner at leisure

WEDNESDAY JULY 1ST

10.30 **Guided City Tour: Meeting point City Hall (Rathaus) Market Place (Marktplatz)**

12.30 **Bus transfer to Travemünde, the port of Lübeck: Meeting point Musik und Kongress Halle (MuK; Music and Congress Hall) Parking lot**

16.30-19.30 **Boat tour from Travemünde to Lübeck with dinner**

THURSDAY JULY 2ND

8.00-8.30 Registration/Information

8.30-10.00 **Session 9: "Viral and Bacterial Diseases"**
Chair: **Gervasio Bechara**, Co-Chair: **Fred Unger**

8.30-8.45 **O38. Nahed H. Ghoneim:** Foot and Mouth Disease in animals and man in Sharkia Governorate in Egypt

8.45-9.00 **O39. Abdel Rahim Mohammed Elhussein:** Studies on Caprine Arthritis Encephalitis Virus in Khartoum State-Sudan

9.00-9.15 **O40. Annalisa Guercio:** Ovine Catarrhal Fever (Bluetongue): analysis of Culicoides species in seropositive farms

9.15-9.30 **O41. Claudia Manno**: Entomological surveillance for Blue Tongue from 2003 to 2008 in Sicily

9.30-9.45 **O42. Fred Unger:** Selected results of a pilot study on RVF and *B. melitensis* in small ruminants in traditional farming systems in regions of The Gambia and Guinea and their public health impact

9.45-10.00 **O43. Hamidou H. Tamboura:** Global prevalence of main pathologies related to dairy production in urban flocks of Hamdallaye (Ouagadougou, Burkina Faso)

10.00-10.30 Coffee break

10.30-12.00 **Session 10: "Natural Products in Veterinary Medicine"**
Chair: **Olivier Sparagano**, Co-Chair: **Maria Eugenia López Arellano**

10.30-10.45 **O45. Adeolu A Adedapo:** Anti-inflammatory and analgesic activities of the aqueous extracts of *Margaritaria discoidea* (Euphorbiaceae) stem bark in experimental animal models

10.45-11.00 **O46. Olivier Sparagano:** Toxicity to the Poultry Red Mite (*Dermanyssus gallinae*) and Yield of Essential Oils Harvested from Wild Growing Plants in Tunisia

11.00-11.15 **O47. Nathesan Punniamurthy:** On Field Assessment of Anthelmintic Activity of Cost Effective (Herbal) Functional- Remedies in Goats for Self Reliance in Livestock Primary Healthcare

11.15-11.30 **O48. Maria E. López-Arellano:** Biochemical Characterization of two Purified Proteins of the IB-16 *Bacillus thuringiensis* Strain and their *In vitro* toxicity against the Sheep nematode *Haemonchus contortus*

11.30-12.30 Lunch

12.30-14.00 **Session 11: "STVM Award Session"**
Chair: **Olivier Sparagano**, Co-Chair: **Julio V. Figueroa**

12.30-13.15 **STVM Dedicatee Award**
O49. Thomas E. Walton: Measures of Success and Standards of Excellence: A Career in Tropical Veterinary Medicine.

13.15-13.35 **STVM Norval-Young Award:**
O50. Ruth C. Galindo: Differential expression of inflammatory and immune response genes in sheep infected with bacteria that target immune cells

13.35-13.55 **STVM Alain Provost Award:**
O51. Youmna M'ghirbi: Serological and Molecular Survey of *Babesia* Parasites in Cattle in Tunisia

13.55-14.25 Coffee break

14.00-16.00 **Session 12: "Poster Session II"**

16.00-19.00 **ConFluTech and Arbo-Zoonet Satellite Meetings**

ConFluTech

16.00-16.20 **Thomas Vahlenkamp:** Swine Influenza

16.20-16.40 **Christian Grund**: Molecular typing of Newcastle Disease virus: Possible consequences for vaccination.

16.40-17.00 **Timm Harder:** Towards a harmonized strategy for molecular diagnosis of avian influenza.

17.00-17.20 **Mohamed H. Hafez:** tba

17.20-17.40 Coffee Break

Arbo-Zoonet

17.40-18.00 **Franco Ruggeri:** Situation in Italy: Risks of importing emerging viruses (BTV, WNF)

18.00-18.20 **Roger Hewson**: tba

18.20-18.40 **Veronique Chevalier**: WP2 of Arbo-Zoonet: Geographical and temporal risk assessment: An integrated approach to assess the risk of introduction, amplification and dissemination of vector-borne zoonotic diseases in the EU

18.40-19.00 **Aysen Gargili**: Lessons from Crimean-Congo Hemorrhagic Fever in Turkey

STVM Business Meeting

20.00-24.00 Farewell Dinner, Schiffergesellschaft (House of the Guild of the Blue Water Captains)

FRIDAY JULY 3RD

8.00-8.30 Registration/Information

8.30-10.10 **Session 13: "Economics/Translational technology"**
Chair: **Dilip Bhandari**, Co-Chair: **Peter-Henning Clausen**

8.30-8.45 **O52. Paul Forster:** The Political Economy of Avian Influenza in Indonesia

8.45-9.00 **O53. Dilip P Bhandari:** Preventing Highly Pathogenic Avian Influenza (HPAI) at the Rural Community Level: A Case Study from Cambodia

9.00-9.15 **O54. Nathalie Vachiéry:** Surveillance of Avian Influenza in the Caribbean through the Caribbean Animal Health Network (CaribVET): Surveillance Tools and Epidemiological Studies

9.15-9.30 **O55. Dinh X.Tung**: Networking between researchers from different countries and backgrounds for collaborative AI research: Lessons from the Asia Partnership on Avian Influenza Research (APAIR)

9.30-9.45 **O56. Edi Basuno:** Socio-economic Impacts of Avian Influenza Outbreaks on Small-scale Producers in Indonesia

9.45-10.00 **O57. Peter-Henning Clausen**: Preventing and Containing Trypanocide Resistance in the Cotton Zone of West Africa

10.00-10.15 **O58. Burkhard Bauer:** Sustainable and Profitable Livestock Production in sub-Saharan Africa needs to be based on the Design of Innovative Vector Control Techniques for Resource-poor Farmers

10.15-10.45: Coffee break

10.45-12.15 **Session 14: "Wildlife diseases/Conservation Medicine"**
Chair: **A. Alonso Aguirre**, Co-Chair: **Ivan Horak**

10.45-11.00 **O59. A. Alonso Aguirre:** Conservation Medicine and One Health: Following the Steps of EcoHealth by Creating a Truly Global Transdiscipline

11.00-11.15 **O60. Rajiv Singh:** Status of Zoo and Wild Animals Diseases in India and Their Management

11.15-11.45 **O61. Ivan G. Horak:** Nematodes and Ticks at the Domestic Stock/Wildlife Interface in South Africa

11.45-12.00 **O62. Maxwell N Opara:** Dietary Influences Of Feed Types On The Haematological Indices Of Captive-Reared Grasscutters Experimentally Infected With *Trypanosoma Congolense*

12.00-12.15 **O63. Maxwell N Opara:** Therapeutic Effect of Berenil^{R} in Experimental Murine Trypanosomiasis using Stocks Isolated from Apparently Healthy Wild Grasscutters (*Thryonomys swinderianus*).

12.15-13.15 Lunch

13.15-14.15 **Session 15: "Trypanosomiasis"**
Chair: **Maxwell Opara** Co-Chair**: Carlos Gutierrez**

13.15-13.30 **O66. Richard J. Selby**: Analysis of cattle based sleeping sickness control in Northern Uganda

13.30-13.45 **O67. Sally L. Wastling:** A New and Improved Molecular Tool for Detecting *Trypanosoma brucei* s.l. in Cattle Blood Samples

13.45-14.00 **O68. Louise C. Hamill:** Domestic pigs as potential reservoirs of human and animal trypanosomiasis

14.00-14.15 **O70. Carlos Gutierrez:** Comparison of PCR and Hematocrit Centrifugation Technique to detect *Trypanosoma evansi* in goats

14.15-14.45 Coffee break

14.45-16.45 **Session 16: "STVM International Affairs Session"**

14.45-15.15 STVM 2011 Bids

15.15-15.45 Reviewing process for the Articles submitted

15.45-16.15 International grants: Future calls for collaboration

16.15-16.45 Closing session: Chaired by Olivier Sparagano, Julio V. Figueroa, Ulrike Seitzer and Jabbar Ahmed.

ABSTRACTS ORAL PRESENTATIONS

Session 1 One Health / One Medicine

O1: "ONE WORLD- ONE HEALTH"

Alain Dehove

Co-ordinator of the World Animal Health and Welfare Fund, OIE (World Organisation for Animal Health)

Session 2 Globalization / Zoonotic Diseases

O2: THE CONTRIBUTION OF TROPICAL VETERINARY MEDICINE TO VETERINARY PUBLIC HEALTH IN THE LIGHT OF GLOBALISATION

Klaus Lorenz

Federal Office of Consumer Protection and Food Safety, Berlin, Germany

Tropical veterinary medicine has a long history of attending to veterinary public health issues in tropical environments and particularly in developing countries. However, the traditional focus has usually been on animal disease control and animal husbandry issues with the aim to improve productivity. This traditional scope of interventions still contributes crucially to facing the current challenges of tropical veterinary medicine but it needs to be complemented by a broader view in the light of recent global developments.

Today we are confronted with a change of paradigms that have been consistent for a long time. Nowadays, tropical animal diseases pose an increasing threat to temperate regions of the world as documented by the incursion of West-Nile-Virus into the US or the Bluetongue outbreak in central Europe, and emerging diseases of animal origin like SARS can spread rapidly along the routes of business travel and tourism. Tropical veterinary medicine as part of a global veterinary public health approach can contribute significantly to sustainable solutions with an intimate understanding of the respective epidemiology and potential control options.

Besides the aspect of infectious diseases, there is a food hygiene aspect to tropical veterinary medicine as well. This is of particular importance as an increasing global demand for animal protein has led to a high degree of interconnectedness in markets all over the world with a potential "transfer of problems" through trade. This is an ongoing development. Potential consequences were highlighted by the recent scandal on melamin contamination of milk powder from China. Similar threats, possibly more related to problems of tropical veterinary medicine, could spread with similar extent and speed and need to be considered in time.

Problems with relevance to international trade that may originate from tropical and subtropical countries are documented in reports by the Food and Veterinary Office of the European Commission and include a general lack of hygiene, residues of pharmaceutical products and contaminants as well as potential threats from infectious diseases including zoonotic agents.

Tropical veterinary medicine should face these new challenges and develop a comprehensive veterinary public health approach that can contribute significantly and in a unique way to the overall concept of "One Health, One Medicine".

O3: THE NEW MASTER PROGRAMME IN TRANSBOUNDARY ANIMAL DISEASE MANAGEMENT (MTADM) FOR AFRICA: AN EXAMPLE FOR A NORTH – SOUTH PARTNERSHIP TO BETTER COPE WITH GLOBALISATION

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Livestock development issues are back on Africa's political and economical agenda with particular attention being paid on livestock trade and export as livestock diseases are the single most essential constraints to trade opportunities arising. Animal health standards imposed by importing countries for international, regional or bi-lateral trade, and through the World Trade Organisation's (WTO) Sanitary and Phytosanitary (SPS) agreement must be met. 12 of the 15 most important transboundary animal diseases persist in Africa.

Disease control under SPS or SPS compatible systems, entailing new standards, regulations and technologies, can and is not covered by conventional veterinary training. This specialist area of its own has to be addressed in a specialised postgraduate course for young personnel already involved and responsible for public, private and hybrid animal disease control services. Visions of a new African livestock sector with changed focus on production, disease, trade, marketing, organisation, delivery and internationality are only realistic with newly trained animal disease control personnel.

To target these issues at the academic level the Addis Ababa University, Ethiopia, with universities of three regional partner countries (Kenya, Uganda, Sudan) and the Freie Universität Berlin, Germany, successfully applied for a grant to establish a Joint Master Course in Transboundary Animal Disease Management (MTADM) for Africa. The 3-year project is funded under the EU - EDULINK Programme of the 9th European Development Funds (EDF) as from 2008 to 2010. The overall objective of the programme is to strengthen the capacity of national veterinary services in Africa to control and manage transboundary and epidemic diseases more effectively in a regional concerted action so as to (a) contributing towards developing or expanding exports markets and trade for animals and animal products and, (b) improve in the longer run the livelihood of livestock keepers as well as consumers demands on quality and safety of animal products.

The specific objectives are to build human resource capacity by producing an effective cadre of professionals in regional/transboundary animal disease control and management and to strengthen the regional network of the veterinary faculties of the participating African countries. This is to be achieved through the development of an innovative and state-of-the-art curriculum for the Joint MTADM Programme and a first MTADM course executed at Addis Ababa University, Ethiopia, Freie Universität Berlin, Germany, and the African home country of the participant.

O4: TRICHINOSIS FOCI IN THAILAND: APPRAISAL OF INFLUENTIAL FACTORS FROM LOCAL BEHAVIOURS

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Trichinosis was first known in Thailand in 1962, when an outbreak affected 53 residents of the north of the country, killing 11 of them. Since this initial outbreak the disease has been continuously reported and surveillance of trichinosis in humans has been established. Data from 1981 to 2007 cluster in the northern region; with spatio-temporal analysis revealing clusters of disease in seven Northern Provinces during 1983 to 1992. A decreasing trend of disease was observed. It could be accounted for by an improvement in pig farm management and the expansion of industrial pig farms.

Despite the declining trend, trichinosis has been repeatedly reported from some provinces. This study looked into the risk-associated behaviour of people in these areas. A study took place in 12 purposively selected villages of Nan Province in which 10 adult males and 10 adult females aged 15-70 years old from each village were requested to participate by the village chief. Age, sex, and occupation of participants were recorded. Pictures of local food and meat; practices such as pig farming, hunting, and eating raw food; news about food poisoning acquired from pork and a trichinosis outbreak were shown or read aloud to participants. Data from individuals and group discussions were collected to indicate their knowledge, attitudes, and practices. Results from individual respondents were analyzed and presented as percentages by gender. Responses to questions related to their attitude toward trichinosis prevention were carefully classified into three groups; favour trichinosis prevention, against trichinosis prevention, and uncertain.

Overall 225 respondents, 112 females and 113 males participated; the majority of men and women were able to identify dishes containing raw meat. About 70% and 67% of men and 77% and 41% of women have heard about illness from eating raw pork and trichinosis, respectively. Despite their knowledge, 67-82% of men and 20- 75% of women eat three local raw foods. About 84%, 81%, 69%, 22%, and 1% of men and 39%, 28%, 37%, 4% and 6% of women eat raw pork, beef, wild boar, bear, and dog. Attitudes of men and women favour trichinosis prevention. However they responded that a quarter or less of residents still eat raw meat or let their pigs scavenge. The association between these respondents' knowledge, attitudes and practices and the presence of trichinosis suggested practices in backyard farms and public awareness should be improved in these areas.

O5: SERODIAGNOSIS OF *TOXOPLASMA GONDII* INFECTION IN DAIRY COWS IN THAILAND

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Toxoplasma gondii is a zoonotic protozoan parasite of both medical and veterinary importance worldwide. The parasite can cause severe complications in immunocompomised individuals such as AIDS patients and transplant recipients; where up 25% of patients will develop toxoplasmic encephalitis. Thailand is a developing agricultural country located in Southeast Asia. Livestock developments particularly in dairy cows of this country have been hampered by low production including milk and slow growth rate due to

many pathogens including *T. gondii*. The objective of this study was to evaluate the serodiagnosis tool to use for detection of *T. gondii* infection in dairy cows of Thailand. During 2006-2007, the sera of 700 cows of 55 small farm holders from the top four highest number of dairy cow population in the northern provinces were collected and analyzed. Antibodies to *T. gondii* were assayed by Latex agglutination test (LAT) and Enzyme Linked Immunosorbent Assay (ELISA) and indirect immunofluorescence antibody test (IFAT). The overall prevalence of *T. gondii* infection was 66 (9.4%) by LAT and 119 (17%) found seropositive for ELISA. Sixty three (95.4%) seropositive samples by LAT and 21 (31.8%) by ELISA were confirmed by IFAT. The result demonstrated that LAT had the high sensitivity and specificity compared to ELISA and indicated that LAT should be used as routine diagnostic tests for detection of *T. gondii* infection of dairy cows in Thailand.

O7: OCCURRENCE OF *TAENIA MULTICEPS* INFECTION IN ISRAEL: NEW ZOONOSIS.

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Taenia multiceps (*Coenurus cerebralis*) infection is a common worldwide problem of small ruminants. Dogs harboring the adult worm (final host) play an important role in spreading the disease. Coenurosis may develop in the brain, spinal cord and in other tissues of a wide range of animals, including sheep, goats and some wild animals. In Israel, the disease was first described by Landau (1957) in herd in central Israel. Since then no new information regarding the prevalence of the disease in Israel was reported. During the period 2000-2008, a prevalence of 1.3 to 9.8% was demonstrated by us in some herds in central and southern Israel, leading to mortality rate of 1.14–24.61% and culling of animals to the extent of 37.4%. High variability was observed regarding the size and cyst locations. Most infections were demonstrated in 0.5-3 years old sheep. Clinical syndromes include vivid types of nervous symptoms with little or no change in hematological and biochemical profile. Treatment of coenuruses in sheep and goats using albendazole, niclosamide and praziquantel is only partially effective. Coenurosis is a rare disease in humans and less than 100 cases were reported from Africa, the United Kingdom, Italy, France, USA and North America. Recently, in the Negev desert area in southern Israel, an unusual case of a huge intraparenchymal cyst in a 4-year-old girl caused by T. multiceps was demonstrated.

Session 3 Vaccines

O8: IMMUNE RESPONSE OF DAIRY COWS IMMUNIZED BY THAI ANTI-TICK VACCINE (KU-VAC1) AGAINST *RHIPICEPHALUS MICROPLUS*

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Rhipicephalus (Boophilus) microplus causes tremendous economic losses in Thailand through direct effects of feeding and as pathogen vectors. Current tick control methods rely on chemical acaricides that result in environmental contamination, acaricide-resistant ticks and high costs for animal owners. Anti-tick vaccines based on concealed antigens have shown promising results in the control of this tick. An anti-tick vaccine against *R. microplus* based on Bm95 antigen has been developed in Thailand (KU-VAC1) as the alternative to control cattle ticks. The objectives of this study were to determine the antibody responses induced by KU-VAC1 using recombinant Bm95 proteins (rBm95) compared to the control (adjuvant and phosphate buffer saline-PBS). Each group contained 6 dairy cows were immunized by KU-VAC1, adjuvant, and PBS as control. These animals were inoculated three times with 3 weeks interval and sera were collected weekly. Enzyme linked immuno-sorbent assay (ELISA) were used to measure the humoral antibody responses and immunoblotting of rBm95 was used to evaluate recognition of anti-Bm95 sera to antigen proteins. Cattle immunized with KU-VAC1 showed antibody production significantly different when compared to the control group ($P<0.05$). The antibody response was increasing tremendously after the second immunization and at peak after the last immunization.
Acknowledgments: This research was facilitated through the Integrated Consortium on Ticks and Tick-borne Diseases (ICTTD-3), financed by the International Cooperation Program of the European Union, coordination action project No. 510561.

O9: VACCINATION WITH RECOMBINANT *BOOPHILUS ANNULATUS* BM86 ORTHOLOG PROTEIN, BA86, PROTECTS CATTLE AGAINST *B. ANNULATUS* AND *B. MICROPLUS* INFESTATIONS

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The cattle ticks, *Boophilus* spp., affect cattle production in tropical and subtropical regions of the world. Tick vaccines constitute a cost-effective and environmentally friendly alternative to tick control. The recombinant *B. microplus* Bm86 protective antigen has been shown to protect cattle against tick infestations. Recently, the gene coding for *B. annulatus* Bm86 ortholog, Ba86, was cloned and the recombinant protein was secreted and purified from the yeast *Pichia pastoris*. Recombinant Ba86 (Israel strain) was used to immunize cattle to test its efficacy for the control of *B. annulatus* (Mercedes, Texas, USA strain) and *B. microplus* (Susceptible, Mexico strain) infestations. Bm86 (Gavac and Mozambique strain) and adjuvant/saline were used as positive and negative controls, respectively. Vaccination with Ba86 reduced tick infestations (71% and 40%), weight (8% and 15%), oviposition (22% and 5%) and egg fertility (25% and 50%) for *B. annulatus* and *B. microplus,* respectively. The efficacy of both Ba86 and Bm86 was higher for *B. annulatus* than for *B. microplus*. The efficacy of Ba86 was higher for B. *annulatus* (83.0%) than for *B. microplus* (71.5%) while the efficacy on *B. microplus* of Bm86 (Gavac; 85.2%) but not Bm86 (Mozambique strain; 70.4%) was higher than that of Ba86 (71.5%). However, the efficacy of Bm86 (both Gavac and Mozambique strain; 99.6%) was higher than that of Ba86 (83.0%) on *B. annulatus*. These experiments showed the efficacy of recombinant Ba86 for the control of *B. annulatus* and *B. microplus* infestations in cattle and suggested that physiological differences between *B. microplus* and *B. annulatus* and those encoded in the sequence of Bm86 orthologs may be responsible for the differences in susceptibility of these tick species to Bm86 vaccines.

O10: EFFICACY OF RHAA86, A HOMOLOGUE OF BM86, AGAINST HOMOLOGOUS CHALLENGE INFESTATIONS OF *HYALOMMA ANATOLICUM ANATOLICUM*

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To develop suitable immunoprophylactic measures against *Hyalomma anatolicum anatolicum*, vector of bovine tropical theileriosis, the Bm86 homologue of *H. a. anatolicum* has been cloned and expressed in prokaryotic expression system and designated as rHaa86[a]. The level of expression was standardized and the expressed protein was bulk purified. Ten crossbred calves (*Bos indicus X B. taurus*) were randomly divided into two groups of five calves each. The calves of group1 were immunized with three inoculations (in monthly intervals) of rHaa 86 emulsified with 10% Montanide in mineral oil as an adjuvant and inoculated intramuscularly in thigh muscle. Suitable control group of calves (Group 2) were inoculated with adjuvant alone. All calves were challenged with 50 laboratory reared normal adults (male and female in equal number) of *H a anatolicum* on day 95.

The vaccinated calves showed sustained anti-rHaa86antibody response after the second immunization till day 32 post challenge thereafter. The major immunological responses in these calves were the elevated count of $CD4^+$ T-lymphocytes and increased level of expression of mRNA of IFN-γ in the peripheral blood mononuclear cells (PBMC) following induction with specific antigen *in-vitro*.

There were significant ($p< 0.05$) reductions in the number of engorged ticks dropped from the vaccinated calves (7.4 ± 1.9) in comparison to control calves (28.25 ± 4.9). Further there were a significant reductions both in the mean weight of the engorged adult ticks (vaccinated calves =263.17 ± 20.9 mg; control= 312.7 ± 9.3mg) and mean weight of the eggs laid by ticks fed on calves of group 1 and 2 (calves of group 1 = 149.3 ± 13.0 mg; group 2 = 217.4 ± 9.5 mg) and both the data were found significant at 5% level. The results indicated effectiveness of rHaa86 antigen in imparting protective immunity against *H a anatolicum* infesting cross-bred cattle.

The research has been facilitated through The integrated consortium on Ticks and Tick borne diseases (ICTTD-3) financed by the International Cooperation Programme of the European union through Coordination Action Project no. 510561

O11: UNDERSTANDING THE FUNCTION OF THE TICK-PROTECTIVE ANTIGEN, SUBOLESIN, DEFINES ITS ROLE AS A VACCINE ANTIGEN FOR CONTROL OF TICKS AND TICK-BORNE PATHOGENS.

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Subolesin is an evolutionarily conserved protein that was discovered recently in *Ixodes scapularis* as a tick protective antigen that impacts blood digestion, reproduction and development. Functional genomics

studies demonstrated that tick subolesin is a regulatory protein involved in control of multiple cellular pathways through interaction with other proteins. Furthermore, subolesin was shown to be an ortholog of akirins, a recently renamed group of proteins that function as transcription factors in *Drosophila* and mice. As demonstrated for insect and vertebrate akirins, subolesin was found to function in the regulation of NF-kB-dependent and independent expression of genes involved in different cellular pathways such as signal transduction and innate immune responses. Collectively, these results provide evidence that subolesin plays a role in control of tick gene expression, thus impacting tick-pathogen interactions. Defining the function of subolesin is fundamental toward understanding the mechanism by which it functions as a vaccine antigen to protect vertebrate hosts against tick infestations and to limit tick-borne pathogen infection and transmission.

Acknowledgments: This research was supported by the Ministerio de Ciencia e Innovación, Spain (project BFU2008-01244/BMC), the Oklahoma Agricultural Experiment Station (project 1669), and the Walter R. Sitlington Endowed Chair for Food Animal Research (K. M. Kocan, Oklahoma State University) and was facilitated through the Integrated Consortium on Ticks and Tick-borne Diseases (ICTTD-3), financed by the International Cooperation Program of the European Union, coordination action project No. 510561.

O12: CHARACTERISATION OF THE IMMUNOLOGICAL RESPONSE TO *D. GALLINAE* INFESTATION IN DOMESTIC FOWL

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Dermanyssus gallinae is a haematophagous ectoparasite of bird adversely affecting their welfare and commercial poultry egg and meat production. Poultry in commercial production systems chronically exposed to *D. gallinae* do not appear to develop immunity to it for unknown reasons. The objective of the current study was to determine the initial immune response in domestic fowl following exposure to *D. gallinae* by measuring mRNA expression (the study of avian cellular immune responses is hampered by the lack of readily available specific antibodies).

Mite chambers were secured to birds in two treatment groups (12 birds/group), Control and Infested. Controls received no mites, whilst Infested birds received 200 unfed female *D. gallinae* on Day 0. Mites were removed on Day 1 or 2 and birds were euthanased on Days 1, 2 and 5. The expression of Th1 (IFN-γ and IL-18), Th2 (IL-10 and IL-13) and pro-inflammatory cytokines (IL-6 and CXCLi2 (the avian equivalent of IL-8)) in spleen samples from 3 out of 4 birds/group at each time point was determined by semi-quantitative PCR. Individual mRNA cytokine expression levels were normalised against the individual house-keeping gene glyceraldehyde 3-phosphate dehydrogenase **(**GAPDH) and data analysed by analysis of variance. Data are presented as the ratio of mean cytokine expression of Infested/Control. A ratio of 1 denotes equivalent gene expression in Infested and Controls.

All mites recovered were engorged. IL-10 and IL-13 expression was not detected in any birds on any day. IFN-γ, IL-18, IL-6 and CXCLi2 expression was increased slightly on Day 1 (1.79, 1.45, 1.26, 1.28, respectively), whilst on Day 2 the expression level of these cytokines was reduced to below that of the Control group (0.53, 0.61, 0.85, 0.54, respectively). On Day 5, IFN-γ, IL-18, IL-6 expression was elevated (1.13, 1.10, 2.43, respectively) whilst CXCli2 was the same between groups (1.04). Despite numerical trends in the data, there were no significant differences between treatments on the same, day although between days differences ($P < 0.05$) were observed.

This study is the first step toward characterising the immunological response of the domestic fowl to *D. gallinae*. Data suggest that *D. gallinae* feeding stimulates Th1 and pro-inflammatory cytokines initially (Day 1) followed by their subsequent down regulation. Removal of mites resulted in increased expression of these cytokines (Day 5). Further research will investigate whether *D. gallinae* stimulates a Th2 response in the host as observed in ticks, or if a different strategy is adopted.

Session 4 Ticks and Tick-Borne Diseases I

O14: TICKS AT THE DOMESTIC PETS, RODENTS/HUMAN INTERFACE IN SOUTH AFRICA

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The species composition and number of ticks collected from a total of 2 439 dogs, 133 cats and 1 486 murid rodents throughout South Africa are compared with the species and numbers of ticks collected from 173 people with tick bites. A total of 103 753 ixodid ticks belonging to 42 species was recovered from the animals and humans, and 4 of these species, namely *Amblyomma hebraeum*, *Haemaphysalis elliptica*, *Rhipicephalus gertrudae* and *Rhipicephalus simus* were collected from dogs, cats, rodents and humans, while a further 7, namely *Amblyomma marmoreum*, *Hyalomma truncatum*, *Ixodes pilosus*, *Rhipicephalus* (*Boophilus*) *decoloratus*, *Rhipicephalus appendiculatus*, *Rhipicephalus evertsi evertsi* and *Rhipicephalus follis* were collected from tick bites on humans and from 2 of the 3 species of animals. The adults of *A. hebraeum* are parasites of cattle and large wild herbivores, while the immature stages infest a large number of hosts. Infestation with this species must have been acquired by the humans affected in a rural environment. The adults of *H. elliptica* infest dogs and domestic and wild felids, while those of *R. gertrudae* and *R. simus* infest domestic and wild canids, felids, equids, suids and larger bovids. The immature stages of these 3 ticks infest murid rodents and hence a suburban or semi rural environment, where dogs, cats and rodents are present, is ideal for human infestation. Furthermore, humans are monogastric, as are the majority of hosts of the adults of these 3 species, and consequently there was a high incidence of human infestation with adult ticks of these species. In contrast, *Rhipicephalus sanguineus*, which was collected in very large numbers from domestic dogs, is a parasite of the latter animals in all its developmental stages. It is dependent upon man-made structures for its life cycle and is thus usually associated with urban and suburban environments, and yet only a single human incident of tick-bite was recorded, confirming the near-strict host-preference of this tick. Tick bites inflicted on humans by the adults of *H. elliptica*, *H. truncatum*, *Rhipicephalus follis*, *R. gertrudae* and *R. simus* are a direct consequence of the numbers of their immature stages present on rodents and only indirectly a consequence of the presence of their adults on domestic pets and livestock.
Possible reasons for human tick-bite by the other tick species recovered are discussed.

O15: MOLECULAR ANALYSIS OF THE BM86 ORTHOLOGUES FROM ARGASID AND IXODID TICKS

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Control of ticks worldwide relies principally on the use of acaricides, but two vaccines targeting *B. microplus* have been commercialized in the 1990s: TickGARD Plus® in Australia and Gavac® in Cuba. Both are based on recombinant Bm86, a glycoprotein of unknown function which is located predominantly on the surface of midgut digest cells. Immunization trials with Bm86-containing vaccines confer heterologous protection against *Boophilus annulatus*, *Boophilus decoloratus*, *Hyalomma anatolicum* and *Hyalomma dromedarii*. However, against *Rhipicephalus appendiculatus* and *Amblyomma variegatum* they were not effective. This varying response suggests that a vaccine based on the Bm86

orthologues of the target tick species may increase the efficacy of vaccination. In this study, the Bm86 orthologues from nine African and European ixodid tick species representing all major tick genera: *Amblyomma variegatum*, *Dermacentor reticulatus*, *Haemaphysalis elliptica*, *Hyalomma detritum, Hyalomma marginatum, Ixodes ricinus, Rhipicephalus appendiculatus, Rhipicephalus bursa* and *Rhipicephalus e. evertsi,* as well as a Bm86-like protein from the argasid tick *Ornithodoros savignyi* were cloned and analyzed. All Rhipicephalinae and Hyalomminae Bm86-like proteins contain eight Epidermal Growth Factor (EGF)-like domains, whereas six of these domains are present in the Bm86-like proteins from the other genera. The ixodid Bm86-like proteins are predicted to contain a C-terminal glycosylphosphatidylinositol (GPI) modification site, which provides linkage of the molecule to the cell membrane. Ovine Bm86 antiserum recognized proteins in the isolated midguts from partially fed *R. appendiculatus* females (with Ra86 sharing 72% identity to Bm86) by Western Blot analysis, but not in *A. variegatum* midguts (with Av86 sharing 27% identity to Bm86) which forms an explanation for the absence of a Bm86 vaccination effect in the latter species. A vaccination trial with recombinant Av86, the Bm86 orthologue from *Amblyomma variegatum*, is scheduled for the near future to assess its vaccination potential against this tick of great economic importance.

Acknowledgements: this research was supported by the Wellcome Trust under the 'Animal Health in the Developing World' initiative through project 075799 entitled 'Adapting recombinant anti-tick vaccines to livestock in Africa', and facilitated through the Integrated Consortium on Ticks and Tick-borne Diseases (ICTTD-3), financed by the International Cooperation Program of the European Union, coordination action project no. 510561.

O16: IMMUNIZATION OF RABBITS WITH SERPIN RECOMBINANT PROTEIN AGAINST CATTLE TICKS (*RHIPICEPHALUS (BOOPHILUS) MICROPLUS*) INFESTATION

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Serine proteases inhibitor (SERPIN) have been recognized by their immune regulatory mechanisms since they are capable of limiting immune activation by curtailing the enzymatic generation of stimulatory signals. TSG proteins have a potential role for application as novel tick control agents. SERPIN from tick salivary glands (TSG) may be involved in facilitation of tick feeding and digestion of blood meal as well as pathogen transmission. Therefore, SERPIN is one of the most interesting candidate target antigens for tick vaccine development. The objective of this study was to determine the immunogenicity of recombinant serpin (r-SERPIN) using rabbits as the host. The r-SERPIN protein expressed in *Pichia pastoris* was used to immunize five rabbits 3 times at 2 weeks interval with 100 µg r-SERPIN per rabbit and challenge infestation with 500 larvae of *Rhiphicephalus (Boophilus) microplus*. Dot blot and SDS-PAGE analysis of r-SERPIN protein shown a distinct 45 kDa band. By ELISA, all immunized rabbits generated antibodies against r-SERPIN on the first week after immunization and reached its peak at the seventh week.Vaccination of rabbits with r-SERPIN conferred significant protective immunity against cattle ticks, resulting in 83% reduction in adult engorgement and 92.2% in egg mass weight. This result indicated that r-SERPIN had the strong immunogenicity and might be candidate for the anti-tick vaccine's antigen against *R. microplus*.

Acknowledgments: This research was facilitated through the Integrated Consortium on Ticks and Tick-borne Diseases (ICTTD-3), financed by the International Cooperation Program of the European Union, coordination action project No. 510561.

O17: COMBINING THE POTENTIAL OF ANTIBODY PHAGE DISPLAY AND PEPTIDE PHAGE DISPLAY TO SELECT PEPTIDES MIMICKING EPITOPES OF THE TICK *BOOPHILUS MICROPLUS*

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In order to select and characterize new proteic targets to be used as immunogens for controlling *Boophilus microplus*, a combinatorial phage displayed library of single chain variable fragments (scFv) was constructed from the repertoire of immunoglobulins from chicken immunized with cattle tick larvae total protein. A protein similar to a vitellin was recognized by a clone from the antibody library and its sequence was determined by N-terminal sequencing. The same clone was used for epitope selection in a recombinant peptide library. Mimetopes of proteins previously described as tick antigens, as for instance vitellin (GP80), serotonin receptor, membrane B antigen, notch-like protein were selected. We further characterized these proteins and their immunogenic regions with affinity to recombinant antibodies. These results demonstrate the efficiency of the utilization of phage displayed scFv and peptide libraries for selection of new proteins epitopes against cattle ticks.
Financial Support: Cappes, FINEP, Vallée S/A, ImunoScan.

O18: PREVALENCE OF TICK-BORNE PATHOGENS IN TICKS IN SICILY

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Sicily represents a typical Mediterranean ecosystem to study tick infestations and the prevalence of endemic tick-borne pathogen. In this study, the prevalence of *Anaplasma*, *Ehrlichia*, *Rickettsia* and *Babesia*/*Theileria* species was analyzed in questing and feeding adult ticks in Sicily. A total of 648 ticks were collected and analyzed for this study. Of them, 29 were questing ticks and 619 were collected from infested cattle, sheep, goats or dogs. Tick species analyzed included *Rhipicephalus bursa*, *R. turanicus*, *R. sanguineus*, *Hyalomma lusitanicum*, *H. marginatum*, *Dermacentor marginatus*, *Ixodes ricinus*, *Boophilus annulatus* and *Haemaphysalis punctata*. With the exception of *B. annulatus* and *H. punctata* for which only 8 and 15 ticks were analyzed, respectively, all tick species were found infected. Most ticks were found infected with a single pathogen group. However, an *I. ricinus* collected from cattle and a questing *R. turanicus* were found infected with *Anaplasma* and *Ehrlichia* spp. *Anaplasma*, *Ehrlichia* and *Rickettsia* spp. and *Rickettsia* and *Babesia*/*Theileria* spp. were detected in two different *H. marginatum* ticks

collected form cattle. To test for differences between tick species in the prevalence of infection for different pathogens, a paired nonparametric Fisher comparison test was used to analyze data obtained from questing ticks (Table 2). Significant differences were found for *Anaplasma* spp. in *R. turanicus* which displayed higher prevalence than *R. sanguineus* (P=0.01) and *D. marginatus* (P=0.02). For *Ricketsia* spp., *D. marginatus* showed statistically higher prevalence than *H. lusitanicum* (p=0.025). For the rest of the tick species and pathogens significant differences were not detected. These results suggested that the most important vectors of pathogens that may affect human and/or animal health in Sicily are *R. turanicus* for *Anaplasma* spp. and *D. marginatus* for *Rickettsia* spp. For *Ehrlichia* spp. and *Babesia*/*Theileria* spp., *R. turanicus*/*D. marginatus* and *H. lusitanicum* may be the most important vectors but additional studies need to be conducted to confirm these results.

O19: DYNAMICS OF NATURAL *EHRLICHIA CANIS* INFECTIONS IN *RHIPICEPHALUS SANGUINEUS* TICKS IN BRAZIL

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Rhipicephalus sanguineus ticks are regarded as one of the main ectoparasites infesting dogs in Brazil. Besides causing direct damage by the blood feeding process, they can also transmit pathogens to dogs and man.

The present study aimed to detect tick-borne pathogen infections in ticks infesting dogs living in Belo Horizonte, Minas Gerais State, Brazil. From August 2006 to July 2007, ticks were collected monthly from 12 adult dogs in nine houses. The climatic seasons in Minas Gerais are not well defined; however, a so-called rainy season occurs from October to March and a dry season occurs from April to September. The climatic data recorded for Belo Horizonte during the experimental period were: mean temperature 18.6ºC; relative air humidity 56.5%, and rainfall 37mm.

The only species of ticks identified from all infested dogs was *R. sanguineus* in all its development stages, with tick population peaks occurring in August, February and June. Specimens of each engorged and non-engorged developmental stages of ticks (larva, nymph, adult male and adult female) collected from each dog were individually pooled for DNA extraction. DNA samples were analyzed by Real-Time PCRs for detection of *Ehrlichia canis* and *Babesia* species affecting dogs (*B. canis* and *B. gibsoni*).

E. canis was detected in both engorged and non-engorged stages of nymphs, females and males from August to March, with highest frequencies occurring in March. No infection was detected from April to June and only engorged females were positive in July. Unexpectedly, there was no evidence of active *Babesia* infections in any developmental stage.

This first comprehensive study on the dynamics of natural pathogen infections in ticks in Belo Horizonte indicates a great potential for transmission of *E. canis* to dogs, particularly during the rainy season. These results point to the need of further investigations regarding the establishment of appropriate measures to prevent tick infestations and transmission of ehrlichiosis to dogs.

Session 5 Ticks and Tick-Borne Diseases II

O20: *ANAPLASMA MARGINALE* EPITOPE MAPPING BASED ON RANDOM PEPTIDES LIBRARIES FUSED IN FILAMENTOUS BACTERIOPHAGES

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Bovine anaplasmosis is caused by *Anaplasma marginale* and *A. centrale*. The most pathogenic and important species for cattle production is *A. marginale*, and is widely distributed in tropical, subtropical and temperate regions of the world. *A. marginale* is an intra-erythrocyte *Rickettsia* of susceptible ruminants, biological and mechanically transmitted by ticks and hematophagous insects. The tick *Rhipicephalus (Boophilus) microplus* is the main vector of *A. marginale* in Brazil. The congenital form of transmission in cattle may occur, causing the neonatal anaplasmosis. The outer membrane of A. marginale includes six well characterized major surface proteins, MSP1a, MSP1b, MSP2, MSP3, MSP4 and MSP5, which play important role in the development of the immune response of infected animals. In this study, we have used the Phage Display technology to identify specific peptides that were immunoreactive to monoclonal antibodies anti-*A. marginale* proteins. Peptide selection was performed using a subtractive selection of a peptide library with 12 random amino acids, Ph.D.-12, expressed on the surface of the M13 filamentous phage concurrently against the anti-MSP1a and anti-MSP2. After four rounds of selection and validation by ELISA, the selected peptides have recognized only the anti-MSP1. *In silico* Analysis identified 49 peptides, which showed the protein consensus sequence STxS that was represented in 78% of selected phages. Due to the multiple motif repeats found in MSP1 protein, the STSSxL motif may become an important biological target, with potential use in diagnostic tests and vaccine for the control of *Anaplasma marginale.*

Financial Support: Cappes, FINEP, Vallée S/A, ImunoScan.

O21: CHARACTERIZATION OF THE APICAL MEMBRANE ANTIGEN 1 IN TWO ITALIAN STRAINS OF *BABESIA BIGEMINA*.

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Babesiosis is one of the most common infections of free – living animals worldwide. It is caused by the infectious intraerythrocytic parasites of the genus *Babesia*. *B. bigemina* is a cattle pathogen and its transmission occurs through the specific tick vector *Rhipicephalus* spp. This parasite is one of the major causes of economic losses in the cattle industry in the tropical and subtropical Countries. The identified or suspected molecules involved in the erythrocyte invasion are many, however in *B. bigemina*, very few are characterized. One of these invasion molecules is the apical membrane antigen 1 (AMA-1), a trans-membrane antigen recently identified. In this work, we characterized the *ama-1* gene from two Italian *B. bigemina* strains, one obtained from Ragusa, Sicily (ITA1) and a second one obtained from Benevento, Campania (ITA2). *Babesia bigemina*-infected blood was used to isolate DNA and this was used to amplify the *ama-1* gene, which was subsequently cloned and sequenced. The sequence of the Naples strain resulted with a high degree of conservation with the sequence of the *ama-1* obtained from the strain coming from Ragusa, Italy (ITA1), the similarity was higher among these two strains than to *ama-1* obtained from a Mexican strain (Mexico) and from that obtained from an Australian strain (sequenced

genome at Sanger). The obtained results confirmed that this newly described *ama-1* gene is highly conserved among Italian and some foreign strains, which has implications for vaccine development. Further investigations would provide more information.
Financed by INCO project no. 003691 and IZSSi 02/2007

O22: IMMUNIZATION OF *BOS TAURUS* STEERS WITH *BABESIA BOVIS* RECOMBINANT ANTIGENS MSA-1, MSA-2C AND 12D3

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Babesia bovis MSA-1 and MSA-2c antigens belong to the variable merozoite surface antigen gene family. These antigenic proteins are present on the merozoite surface and are involved in the parasite invasion to the bovine erythrocyte. 12D3 is a glycoprotein localized in the apical complex of the merozoite which is released in soluble form in the cytoplasm of the infected erythrocyte during parasite division. The characterized *msa-1* gene locus describes an intronless single-copy gene with an open reading frame of 961 bp that codes for a 42-kDa membrane glycoprotein. *msa-2c* is an intronless single-copy gene and contains a 795-bp-long coding sequence that is translated into a 30-kDa protein. The *12d3* gene contains a 1038 bp coding sequence that codes for a 41-kDa protein. Previous studies have shown that the genes coding for MSA-1, MSA-2c and 12D3 are widely conserved on at least 14 different *B. bovis* isolates from distinct geographic regions in Mexico. Sequencing of *msa*-1 genes from the various *B. bovis* populations revealed that the *msa*-1 gene product varies from 51 to 99.7% in sequence identity. Sequence analysis and multiple alignment of deduced MSA-2c, demonstrated a high degree of sequence identity (90-100%) among the Mexican *B. bovis* isolates. Similarly, BLASTX analysis of deduced 12D3 amino acid sequences revealed 94-99 % identity in 20 different *B. bovis* isolates tested. The elevated sequence identity conservation in the Mexican *B. bovis* isolates analyzed made it amenable for MSA-1, MSA-2C and 12D3 antigens worth of evaluating as a combined recombinant immunogen. Recombinant MSA-1, MSA-2c and 12D3 proteins were obtained by cloning the corresponding gene's coding sequences from *B. bovis* (RAD strain) into the pBAD/TOPO thio-expression system (Invitrogen), and expressed as a fusion protein with thioredoxin. The recombinant version of the *B. bovis* antigens were purified by affinity chromatography in NI-NTA resin columns. The recombinant proteins were homogenized with Montanide 75 and used to immunize five, 12 months old steers with 50 µg of each recombinant protein, on two occasions two weeks apart. A control group of 5 animals received PBS-adjuvant only. Both groups of animals were weekly bled to collect sera and samples were tested for seroconversion to *B. bovis* by using an indirect fluorescence antibody test and an ELISA. Three out of five steers immunized with the combined recombinant *B. bovis* proteins seroconverted with moderate antibody titers at day 14 post-immunization. All five immunized steers presented with strong seropositivity to *B. bovis* antigens at day 21 post-initiation of study (7 days after boosting). The experimental animals will be challenged with a virulent field isolate of *B. bovis* and the clinical outcome will be discussed in terms of protection, if any, against development of bovine babesiosis.
Financed by SAGARPA-CONACYT Project C01-2005-12023; and INIFAP project PRECI No. 6216596P.

O23: RECOMBINATION IN BUFFALO-DERIVED *T. PARVA* ISOLATES TRAIT TO LIVESTOCK OF SOUTH AFRICA?

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East Coast fever (ECF) is a protozoan disease, caused by *Theileria parva* and transmitted by the brown eartick *Rhipicephalus appendiculatus* with high mortality, especially in improved cattle breeds. This causes serious economical losses in the agricultural sector of eastern and southern Africa corresponding with the occurrence of the vector. Control is difficult, but South Africa succeeded in eradicating the disease in the 1950's by a strickt slaughter policy. It is believed that classical ECF in cattle originated through a speciation event from buffalo parasites being the natural reservoir of *T. parva* parasites. Corridor disease is related syndrome caused by *T. parva* parasites from buffalo transmitted to cattle in those areas where cattle are using the same grazing grounds as buffalo. But this parasite is not transmitted from cattle to cattle. The syndrome is present throughout eastern and southern Africa where cattle and buffalo contact is likely to occur, also in specific parts of South Africa. The frequency and likelihood of a new speciation event (transformation from buffalo-derived to classical cattle to cattle transmissable *T. parva*) under these conditions is not known.
The concern of ECF reoccurring in South Africa is becoming a major issue as buffalo-cattle contacts are ever increasing giving eventually more chances to *T. parva* to get adapted (new speciation event) to cattle. In order to check this and another hypothesis stating that buffalo are still carrying the classical ECF parasite (pre-eradication) more than 100 samples from buffalo were characterized using Multi-Locus Genotyping (MLG) based on PCR-RFLP assays of 3 different antigenic loci. Sequence analysis of the Polymorphic Immunodominant Molecule (PIM), one of the 3 loci used in the MLG, identified 3 different clusters corresponding to respectively cattle-derived, buffalo-derived isolates and recombinants between the two types. Results show that frequent intra-genic recombination between buffalo-derived and cattle-derived parasites had occurred, and that there is currently no marker that can be used to differentiate these. The importance of parasites showing recombination is not well known but there is evidence that recombinants are causing classical East Coast fever in Eastern Africa. The role of recombinants in the epidemiology of ECF will be further investigated by the use of a newly developed glass based micro-array as a routine test. It is important that these results of the South African isolates be further compared with buffalo and bovine samples from these areas in eastern Africa where these animals share common grazing grounds.

O24: CORRIDOR DISEASE (*THEILERIA PARVA* BUFFALO-ASSOCIATED INFECTION IN CATTLE) IN SOUTH AFRICA: THE CARRIER-STATE IN CATTLE

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Breeding and trading in buffaloes have escalated in South Africa to supply "disease-free buffaloes" to meet the increasing demands of the eco-tourism industry. In spite of strict regulation and control of Corridor disease CD (*Theileria parva* infection in cattle) by the veterinary authorities, outbreaks in cattle have increased in the areas where the disease is endemic in buffalo populations. The risk that infected cattle may recover to become carriers of the infection, poses a threat of re-emergence of cattle to cattle transmission. The carrier-state of buffalo derived *T. parva* in cattle has been demonstrated in other African countries.

The main objective of this study was to demonstrate the carrier-state in cattle resulting from buffalo associated infections under controlled conditions. Naturally and experimentally infected cattle which had recovered from the disease were used for xenodiagnoses and tissue culture isolation of the *Theileria* parasites.
The results obtained were**:** 1. Pooled ticks derived from two buffaloes (Welgevonden) transmitted *T. parva* infection to a susceptible splenectomized bovine which reacted severely but recovered without treatment and became a carrier; 2. Ticks fed on this animal transmitted a fatal infection to another susceptible bovine; 3. Ticks fed on three buffaloes from two different game parks (Ithala & Marakele) transmitted *T. parva*; one resulted in a mild infection, the other caused a severe reaction but the animal recovered spontaneously and the third attempt resulted in a fatal infection. 4. A spelenectomizd bovine which received blood from a *T parva* (buffalo-derived) carrier bovine developed a piroplasm-carrier state for over 4 years. Ticks fed on this bovine were used to challenge the same animal together with a susceptible control. The carrier animal died to acute infection and the control reacted severely but recovered after treatment. 5. Six cattle which recovered from an apparent severe *T. parva* infection in the field and confirmed to be positive by PCR, all became negative before they were used in the transmission experiments. Ticks derived from these cattle were used to infect susceptible bovines but only *T. taurotragi w*as transmitted.
The buffalo-derived *T parva* carrier-state in cattle has been demonstrated under controlled laboratory conditions but it still has to be shown to occur, naturally in the field. Failure to confirm the carrier-state in the field cases may be due to the fact that it was clinically sick and received chemotherapeutic treatment.

O25: SPECIES IDENTIFICATION OF *THEILERIA EQUI* AND ITS DIFFERENTIATION FROM *BABESIA CABALLI* BY HIGH RESOLUTION MELTING (HRM) ANALYSIS

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Theileria equi and *Babesia caballi* are tick-borne protozoan parasite responsible for an endemic disease of horses in tropical and subtropical areas of the world referred to as equine piroplasmosis. The disease is of great economic importance to horse trading and many countries restrict the importation of horses that are serologically positive for *Babesia* species. In Sudan, limited work has been carried out to understand the epidemiology of equine piroplasmosis and the distribution of *T. equi* and *B. caballi* (Abdoon 1986, Salim et al., 2008). High Resolution Melting (HRM) analysis is a novel tool for genotyping, mutation scanning and sequence matching. The method eliminates the need for sequencing and sequence analysis and has been applied to type single nucleotide polymorphisms (SNPs) (Liew et al., 2004; Reed et al., 2007). Here, we attempted to investigate the utility of HRM as a possible epidemiological tool for genotyping and SNP typing of Sudanese field isolates of *T. equi* and *B. caballi*. HRM analysis methods were established to type *T. equi* by the application of primers targeting a polymorphic region of the *T. equi* EMA-1 gene. DNA samples, which were prepared from blood collected on filter papers, were successfully genotyped and single nucleotide polymorphism SNP was determined for Sudanese isolates of *T. equi*. In addition, HRM to differentiate between *T. equi* and *B. caballi* was established based on the 18S rRNA gene polymorphism. Thus we were able to show distinct separation of the two parasites and the normalized curve and derivative plots revealed distinct melting curves and melting peaks. Samples were subsequently sequenced and polymorphism was further confirmed by sequence alignment using Genetyx software and ClusralW to verify the location of SNPs. Our results suggest HRM as an ideal format for rapid genotyping, which could be useful to promptly determine a drug of choice or to administer an appropriate type of vaccine during outbreaks.

Session 6 Host-vector-pathogen interactions

O27: SILENCING OF GENES INVOLVED IN *ANAPLASMA MARGINALE*-TICK INTERACTIONS AFFECTS THE PATHOGEN DEVELOPMENTAL CYCLE IN *DERMACENTOR VARIABILIS*

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The cattle pathogen, *Anaplasma marginale*, undergoes a developmental cycle in ticks that begins in gut cells. Transmission to cattle occurs from salivary glands during a second tick feeding. At each site of development two forms of *A. marginale* (reticulated and dense) occur within a parasitophorous vacuole in the host cell cytoplasm. However, the role of tick genes in pathogen development is unknown. Four genes, found in previous studies to be differentially expressed in *Dermacentor variabilis* ticks in response to infection with *A. marginale,* were silenced by RNA interference (RNAi) to determine the effect of silencing on *A. marginale* developmental cycle. These four genes encoded for putative glutathione S-transferase (GST), salivary selenoprotein M (SelM), H+ transporting lysosomal vacuolar proton pump (vATPase) and subolesin. The impact of gene knockdown on the *A. marginale* infections both after acquiring infection and after a second transmission feeding was determined and studied by light microscopy. Silencing of these genes had a different impact on *A. marginale* development in different tick tissues by affecting infection levels, the densities of colonies containing reticulated or dense forms and tissue development. Salivary gland infections were not seen in any of the gene-silenced ticks, raising the question of whether these ticks were able to transmit the pathogens. The results of this RNAi and light microscopic analyses of tick tissues infected with *A. marginale* after the silencing of genes functionally important for pathogen development suggest a role for these molecules during pathogen life cycle in ticks.

O28: *ANAPLASMA PHAGOCYTOPHILUM* INFECTION IN *IXODES SCAPULARIS* (ISE6) CULTURED CELLS MODIFIES TICK GENE EXPRESSION

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Anaplasma phagocytophilum is an obligate intracellular pathogen of the genus *Anaplasma* (Rickettsiales: Anaplasmataceae) found exclusively within membrane-bound inclusions in the cytoplasm of both vertebrate and tick host cells. Recently, we showed that infection of tick cells with the related pathogen, *A. marginale,* affected expression of tick genes that are essential for both tick survival and pathogen infection and multiplication. While *A. phagocytophilum* undergoes a similar developmental cycle in cultured tick cells, gene expression profiles in response to infection have not been well characterized. Therefore, the objective of this study was to characterize tick gene expression profiles in cultured *Ixodes scapularis* ISE6 cells in response to infection with *A. phagocytophilum.* By use of microarray and real-time RT-PCR analyses the results of these studies demonstrated that infection of ticks cells with *A. phagocytophilum* resulted in differential expression of several tick genes. Genes found to be upregulated in the ISE6 cells included U2A8 (signal sequence receptor delta), 1I5B9 (ixodegrin-2A RGD containing protein), and C4B10 (von Willebrand factor), while other genes, such as 2I3A7 (NADH-ubiquinoe oxidoreductase), C3B2 (aspartic protease) and 1I1H6 (glutathione S-transferase (GST), were found to be downregulated in response to infection. The genes of most interest would be those that are involved in both tick biology and pathogen infection and multiplication in tick cells. Characterization of these key genes may contribute to development of dual target vaccines that both reduce tick infestations and tick vector competence.
Acknowledgments: This research was supported by the Ministerio de Ciencia e Innovación, Spain (project BFU2008-01244/BMC), the Oklahoma Agricultural Experiment Station (project 1669), and the Walter R. Sitlington Endowed Chair for Food Animal Research (K. M. Kocan, Oklahoma State University) and was facilitated through the Integrated Consortium on Ticks and Tick-borne Diseases (ICTTD-3), financed by the International Cooperation Program of the European Union, coordination action project No. 510561.

O29: DIFFERENTIAL EXPRESSION OF GENES IN SALIVARY GLANDS OF MALE *RHIPICEPHALUS* (*BOOPHILUS*) *MICROPLUS* IN RESPONSE TO INFECTION WITH *ANAPLASMA MARGINALE*

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Rhipicephalus (*Boophilus*) *microplus* is considered to be the main vector of bovine anaplasmosis in tropical and subtropical regions of the world. *A. marginale,* the causative agent of bovine anaplasmosis, undergoes a complex developmental cycle in ticks which results in infection of salivary glands from where the pathogen is transmitted to cattle. In this study we have used functional genomics approach to identify genes differentially expressed in *R. microplus* salivary glands in response to *A. marginale* infection. Additionally, a *R. microplus*-derived cell line, BME26, was used for the first time to also study tick cell gene regulation in response to *A. marginale* infection. Suppression subtractive hybridization (SSH) libraries were constructed from infected and uninfected *R. microplus* ticks and used to identify genes differentially expressed in male *R. microplus* salivary glands infected with *A. marginale*. A total of 279 ESTs were identified as candidate differentially expressed genes. Of these, five genes down-regulated in tick salivary glands in response to *A. marginale* infection were confirmed by real-time RT-PCR and these genes encoded for putative histamine-binding protein (22Hbp), von Willebrand factor (94Will), flagelliform silk protein (100Silk), Kunitz-like protease inhibitor precursor (108Kunz) and proline-rich

protein BstNI subfamily 3 precursor (7BstNI3). The impact of selected tick genes on *A. marginale* infections in tick cells was characterized by RNA interference (RNAi). Genes encoding for putative flagelliform silk protein (100Silk) and the positive control, subolesin, resulted in reduced *A. marginale* infection in both tick salivary glands and cultured BME26 cells, while silencing of the gene encoding for putative von Willebrand factor (94Will) significantly reduced infection only in tick salivary glands. Characterization of differential gene expression in salivary glands of *R. microplus* salivary gland genes in response to *A. marginale* infection expands our understanding of the molecular mechanisms of the tick-pathogen interface.

Acknowledgements This research was supported by grants from the European Community, INCO-DEV program (project no. 003713), entitled 'Epidemiology and new generation vaccines for *Ehrlichia* and *Anaplasma* infections of ruminants', the Ministerio de Ciencia e Innovación, Spain (project BFU2008-01244/BMC) and was facilitated through the Integrated Consortium on Ticks and Tick-borne Diseases (ICTTD-3), financed by the International Cooperation Program of the European Union, coordination action project No. 510561. FAPESP (Fundação de Amparo a Pesquisa do Estado de São Paulo, Brazil), and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil).

O30: VECTOR-PATHOGEN INTERACTIONS: THE IMPACT OF TICK ECOLOGY ON THE EVOLUTION OF *ANAPLASMA MARGINALE*

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Vector-pathogen interactions involve genetic traits of both the vector and the pathogen. Defining the factors that affect these interactions is important toward understanding the pathogen life cycle, pathogenesis and evolution. The tick-borne pathogen *Anaplasma marginale,* which is endemic worldwide, is the type species of the genus *Anaplasma* (Rickettsiales: Anaplasmataceae). *Rhipicephalus* (*Boophilus*) *microplus* is the most important biological vector of *A. marginale* in tropical and subtropical regions of the world. Despite extensive characterization of the genetic diversity in *A. marginale* geographic strains using major surface protein (MSP) sequences, little is known about the biogeography and evolution of *A. marginale* and other *Anaplasma* species. For *A. marginale*, MSP1a was shown to be involved in vector-pathogen and host-pathogen interactions and to have evolved under positive selection pressure. The MSP1a of *A. marginale* strains differs in molecular weight because of a variable number of tandem 23-31 amino acid repeats and has proven to be a stable marker of strain identity. While phylogenetic studies of MSP1a repeat sequences have shown evidence of *A. marginale*-tick co-evolution, these studies have not provided phylogeographic information on a global scale because of the high level of MSP1a genetic diversity among geographic strains.

In this study we hypothesized that the phylogeography of *A. marginale* MSP1a sequences is associated with world ecological regions (ecoregions) resulting from different evolutionary pressures related to regional vector ecology. The results demonstrated that the MSP1a first (R1) and last (RL) repeats and microsatellite sequences were associated with world ecoregion clusters with specific regional signatures. The evolution of R1 repeat sequences was found to be under positive selection pressure and linked to tick ecology. The results reported herein provided the first evidence that the evolution of *A. marginale* was linked to ecological traits influencing the tick vector, most notably *R. microplus*. These results suggested that some strains have evolved under conditions that support biological transmission of *A. marginale* by *R. microplus,* while the evolution of other strains may be linked to transmission by other tick species or to mechanical transmission in regions where *R. microplus* is currently absent. The information derived from this study is fundamental toward understanding the evolution of other vector-borne pathogens.

This research was facilitated through the Integrated Consortium on Ticks and Tick-borne Diseases (ICTTD-3), financed by the International Cooperation Program of the European Union, coordination action project No. 510561.

Session 7 Genetics / Genomics / Transcriptomics

O32: CHARACTERIZATION OF THE CHROMOSOMAL LOCUS FOR THE *IXODES SCAPULARIS* TICK PROTECTIVE ANTIGEN, SUBOLESIN

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Subolesin was discovered as a tick protective antigen in *Ixodes scapularis* and has been shown by both RNA interference gene knockdown and immunization trials using the recombinant protein to protect hosts against tick infestations, reduce tick survival and reproduction, and cause degeneration of gut, salivary gland, reproductive tissues and embryos. In addition, subolesin was shown to function in the control of gene expression in ticks. In this work, the predicted gene sequence encoding the tick protective antigen, subolesin was located in the *Ixodes scapularis* (Wikel strain) assembled whole genome shotgun sequence data (GenBank project accession ABJB010000000) after alignment with the cultured IDE8 tick cells-derived cDNA sequence (Genbank accession No. AY652654.1). The cDNA contained 79 bp of 5´UTR, 555 bp of translated region coding for a protein of 185 amino acids and 2,030 bp of 3´UTR. Three introns were located in the gene coding for subolesin, between positions 181-182 (10,158 nt), 472-473 (9,730 nt) and 544-545 (2,386 nt) (position 1 = A in the translation start codon of AY652654.1). Subolesin coding sequence alignment demonstrated the presence of SNPs and indels between different *I. scapularis* strains. Approximately 5.5 kb of sequence data upstream of the subolesin gene was used to characterize the subolesin promoter region and predict transcription factors involved in the regulation of subolesin expression. These results advance the characterization of gene structure and regulation of transcription in ticks and contribute to the annotation of the *I. scapularis* genome.

O33: SELECTIVE CAPTURE OF TRANSCRIBED SEQUENCES METHOD OF *EHRLICHIA RUMINANTIUM* FOR TRANSCRIPTOMIC STUDY

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Whole genome transcriptomic analysis is a powerful approach to elucidate the molecular mechanisms controlling the pathogenesis of obligate intracellular bacteria. However, the major hurdle resides in the low quantity of prokaryotic mRNAs extracted from host cells. Thus, we successfully adapted the SCOTS method (Selective Capture of Transcript Sequences) on the model Rickettsiales, *Ehrlichia ruminantium (ER)* to capture mRNAs. Southern Blots and RT-PCR revealed an enrichment of *ER* cDNAs and a diminution of ribosomal contaminants after three rounds of capture. qRT-PCR and whole-genome *ER*

microarrays hybridizations demonstrated that SCOTS method introduced only a limited bias on gene expression. Indeed, we confirmed the differential gene expression between poorly and highly expressed genes before and after SCOTS captures. The comparative gene expression obtained from *ER* microarrays data, on samples before and after SCOTS at 96 hpi was significantly correlated (R^2=0.7). Moreover, SCOTS method is crucial for microarrays analysis of *ER*, especially for early time points post-infection. There was low detection of transcripts for untreated samples whereas 24% and 70.7% were revealed for SCOTS samples at 24 and 96 hpi respectively. Gene expressions of attenuated and virulent Gardel strains were compared at different time post infection in order to identify their role in pathogenicity mechanisms. About 30 genes were expressed specifically for attenuated or virulent Gardel strains. Comparative genomic studies on these genes provided hypothesis for their involvement in attenuation.
The adaptation of SCOTS method to our model *ER* could be used widely for other obligate intracellular pathogen. Moreover, this study will facilitate a better understanding of both the *ER* pathogenicity and the adaptation of obligate intracellular bacteria to their environment. This strategy paves the way for new insights in pathogenicity of obligate intracellular pathogens.

O34: *BABESIA BIGEMINA*: EXPRESSED SEQUENCE TAG ANALYSIS OF THE INTRAERYTHROCYTIC STAGE

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Expressed sequence tags (ESTs) generation and analysis provide an option for identification of a number of expressed genes in different stages of an organism's lyfe cycle. Strategies based upon ESTs have been useful in medical parasitology for discovering of genes coding for proteins of biological importance in pathogenic Apicomplexan parasites such as *Plasmodium, Toxoplasma, Neospora, Cryptosporidium, Eimeria spp* and *Babesia bovis*. ESTs analysis has facilitated the identification of several candidate antigens for vaccine development in Apicomplexa parasitology with significant results. There is a lack, however, of expressed sequence information for *Babesia bigemina*, another economically important etiological agent of bovine babesiosis in the tropics. Thus, the objective of this study was to initiate the generation of a ESTs database based on the analysis of expressed genes on the intraerythrocytic stage of *Babesia bigemina*, knowledge considered necessary for establishing new and improved control strategies for bovine babesiosis.
In this study, ESTs were obtained and analyzed from 940 randomly selected clones containing cDNA inserts derived from a *Babesia bigemina* library produced in pBluescript vector. The recombinant pBluescript plasmids were purified and cDNA inserts were single-pass sequenced by the cycle sequencing reaction using T7 as primer and fluorescent dideoxynucleotides as terminators. The obtained sequences were extracted and subject to BLAST homology search in the genbank databases (http://www.ncbi.nlm.nih.gov/blast/). Sequence homology analysis of the 940 selected recombinant clones provided the following information: 231 clones (grouped in 71 distinct clusters) contained ESTs with no significant sequence identity with Babesia sp genes or other Apicomplexan parasites. Presumably, these ESTs would correspond either to new, unreported *B. bigemina* transcribed genes present in the erythrocyte stages of the parasite, or to non-translated sequences of the putative genes. 17 clones were identified which contained ESTs corresponding to 5 genes coding for *B. bigemina* antigens already reported in the literature; 682 clones (grouped in 130 clusters of distinct sequences) had significant sequence identity with genes coding for hypothetical proteins previously identified in the *Babesia bovis* genome. Moreover, 4 clones had ESTs corresponding to 4 different *Theileria annulata* genes; 1 clone had high sequence similarity with a *Theileria parva* gene; 4 clones (3 distinct sequences) showed ESTs with sequence similarity to 3 genes of *Plasmodium berghei*, whereas one clone contained an EST with higher percent identity for a *Plasmodium falciparum* gene. The results obtained, in addition to EST analysis of a larger number of *B. bigemina* cDNA clones, will allow the characterization and, eventually, the manipulation of gene coding regions, essential for the establishment of improved control strategies for cattle babesiosis caused by *B. bigemina*.
Financially supported by SEP-CONACYT, Project No. 2004-C01-47739

O35: MICROSATELLITE MULTILOCUS POLYMORPHISM ANALYSIS TO CHARACTERIZE *LEISHMANIA INFANTUM* STRAINS ISOLATED IN SICILY

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The *L. infantum belonging to Leishmania donovani* complex, is responsible for cutaneous and visceral manifestations of leishmaniasis in Mediterranean area. Isoenzyme electrophoresis is the standard method for the characterization and identification of strains of *Leishmania and* zymodeme MON-1 despite their very wide geographical distribution is spreading in Europe and Mediterranean bacine. Different approach are been developed to improve the discrimination *Leishmania* genus using biochemical and molecular methods. The authors optimized an experimental protocol to discriminate the isolated and stored strains at the Italian National Reference Centre (C.Re.Na.L.). The used method permitted us to amplify microsatellites DNA target at multilocus level. Microsatellites are DNA tandem repeats sequences of a simple nucleotide motif distributed abundantly in the eukaryotic genomes and may reveal important strain polymorphisms. The discriminative power of microsatellite analysis permit to separate MON-1 strains into different closely related genotypes. Other zymodeme also give various related or divergent genotypes. The authors use genotyping system to define fingerprinting models in the isolated strains from dogs, cats and humans in Sicily. The PCR was employed to amplify the micosatellites contained in the DNA regions of 12 markers selected from among more investigated locus. A number of 50 isolates of *L. infantum* were tested by using the same locus panel. The products were successively analysed using 3130 AbiPrism sequence detector (Applied Biosystems) to discover relevant microsatellite polymorphisms trough the collected strains. It was possible to discriminate between non-MON-1 group, and MON-1 group of *L. infantum* isolates. Within MON-1, geographical correlations became apparent analysing polymorphic patterns. The frequency of heterozygosity in the alleles analyzed varied extremely between different groups of isolates. Isoenzymatic typing methods don't permit the establishing correlations between clinical feature and preferential host (animals, immunocompetent and human immunodeficiency patients) while microsatellite polymorphism investigation could be useful for the high discriminative power. The main clusters described are not consistent with the definitions based on isoenzyme analysis but confirm the results of former PCR-based investigations. It exhibiting a high level of discrimination, is suitable for characterization of closely related strains in epidemiological studies.

O36: QUANTIFICATION OF MRNA CYTOKINE USING SPECIFIC QRT-PCR DURING AN EXPERIMENTAL TRYPANOSOME INFECTION ON AFRICAN TRYPANOTOLERANT AND TRYPANOSUSCEPTIBLE BOVINE.

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African trypanosomosis are parasitic diseases transmitted by tse-tse flies considered as the main sanitary constraint to animal production development in sub-Saharian Africa. These parasitic diseases, due to protozoan parasite belonging to trypanosomatidae family, hamper and prevent breeding over more than 7 millions of km² with high forage and agricultural potential. More than 50 million of cattle and 100 millions of small ruminants are concerned. Economic consequences are disastrous for human populations already extremely poor. However, if trypanosomosis have dramatic consequences on zebu populations (*Bos indicus*), they present a weaker impact on western African taurine (*Bos taurus*) which are known to be naturally tolerant to trypanosomes infection.

Mechanisms governing trypanotolerant trait are still badly known but thesis of genetic determinism is today well accepted. Our team has first developed a transcriptomic approach using SAGE technology to investigated Trypanotolerance character in which some transcripts (TAGs) have been found to be differentially expressed (Berthier et al. ANYAS 2008). Unfortunately, differential expression of cytokine failed to be investigated using SAGE may be due to transitory or short half life of the respected transcripts. In complement to this study, we propose here to investigate the expression levels of mRNA cytokine (IL1, IL2, IL4, IL6, IL8, IL10, TNFa, IFNg, TGFb) from Total White Blood Cells of trypanotolerant and trypanosuceptible African Bovine during a new experimental *Trypanosoma congolense* infection. Changes in expression profiles were monitored with bovine cytokines dedicated qRT-PCR using several points during the infection (before infection, maximum of parasitaemia, maximum of anaemia, end of experiment after value normalization). Several cytokines showed significant variation during the infection kinetic. We discussed here the results obtained.

O37: USE OF QRT-PCR METHOD TO VALIDATE SAGE EXPRESSION DATA IN EXPERIMENTAL *TRYPANOSOMA CONGOLENSE* INFECTION.

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Infectious diseases are a permanent threat to animal breeding, resulting in direct and indirect economic losses. Among these diseases, zoonoses endanger human health. Infectious diseases are controlled by vaccination, the use of antibiotics or anti-parasite medications, the eradication of vectors, the limitation of animal displacement, even animal slaughter. However, effective vaccines are not available for all diseases, notably those that are instigated by eukaryote parasites, resistance to medications appears and the constraints in terms of public and environmental health impose restrictions in the use of antibiotics and insecticides. It is the case in Animal African Trypanosomosis (AAT). These Tse-Tse flied transmitted diseases concern more than 50 million of bovine and at least 100 millions of small ruminants over seven African countries. Trypanotolerance exist in some breed which exhibit better control of parasitaemia and preservation of hematological parameters as Packed Cells Volume (PCV) under natural field conditions. An alternative method to fight theses diseases makes use of the genetic variability of the host towards the sensitivity to a disease. The genetic determinisms involved in tolerance to an infection are not well known, are under multigenic controls, and they often stem from adaption of a breed subjected to permanent pressure of the pathogenic agent. In order to study trypanotolerance, we have chosen to develop transcritpomic analyses using SAGE technology on Trypanotolerant and Trypanosusceptible African cattle under experimental infection with *Trypanosoma congolense*. Twelve Libraries are now available and differential expression of several transcripts showed that differences exist between animals that present variable degree of susceptibility/tolerance to trypanosome infection (Berthier et al. ANYAS 2008). In order to validate the variation observed we quantified the corresponding genes by qRT-PCR. Several points (before infection, maximum of parasitaemia, maximum of anaemia, end of experiment after value normalization) were used to analyse gene expression variation in the course of infection. Comparisons with previous SAGE data were performed. We discussed here of the results obtained and on the complementarity of the both methods.

Session 9 Viral / Bacterial diseases

O 38: FOOT AND MOUTH DISEASE IN ANIMALS AND MAN IN SHARKIA GOVERNORATE IN EGYPT.

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This present study is carried out to determine the current state of FMD in different animal species in Sharkia governorate in Egypt. In addition, we investigated the spreading of the virus through water and soil in the animal environment as well as by rodents and to human contact. Oropharyngeal swabs, feces, milk and blood samples were collected from animals (cattle, buffalo,, sheep and goat), from animal drinking water and from soil from animal yards for both the detection of the FMDV by RT-PCR techniques and isolation of the virus on BHK then typing of isolates by ELISA. Moreover, rat tissues collected from the same yards were examined by both techniques, whereas serum samples from people in intimate contact with the examined animals were tested by serum neutralization test for the detection of antibodies against FMD. The isolation rates of FMD virus on BHK were 39.6%, 11.4%, 41.2% and 100% for cattle, buffalo, sheep and goat respectively. The same results were obtained when animals were examined by RT-PCR technique. All animals did not show any clinical signs for FMD. Most isolates were type O, however, few were type A. The positivity for non-structure protein was 21.2% for cattle. 22.9% for buffalo, non for sheep and 1oo% for goats. Whereas, neither rodent nor human samples was positive. In addition, the virus was isolated from the milk of one animal as well as from one water sample while all soil samples were negative.
There was a high incidence of FMDV in asymptomatic animals especially goats indicating the potential role of asymptomatic animals in the spreading of the virus with special consideration for goats as grazing animals passing long distances daily and hence capable of spreading the virus in many areas as well as their possibility of contaminating surface water. The isolation of the virus from animal drinking water denotes the shedding of the virus in the saliva of asymptomatic animals and highlights the possibility of water-borne transmission of the virus while the role of rats and man in the epidemiology of FMD requires further studies.

O39: STUDIES ON CAPRINE ARTHRITIS ENCEPHALITIS VIRUS IN KHARTOUM STATE-SUDAN

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Caprine arthritis-encephalitis virus (CAEV) infections of goats have a worldwide distribution with importation of live animals being the main reason for the widespread of the disease.
In Sudan, the disease has never been diagnosed. The objective of this study was to investigate CAEV infection among the imported foreign animals and their crossbreds in the Sudan, to establish serological and molecular methods for routine diagnosis of CAE, and to study the molecular epidemiology of the disease as base line for further epidemiological and virological studies. 177 samples (blood and serum) were collected from different goats in areas of Khartoum State and CAEV-specific serum antibodies were detected by means of ELISA and Polymerase chain reaction (PCR) was performed to detect CAEV in

DNA prepared from blood (Buffy coats) samples. Sequencing and phylogenetic analysis for PCR product were subsequently carried out.
Out of 177 samples, 19 (10.7%) were found serologically positive to CAEV infection and 41 (23.2%) were found positive by PCR. A fragment for the gag gene covering part of the coding sequences for the capsid (CA) P25 protein was sequenced and sequences were aligned with those from other ovine/caprine lentiviruses isolates. The presented data showed that the sequences of Sudanese lentiviruses under study were closely related to prototypic CAEV-Co.
This study represents the first report on existence of CAEV in goats in Sudan.

O40: OVINE CATARRHAL FEVER (BLUETONGUE): ANALYSIS OF CULICOIDES SPECIES IN SEROPOSITIVE FARMS

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In Sicily, the first Bluetongue outbreak occurred in October 2000, until 76 outbreaks have been registered. The National Surveillance Plan, based on European Union Commission Decision 138/2001/CE, suggests a serological and entomological survey. This Plan consists of controls of seronegative cattle, called "sentry" of its role as indicators of presence and circulation of virus in definite areas. In order to check the seroconvertions and the serotype of the specific viruses, the regional territory has been subdivided into 400 Km^2 areas including 58 seronegative cattle periodically checked by serological tests using a competitive ELISA. All positive sera have been tested to detect the serotype by the National Reference Centre (CESME).
Moreover, in the positive farms, entomological captures were also performed to investigate about the presence of insect vectors belonging to *Culicoides* genus, using black light traps activated for two consecutive nights near cattleshed.
The goal of the present communication is to report the different species of Culicoides founded in the farms with Bluetongue virus circulation. This study investigated the dates from 2003 to 2008; in this period, the farms controlled were nr. 321 and the relative captures were nr. 581. We observed that 82,2 % of checked farms were positive for *Culicoide*s spp, and only 10 % of the farms were positive for *C. imicola*. The small number of positive farms for *C. imicola* suggested to investigate, for each capture, the presence of *C. obsoletus*, *C. pulicaris* and other species in order to evaluate the probably vectors cause- of BTV infection.

O41: ENTOMOLOGICAL SURVEILLANCE FOR BLUE TONGUE FROM 2003 TO 2008 IN SICILY

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Entomological surveillance for Blue Tongue (BT) started in Sicily in October 2000 with the beginning of epidemic and first clinical cases observed in sheep. Since now is regulated by Italian O.M. of 11 May 2001 and further updates. Data reported in this report concern monitoring activity on vector distribution utilising fixed traps captures distributed in each Sicilian provinces and carried out from January 2003 to December 2008. Black light traps were weekly armed, located close to livestock areas, cattle and sheep farm in order to monitor presence of "at risk" vectors. In eight Sicilian provinces (AG, CL, CT, EN, PA, RG, SR and TP) a total number of n° 1875 entomological catches were collected, these were positive for presence of *Culicoides spp*. (Diptera: Ceratopogonidae) with a prevalence ranging from 35,1% (CT) to 97,0% (CL). Among total captures collected *C.imicola* (Kieffer, 1913) specie showed a prevalence ranging from 0,0% (AG, CL, EN, RG,) to 37,2% (PA). Medium numbers of *Culicoides* monthly trapped

in Sicily showed their major value in the month of August gradually decreasing to spring time for reaching the lowest population in winter. A different behaviour was observed for the *C.imicola* specie which gives its higher prevalence in autumn from September to November with a pick in the month of October and rapidly decreasing, almost disappearing (medium value around 1.0), in the month of December. Another consideration concerns average number of *C.imicola* captured in this study which has never overcame more than 57 insects per trap. This limited number of insects could mean that there is low parasitic pressure to cause a severe epidemic and that other vectors could be involved in the maintenance of the disease. Analysis of trapping activity applied to geographical data showed significant difference relate to altitude: all farms located above 350 meter on sea level (EN, CL and AG) were characterised by higher number of insects collected with one only major peak in summer. These samplings were always found negative for *C. imicola* presence. All other farms (TP, SR, CT, PA, and RG districts) showed two major peaks (spring and autumn) and lower population density including *C. imicola* in three districts (TP, SR and PA). We have described results obtained on entomological surveillance mainly relate to temperature and altitude but further work should be carried out to clarify the role of other environmental factors and their influence in vector ecology (wind, water reservoir, geological aspects, vegetation features) for a prevision of risk for Blue Tongue.

O42: SELECTED RESULTS OF A PILOT STUDY ON RVF AND *B. MELITENSIS* IN SMALL RUMINANTS IN TRADITIONAL FARMING SYSTEMS IN REGIONS OF THE GAMBIA AND GUINEA AND THEIR PUBLIC HEALTH IMPACT

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Small ruminant (SR) populations in The Gambia and Guinea are well adapted to the environment with potential for high reproduction rates. However, relatively high rates of abortion in village herds were identified in recent years as a major constraint, which causes are often not diagnosed. Likely causing diseases are trypanosomosis, brucellosis, PPR and Rift Valley Fever (RVF), known or suspected to be endemic in the region. Besides the impact on herd productivity, some of the causing infections are zoonoses, like brucellosis and RVF.

In West Africa, RVF disease outbreaks have been reported for some years with irregular virulence and frequency (e.g. Senegal, Mauretania and The Gambia). Brucellosis is expected to be endemic in both study countries. However, little is known about the specific situation for The Gambia and Guinea. Therefore a study based on serological surveys was carried out to estimate the prevalences of brucellosis and of RVF in small ruminants in selected parts of The Gambia and Guinea in 2006. In addition, a survey in people at risk was initiated for both diseases. This part was carried out in collaboration with national health authorities. Study areas were selected in view of ecologically predisposing features for RVF. In each country samples were taken from two Districts, 14-15 villages per District and up to 59 SR per village. In each region human samples were taken from herd owners and "patients with malaria like symptoms" admitted to the health centres.

Individual animal prevalence varied between countries, regions and species (RVF: IgG 13.2% to 25.4, The Gambia; 3.8% to 10.7%, Guinea and *B. melitensis*: 0% to 0.3%, The Gambia; 1.9% to 11.3%, Guinea). Abortion history correlates with sero-positivity for brucellosis but not for RFV. High prevalences for Brucellosis in humans (up to 18%) in sampled regions of Guinea indicate the related public health risk.

O43: GLOBAL PREVALENCE OF MAIN PATHOLOGIES RELATED TO DAIRY PRODUCTION IN URBAN FLOCKS OF HAMDALLAYE (OUAGADOUGOU, BURKINA FASO)

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The aim of this study was to assess prevalence of three main diseases related to dairy production in urban cattle herds: brucellosis, tuberculosis and mastitis. Serum samples collected from 209 bovines have been tested for brucellosis using antigen buffered test. A global prevalence of 13.2 % has been observed; females were significantly ($p<0.05$) more infected than males (14.3% *vs* 5.6%). Simple Intra Dermo Tuberculination (IDT) test with the PPD tuberculin was used on 325 cows. The global prevalence of this infection was 27.7%, with a very high significant variation ($p <0.01$) between two years old animals and those over 6 years. From 98 individual milk samples submitted to California Mastitis Test (CMT), mean cellular concentration was 5385. 10^3 ± 1061. 10^3 TCN/ml. The prevalence of the three studied diseases is important enough to retain attention of farmers as well as technicians and authorities. Maximum care and actions are to be taken particularly for tuberculosis and brucellosis which are major zoonosis and can seriously damage consumer's health.

Session 10 Natural products in Veterinary Medicine

O45: ANTI-INFLAMMATORY AND ANALGESIC ACTIVITIES OF THE AQUEOUS EXTRACTS OF MARGARITARIA DISCOIDEA (EUPHORBIACEAE) STEM BARK IN EXPERIMENTAL ANIMAL MODELS.

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Margaritaria discoidea is a medicinal plant used for the treatment of various body pains in central, eastern and southern Africa. Inflammation has become the focus of global scientific research because of its implication in virtually all human and animal diseases. The conventional drugs used to ameliorate this phenomenon are either too expensive or toxic and not commonly available to the rural folks that constitute the major populace of the world.The anti-inflammatory activity of the stem extract of this plant was assessed using carrageenan-induced paw oedema and histamine-induced paw oedema. The analgesic effect was determined using the acetic acid writhing method as well as formalin test. Acute toxicity test to determine the safety or otherwise of this plant was also carried out in animal models. The extract at 50, 100 and 200 mg/kg body weight reduced significantly, the formation of oedema induced by carrageenan and histamine. In the acetic acid-induced writhing model, the extract showed a good analgesic effect characterized by reduction in the number of writhes when compared to the control. The extract caused dose-dependent decrease of licking time and licking frequency in rats injected with 2.5% formalin, signifying its analgesic effect. These results were also comparable to those of indomethacin, the reference

drug used in this study. Acute toxicity test showed that the plant may be very safe for pharmacological uses. Since the plant extract reduced significantly the formation of oedema induced by carrageenan and histamine as well as reduced the number of writhes in acetic acid-induced writhing models, this study has provided some justification for the folkloric use of the plant in several communities for conditions such as stomachache, pain and inflammations.

O46: TOXICITY TO THE POULTRY RED MITE (DERMANYSSUS GALLINAE) AND YIELD OF ESSENTIAL OILS HARVESTED FROM WILD GROWING PLANTS IN TUNISIA

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With changes in legislation and consumer demand, alternatives to synthetic acaricides to manage the poultry red mite *Dermanyssus gallinae* (De Geer), a serious pest in laying hen flocks, are urgently needed. Plant-derived products are being increasingly studied for their potential as acaricides for *D. gallinae* and work from both Korea and the UK has suggested that certain commercially-available essential oils show promise to this end. Work elsewhere has shown that wild plants may offer a useful source of novel material from which to produce essential oils with pesticidal potential.

A laboratory experiment was conducted with *D. gallinae* to assess the toxicity of a range of Tunisian essential oils, obtained by hydro-distillation from plants harvested in the wild from the Kef region in 2007-2008. Details of the percentage essential oil yield from these plant species were also recorded. For comparison, commercially-sourced thyme essential oil (from *Thymus vulgaris* (L.) was also tested against *D. gallinae* after work elsewhere found this product to be among the most acaricidal from a range of 50 essential oils tested. Recently-fed adult female *D. gallinae* were exposed to the eight essential oils at 0.1 mg oil / cm² in Petri-dishes at 22°C over a period of 24 hours.

Results showed that the yield of essential oil varied considerably depending upon the source plant. Whilst maximum yields of 0.5% were achieved with the essential oils of *Thymbra capitata* (L.) Cav. and *Pteranthus dichotomus* Forssk., oils of *Juniperus phoenicea* L., *Myrtus communis* L. and *Santolina africana* Jord. & Fourr. provided yields of less than 0.1%. Similar variability was recorded with respect to the toxicity of the essential oils to *D. gallinae*. Although essential oils from *Seriphidium herba-album* (Asso) J. Soják, *Juniperus phoenicea* L., and *P. dichotomus* did not cause significant *D. gallinae* mortality (in comparison to the control), all other selected oils provided mortality levels statistically similar to that achieved with commercial thyme oil. The essential oil of *Pelargonium graveolens* L'Hér. was particularly acaricidal, with this oil being the only product tested to provide 100% mortality of *D. gallinae*.

The results suggest that the essential oil of *P. graveolens,* in particular, may warrant further attention in the search for a plant-based *D. gallinae* acaricide. This could be especially true if a widely available commercial form of this oil could be shown to be as effective against *D. gallinae* as oil from wild-growing plants in Tunisia.

O47: ON FIELD ASSESSMENT OF ANTHELMINTIC ACTIVITY OF COST EFFECTIVE (HERBAL) FUNCTIONAL- REMEDIES IN GOATS FOR SELF RELIANCE IN LIVESTOCK PRIMARY HEALTHCARE

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Five groups (n=6) of local goats (12-15 kg) from Thanjavur district in Tamil Nadu, India, were used for the assessment of anthelmintic activity of locally available natural products in comparison with

fenbendazole. On zero day except Group I (untreated control), other four groups were treated once (*per os)* as follows: Group II (*Aloe vera* @ 50 grams/animal), group III (*Cissus quadrangularis* -5 stems, *Leucas aspera* -10 leaves and *Azadirachta indica* -10 leaves/animal), Group IV (cummins-10 grams, garlic-5 bulbs, mustard- 10 grams, pepper-5 pearls and turmeric-5 grams) and group V (fenbendazole @ 7.5 mg/kg). On day zero and fifteen, the blood and faecal samples were collected and body weight measured.

Eggs per gram (EPG) of faeces, haemoglobin, white blood cells and total protein levels were estimated. The average EPG levels in each treatment group were shown to be significantly reduced on fifteenth day when compared to that of the untreated control. Among treatment groups, group III showed profound reduction in EPG levels (95.45%) and was comparable to group V (95.83 %), treated with fenbendazole. There were no significant differences among the groups in respect of body weight gain. The haemoglobin and total protein levels in all treated groups were significantly higher ($P \leq 0.05$) while control (group I) showed reductions in the respective levels. The white blood cell levels in all treated groups were found to be increased whereas the control group showed no improvement on fifteenth day. *Aloe vera* treated goats showed profound increase in the levels of white blood cells as compared to other groups.

The study revealed the anthelmintic potential of above natural products in domestic goats under field conditions, as herbal functional- remedies for self-reliance in livestock primary healthcare. Fresh herbal preparations for control of mastitis, foot and mouth disease, fowl Pox, endo and ecto parasites in livestock or poultry are successfully used by hundreds of farmers in the field conditions for nearly a decade under the guidance of the EVM centre. Further work is under way to evaluate functional remedies under field conditions.

O 48: BIOCHEMICAL CHARACTERIZATION OF TWO PURIFIED PROTEINS OF THE IB-16 *BACILLUS THURINGIENSIS* STRAIN AND THEIR *IN VITRO* TOXICITY AGAINST THE SHEEP NEMATODE *HAEMONCHUS CONTORTUS*

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The main aim of this study was to characterize the biochemnical composition of two main IB-16 *Bacillus thuringiensis* proteins of 25 kDa and 70 kDa and to determine their toxicity on the blood-feeder nematode, *H. contortus*. The IB-16 soluble toxin is conformed by two main proteins, which were purified by HPLC chromatography using a an hydrogel column and sephadex-beads G-50. Biochemical analysis was carried out to determine protein pI, native-mass molecular fracction, enzyme and carbohydrate molecules. Beside, the *in vitro* lethal effect was assessed using *H. contortus* histiotropic larvae (L_4) and protein fractions of 25 kDa and 70 kDa, respectively. Our results showed a pI of 8 and 10 pH fracctions and an active enzyme action from a 25 kDa protein. In contrast, non-enzymatic activity was observed by the 70 kDa protein. These results suggest the role of Cry and Cyt δ-proteins involved on IB-16 *B. thuringiensis* strain with nematicidal activity, since 70 kDa and 25 kDa proteins fracctions showed 68% and 30% of toxicity on *H. contortus* L_4, respectively. Alternatively, lethal effect caused by the *B. thuringiensis* IB-16 strain appeared to be driven by one protein fracction, the 70 kDa.

This project was supported by CONACYT-México.

Session 11 STVM Award Session

O49: Measures of Success and Standards of Excellence: A Career in Tropical Veterinary Medicine.

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How do **you** measure success in life and success in a career? It is uncomfortable for many of us to discuss our careers without feeling boorish and boastful and it is difficult to present an honest appraisal of one's achievements. The response to this rhetorical question varies with our age, our station in life, our jobs, our awards and honors, our salaries and financial rewards, our happiness, our perceived and actual achievements, and our personalities. But, most of all, success for me is set by a very high standard of excellence-- my reputation, my dignity, my integrity, the happiness of my family, the welfare and recognition of and credit to my colleagues, and my expressions of gratitude to my friends.

My career in veterinary medicine and tropical veterinary medicine has been challenging, rewarding, productive, and fun. While we have all had boring and stressful jobs, I believe that I can say to you with all sincerity, that I have never been bored with veterinary medicine and my interactions with my learned and respected colleagues.

I hope to weave a tapestry of my career that will interest, or at least, not bore, you. My professional career has highlighted the concepts and philosophies embraced in the theme of our biennial conference, "One Medicine." I have experienced many serendipitous moments. I have been blessed to have many supportive and attentive mentors, have had the good fortune to have worked with numerous talented colleagues, and have been humbled by the talents and expertise of the many students with whom I have had the pleasure of interacting.

The foundation of my tapestry is characterized by the goals and philosophies of the Society for Tropical Veterinary Medicine. The goals and achievements of the STVM epitomize the ideals of my life and my career. My tapestry will weave some career experiences with the notable scientists, colleagues, and friends with whom I have shared ideas, successes, disappointments, and laughs. I am privileged to have been able to serve the STVM. I cherish the memories of my friends and colleagues in the STVM, and am flush with happiness and appreciation for my Wife, my children, and my retirement.

Thanks for the honor you have conferred on me.

O50: Differential expression of inflammatory and immune response genes in sheep infected with bacteria that target immune cells.

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Anaplasma phagocytophilum and *Brucella ovis* both infect immune cells which results in diseases that affect humans and animals. *A. phagocytophilum* infects neutrophils in a wide variety of host species and causes tick-borne fever (TBF) in ruminants and granulocytic anaplasmosis in humans, horses and dogs, while *B. ovis* replicates and persists primarily within macrophages and causes ovine brucellosis, a disease characterized by infertility in rams, abortion in ewes and increased perinatal mortality in lambs. Infection

of immune cells with these bacteria modifies host gene expression and the host immune response. The objective of this research was to characterize differential gene expression in sheep infected with *A. phagocytophilum* or *B. ovis* by microarray hybridization and real-time RT-PCR. The results of these studies demonstrated both similarities and differences in the host response to infection with *A. phagocytophilum* and *B. ovis*. Analysis of differentially expressed genes in infected animals demonstrated activation of inflammatory and innate immune pathways. *A. phagocytophilum* infection also resulted in the impairment of adaptive immunity, while infection with *B. ovis* resulted in upregulation of genes involved in phagocytosis and downregulation of host protective responses, mechanisms that may contribute to the pathogenicity and persistence of these bacteria. The comparative analysis of the genes and their expression profiles in sheep in response to infection with bacteria infecting immune cells advances our understanding of the molecular mechanisms of infection and pathogenesis and reveals common and distinctive host responses to infection.

Acknowledgments: This research was supported by the Ministerio de Ciencia e Innovación, Spain (project BFU2008-01244/BMC), Grupo Santander and Fundación Marcelino Botín, the Ministerio de Educación y Ciencia (project AGL2005-07401), FEDER, the Oklahoma Agricultural Experiment Station (project 1669), and the Walter R. Sitlington Endowed Chair for Food Animal Research (K. M. Kocan, Oklahoma State University) and was facilitated through the Integrated Consortium on Ticks and Tick-borne Diseases (ICTTD-3), financed by the International Cooperation Program of the European Union, coordination action project No. 510561.

O51: SEROLOGICAL AND MOLECULAR SURVEY OF *BABESIA* PARASITES IN CATTLE IN TUNISIA

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Babesia species are tick-borne intracellular protozoan parasites belonging to the phylum Apicomplexa, which affect many domestic and wild animals. In North Africa, three species affecting cattle have been described: *Babesia bovis*, *B. bigemina* and *B. divergens*. Unfortunately, the epidemiology of babesiosis in Tunisia has not been extensively investigated. In this study, we conducted a cross-sectional study to determine the seroprevalence of *Babesia bovis* and *B. bigemina* in cattle and to correlate our findings with reverse line blot hybridisation results.

A total of 152 cattle (21 males and 131 females) were bled once between October and November 2006, belonging to six Tunisian sites located in three bioclimatic zones. *B. bovis* and *B. bigemina* was investigated first using species-specific immunofluorescence anti-body (IFA) test; and, second, using species-specific oligonucleotide hybridisation (RLB assay). Randomly chosen PCR products were then purified and sequenced.

Babesia DNA was detected in 18 blood samples (11.8%), including nine (6%) cattle infected by *B. bigemina* and eleven infected by *B. bovis* (7.2%). The 18 cattle had antibodies reactive to *B. bovis* and/or *B. bigemina*. In contrast, 76 cattle were negative by PCR/RLB but had antibodies reactive to *B. bovis* and 59 cattle had antibodies reactive to *B. bigemina*. In addition, tow PCR/RLB, *B. bovis* and *B. bigemina*, positive cattle had antibodies reactive with the two pathogens. The partial sequence of the amplicon from one randomly selected *B. bovis* positive cattle (338 nucleotides sequenced) demonstrated a 96% similarity with the Mexican *B. bovis* isolate (EF643472).

This work is the first molecular report of infected cattle by *B. bovis* and *B. bigemina*. The epidemiology of these diseases was preliminary studied for the implementation of successful control programmes that include effective treatment of malignant and/or pathogenic babesiosis.

Session 13 Economics / Translational Technology

O52: THE POLITICAL ECONOMY OF AVIAN INFLUENZA IN INDONESIA

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Why is the response to H5N1 highly pathogenic avian influenza (HPAI) so challenged in Indonesia? Why did the virus spread so fast, and why has the disease persisted? Are there features of the country and its culture that encourage or inhibit the disease? Is the internationally led response appropriately sensitive to local contexts? This presentation suggests that distinctive social, cultural, economic and political factors work against a technocratic response such as has been employed in Indonesia. The presentation explores the interactions between global bio-medicine, a mesh of power relations linking health, industry, institutionalism and governance, and Indonesia's diverse and complex political and social contexts. How is an infectious zoonotic disease controlled in a dynamic environment where modernist models of authority and rationality are unproven?

Since H5N1 was first detected in central Java in mid-2003, it has spread to 31 of Indonesia's 33 provinces, caused the death or destruction of at least 150 million poultry birds, and killed over 110 humans. The international response, which began in mid-2005, has focused on animal surveillance, control and vaccination, human health system capacity building, and information and behaviour change communications. The response is challenged by the size, geography and infrastructure of the country, an exuberant democracy and extensive decentralization. Other diseases, sectarian tensions and regular natural disasters overshadow the threat of HPAI to human health and food security. Nevertheless, issues of trust between science, government, business and civil society, and nationalism, are shown to be key, as are the varying constructions of risk, public goods and governance associated with the international organizations driving the response, and the people affected by the disease.

O53: PREVENTING HIGHLY PATHOGENIC AVIAN INFLUENZA (HPAI) AT THE RURAL COMMUNITY LEVEL: A CASE STUDY FROM CAMBODIA

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Poultry is an integral part of rural livelihoods in Cambodia, with more than half of households keeping poultry in their backyards. Small-scale, traditional and extensive backyard production is one of the many activities in diversified rural farming systems in Cambodia.

Despite Cambodia's long poultry tradition, monitoring poultry stocks is very difficult. This is because Cambodia has numerous small farms where chickens roam freely, and transportation and communications links poorly developed.

More than 20 HPAI outbreaks have been reported since 2004 with deaths of over 21,000 birds.

During the HPAI outbreaks, some of the flocks in the rural area were culled without compensation and producers were not allowed to sell outside of the community.

Heifer International worked with 2,000 rural families through local project partners in the target communities to develop an effective intervention mechanism to mitigate the impact of the HPAI crisis. Heifer International provided training, public education and networking as well as promoting model farms based on improved scavenging poultry management. Each community selected one farm family to serve as a model farm. They were trained in Heifer's working approach and committed to practicing integrated farming systems based on scavenging poultry management. Baseline data were collected. One Village Animal Health Worker (VAHW) in each community participated during project implementation, playing a key role in information exchange and interaction between the communities and Avian Influenza experts.

Formal and informal trainings were conducted for all project partners and project recipients through experts and VAHWs respectively.
There have been no outbreaks reported in the communities in the project areas. Farmers have started using appropriate techniques to maintain biosecurity. They are passing on the knowledge and skills to surrounding communities. This participatory approach in educating rural farmers can serve as a model to mitigate HPAI in developing countries around the world.

O54: SURVEILLANCE OF AVIAN INFLUENZA IN THE CARIBBEAN THROUGH THE CARIBBEAN ANIMAL HEALTH NETWORK (CARIBVET): SURVEILLANCE TOOLS AND EPIDEMIOLOGICAL STUDIES.

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The Caribbean region is considered to be at risk for avian influenza (AI) due to backyard poultry system, important poultry production, migratory birds and disparities in the surveillance systems. The Caribbean animal health network (CaribVET) has developed tools to implement AI surveillance in the region: 1/ a regionally harmonized surveillance protocol, 2/ specific web pages for AI surveillance on www.caribvet.net. 3/a diagnostic network for the Caribbean including AI virus molecular diagnostic capability in Guadeloupe and technology transfer. Altogether 303 samples from 4 Caribbean countries were tested between June 2006 and March 2009 by real time PCR both for importation purpose or following clinical suspicion.
In addition, a wild bird survey was conducted in Guadeloupe mainly on waders in the southward and northward migration periods of 2007-2008. A total of 324 birds were tested by real time PCR for AI virus matrix (M) gene. None of the sample tested were positives suggesting a limited ecological role of these species in the AI virus ecology in the Caribbean.
Following H5N2 outbreaks in Dominican Republic in 2007, a questionnaire was developed to collect data for risk analysis of AI spread in the region through fighting cocks. The infection pathway of Martinique professional poultry sector by AI through introduction of infected cocks was designed and recommendations were provided to the Caribbean veterinary services to improve cock movement control and biosecurity measures.
Altogether these CaribVET activities contribute to strengthen surveillance of AI in the Caribbean region and may allow the development of research studies both on AI risk analysis and on AI virus ecology.

O55: NETWORKING BETWEEN RESEARCHERS FROM DIFFERENT COUNTRIES AND BACKGROUNDS FOR COLLABORATIVE AI RESEARCH: LESSONS FROM THE ASIA PARTNERSHIP ON AVIAN INFLUENZA RESEARCH (APAIR)

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The Asian Partnership on Avian Influenza Research (APAIR) was formed in 2006 by building a joint work among different research institutions from five Asian countries to control more effective Avian

Influenza (AI) in the region. The goals of this partnership are to (i) to manage collaboration research on AI; (ii) to support research capacity building; (iii) to share relevant information; (iv) to build networking among researcher/institution and (v) to advocate for changes in practice, tools and policy. APAIR was initially initiated as a high-level, multi-country, multi-disciplinary initiative with key institutions, researchers and champions in AI-related domains. Currently the research frame is extended towards EIDs.
Until now 88 key researchers, representing 24 institutions from China, Cambodia, Indonesia, Thailand and Vietnam formed several project groups to develop and implement their multi-country and multi-institutional research projects. A first set of five projects was funded and implementing in 2008. They consist of: (a) Forming a regional network for surveillance and monitoring of AI viruses in migratory birds; (b) Socio-Economic Impact of HPAI outbreaks and control measures on small-scale and backyard poultry producers; (c) Characteristics and dynamics of backyard poultry systems in relations to reduce AI risks; (d) Policy Analysis for Pandemic Influenza Preparedness and (e) Studies on the effectiveness of AI control measures.
APAIR has established an informal network, based on trust and shared interest through multi-country collaborative projects. Through mobilizing of joining researchers and institutions with different skills and expertise (e.g. by enabling mutual learning and knowledge sharing) relevant problems were addressed. Within country and regionally APAIR contributes to collaboration among the research funding agencies, research institutes, research users and policy makers. In particular bridges between animal and human health were established or strengthened. Jointly developed new or follow-up research proposals donate to the increased R&D capacity.
Main challenges are how to synthesis best research findings for cross-country comparisons and how to use results most effective as evidence for policy advice and changes in AI control. As further challenges remain the continuously funding of the network and the facilitation of collaboration between researchers/institutions also under the perspective of widening on-going AI research towards the EID context including the identification of new specific research areas that address country and regional needs.

O56: SOCIO-ECONOMIC IMPACTS OF AVIAN INFLUENZA OUTBREAKS ON SMALL-SCALE PRODUCERS IN INDONESIA

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Since it first introduction in 2003 until January 2009 HPAI was reported in 31 of 33 provinces of Indonesia. In addition 115 fatal human cases have been reported in the same period and about 11 million chickens had been died or culled, while in 2005 alone, about 60 percent of farms stop their operations. HPAI in Indonesia had thus significantly impacted the poultry industries and particularly small scale producers.
The objective of this paper is to describe socio-economic impacts of HPAI on small producers in Indonesia. For this simultaneous survey had been conducted in three provinces which represented low, medium and high incidences areas respectively, with total respondents of 720 farms involved.
Results indicated that the number of poultry raised decreased due to HPAI by 25 to 80 percent for broiler, 7 to 93 percent for layer and 48 percent for ducks. Overall, the number of farms which stopped their operations was 30 percent and in the high incidence area even nearly 70 percent. The proportion of income from poultry for daily household expenditure decreased from 75 to 91 percent before to 38 to 82 percent after HPAI. HPAI caused also more loan requests and less saving in the infected farms. Direct impact of HPAI was also seen from decrease of expenditures for education and daily consumption in particular in the high incidence farms. The high proportion of income before HPAI indicated that poultry enterprise was the main income for respondents. HPAI caused significant losses for respondents in all study areas which consisted of high mortality, lower production and lower demand for poultry products. There is also an impact on existing village economy, such as increase in unemployment and migration to

nearby cities looking for temporary jobs. However, levels of social relationship, social networking, social trust, social organization and decision making remains relatively unchanged by HPAI outbreak.
In order to maintain or re-establish the shown heavily affected poultry business due to HPAI it is expected that recovery of poultry enterprise can be best targeted in low incidence areas. An option might be to re-allocate small scale poultry producers to villages in less densely populated areas (human and poultry).

O57: PREVENTING AND CONTAINING TRYPANOCIDE RESISTANCE IN THE COTTON ZONE OF WEST AFRICA

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Trypanocidal drugs are the most commonly purchased and used livestock input by resource-poor farmers in sub-Saharan Africa. In West Africa, these drugs are critical in protecting the 17 million head of cattle from trypanosomosis. The effective use of trypanocidal drugs by smallholder farmers is threatened by the development of widespread resistance. This is a particular concern for smallholder crop-livestock farmers in the cotton-zone of West Africa. A recent BMZ-funded project has confirmed significant resistance to trypanocidal drugs in villages with high trypanosomosis risk in Burkina Faso and Mali. Strategies for resistance prevention were investigated. Keeping trypanotolerant cattle was found to be an effective disease management strategy, but farmers' preference for trypano-susceptible breeds, for reasons unrelated to animal health, suggest the intromission of zebu genotype will continue. Community vector control was found to be effective in managing trypanosomosis in the presence of resistance and the high-level participatory approach tested was found to be more sustainable than the low-level approaches previously used in the region. This suggests that participatory vector control with appropriate external support is likely to be a viable option for implementing resistance 'clean-up'. Promoting Rational Drug Use (RDU) emerged as a promising prevention strategy, with clear improvements in farmer knowledge, farmer practice and animal health outcomes. However, policy studies showed low understanding of the problem of resistance and the absence of an enabling environment for RDU. Engagement was initiated with actors involved in the problem of resistance and for its solution, including manufacturers, sellers and users of drugs, regulators and extension providers.

O58: SUSTAINABLE AND PROFITABLE LIVESTOCK PRODUCTION IN SUB-SAHARAN AFRICA NEEDS TO BE BASED ON THE DESIGN OF INNOVATIVE VECTOR CONTROL TECHNIQUES FOR RESOURCE-POOR FARMERS

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The increasing demand for meat and dairy products – notably in the vicinity of expanding urban centres in many African countries – requires the foreseeable intensification of livestock production systems. A concomitant and inevitable increase of vector-borne diseases needs to be countered by integrated pest management (IPM) practices. Arguably, tsetse-transmitted trypanosomosis is one of the most devastating diseases, affecting human and livestock health, agricultural production and rural development (1).Trypanocidal drugs still constitute the mainstay and are the most commonly purchased and used livestock input by resource-poor farmers in sub-Saharan Africa. The increasing occurrence of wide-spread resistance against trypanocidal drugs is warranting new approaches, particularly targeting vector control methods. There are now efforts to eradicate tsetse and the diseases on a Pan-African scale (2). So far, a

lack of sustainability has led to the reappearance of tsetse once the external funding had come to an end. Habitat modification through an encroachment of human activities has led to the disappearance of tsetse from many areas ("autonomous control") although some tsetse species have a capacity for adapting to peri-domestic sites. In the absence of tsetse other vectors are becoming increasingly relevant. Flies from the sub-family *Muscinae* are known to transmit more than 100 pathogens of potential risk for human and animal health. The uncontrolled use of insecticides has led to an increase in insecticidal resistance. The recent design of a new vector control method is offering a promising alternative to the wide-spread use of trypanocides and insecticides. Insecticide-treated mosquito fences were found to effectively control tsetse and nuisance flies when circumventing pens or other enclosures for livestock at a height of 1 – 1.50m. This new vector control approach is considered to offer considerable advantages insofar that it has not only a private but also public goods character, since spin-offs can be expected that are beneficial due to a control of vectors, which might otherwise inflict damages to neighbouring farmers or livestock.
(1) FAO, The Programme against African trypanosomiasis (PAAT)
(2) Pan-African Tsetse and Trypanosomosis Eradication Campaign (PATTEC)

Session 14 Wild Life Diseases / Conservation Medicine

O59: CONSERVATION MEDICINE AND ONE HEALTH: FOLLOWING THE STEPS OF ECOHEALTH BY CREATING A TRULY GLOBAL TRANSDISCIPLINE

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Transdisciplinarity or transdisciplinary thinking (TD) can be defined as a complex system at all scales that includes perspectives transcending traditional disciplines going beyond interdisciplinary approaches. It integrates and requires cooperation and a common language linking the natural, social, physical, biological and health sciences to produce a comprehensive account for health problems in a given ecosystem. This shared language attempts to express all the relevant terms of the involved disciplines to develop a metalanguage. TD reflects real-world system complexity. At its last biennial conference, the membership of the Society for Tropical Veterinary Medicine adopted a resolution in support of "One Health," an initiative that promotes collaboration between veterinary and human medical professionals and allied health scientists. The Resolution by the Society for Tropical Veterinary Medicine in Support of "One Health" recently published (Bokma et al. PNYAS 2008). Conservation Medicine and more recently EcoHealth have emphasized the need to bridge disciplines, thereby linking human health, animal health, and ecosystem health under the paradigm that "health connects all species on the planet" with the urgent need to address the rapid deterioration of the planet. The recent convergence of global problems including climate change, biodiversity loss, habitat fragmentation, globalization, infectious disease emergence and ecological health demanded integrative approaches breaching disciplinary boundaries and naturally leading to "One Health". This integration requires commitment not only from government agencies, universities and other organizations but eventually will attempt to generate new international structures. Launched in 2004, EcoHealth is an international peer-reviewed journal. This journal springs from these efforts and focuses on the integration of knowledge at the interface between ecological and health sciences, addressing human health, conservation medicine, and ecosystem sustainability. The journal and supporters of this field led by default to the creation of the International Association of Ecology and Health. A major task is to get developing countries more involved. Only about 20% of articles submitted have authors from the developing world. These regions represent most of the world population, poverty,

biodiversity, and environmental health challenges. “One Health” need to orient itself toward research that accounts for these global changes and contextualize it in terms of human development. The challenges faced today and how to overcome them at a pivotal time in the environmental history of humanity require true regionalization of Conservation Medicine, One Health and EcoHealth. Perhaps most importantly, not only research needs expansion to all sciences but also truly be geographically and culturally participatory.

O60: STATUS OF ZOO AND WILD ANIMALS DISEASES IN INDIA AND THEIR MANAGEMENT.

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India has an impressive mammalian, avian and reptilian fauna distributed in natural habitats and managed in capitivity. Wildlife diseases are given more priority whether it occurs in wild animals reared in captive wild animal places like zoos, zoological parks and zoological gardens or in the free ranging conditions. Diseases in zoo and free ranging wild animals are more or less the same as in the domestic canids, felids, herbivores, birds etc. However, the difference of these wild animal species should not be ignored because of their unique biology, nature, habitat related factors, capture myopathy related pathogenesis etc. Further, the treatment of wild animals of free ranging regions, maintained by protected area managers and custodians of forests, is very difficult. The information regarding diseases in wildlife is generally based on the reports and experiences of a few veterinarians who practice on wild life in captivity. Diseases like Haemorrhagic septicaemia, anthrax, parasitic conditions, FMD, Kyasanur Forest Disease and pox virus infections are the notable ones documented in free ranging wild animal species. Similarly, tuberculosis is a major threat to conservation programme for wild animals. Anthrax has been documented in gaurs, chitals, deer, sambar, antelopes, wild pigs, monkeys, rhinoceros, elephants, felids, canids, etc. Tetanus has been documented in elephants, monkeys, Bonnet macaque, wild ass and rhinoceros kept under captivity in India. Trypanosomiasis, caused by blood protozoa *T. evansi* and transmitted by biting flies is common in felids and documented in wild dogs, elephants, chitals and sambars. Ehrlichiosis and dirofiliariasis are the emerging disease problems in captive carnivores like fox, jackals, leopard. An attempt has been made to highlight some of the common diseases prevalent in zoo and wild animals and their treatments, which are being commonly practiced in India.

O61: NEMATODES AND TICKS AT THE DOMESTIC STOCK/WILDLIFE INTERFACE IN SOUTH AFRICA

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A total of 427 animals on 3 properties were killed in various sets at monthly intervals over a period of 1 or 2 years and processed for the recovery of internal and external parasites.

Cattle and impalas, *Aepyceros melampus* were examined for nematodes and ticks in a nature reserve in Limpopo Province. Thirteen nematode species were recovered and the cattle and the impalas shared 5 of these, while 3 species were present only in the cattle and 5 only in the impalas. Eight tick species were recovered of which all were present on the cattle and 6 on the impalas. The cattle harboured both adult and immature ticks whereas the impalas were infested mainly with immature ticks.

Sympatric Angora and Boer goats, common duikers, *Sylvicapra grimmia*, and Cape grysbok, *Raphicerus melanotis*, were examined for nematodes, and the same animals plus greater kudus, *Tragelaphus strepsiceros*, and scrub hares, *Lepus saxatilis* were examined for ticks on a farm in a south-western region of Valley Bushveld. The adults of 12 nematode species were collected of which the goats harboured 11, the common duikers 6, and the Cape grysbok 9. Seven tick species were present and the adults of all of these infested the goats, 4 infested the kudus, 4 the duikers, 4 the grysbok and 2 the scrub hares. The scrub hares were also infested with the immature stages of 6 of the tick species.

Dorper sheep, Angora goats and greater kudus were examined for nematodes, and the same animals plus cattle, scrub hares and helmeted guineafowls, *Numida meleagris* were examined for ticks on a farm in a central region of the Valley Bushveld. The sheep were infected with the adults of 12 nematode species, the goats with 11, whereas the kudus harboured only 1 nematode species entirely different to any of those infecting the sheep or goats. Eleven tick species were recovered of which the cattle were infested with the adults of 9, the sheep with the adults of 8, the goats with those of 9, the kudus with 8, and the scrub hares with the adults of 2, while the guineafowls harboured no adult ticks. The immature stages of 10 tick species were collected, of which the scrub hares were infested with all 10 and the guineafowls with 8 species.

O62: DIETARY INFLUENCES OF FEED TYPES ON THE HAEMATOLOGICAL INDICES OF CAPTIVE-REARED GRASSCUTTERS EXPERIMENTALLY INFECTED WITH *TRYPANOSOMA CONGOLENSE*

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The influence of feed types on the hematological indices of captive – reared grasscutters (*Thryonomys swinderianus*) experimentally infected with *Trypanosoma congolense* was done. Sixty grasscutters were intensively reared and separated into three treatment groups, each consisting of twenty animals. Treatment O (TO), served as control which were fed forages but not experimentally infected, TA were fed on concentrates and infected with *T. congolense*, while TB were fed on forages and also infected with *T. congolense*. Results showed no significant difference ($P > 0.05$) in weight gain among the animals. However, there were significant differences ($P<0.05$) in their temperature and hematological indices. This suggests that the animals could not gain weight, despite the variations in their diet. However, diet had positive influence on the hematological indices of the captive-reared grasscutters but could not reduce the increase in rectal temperature arising from trypanosomiasis, which however, later decreased and returned to normal.

O63: THERAPEUTIC EFFECT OF BERENIL^{R} IN EXPERIMENTAL MURINE TRYPANOSOMIASIS USING STOCKS ISOLATED FROM APPARENTLY HEALTHY WILD GRASSCUTTERS (*THRYONOMYS SWINDERIANUS*).

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We investigated the effects of a trypanocide (Berenil ^{R}) on Swiss albino mice infected with *Trypanosoma congolense* and *Trypanosoma vivax* stocks isolated from apparently healthy wild grasscutters. The two trypanosome organisms elicited severe parasitaemia and anaemia in the mice after a pre- patent period of 6 to 10 days and 4 to 7 days for *T. congolense* and *T. vivax* respectively. *T. vivax* produced a more severe anaemia than *T. congolense.* At 10 days post infection, the red blood cell (RBC) indices, packed cell volume (PCV) and hemoglobin concentration (Hb) were significantly
($P< 0.01$) lower in mice infected with *T. vivax* than those infected with *T. congolense*. Curative treatment of the infected rodents using 1mg / kg Diminazene aceturate (Berenil ^{R}) given on the 10th day of infection resulted in complete recovery of the animals from the parasitaemia and anaemia. It appears that the grasscutter is trypano- tolerant and this is note- worthy for possible vaccine development in the future.

Session 15 Trypanosomiasis

O66: ANALYSIS OF CATTLE BASED SLEEPING SICKNESS CONTROL IN NORTHERN UGANDA

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In October 2006 an emerging intervention was initiated to arrest the continuing northerly advance of the zoonotic parasite *Trypanosoma brucei rhodesiense* in Uganda which was threatening to result in the unprecedented geographical overlap with *T. b. gambiense* foci in the north of Uganda.
The Stamp Out Sleeping sickness (SOS) programme treated the cattle reservoir of *T. b. rhodesiense* to interrupt transmission to humans, by treating 180,000 heads of cattle with trypanocides in the most recently affected districts. Simultaneous spraying of cattle with deltamethrin was aimed at suppressing the vector tsetse population.
Prior to the treatment programme the prevalence of *T. b. rhodesiense* was established within the cattle population of the programme area. After treatment of the cattle with the trypanocidal drugs follow up sampling was conducted at the time points of three, nine and eighteen months in twenty four pre-determined villages throughout the treatment area. These samples were analysed for the prevalence of the zoonotic *T. b. rhodesiense* parasite within the cattle population.
Analysis showed that the prevalence of *T. b. rhodesiense* decreased after the initial treatment, however due to complications with the supply of deltamethrin spray the prevalence of *T. b. rhodesiense* in cattle returned to the baseline prevalence. The spatial distribution of the positives showed that a definite focus of the parasite remained.
Along with analysing cattle borne *T. b. rhodesiense*, case records from local sleeping sickness treatment hospitals were examined to identify the village from which patients have originated. Through combining human case data along with the locations at which *T. b. rhodesiense* was identified in the cattle population it is possible to gain insight to the effect of the SOS intervention within areas of the treatment area and direct any necessary further treatment

O67: A NEW AND IMPROVED MOLECULAR TOOL FOR DETECTING *TRYPANOSOMA BRUCEI* S.L. IN CATTLE BLOOD SAMPLES

Sally Wastling, Louise Hamill, Kim Picozzi, Sue Welburn

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Molecular methods for the detection and identification of trypanosomes in their human and livestock hosts are now well established. In particular the *Trypanozoon* specific PCR has been available for almost 20 years, and continues to be used as part of epidemiological studies and to guide rational disease control strategies.
Recently a novel DNA amplification method – Loop-Mediated Isothermal Amplification (LAMP) – has been developed which offers significant advantages over traditional PCR. The reaction is performed isothermally, so a high precision thermocycler is not required. LAMP is reported to be more sensitive and specific than PCR, and it is much quicker, taking only 1 hour.
Two *Trypanozoon* specific LAMP assays have been published, but, to date, have not been used for large scale analysis of field samples. One targets the single copy *PfrA* gene, one the multi-copy RIME element. Here we compared the well established *Trypanozoon* specific PCR, to the two new LAMP methodologies, using a large (n>500) set of cattle blood samples, collected in Uganda during 2008. All samples had been

collected onto Whatman FTA cards; this is a standard method, well suited for collection at remote, temporary or poorly-resourced field sites, easy transportation and long term storage without refrigeration.
This study assessed whether the LAMP assays could be used as simpler, more sensitive and more rapid alternatives to the PCR. In the absence of a true gold standard, we constructed a reference standard – we counted a sample as positive if it was positive by any one of the assays. We then compared the sensitivity of each assay against our standard. The LAMP for RIME assay far out performed its PCR counterpart, with 66.8% sensitivity (95% confidence interval 61.2%-72.4%) as compared to 38.0% (95% confidence interval 32.3% - 43.7%) for the PCR. However, the LAMP for *PfrA* assay was poor, detecting only 12.0% (95% confidence interval 8.2%-15.8%) of positive samples.
We recommend that LAMP for RIME be considered as best practice for the molecular detection of *T. brucei* s.l. in large sets of field samples. It detects a higher proportion of positive samples and is also more efficient, taking only 1 hour. Furthermore, since the reaction can be performed at one temperature in a simple water bath, this technology could be more easily applied in low-technology, local laboratories, enabling in-country monitoring of an in-country problem. This has obvious potential benefits for disease control.

O68: DOMESTIC PIGS AS POTENTIAL RESERVOIRS OF HUMAN AND ANIMAL TRYPANOSOMIASIS

Louise Hamill, Kim Picozzi, Sue Welburn

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Trypanosomiasis affects a number of mammalian species, including domestic livestock and humans, causing considerable morbidity, mortality and economic loss. Much work has been done elucidating the epidemiology of trypanosomiasis in domestic and wild animals, however very few studies have been focused on the involvement of pigs. In this study we look at the levels of trypanosome infection in domestic pigs in Tanzania, and suggest the role that these animals may play as potential reservoirs of cattle and human infective trypanosomes.
Blood samples were collected on Whatman FTA cards from 168 domestic pigs from the Arusha region of Northern Tanzania.PCR analysis identified 28 (16.7%) pigs infected with one or more species of trypanosome, 5 of which were multiple infections. The parasites identified as circulating in this area include *T. vivax* ,*T. simiae*, *T. b. brucei*, *T. b rhodesiense*, and some suspected *T. godfreyi* infections.
The results of this study suggest that domestic pigs should be seriously considered as a potential reservoir species for *T. b. rhodesiense*. This parasite, which is the causative agent of acute human sleeping sickness, was found in 4.76% of domestic pig samples overall. However, the *T. b. rhodesiense* prevalence was not evenly distributed across the 4 districts from which samples were collected. Notably, prevalence reached 10.8% in Arumeru district, a heavily populated area where tsetse species with a predilection for feeding on both porcine and human hosts are also found. These results also suggest domestic pigs may play a role as reservoirs for *T. vivax* and *T. brucei* s. l. infection in cattle, and while these parasites cause mild or asymptomatic infection in porcine species, *T. vivax* is known to cause serious disease in cattle and other economically important livestock.

O70: COMPARISION OF PCR AND HEMATOCRIT CENTRIFUGATION TECHNIQUE TO DETECT *TRYPANOSOMA EVANSI* IN GOATS

Maria T. Tejedor-Junto[1], Margarita González[1], Noé F. Rodríguez[2], Juan A. Corbera[2], **Carlos Gutierrez C**[2].

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Natural *Trypanosoma evansi* infection in the Canary Islands has only been diagnosed in the camel

population, but dissemination of the disease in other hosts has not been excluded. Goat population is usually concentrates very close to camel herds, and goats could play an important role in the dissemination of the disease. The objective of this work was to assess the performance of a Polymerase Chain Reaction (PCR) with a primer targeting a repetitive region specific for *Trypanozoon* subgenus used to amplify a 227 bp fragment from the genomic DNA, to detect *T. evansi* in blood samples of infected goats. Ten Canarian goats were tested for *T. evansi* detection by PCR and two additional methods (CATT/*T. evansi* and hematocrit centrifugation technique). Six goats were inoculated intravenously with at least 1 x10^5 *T. evansi*., and four goats were used as control. Blood samples from control goats were parasitologically and serologically negative for *T. evansi.* Blood samples obtained from experimentally infected goats showed DNA amplification of the specific 227 bp band and they were positive for *T. evansi*. They resulted also positive by serology and by hematocrit centrifugation technique. Remaining four goats were found negative by all techniques used. We conclude that this PCR could be adequate for assessing infection of goats with *T. evansi*.

Posters Program

Session 8: "Poster session I" Tuesday June 30th, 2009 (15:00-17:00)		
1	**Avelino J. Bittencourt**	Laboratory Rearing and Behaviour of *Stomoxys calcitrans* (Diptera: Muscidae)
2	**Avelino J. Bittencourt**	Antifungals for Rearing Medium of *Stomoxys calcitrans* (Diptera: Muscidae)
3	**Avelino J. Bittencourt**	*Metarhizium anisopliae* for Biological Control of *Stomoxys calcitrans*
4	**Avelino J. Bittencourt**	Hematological Changes in Rabbits Associated with *Stomoxys calcitrans* Exposure
5	**Avelino J. Bittencourt**	Histopathological Lesions Caused by *Stomoxys calcitrans* (Linnaeus, 1758) on Rabbit Skin
6	**Matias P. J. Szabó**	Fauna, Seasonal Activity and Prevalence of Ticks within Various Phytophysiognomies in a Savannah Reserve in Uberlândia, Minas Gerais, Brazil
7	**Matias P. J. Szabó**	Ticks (Acari: Ixodidae) on Dogs from Uberlândia, Minas Gerais, Brazil
8	**Olivier A. E. Sparagano**	Static and dynamic systems in *Rickettsia slovaca* life cycle evaluated by quantitative real time PCR
9	**Marinda C. Oosthuizen**	Identification of novel *Theileria* genotypes of the Africa Buffalo (*Syncerus caffer*) by means of the Reverse Line Blot hybridization assay and 18S rDNA sequence analysis
11	**Youmna M'ghirbi**	*Theileria* and *Babesia* Parasites in Ticks in Tunisia
12	**Gervásio H. Bechara**	Innate Immunity in Wooless Lamb to Larvae of *Amblyomma cajennense* Tick (FABRICIUS, 1787) (ACARI:IXODIDAE)
13	**Gervásio H. Bechara**	Localization of Antigenic Sites in Unfed Nymphs of *Amblyomma triste* Koch 1844 (Acari: Ixodidae) Ticks by Immunohistochemistry
14	**Erich P. Zweygarth**	Experimental Use of the Attenuated *Ehrlichia ruminantium* (Welgevonden) Vaccine in Friesian Cattle – a Pilot Study
15	**Carlos Gutierrez**	Seroprevalence of *Coxiella burnetii* in domestic ruminants in Gran Canaria island, Spain
16	**Vânia Bittencourt**	Qualitative and Quantitative Analyses of Two Different Methods for Collecting Hemolymph from *Rhipicephalus* (*Boophilus*) *microplus* Tick
17	**Vânia Bittencourt**	Evaluation of the Effects of Destruxin A to *Rhipicefalus (Boophilus) microplus* Engorged Females
18	**Vânia Bittencourt**	Investigation on Lipids Presence in Hemolymph of *Rhipicephalus* (*Boophilus*) *microplus* Infected with Fungi
19	**Vânia Bittencourt**	*Lecanicillium lecanii* conidial oil-based suspension for *Rhipicephalus* (*Boophilus*) *microplus* biocontrol
20	**Vânia Bittencourt**	Protein Profile of Hemolymph of *Rhipicephalus (Boophilus) microplus* Infected with Fungi
21	**Vânia Bittencourt**	Susceptibility of Unfed Larvae of *Rhipicephalus (Boophilus) microplus* to the Fungi *Beauveria bassiana and Metarhizium anisopliae*
22	**Patrícia R. de Oliveira**	Ultrastructural Changes Induced by Fipronil on Ovary Cells of Semi-engorged *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae) Females
23	**Patrícia R. de Oliveira**	Fipronil Induces Microscopic Changes on Salivary Gland Cells of Unfed and Semi-engorged *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae) Females

24	**Julio V. Figueroa**	Using *msa-2b* as a Molecular Marker for Genotyping Mexican Isolates of *Babesia bovis*
25	**Julio V. Figueroa**	Sequence conservation of 12D3 gene in Mexican isolates of *Babesia bovis*
26	**Julio V. Figueroa**	Tick Transmissibility Studies of *Babesia bigemina* and *Babesia bovis* Attenuated Strains derived from *in vitro* Culture
27	**J. Antonio Álvarez**	Validation of an attenuated live vaccine against babesiosis in native cattle in an endemic area
28	**Eliana Guillemi**	Multilocus Sequence Typing of *Anaplasma marginale* Isolates
29	**Paula Ruybal**	*Babesia* sp. and *Anaplasma marginale* Co-infection Appraisal in Cattle
30	**Umberto Vesco**	Modelling the different distribution of *Amblyomma cajennense* and *A. triste* in South America, the main vectors of Spotted Fevers to humans
31	**Umberto Vesco**	Ticks and Tick-borne Zoonoses in the (Sub-) Tropics: the Use of an Integrated Database as a Tool for Risk Assessment
32	**Agustín Estrada-Peña**	A data set of landscape, climate and host features to map distribution of tick species.
33	**Maryna Golovchenko**	Delineation of a New Species in *Borrelia burgdorferi* Sensu Lato Complex from Atypical American Strains.
34	**Nataliia Rudenko**	Immune Proteins of *Ixodes ricinus*
35	**Worawidh Wajjwalku**	Mixed Infection of Benign *Theileria* sp. in Sambar Deer *(Cervus unicolor)*
36	**Rosangela Zacarias Machado**	Phylogenetic analysis of *Ehrlichia sp.* from Brazilian wild felids based on 16S rRNA and *omp-1* genes
37	**Rosangela Zacarias Machado**	Seroepidemiology of *E. chaffeensis* and *A. phagocytophilum* in brazilian marsh deer (*B. dichotomus*) from Porto Primavera hydroeletric power station, Parana river, Brazil
38	**Mehdi Namavari**	Use of MTT colorimetric assay to measure the virulence of five local strains of *Theileria annulata*
39	**Mehdi Namavari**	The first finding of a natural infection of *Theileria equi* in an ewe: an unusual host
S1	**Parviz Shayan**	Comparative study of PCR and Giemsa staining analysis for detection of *Anaplasma spp*. in reservoir cattle
S2	**Lesley Bell-Sakyi**	Establishment and Maintenance of a Global Tick Cell Line Collection
S3	**Maria Kazimírová**	Effects of the tick-derived antigen 64P on transmission of *Borrelia afzelii* in laboratory C3HN mice
S4	**Heike Müller**	A Loop- mediated Isothermal Amplification (LAMP) Assay for detection of *Babesia vogeli*- infections in dogs
S5	**Awadia Ali**	Development of a loop-mediated isothermal amplification method for detection of *Theileria lestoquardi*
S6	**Diaeldin Salih**	Comparison between Reverse Line Blot and Enzyme-linked Immunosorbent Assay in Diagnosis of Major Tick-borne Diseases of Cattle in Southern Sudan
S7	**Zhijie Liu**	Identification of *Theileria uilenbergi* immunodominant protein for development of an indirect ELISA
S8	**Stefanie Renneker**	Validation of a competitive ELISA for detection of *Theileria annulata* infection
S9	**Jassim Abdo**	Development of a recombinant indirect ELISA for the diagnosis of *Theileria uilenbergi* infection in small ruminants
S10	**Ana Domingos**	Identification of babesipain-1, a cysteine protease from the bovine piroplasm *Babesia bigemina*

S11	**Jasim Abdo**	Application of Polymerase Chain Reaction (PCR) for the Detection of Theileri annulata among Cattle in Kurdistan Region / Iraq
40	**Elida M. Rabelo**	Establishment of the RNA Interference Technique to a Nematode Parasite, Member of the Ancylostomatidae Family
41	**Ma. Eugenia Lopez-Arellano**	Interferon g Haplotype B Gene Associated to a *Haemonchus contortus* Infection on Pelibuey Sheep
Session 12: "Poster session II" Thursday July 2nd, 2009 (15:00-17:00)		
43	**Louise Hamill**	The effectiveness of a targeted re-treatment intervention programme in reducing the incidence of Trypanosomiasis in cattle in Uganda
45	**Matilde Jimenez-Coello**	In vivo Activity Of (8-hydroxymethylen)-trieicosanyl acetate Against *Trypanosoma cruzi* During Acute Phase Of The Infection
46	**Matilde Jimenez-Coello**	Cardiac Lesions in Dogs Naturally Infected with *Trypanosoma cruzi*
47	**Matilde Jimenez-Coello**	Serological Survey of American Tripanosomiasis in Dogs and their Owners from an Urban Area of Merida Yucatan, Mexico
48	**Matilde Jimenez-Coello**	Serological survey of Toxoplasmosis in domestic cats from Merida, Yucatan, Mexico
49	**Matilde Jimenez-Coello**	Presence of IgM and IgG Antibodies against *Toxoplasma gondii* in Pig Farms from Yucatan, Mexico.
50	**Miliane M. Soares**	Theoretical Model of Inhibition of *Leishmania* spp. Trypanothione Reductase
51	**Miliane M. Soares**	Resistance Pattern of *Staphylococcus* spp From Bovine Mastitis in Rio de Janeiro, Brazil
52	**Miliane M. Soares**	*Staphylococcus aureus* Virulence Factors From Bovine Mastitis at Rio de Janeiro, Brazil
53	**Miliane M. Soares**	Potential Pathogenical Bacteria Isolated from Bivalve Mollusks at the Archipelagus of Santana – Macaé
54	**Richard Bishop**	Bovine Tuberculosis in the Developing World
55	**Carlos J. De Luna**	Emergence of Bluetongue virus in northern Europe and its current situation in Great Britain
56	**Maria Vitale**	Screening for *Leptospira* spp. in Different Animal Species in Sicilian Island, Italy
57	**Avelino J. Bittencourt**	Genetic Characterization of Dairy Mastitis *Escherichia coli* Strains from Rio de Janeiro Sate, Brazil
58	**Avelino J. Bittencourt**	Enterobacterial microbiota from *Stomoxys calcitrans* (Linnaeus, 1758) segments
59	**Avelino J. Bittencourt**	Ocurrence of Shiga-toxigenic *Escherichia coli* in *Stomoxys calcitrans* (Linnaeus, 1758)
60	**Carlos Gutierrez**	Identification, antimicrobial susceptibility and virulence genes of *Enterococcus* isolated from Camels
62	**Worawidh Wajjwalku**	Identification of *Leptospira* species by flaB-Polymerase Chain Reaction-Based Techniques
63	**Edi Basuno**	Restructuring Policy for Small Scale Poultry Producer to Control HPAI in Indonesia

65	**Fernando Diaz**	Negative Regulation in the Immune Response by Exogenous Interferon gamma Application in Newborn Calves Vaccinated with the Culture Filtrate Protein of *M. bovis*
66	**Fernando Diaz**	Presence of Glycoproteins in *Mannheimia haemolytica* and *Pasteurella multocida*
67	**Carlos R. Prudencio**	Inter Transmission of Leptospirosis in Bovine, Canine, Goats, Horses and Sheep in One Farm with Losses in Reproductive Efficiency
68	**Carlos R. Prudencio**	Transmissibility of *Leptospira interrogans* Among Cattle, Horses and Swine
69	**Yahya Tahamtan**	Sheep Carcasses Could Be Contaminated During Slaughtering Practice
70	**Yahya Tahamtan**	O157 and Non-O157 STEC Major Gene from Cattle in Southern Iran
71	**Yahya Tahamtan**	*Escherichia coli* O157:H7 Strains Associated With Diarrhea and Hemolytic Uremic Syndrome But Do Not Produce Shiga Toxin
72	**Yahya Tahamtan**	Prevalence of the *stx2* Gene in Coliform Populations
73	**Khalid A. Enan**	Evaluation of Human Cytomegalovirus (HCMV) Viral Load Using Real-time PCR in Comparison With HCMV ELISA in Renal Transplant Patients in Khartoum State-Sudan
78	**Maryam Mansourian**	Comparison between the pathogenesis of low and high passage of *Neospora caninum* tachyzoites in chicken embryonated eggs
79	**Maryam Mansourian**	Histopathological and molecular investigations in *Neospora caninum* experimentally infected embryonated quail eggs
80	**Maryam Mansourian**	Comparison between *Neospora caninum* infection in broiler and egg-laying chicken embryonated eggs
81	**Ahad Oliaee**	Prevalence of *Cryptosporidium* Infection in calf in Kazeroon southwestern Iran
82	**Ahad Oliaee**	The Survey of *Dirofilaria immitis* Infection Rate of Stray Dogs in kazeroon , southwestern Iran
83	**Yemisi Adesiji**	Detection of Arcobacters in Faecal Sample of Healthy Chickens in Osogbo, Nigeria
84	**Nader G. Golab Behbahan**	Isolation and Identification of *Salmonella* spp. in Chicken Eggs
85	**Nader G. Golab Behbahan**	Comparison of the Effects of Ultrasonic Waves, Formaldehyde and b-Propiolactone on Inactivating of Newcastle Disease Virus

ABSTRACTS POSTER PRESENTATIONS

Session 8 Poster session I

ECTOPARASITES

P1: LABORATORY REARING AND BEHAVIOUR OF STOMOXYS CALCITRANS (DIPTERA: MUSCIDAE)

Ana Paula Rodrigues Moraes, **Avelino José Bittencourt**
Federal Rural University of Rio de Janeiro, Seropedica, RJ – Brazil

The absence of an updated methodology for the *Stomoxys calcitrans* rearing difficult the establishment of the colony in laboratory. This study aimed to describe the methodology used to establish *S. calcitrans* colony in laboratory, at the Laboratory for Diptera Haematophagous Research of Rural Federal University of the Rio de Janeiro.
The flies were caught with entomological net, stored in plastic cage for transport and identification. Some modifications were realized in relation to the used materials, disposition and size of the cages, water and blood supply to the adults, as well as the medium rearing composition of immature stages. Plastic cages were adapted to the fly's behavior, favoring the daily management, as well as the obtaining of eggs. Transparent plastic cages (60 cm x 40 cm x 50 cm) were destined to the flies rearing, while others with smaller dimensions (15 cm X 15 cm X 20 cm) were used in the transport or employed to adult flies in the B.O.D. chamber. Six cotton strings (20 cm) were fixed on the top of the creation cages to simulate the flies rest sites in the environment. Half of the cage on the top part was covered with brown paper (80 x 30 cm) to create shaded places.
The new cages allowed transfer the great number of flies, because it made possible to connect the two cages models. Incandescent lamp of 200W was used for the temperature maintenance of the creation cages and to stimulate the oviposition. The brown paper, put in the bottom of the cage, favored the daily eggs collect and the cleaning. Humid sponge and paper towel were used to cleaning the plastic cages. The sterilization of the rearing medium reduced the proliferation of contaminants. The citrated bovine blood (0.38%) was supplied to the flies inside the cage in gauze cushion, while the storage was in plastic bags (4 cm x 20 cm). The transference of pupae for Petri plates into plastic cages contributed to the fly's emergency, and available water in Erlenmeyer with gauze avoided the adult flies' death by submersion. The creation of the stables fly should be adapted to its behaviour, in order to reduce stress and contaminants.

P2: ANTIFUNGALS FOR REARING MEDIUM OF STOMOXYS CALCITRANS (DIPTERA: MUSCIDAE)

Ana Paula Rodrigues Moraes[1], Paula Sant'ana Alves[1], Vanilla Gomes do Carmo Pena[1], Vânia Rita Elias Pinheiro Bittencourt[1], **Avelino José Bittencourt**[1]

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The rearing medium used for immature stages of *Stomoxys calcitrans* contain several nutrients that contribute to proliferation of fungus, harming the colony in laboratory. This work aimed to verify if the antifungals dodine and benomil can be used in the rearing medium of *S. calcitrans*.

Assay tubes (200 mm X 25 mm) containing rearing medium composed by sugar cane (66g), bran (25g), sodium bicarbonate (1g), distilled water (127ml) and bovine milled meat (8g) were previously autoclaved (120 ^{o}C/20 min.). The control group just contained rearing medium, while in the other group the antifungals dodine and benomil were added (0,46 g/L and 0,38 g/L of distilled water, respectively). Eighteen tubes were used for each treatment. For the fungus inoculation in rearing medium, ten *S. calcitrans* eggs were deposited in each assay tube and maintained in BOD chamber (25 ± 1 °C, 70-80% R.H.) for 15 days. The contaminant fungus were inoculated in potato dextrose agar and cultivated on glass slides for macro and microscopic characteristics evaluation. The evaluation of the assay tubes was accomplished 15 days after the experiment. For the evaluation of the larvae exposed to the antifungals, 30 *S. calcitrans* larvae were immersed two minutes in distilled sterile water added with two antifungals in the same concentration. Soon afterwards, they were transferred for Petri plate (9 mm) containing humid filter paper (0.1 ml of citrated bovine blood at 0.38% and 2 ml of fungicide solution). Same procedure was accomplished for the control group, just using distilled sterile water. The larvae counting were accomplished five days after exhibition and the data were analyzed using Qui-square Test.
In the assay tubes experiment, 88.88% of the tubes without antifungals showed *Penicillium* sp. and *Monascus* sp. fungus, they are very common in the natural environment. However, there was not fungus development in the tubes with dodine and benomil added. It was obtained 80.00% of larval viability in the group with antifungals, and 70.00% in the control group. There was no statistical difference among the treatments, indicating that *S. calcitrans* larvae are tolerant to the antifungals used. The preliminary results suggest that dodine and benomil can be used to avoid the proliferation of contaminants fungus in the rearing medium of *S. calcitrans* immature stages.

P3: METARHIZIUM ANISOPLIAE FOR BIOLOGICAL CONTROL OF STOMOXYS CALCITRANS

Ana Paula Rodrigues Moraes[1], Isabele da Costa Angelo[1], Everton Kort Kamp Fernandes[2], Vânia Rita Elias Pinheiro Bittencourt[1], **Avelino José Bittencourt**[1]

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Stomoxys calcitrans, a hematophagous dipterous, cause stress and may transmit several pathogenic agents to parasitized animals, resulting in severe losses to cattle production. The resistance of *S. calcitrans* to chemical products has increased in the last years. Accordingly, microorganisms have been evaluated as biological control agents of *S. calcitrans* populations, reducing economic losses.
Larvae of the fly were immersed in 0.01% Tween 80 aqueous conidial suspension of *Metarhizium anisopliae* at 2×10^8, 10^7, 10^6 and 10^5 conidia ml^{-1}. One control group was immersed in 0.01% Tween 80 solution (with no conidia), while another control group was not treated.
We observed 45.90%, 34.43%, 44.26%, 39.35%, 34.43% and 27.87% mortality, respectively. Chi-square test was used to analyze the results. Dead larvae and pupae were transferred to humid chamber to check fungal growth. No significant statistical difference was observed among the groups; however, some dead larvae and pupae from groups treated with 10^7 or 10^8 conidia ml^{-1} were mummified or deformed. Also, in those groups, we observed disorders of pupal formation or flies that did not emerged completely. Fungal growth was observed over the larvae and pupae held in humid chamber. The results suggests that stable fly, *S. calcitrans*, is susceptible to the fungus *M. anisopliae*, isolate ESALQ 959; however, this fungal isolate do not demonstrate efficacy to control *S calcitrans* population. In contrast, *M. anisopliae* has potential to control populations of other fly species. Previous studies reported that *M. anisopliae* efficaciously penetrates the cuticle of *Musca domestica*, colonize internal organs, and externalize hyphae over the surface of larvae and pupae. Further studies shall evaluate the efficacy of other fungal isolates to control immature stages of *S. calcitrans*.

P4: HEMATOLOGICAL CHANGES IN RABBITS ASSOCIATED WITH *STOMOXYS CALCITRANS* EXPOSURE.

Walker Nunes Chagas[1], Ana Paula Rodrigues Moraes[2], **Avelino José Bittencourt**[2]

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The experiment aimed to evaluate haematology changes in rabbits caused by different levels of *Stomoxys calcitrans* infestation. The experiment was conducted at the Technical College of Federal Rural University of Rio de Janeiro, at Seropédica county, Rio de Janeiro – Brazil. Thirty male New Zealand White rabbits, with 96 to 105 days of age were divided into five groups, containing six animals each. The rabbits were exposed to *S. calcitrans* bites in one ear, with three, seven and 14 flies, respectively (Group I, II and III). Another group was submitted to repeated bites with 14 flies, 15 days after first exposure (Group IV), and the fifth group was not submitted to bites by *S. calcitrans* (control). Forty-eight hours after exposure, blood samples were collected for hematology.

The infested animals showed reduction of lymphocites and eosinophyls, however this reduction could not be related with the stress of the *S. calcitrans* bites, because the animals where submit to anaesthesia during the blood samples collected. The increase of neutrophyls and monocytes can be related with specific inflamatory response due flies bites. The bites and the intensity of *S. calcitrans* infestation caused leukometry changes in rabbits. The animals submitted to re-exposure (group IV) showed reduction of leukocytes and other leukometry changes had compatible to chronical responses.

P5: HISTOPATHOLOGICAL LESIONS CAUSED BY *STOMOXYS CALCITRANS* (LINNAEUS, 1758) ON RABBIT SKIN.

Walker Nunes Chagas[1], Ana Paula Rodrigues Moraes[2], **Avelino José Bittencourt**[2]

[1]Plínio Leite University, Itaboraí, RJ – Brazil, [2]Federal Rural University of Rio de Janeiro, Seropedica, RJ – Brazil

Histopathological alterations caused by *S. calcitrans* bites on rabbit skin were evaluated regarding different intensity of infestation and reinfestation. Rabbits were divided into five groups of six animals each. Each animal had one of the ears exposed to bites of three (group I), seven (group II), 14 (group III) flies, or submitted to reinfestation with 14 flies 15 days after the first exposure (group IV). A control group (group V) was exposed to 7 flies with extirpated buccal apparatus. After 48 hours, skin biopsies were collected from the rabbits and sent for histopathological evaluation.

Groups I, II and III presented compact orthokeratotic hyperkeratosis (83.33%). Acanthosis and serocellular crusts were observed in groups II and IV. All bite-exposed groups presented interstitial edema, associated with lymphangiectasis, differing from the control. Discrete edema was observed in 50, 50 and 83.33% of rabbits from groups I, II and III, respectively. Groups III and IV had moderate edema (16.67% and 33.33%). Lymphangiectasis was predominant on group IV, while no difference was shown among groups I, II and III. All bite-exposed groups presented discrete hemorrhage, differing from the control. Exclusively groups II and IV presented moderate hemorrhage. Discrete congestion was diagnosed in 88.33, 88.33, 100, and 33.33% of groups I, III, IV, and V, respectively. 16.67% of the rabbits from groups II and III presented moderate congestion. No inflammatory infiltrate was observed on group V. The predominance of discrete polymorph infiltrate was observed on group I (66.67%). No difference was demonstrated between groups II and IV. Moderate polymorph infiltrate was observed on only 16.67% of rabbits from group III. No degranulated or collagenolysis-associated eosinophils were observed. Group I presented 66.67% of discrete mononuclear infiltrate. Moderate mononuclear infiltrate was verified on groups II, III and IV, predominating on group III. In contrast, severe mononuclear infiltrate was observed only on group IV (16.67%). Histiocytes were predominant, while no mastocytes were observed.

Histopathological findings indicated that the infestations determined the formation and development of perivascular and interstitial dermatites, thus being compatible with hypersensibility type-1 reaction. Additionally, was observed the existence of an evident correlation between the intensities of histopathological alterations and *S. calcitrans* infestation.

TICKS AND TICK-BORNE DISEASES

P6: FAUNA, SEASONAL ACTIVITY AND PREVALENCE OF TICKS WITHIN VARIOUS PHYTOPHYSIOGNOMIES IN A SAVANNAH RESERVE IN UBERLÂNDIA, MINAS GERAIS, BRAZIL

Viviane A.Veronez[1], Beatriz Z. Freitas[2], Maria Marlene M. Olegário[2] William M. Carvalho[2], Graziela V. T. Pascoli[2], Khelma Thorga[2], Marcos V. Garcia[1], **Matias P. J. Szabó[2]**

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Brazil's tropical savannah, the Cerrado biome covers about 2 million km^2 and is considered a biodiversity hot spot. Information about ticks from the Cerrado biome is overall scarce. In this report species, seasonal distribution and relative distribution within the various Cerrado phytophysiognomies of free-living ticks from a reserve in Uberlândia municipality, Minas Gerais, Brazil is presented. Overall 2694 free living ticks were found during a two years sampling period with CO_2 traps and cloth dragging. From these 73.5% were *Amblyomma cajennense* and 0.6% *A.dubitatum*. All other ticks were retained as *Amblyomma* spp. (25.9%). Adults of *A. cajennense* peaked in spring whereas nymphs in winter of both years. *Amblyomma* larva clusters were found in autumn and winter. Most of adult ticks (46.7%) and nymphs (39.5%) were found in woodlands whereas most larva clusters were found in valley-side marshes (39%). *Anocentor nitens*, *Boophilus microplus* and *Rhipicephalus sanguineus* ticks were found on domestic animals from neighboring properties. Search for *Rickettsia* in the hemolymph of 497 *A. cajennense* and one *A. dubitatum* ticks yielded negative results. Results confirmed earlier reports on the overwhelming prevalence of *A. cajennense* ticks in the Cerrado biome of Brazil and add information to habitat preferences of this tick species, a major vector of the Brazilian spotted fever.
Financial support: FAPEMIG, CNPq and CAPES

P7: TICKS (ACARI: IXODIDAE) ON DOGS FROM UBERLÂNDIA, MINAS GERAIS, BRAZIL

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Uberlândia in Minas Gerais State, southeastern Brazil, has 622,000 inhabitants and is located in the Cerrado biome, the South American savannah. The municipality has over 4,000 km^2 whereas the urban area covers, approximately, 135 km². The city dog population is estimated at 82,000 and up to our knowledge identification of tick species and infestation prevalence has not been determined. A major infectious disease of dogs in the city, canine ehrlichiosis, is transmitted by *Rhipicephalus sanguineus* ticks. At the same time autochthonous leishmaniosis has been recently described in the city and a role for dog ticks in the disease transmission has been supposed in Brazil. In this work we present general information on dog ticks in Uberlândia municipality and region. Dogs from 33 farms and 31 districts were examined for ticks from July 2007 to February 2009. Most of the urban dogs were examined during the

rabies vaccination campaigns in July and August 2007. Whenever possible all ticks were collected, stored in alcohol and identified in the Laboratory. On the whole 413 dogs were examined, 311 (75.66 %) from the city and 102 (24.81 %) from rural area. Overall infestation rate of dogs from Uberlândia was of 37.46% and the mean infestation intensity of 3.25 parasites per dog. In the urban area 100 dogs (32.15%) had ticks whereas 54 dogs (52.94%) from rural areas were infested. Four tick species were found *Rhipicephalus sanguineus, Amblyomma cajennense, Amblyomma ovale and Boophilus microplus.* In the city only *R. sanguineus* was found on dogs and *R. sanguineus* and *A. cajennense* were the main dog ticks in rural areas. Ongoing research is looking for tick-borne disease agents in these dogs.
Financial support: CNPq

P8: STATIC AND DYNAMIC SYSTEMS IN *RICKETTSIA SLOVACA* LIFE CYCLE EVALUATED BY QUANTITATIVE REAL TIME PCR

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Ticks transmit many different pathogens to animals, humans and their pets. Tick-borne pathogens and diseases caused by them vary geographically and are determined by the climate, environment and the presence of their reservoirs, hosts and vectors. Spotted fever group (SFG) rickettsiae, with considerable variation in vertebrate host pathogenicity, are spread by ticks. *Rickettsia slovaca*, as a member of SFG rickettsiae is an agent of human disease called TIBOLA/DEBONEL with occurrence from Mediterranean to central Europe transmitted by *Dermacentor reticulatus* and *D. marginatus* (Acari: Ixodidae).
In this study, quantitative real time PCR was used to characterize the growth of *Rickettsia slovaca*, strain B in static and dynamic cultivation systems. Eukaryotic host cell lines L929 and Vero represented static cultivation, where the growth medium was not replaced and cell cultures were daily examined during the 14 days. *D. marginatus* and *Ixodes ricinus* ticks presented dynamic cultivation. Ticks were infected by capillary feeding and tested every three days during 36 days.
Curves of bacterial growth in static cultivations had exponential, stationary and death phases, whereas in dynamic systems the stationary phase was absent and the lag phase was observed. The highest point of multiplication of *R. slovaca* was recorded on the 4th day post infection and the rickettsial DNA copy numbers at this point was 21 times in L929 cells and 27 times in Vero cells greater than the rickettsial DNA copy number of the inoculum. In dynamic system, the highest point of multiplication was on 21th and 12th day after feeding of ticks and rickettsial DNA copy numbers were 7,482 times and 865 times greater than the inoculum in *D. marginatus* and *I. ricinus*, respectively.
Life cycle of *R. slovaca* in L929 and Vero cell lines was shorter; supposedly, bacteria destroyed eukaryotic cells and ticks were considered a more appropriate environment. Our results confirm the important role of ticks in viability of *R. slovaca* and the competence of *D. marginatus* as a vector for *R. slovaca*.
The study was financially supported by VEGA grant No. 2/0065.

P9: IDENTIFICATION OF NOVEL *THEILERIA* GENOTYPES OF THE AFRICA BUFFALO (*SYNCERUS CAFFER*) BY MEANS OF THE REVERSE LINE BLOT (RLB) HYBRIDIZATION ASSAY AND 18S RDNA SEQUENCE ANALYSIS

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The African buffalo (*Syncerus caffer*) is the natural reservoir host of both pathogenic and non-pathogenic *Theileria* species. Corridor disease, caused by *Theileria parva,* is a controlled disease in South Africa.

Theileria parasites usually occur as mixed infections in infected animals, and although the non-pathogenic forms do not have any significant economic importance, their presence interferes with the diagnosis of *T. parva*. In this study, the phylogenetic relationships of pathogenic and non-pathogenic *Theileria* species obtained from buffalo blood samples originating from different geographical regions in South Africa were investigated using 18S rRNA gene sequence analysis. DNA was extracted from buffalo blood; the V4 hypervariable region of the parasite 18S rRNA gene was amplified and subjected to the Reverse Line Blot (RLB) hybridization assay using *Babesia* and *Theileria* genus- and species-specific probes. Results of the RLB revealed the presence of the pathogenic *T. parva*, benign *T. mutans*, and the non-pathogenic *T. velifera*, *T. buffeli* and *Theileria* sp. (buffalo). In some samples, the PCR products hybridized only with the genus-specific probes, and not with any of the species-specific probes, suggesting the presence of novel species or genotypes. The full length 18S rRNA gene of selected samples was amplified, cloned and the recombinants sequenced. Sequence and phylogenetic analyses indicated that novel *T. mutans*, *T. velifera* and *Theileria* sp. (buffalo) genotypes occur in buffalo. This could have serious implications, since such sequence variants could compromise the specificity of the real-time PCR test currently used to detect *T. parva* infections in buffalo and cattle in South Africa.
(Acknowledgements: This research was supported by South African National Research Foundation (NRF ICD2006072000009) and UP Research Development Programme grants awarded to Dr Marinda C Oosthuizen. This work also falls under the Belgian Directorate General for Development Co-operation Framework agreement ITM/DGCD. We thank Dr Roy Bengis, Dr Dave Cooper and Dr Fred Potgieter for providing buffalo blood samples.)

P11: *THEILERIA* AND *BABESIA* PARASITES IN TICKS IN TUNISIA

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Theileria and *Babesia* species are tick-borne intracellular protozoan parasites which affect domestic and wild animals. In North Africa, five species affecting cattle have been described: *T. annulata*, transmitted by the Ixodid tick, *Hyalomma detritum*; *T. buffeli*/*orientalis*, whose vector is not yet known; the causative agents of bovine babesiosis, *Babesia* bovis and *B. bigemina*, which are both transmitted by *Boophilus annulatus* and *Rhipicephalus bursa*; and *B. divergens*, transmitted by *Ixodes ricinus*. Unfortunately, *Theileria* and *Babesia* species infecting ticks in Tunisia has not been extensively investigated. In this study, we detect piroplasm infections in ticks removed from cattle in three different regions based on reverse line blot hybridisation.
A total of 576 unfed adult tick samples removed from cattle, between march and December 2006, belonging to six Tunisian sites located in three bioclimatic zones, were used. *Theileria* and *Babesia* DNA were detected using species-specific probes. Randomly chosen PCR products were then purified and sequenced.
Seven Ixodid tick species (*Boophilus annulatus*, *Ixodes ricinus*, *Hyalomma marginatum*, *Hyalomma excavatum*, *Hyalomma detritum*, *Haemaphysalis punctata* and *Haemaphysalis sulcata*) were collected from examined cattle. A polymerase chain reaction with hybridisation detected *Theileria annulata*, *T. buffeli*, *Babesia bovis*, *B. ovis*, *B. motasi, B. major* and *B. bigemina* in ticks accounting for 22.7% of positive samples. Sample prevalence of single infections was 17% (n=98), while mixed infections were detected in 33 samples (5.7%), accounting for 14 different combinations of species. We confirm by sequencing the naturally infection of *R. bursa* tick by *B. bigemina* and *B. ovis*.
This work is the first report of naturally infected ticks, removed from cattle, by *Theileria* spp. and *Babesia* spp.

P12: INNATE IMMUNITY IN WOOLESS LAMB TO LARVAE OF *AMBLYOMMA CAJENNENSE* TICK (FABRICIUS, 1787)(ACARI:IXODIDAE)

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The Cayenne tick *Amblyomma cajennense* is widely distributed throughout the American continent and of human health importance as the main vector of *Rickettsia rickettsii*, the Brazilian spotted fever pathogen. It infests preferably horses in its adult form but other mammal species in its immature stages. As wooless lambs often raise on pasture together with horses, it was aimed to investigate their possible acquisition of resistance to *A. cajennense* after successive infestations.
Seven naïve wooless lambs (2 males and 5 females), 3 months old, were infested thrice at 60 days interval with 130 larvae, 100 nymphs and 20 adults (10 males + 10 females) of *A. cajennense* in the first infestation and 10 adults (5 males + 5 females) in the reinfestations. The ticks were from a colony maintained under controlled conditions (28°C, 80% humidity and 12:12 photoperiod) on a BOD incubator at the Laboratory of Immunopathology at the São Paulo State University, Jaboticabal, Brazil. The ticks were released in separate within three feeding chambers glued on the shaved flank of the lambs being determined the following biological parameters: engorgement weight and period, % recovery or death, hatch period and % and egg mass weight. Biopsies of the tick bite lesion were processed according to routine histology to investigate the inflammatory cell influx.
Nearly 100% of larvae died in all infestations, while nymphs and adults fed normally during the re-infestations when compared with the first infestation. Microscopic features of adult tick bite lesions revealed predominance of neutrophils (38%) in the first infestation and of eosinophils (36.8%) in the second one, when the number of neutrophils dropped to 4.4%. In the third infestation it was found 43.6% of mononuclear cells and about 31% of eosinophils. On the other hand, nymph bite lesions revealed in all three infestations a great number of eosinophils, increasing from 36% in the first infestation to 50.5% in the third one.
It was concluded that wooless lambs present remarkable innate resistance against larvae of *Amblyomma cajennense*, but marked susceptibility to the other tick instars despite the great number of inflammatory cells influx.
Supported by the Brazilian agencies FAPESP (Grants 07/57587-2 and 08/00732-3) and CNPQ (Grants 300772/06-8 and 500329/08-7).

P13: LOCALIZATION OF ANTIGENIC SITES IN UNFED NYMPHS OF *AMBLYOMMA TRISTE* KOCH 1844 (ACARI: IXODIDAE) TICKS BY IMMUNOHISTOCHEMISTRY

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Amblyomma ticks are well distributed in South America with over 50 species being 21 including *A. triste* collected from humans. This tick is one of the most widespread *Amblyomma* species from Argentina to Mexico and vector of *Rickettsia rickettsii* to man in South America, the biopathogen of the Brazilian spotted fever. Free-living specimens were recently collected on a lea alongside Parana River, Brazil, a natural habitat of the cavy *Cavia aperea.* In the present study we investigated the ability of sera collected from guinea pigs after three infestations with *A. triste* nymphs to identify through immunohistochemistry potential target cells and tissues on histological sections of the same tick species.
Six guinea pigs were infested three times, at 30 days interval, with 30 nymphs of *A. triste* per host in each infestation. The ticks were released inside a feeding chamber fixed with a non toxic glue to the shaved

back of the hosts. Guinea pig blood samples were collected 15 days after each infestation for serum separation; normal serum was obtained before the first infestation as control. Unfed *A. triste* nymphs histological sections 5 μm thick were firstly incubated with either guinea pigs normal or hyperimune serum (primary antibody) diluted 1:20 in normal serum from species used to produce the conjugate, and secondly with the conjugate (goat IgG-alkaline phosphatase-APase) (secondary antibody) diluted 1:20 in TBS. APase activity was evidenced as a pink to reddish colour on tick cells and tissues, recorded respectively as weak and strong labelling.
It was not observed APase activity at all on unfed *A. triste* nymphs incubated with guinea pigs naive serum. On the other hand, a weak to moderate APase activity was observed in cells of salivary gland types II and III acini, midgut and hemolymph of unfed nymphs incubated with serum from three infested guinea pigs.
In conclusion, the immunohistochemistry revealed probable antigenic sites on some cells and tissues of unfed *A. triste* nymphs. It should be stressed however that other cells/tissues were not marked probably because they are not normally presented to the host in significative amount in natural infestations.
Financial support by the Brazilian agencies FAPESP (Proc. nº 05/57672-4) and CNPq (Proc. nº 300772/06-8)

P14: EXPERIMENTAL USE OF THE ATTENUATED *EHRLICHIA RUMINANTIUM* (WELGEVONDEN) VACCINE IN FRIESIAN CATTLE – A PILOT STUDY

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Heartwater is an infectious, non-contagious, tick-borne disease, caused by the intracellular rickettsial organism *Ehrlichia ruminantium*. The only commercially available immunization procedure involves infecting animals with cryopreserved sheep blood containing virulent *E. ruminantium* organisms, followed by chemotherapeutic treatment when fever develops. An attenuated *E. ruminatium* (Welgevonden) stock provides protection against a virulent homologous needle challenge in Merino sheep, Boer goats and partial protection in Angora goats. In this study, two groups, each of 3 Friesian cattle, were inoculated i.v. with attenuated organisms, 1.7×10^5 or 2.2×10^6 per animal. Vaccination did not produce clinical disease in any of the animals and no rise in body temperature was observed. Upon challenge with a virulent *E. ruminantium* stock (Gardel) (1×10^5 life organisms per dose) all but one animal were fully protected. One of the two challenge control animals became clinically sick and was successfully treated with oxytetracycline, and although the other challenge control only showed a slight temperature reaction for two days, it died due to heartwater 21 days after challenge, which was confirmed by histopatholgy.

P15: SEROPREVALENCE OF *COXIELLA BURNETII* IN DOMESTIC RUMINANTS IN GRAN CANARIA ISLAND, SPAIN.

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Coxiella burnetii, is the causative agent of Q fever, a zoonosis with worldwide occurrence. The reservoir of this bacterium is extensive and includes arthropods (mainly ticks) as well as many wild and domestic mammals and birds. In the Canary Islands, the overall seroprevalence in humans has been estimated to be

21.5%. Gran Canaria island concentrates the highest ruminant population in the archipelago and the prevalence of the human infection is estimated to be 23.5%. Thus, a cross-sectional study was conducted in a statistically representative domestic ruminant population in Gran Canara to evaluate the seroprevalence and the affected areas in the island. A total of 1.249 ruminants were randomized selected for this study, including 733 goats, 369 sheep and 147 cattle. An indirect ELISA Kit was used (Ruminants Serum Q fever. LSI, Lissieu-France) and the procedures were performed according to the manufacturer's recommendations. Results were expressed as a percentage of the optical density reading of the test sample calculated as %OD= 100 x (S-N)/(P-N) where S, N and P are the OD values of the test sample, the negative and positive controls , respectively. Sera was considered to be positive if %OD >40. Results showed seroprevalences of 60.4%, 31.7% and 12.2% in goats, sheep and cattle respectively. Based on these results, Q fever could be considered as endemic in Gran Canaria island. Distribution of the disease by areas and animal species are discussed. Sanitary measures should be taken at farm level in order to minimize the risk of exposure of *Coxiella burnetii* to humans
Study supported by INIA, project reference: FAU2006-00002-C04-00.

P16: QUALITATIVE AND QUANTITATIVE ANALYSES OF TWO DIFFERENT METHODS FOR COLLECTING HEMOLYMPH FROM *RHIPICEPHALUS* (*BOOPHILUS*) *MICROPLUS* TICK

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The current study investigates two different methods for collecting hemolymph from *R.* (*B.*) *microplus* tick. Sixty engorged females were divided in two groups of thirty ticks. In the first group, hemolymph samples were collected from a restricted cut on the cuticle, in the posterior dorsal region of the tick, performed with an insulin needle. Applying gentle pressure on the tick's body, the hemolymph was drawn into a capillary tube. In the second group, hemolymph samples were collected by cutting off the first leg at the coxal-trochanteral joint. The hemolymph samples were immediately placed in a tube containing 30 µl protease inhibitors cocktail and 82 µl saline buffer (1.5M NaCl, 50mM EDTA, phenylthiourea). The samples were centrifuged at 5040×g for ten minutes; the supernatant (plasma portion) was retained. The hemocytes were briefly re-suspended in 100 µl phosphate buffer. Both hemocytes suspension and plasma were stored at –80°C. Sixty-eight-µl hemolymph was obtained by the needle-perforated cuticle method (first group), while 8µl hemolymph was obtained by the leg-cutting method (second group). The protein amount in hemolymph samples was determined using a modified Lowry method. Samples were analyzed by spectrophotometer at 660 nm using bovine serum albumin as a standard. The protein content in cell-free hemolymph obtained from the first and second group was 15.34 µg/µl and 10.27 µg/µl, respectively. The hemocytes protein content from the first group was 1.70 µg/µl, while in the second group was 6.01 µg/µl. The thin-layer chromatography presented variations in the content of neutral lipids between the two collection methods analyzed. The cell-free hemolymph obtained by the leg-cutting method showed triacylglycerol and fatty acid reductions in relation to the other method. On the other hand, the hemolymph collected by needle-perforated cuticle presented a reduction in the amount of cholesterol ester in comparison with second method. Furthermore, the hemolymph obtained from the first group had a darker color in comparison with hemolymph collected from the second one. In conclusion, the methodology applied to the first group is more appropriate to studies that require a large volume of hemolymph samples. The higher hemocytes protein content from the second group suggests a higher number of cells; however, this will be confirmed in further studies.

P17: EVALUATION OF THE EFFECTS OF DESTRUXIN A TO RHIPICEFALUS (BOOPHILUS) MICROPLUS ENGORGED FEMALES

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Destruxins are toxic secondary metabolites produced by entomopathogenic fungi, and characterized as insecticidal compounds. This group of cyclic depsipeptides consists of five amino acids and an α-hydroxy acid. The affirmative that destruxin production increases fungal pathogenicity is not totally understood. Studies have reported that destruxins may be involved with insect-cuticle dissolution, suppression of immune system and interference in host ions channels and other cell dysfunctions. This study evaluates the effects of destruxin A to Rhipicephalus (Boophilus) microplus engorged females. Bioassays were composed of eleven treatment groups, each one with eight repetitions. In four treatment groups, ticks were immersed in destruxin solution at 5, 10 and 20 ppm for five minutes. Ticks from the control group were immersed in distilled water for the same period of time. The other seven groups were inoculated with destruxin solution. Inoculation was conducted at the insertion of the fourth leg. The inoculum was individually calculated based on the weight of each female at 0.075, 0.15 and 0.3 µg of destruxin per gram of tick. Three control groups were inoculated with physiological solution at the same dose. Another control group was composed of engorged females injured by a needle, with no inoculation. After treatments, ticks were incubated at 27 ± 1°C and RH ≥ 80%. The main studied parameters were: egg production index, percentage of hatch, nutrient index and percentage of control. The highest value of control was 20.71%, observed in the group immersed in destruxin solution at 20 ppm. The results suggest that destruxin A did not significantly affect biological parameters of R. (B.) microplus engorged females. In conclusion, the production of destruxin A possibly does not determine the virulence level of entomopathogenic fungal strains.

P18: INVESTIGATION ON LIPIDS PRESENCE IN HEMOLYMPH OF *RHIPICEPHALUS* (*BOOPHILUS*) *MICROPLUS* INFECTED WITH FUNGI

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The current study investigates the lipidic profile of hemolymph from *Rhipicephalus* (*Boophilus*) *microplus* engorged females infected with entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana*, or exposed to the non-entomopathogenic fungi *Fusarium oxysporum*. The fungal isolates were cultured on PDA at 25 ± 1°C and UR ≥ 80% for 15 days. After this period, conidia were harvested from the medium surface and suspended in sterile distilled water plus 0.1% Tween 80. Conidial suspension was adjusted to 10^8 conidia ml^{-1}. Ticks were immersed in fungal conidial suspension for three minutes, or inoculated with 5 µl conidial suspension at 10^8 conidia ml^{-1}. Inoculation were performed on the foramen localized between basis capituli and scutum. Three groups of 90 ticks each were immersed in different conidial suspension, while a control group was immersed in a control solution (0.1% Tween 80, with no conidia). Also, three groups of 90 ticks each were inoculated with conidial suspension; in one control group, ticks were injured by a needle (with no inoculation), while in another control group, ticks were inoculated with control solution. Hemolymph was collected from the dorsal surface of engorged females 24 and 48 hours after treatment. Hemolymph samples were stored at -70ºC with 30µl protease inhibitors cocktail and 82µl saline buffer (1.5M NaCl, 50mM EDTA, phenylthiourea). Hemolymph samples were centrifuged at 5040×g for 10 minutes. The concentration of plasma proteins was determined using the Bradford method. Lipids were extracted using the Bligh and Dyer method, and analyzed by thin-layer chromatography (TLC). The results showed that 24 hours after treatment, an increased cholesterol ester amount was

registered in hemolymph of ticks immersed in *B. bassiana* and *F. oxysporum* conidial suspension in comparison with the control group. In addition, 48 hours after treatment, hemolymph of ticks immersed in *B. bassiana* conidial suspension had a higher cholesterol ester concentration in comparison with 24 hours. The concentration of cholesterol ester was not altered after 24 or 48 hours in hemolymph from ticks immersed in *F. oxysporum* suspension. No difference in lipidic profile was observed among the groups inoculated with conidial suspension. Possibly, after inoculation of conidial suspension in hemolymph, the tick metabolism response was similar in the three treatment groups. In contrast, difference among treatment groups was detected when ticks were immersed in conidial suspension. Further studies will confirm these results in a near future.

P19: *LECANICILLIUM LECANII* CONIDIAL OIL-BASED SUSPENSION FOR *RHIPICEPHALUS* (*BOOPHILUS*) *MICROPLUS* BIOCONTROL

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This study evaluates the *in vitro* effects of *Lecanicillium lecanii* water- or oil-based conidial suspensions to engorged female, egg and larva of *Rhipicephalus* (*Boophilus*) *microplus* tick. The entomopathogenic fungus *L. lecanii*, isolate CG 420, was cultivated on Malt Extract Medium at 25 ± 1ºC and relative humidity ≥ 80% for 15 days. Afterwards, conidia were harvested, suspended in 0.1% Tween 80 aqueous solution and quantified in hemacytometer. Also, *L. lecanii* conidia were suspended in oil solution containing 84% sterile distilled water, 15% sterile mineral oil and 1% Tween 80. The specimens were immersed in 1 ml aqueous conidial suspensions at 10^5, 10^6, 10^7 or 10^8 conidia ml^{-1}. Also, specimens were immersed in oil-based conidial suspensions at 10^8 conidia ml^{-1}. The control groups were treated with aqueous or oleaginous solutions with no conidia. To test aqueous conidial suspensions, bioassays were conducted with 10 repetitions (specimens) per group, whereas 30 repetitions were used to test the oleaginous conidial suspension. The two bioassays were repeated twice in different days with different batches of conidia. After treatment, biological parameters of engorged females were evaluated, while eggs and larvae were evaluated considering hatch and mortality rates. The results indicate that *L. lecanii* is a potential microbial agent to control engorged females, eggs and larvae of *R.* (*B.*) *microplus*. Better results were observed when an oil-based conidial suspension was used. As far as we know, this is the first report of *L. lecanii* effects to *R.* (*B.*) *microplus* tick.

P20: PROTEIN PROFILE OF HEMOLYMPH OF *RHIPICEPHALUS (BOOPHILUS) MICROPLUS* INFECTED WITH FUNGI

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The current study evaluates the protein profile of hemolymph of *Rhipicephalus* (*Boophilus*) *microplus* engorged females infected with entomopathogenic fungi (*Metarhizium anisopliae* and *Beauveria bassiana*) and a non-entomopathogenic fungus (*Fusarium oxysporum*). Ticks were immersed in fungal conidial suspension for three minutes, or inoculated with 5 µl conidial suspension at 10^8 conidia ml. Inoculation were performed on the foramen localized between basis capituli and scutum. Three groups of 90 ticks each were immersed in different fungal conidial suspension, while a control group was immersed in a control solution (0.1% Tween 80, with no conidia). Also, three groups of 90 ticks each were inoculated with conidial suspension; in one control group, ticks were injured by a needle (with no inoculation), while in another control group, ticks were inoculated with control solution. The fungal

isolates were cultured on PDA at 25 ± 1°C and UR ≥ 80%. Hemolymph was collected from the dorsal surface of engorged females 24 and 48 hours after treatment. Hemolymph samples were stored at -70ºC with 30µl protease inhibitors cocktail and 82µl saline buffer (1.5M NaCl, 50mM EDTA, phenylthiourea). Hemolymph was centrifuged at 5040×□ for 10 minutes. The protein amount was determined using the Bradford method and a Coomassie blue-stained SDS polyacrylamide gel electrophoresis (SDS-PAGE) analysis. The results showed that 24 hours after treatment, 20% reduction of total protein was observed in hemolymph of ticks immersed in entomopathogenic fungi conidial suspension; in contrast, ticks immersed in *F. oxysporum* conidial suspension increased 3% the amount of total protein in hemolymph. Moreover, 48 hours after treatment, entomopathogenic fungi reduced 23% the amount of total protein, whereas *F. oxysporum* reduced 26%. Forty eight hours after treatment, all groups inoculated with fungal conidial suspension increased the amount of total protein in tick's hemolymph, varying from 10 to 50%; an exception was the group treated with *B. bassiana*, in which reduced 30% the amount of total protein. No significant difference was observed on electrophoretic profile of hemolymph samples. Hemolymph collected form ticks immersed in *M. anisopliae* or *B. bassiana* conidial suspension were examined by HPLC. Accordingly, a considerable peak was observed at 45 minutes. This peak was not observed in hemolymph samples from non-treated ticks (control group). Albumin peak was observed at 28.2 minutes. Further studies will determine the composition of the HPLC peak at 45 minutes.

P21: SUSCEPTIBILITY OF UNFED LARVAE OF RHIPICEPHALUS *(*BOOPHILUS*)* MICROPLUS *TO THE FUNGI* BEAUVERIA BASSIANA *AND* METARHIZIUM ANISOPLIAE

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Rhipicephalus (*B.*) *microplus* causes severe economic losses to the Brazilian cattle-raising industry. Currently, the control of this tick species is mainly based on the use of chemical acaricides. Alternative methods to chemical acaricides add efforts for controlling *R.* (*B.*) *microplus* populations as an integrated management. The use of entomopathogenic fungi has been studied to reduce tick populations; however, there is a speculation regarding the variation in susceptibility of different populations of *R.* (*B.*) *microplus* to entomopathogenic fungi. The current study investigates the *in vitro* effect of *Metarhizium anisopliae*, isolate Ma 959, and *B. bassiana*, isolate Bb 986, to *R.* (*B.*) *microplus* unfed larvae. Ticks were obtained from populations originated from two different rural properties. The fungal isolates were cultivated on PDA at 25 ± 1°C and relative humidity (RH) ≥ 80% for 15 days. Conidia were harvested from Petri plates and suspended in distilled water plus 0.1% Tween 80. Conidial suspensions were quantified in hemacytometer and adjusted to 10^8 or 10^7 conidia ml^{-1}. Each treatment or control group was composed of 10 test tubes with approximately 1000 larvae each. Larvae were immersed in 1ml of conidial suspension or control solution (0.1% Tween 80 with no conidia) for three minutes. Percentage mortality and median lethal time was estimated every five-day interval for 30 days. A significant percent mortality was observed in the treatment groups. In addition, significant mortality was observed between larvae originated from the two properties. Percentage mortality of larvae varied from 91.8 to 98.7%, and differed from percentage mortality of larvae originated from a distinct population, 71.0 to 94%. No mortality was observed in the control groups. The median lethal time LT_{90} was reduced in all treatment groups, varying from 19.52 to 27.51 days, whereas larva originated from a different property varied from 22.89 to 37.31 days. In conclusion, the entomopathogenic fungal isolates investigated in the current study are potential biocontrol agents for *R.* (*B.*) *microplus*. However, larva from different geographical origins may diverge in susceptibility to *B. bassiana* and *M. anisopliae* infection.

P22: ULTRASTRUCTURAL CHANGES INDUCED BY FIPRONIL ON OVARY CELLS OF SEMI-ENGORGED *RHIPICEPHALUS SANGUINEUS* (LATREILLE, 1806) (ACARI: IXODIDAE) FEMALES.

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Ticks are ectoparasites of great medical and veterinary importance. The dog tick *Rhipicephalus sanguineus* causes significant blood losses in the host and transmits several pathogens. Acaricides are still considered the most efficient method of tick control. New drugs are being used, such as fipronil, however its effects are not yet completely known. In the present study, semi-engorged adult females of *R. sanguineus* were immersed for two minutes in either 1, 5 or 10 ppm (LC_{50}) of fipronil (Frontline®) aiming to evaluate the toxic effects of the acaricide on tick ovary. Sixty semi-engorged females of *R. sanguineus* tick were distributed into the following groups of 15 specimens each: I- non-treated; II, III and IV- treated with 1, 5 and 10 ppm of fipronil**,** respectively. Tick females of the group I were immersed in distilled water as control. In the sequence, tick ovaries were removed, fixed, dehydrated, included in resin, sectioned and photographed in a transmission electron microscope (TEM). In group I (control group), the ovaries of semi-engorged *R. sanguineus* exhibited oocytes in five developmental stages: I- homogeneous cytoplasm but devoid of granules; II- fine and homogeneous granulation in the cytoplasm; III- intermediate in size with smaller yolk granules in their central region and larger ones in the periphery; IV- several yolk granules of various sizes randomly distributed throughout the cytoplasm; V- the largest cells with large yolk granules completely filling the cytoplasm, chorium very thick. The fipronil induced several structural changes in the oocytes of semi-engorged females of *R. sanguineus* in every treated group (II, III, IV), ranging from damaged cellular components such as plasmic membrane, mitochondria and proteic yolk granules (due to alteration in the protein synthesis), cellular defense mechanisms such as increase in the amount of cytoplasmic microtubules, different degree of vacuolation and myelin figures, until interruption of vitellogenesis and cell death. These data demonstrate that fipronil is an efficient acaricides agent by reducing the fertility of *R. sanguineus* females.
Supported by the Brazilian agencies FAPESP (Proc. nº 06/52599-0 and 08/59020-0) and CNPQ (Grant nº 308733/2006-1).

P23: FIPRONIL INDUCES MICROSCOPIC CHANGES ON SALIVARY GLAND CELLS OF UNFED AND SEMI-ENGORGED *RHIPICEPHALUS SANGUINEUS* (LATREILLE, 1806) (ACARI: IXODIDAE) FEMALES

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Ticks are ectoparasites of great medical and veterinary importance. The dog tick *Rhipicephalus sanguineus* causes significant blood losses in the host and transmits several pathogens. Acaricides are still considered the most efficient method of tick control and new drugs are being used, such as fipronil, however its effects are not yet completely known. The present study aimed to investigate possible effects of fipronil on the morphophysiology of salivary gland cells of unfed and semi-engorged females of *R. sanguineus.* A total of 90 ticks were divided into six groups of 15 specimens each, as follows: 1 and 2- unfed females immersed in distilled water (control) and fipronil (Frontline®, 1ppm), respectively; 3 to 6- semi-engorged females immersed in distilled water (control) and fipronil (1, 5 and 10 ppm). Fragments of tick salivary glands were fixed, dehydrated, included in Leica resin, sectioned, stained with hematoxylin and eosin according to routine histology. Other fragments were prepared for histochemical techniques

(bromophenol blue and PAS). Slides with stained sections were photographed at a MOTIC BA 300 light microscope. In the unfed female group 2, the effects of fipronil were strictly observed in salivary gland type I acinus, with increase in size and diameter of its lumen. These changes were probably associated with the excretory function, indicating that type I acinus might be responsible for eliminating this toxic substance from the system of the ectoparasite. In semi-engorged females (groups 4, 5 and 6), fipronil did not interfere in the process of cell death, however it accelerated the salivary gland degenerative processes as the extent of damage increased in a dose-response manner. These results demonstrate that fipronil may interfere with the engorgement process in tick females as revealed by the salivary gland microscopic changes with possible consequences to the reproductive process, decreasing or even halting egg laying.
Supported by the Brazilian agencies FAPESP (Proc. nº 07/58633-8 and 08/59020-0) and CNPq (Grant nº 308733/2006-1).

P24: USING *MSA-2B* AS A MOLECULAR MARKER FOR GENOTYPING MEXICAN ISOLATES OF *BABESIA BOVIS*

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To determine the sequence variation among *B. bovis* Mexican isolates using *msa-2b* as a genetic marker, PCR amplicons corresponding to *msa*-2b were cloned and plasmids carrying the corresponding inserts were purified and sequenced. Comparative analysis of nucleotide and deduced amino acid sequences revealed distinct degrees of variability and identity among the coding gene sequences obtained from 16 geographically different Mexican *B. bovis* isolates and a reference strain. Clustal-W multiple alignments of the MSA-2b deduced amino acid sequences performed with the 17 *B. bovis* Mexican isolates, revealed the identification of 3 genotypes with a distinct set each of amino acid residues present at the variable region: Genotype I represented by the MO7 strain (in vitro culture-derived from the Mexico isolate) as well as RAD, Chiapas-1, Tabasco and Veracruz-3 isolates; Genotype II, represented by the Jalisco, Mexico and Veracruz-2 isolates; and Genotype III comprising the sequences from most of the isolates studied, Tamaulipas-1, Chiapas-2, Guerrero-1, Nayarit, Quintana Roo, Nuevo Leon, Tamaulipas-2, Yucatan and Guerrero-2. Moreover, these 3 genotypes could be discriminated against each other by using a PCR-RFLP approach. The results suggest that occurrence of indels within the variable region of *msa-2b* sequences can be useful markers for identifying a particular genotype present in field populations of *B. bovis* isolated from infected cattle in Mexico.
Partially financed by: SAGARPA-CONACYT, Project CO1-2005-12023; PAIEPEME, A.C.; and INIFAP PRECI No. 6216596P.

P25: SEQUENCE CONSERVATION OF THE 12D3 GENE IN MEXICAN ISOLATES OF *BABESIA BOVIS*

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The 12D3 antigen present in *Babesia bovis* has been evaluated as a recombinant vaccine candidate and the *12d3* coding sequence has been reported for an Australian and an USA (Texas) isolates of *B. bovis*. However, no approach has been conducted to perform analysis of *12d3* sequence conservation on a larger number of *B. bovis* isolates from Mexico. This could provide important information to determine whether

a recombinant vaccine containing this antigen could be widely used. This study reports the cloning and sequencing analysis of the *12d3* coding region in 20 different *B. bovis* isolates collected from various geographical regions in the tropical and subtropics of Mexico. The general strategy utilized in the study included: Genomic DNA extraction from infected erythrocytes of the 20 isolates; PCR amplification of the 12d3 open reading frame using primers 12d3F 5'-ATGTTGGCTACACGTTTTGTTTTTTAG-3' and 12d3R 5'- AAGCTCCTGCCTTTCGCTC-3'; Cloning of the expected size amplicon (about 1040 bp) in pCR 2.1-TOPO vector and bacterial transformation in *E. coli* TOP 10 competent cells; Clone selection and recombinant plasmid purification; Sequencing of two independent clones (plus and minus strands) derived from each *B. bovis* isolate; and sequence analysis with CLC Sequence Viewer v4.6.4 and BLAST (http://www.ncbi.nlm.nih.gov/BLAST). Agarose gel electrophoresis analysis of the PCR products showed *12d3* amplicons obtained for all *B. bovis* isolates tested. Comparative analysis of the consensus nucleotide sequences obtained for each isolate revealed a high degree of conservation (94-99% sequence identity) among the *12d3* alleles present in the Mexican isolates when compared to the *12d3* ORF sequences from the Texan (T2Bo) y Australian *B. bovis* isolates. Similarly, BLASTX sequence homology search showed a high percent identity (93-99%) of the deduced amino acid 12D3 sequence as compared to the T2Bo and Australian isolates sequences. The high level of sequence conservation in *12d3* among the 20 *B. bovis* isolates collected from geographically distant locations in Mexico suggests that there exists a minimal bovine-host immunologic pressure which could be translated in antigenic diversity or variation, and most probably this is reflected in the non- inmunodominant characteristic of the 12D3 antigen as it has been previously described in the literature. Thus, the 12D3 antigen can be considered as a viable candidate for inclusion in a recombinant vaccine for cattle babesiosis caused by *B. bovis* in Mexico.
Financially supported by Projects SAGARPA-CONACyT, C01-2005-12023; INIFAP, PRECI No. 6216596P; and CONACyT SNI-Licenciatura No. 103119.

P26: TICK TRANSMISSIBILITY STUDIES OF *BABESIA BIGEMINA* AND *BABESIA BOVIS* ATTENUATED STRAINS DERIVED FROM IN VITRO CULTURE

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This work was conducted to assess transmissibility by *Boophilus microplus* ticks of attenuated *Babesia bigemina* and *Babesia bovis* strains, as well as reversion to virulence after one, two and three passages in susceptible cattle. Initially, for each strain, six non-splenectomized steers from a tick-free area were divided in two groups and used in the first part of this experiment. Up to 20,000 *B. microplus* tick larvae were deposited in the back of each bovine at different intervals. One animal from each group was infected with $1x10^9$ infected erythrocytes of the *B. bigemina*- and *B. bovis*-*in vitro* culture derived attenuated strain. Two additional steers were each similarly inoculated with $1x10^8$ infected erythrocytes of a *B. bigemina* or *B. bovis* field strain. At peak parasitemia in each inoculated steer, blood was collected and transferred to a second and third steer for each parasite population. Engorged, female *B. microplus* ticks were also collected at peak parasitemia; they were allowed to lay eggs and were incubated until larvae emerged. Additional groups of four susceptible animals for *B. bigemina* and six animals for *B. bovis,* also from a tick-free area, were used as recipients of the larval progeny obtained from the detached engorged female, and were allowed to feed on the recipient animals (three recipients for each of the bovine passages of the attenuated strains, and the remainder for the field strains). All needle passages were successful for both parasite population, and *Babesia* sp strains. *B. bigemina* and *B. bovis* field strains were detected in the hemolymph of the collected engorged female ticks; however, none was detected by the hemolymph test in samples collected from ticks fed on attenuated strain´s animals. Similarly, recipient animals that were infested with larvae obtained from female ticks that fed on the first needle-passed animals with the field strain became positive in Giemsa-stained blood smears. On the other hand, Giemsa-stained smears

from animals infested with tick larvae from engorged ticks that fed on attenuated strains-infected animals were negative, except for the recipients of the third passage´s strains. It was concluded that these attenuated strains would be considered safe for use in animals as a life immunogen, which cannot be tick-transmitted in nature, at least after two passages in cattle.
Financially supported by Projects: SAGARPA-CONACyT, C01-2005-12023; INIFAP, PRECI No. 6216596P; and PAIPEME, A.C.

P27: VALIDATION OF AN ATTENUATED LIVE VACCINE AGAINST BABESIOSIS IN NATIVE CATTLE IN AN ENDEMIC AREA

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In México bovine babesiosis still being an important difficulty for the cattle industry development; the endemic stability changes are causing babesiosis outbreaks in local cattle within endemic areas. The attenuated live vaccine originally designed for naive animals moved from free tick areas to infested areas, nowadays may perhaps be recommended also for native animals. The objective of this study was to evaluate the use of a bivalent, attenuated live vaccine (*Babesia bovis* - *Babesia bigemina*) derived from an in vitro culture, in native cattle in three commercial farms located in a tropical region in Chiapas State, Mexico. For each ranch 40 animals were selected as negative to *Babesia* spp. by using immunofluorescent antibodies test (IFAT) and PCR, and were distributed by age into four groups with 10 animals each one: I) < 9 months old, II) 9-18, III) 18-36 and IV) > 36 months old. From each group, two subgroups were formed 5 animals each; one vaccinated and one control group, without vaccination. Monitoring was carried out initially on day 0 (vaccination), day 7 and then every 4 weeks for 12 months, allowing for rectal temperature (°C), packed cell volume (Ht %) and percentage of erythrocytes parasitized (PEP), furthermore IFAT and PCR were performed. Prevalence rate at the beginning was 83% by IFAT. From 120 selected animals 26(22%) non-vaccinated showed clinical symptoms as babesiosis, confirmed by stained smears vs. only 4 (3.3%) vaccinated. All *Babesia*-affected animals were specific treatment required. Vaccinated cattle by IFAT showed titles up to 1:1840 and 1:1027 for *B. bovis* and *B. bigemina*, respectively. The specific age stratum protection will be discussed. The vaccine induced protection for 93% of the immunized animals, thus it is suggested to incorporate the bivalent (*B. bovis*-*B. bigemina*) attenuated live vaccine as part of the health programs of herds located in tropical conditions in order to reduce the number of outbreaks. This vaccine should not be just used in cattle coming from babesiosis free zones, but also in native cattle maintained in hyperendemic areas.
Financed by Fundación Produce Chiapas A.C.

P28: MULTILOCUS SEQUENCE TYPING OF *ANAPLASMA MARGINALE* ISOLATES.

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A highly reproducible and discriminative typing system is essential for better understanding of the epidemiology of *Anaplasma marginale*. Only two molecular markers have been described for *A. marginale* so far. Major surface protein 4 (MSP4) nucleotide variations provides phylogeographic resolution for *A. marginale* isolates while MSP1a variable number of tandem repeat units in the 5'-end region of the gene has been used to discriminate between genotypes. In addition, *A. marginale* has a reduced genome that render low frequency of variable number of tandem repeats after Tandem Repeat

Finder software analysis. This means that VNTR would not represent an accurate approach for *A. marginale* characterization.
The aim of this study was to develop the MLST strategy to discriminate between *A. marginale* isolates. For this purpose, 14 housekeeping candidate genes were initially amplified and sequenced. These genes were selected mainly because of their use in previous bacterial MLST studies. After testing specific amplification against *Babesia sp.* and *Bos taurus* DNA and comparing the number of polymorphic sites in each gene, we selected 8 genes for further MLST studies. These genes were: *dnaA*, *groEL*, *ftsz*, *lipA*, *sucB*, *sodB*, *recA*, *secY*. Genetic variation among these 8 genes sequences was evaluated using the recently available genomes of *A. marginale* strains (str. Mississippi, str. Puerto Rico, str. Florida, and str. Virginia) and sequences from 4 other isolates (Mercedes, Salta, Quitilipi and South Idaho). Phylogenetic analysis was conducted using the program MEGA version 3.1. Neighbor-joining trees were generated selecting Kimura-2-parameter model; confidence values were determined by bootstrap analysis with 1000 replicates. *Anaplasma phagocytophylum* was considered as outgroup. Notably, two well supported clusters were found, where Argentinean isolates cluster split from North American one. Taking into account that more isolates must be included in the MLST analysis, these results show that this strategy, in accordance to the discriminatory power described for other bacteria, seems to be a promising tool for clearly distinguish *A. marginale* isolates.

P29: *BABESIA SP.* AND *ANAPLASMA MARGINALE* CO-INFECTION APPRAISAL IN CATTLE.

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In northern Argentina, where climatic conditions support tick development, bovine babesiosis and anaplasmosis are considered an important economic constrain to livestock production. The aim of this study was to analyze *Babesia sp.* and *Anaplasma marginale* co-infection within Argentine cattle.
During 2005 to 2007, 348 blood samples were collected from 12 herds (182 samples) in the northeastern (NEA), and from 10 herds (166 samples) in the northwestern (NWA) regions of the country.
Specific detection of *B. bovis, B. bigemina.* and *A. marginale* was carried out by Reverse Line Blot Hybridization. Two hundred and forty six samples (70.69%) were positive to *A. marginale*, whereas 203 (58.33%) were positive to *Babesia sp*. The proportion of cattle co-infected by both agents (47.7%) was significantly higher (P<0.001) than the proportion of animals positive to either *Babesia sp*. (10.6%) or *A. marginale* (23.0%). Association between status of co-infection (Babesia-positive, Anaplasma-negative; Babesia-negative, Anaplasma-positive; Babesia-positive, Anaplasma-positive) and factors hypothesized to influence the risk of co-infection was explored for the 283 cattle that were positive to either or both agents. Association was explored using a multinomial multivariate regression model while accounting for the dependence associated to herd of origin. Factors supposed to influence the risk of co-infection were region of origin (NEA, NWA) and age of cattle (<12 months, >12 months). Cattle from NEA were at 10.2 (CI95%=4.02-25.97) and 2.2 (CI95%=1.2-4.1) higher risk of being infected by, respectively, *Babesia sp.* and *A. marginale* alone, compared with the baseline risk of being co-infected by both agents and located in NOA. After accounting for the effect related to region of origin, the risk of being infected with *A. marginale* alone was 5.6 higher for >12 months animals compared to the baseline risk of being co-infected by both agents and <12 months old.
These results showed that co-infection status is significantly frequent (P<0.001) among cattle in which *A. marginale* and *Babesia sp.* are prevalent, ruling out infection-exclusion phenomena. Furthermore, the

probability of co-infection is likely affected by environmental, biological and demographic factors such as region of origin and biological dynamics of infection.

P30: MODELLING THE DIFFERENT DISTRIBUTION OF *AMBLYOMMA CAJENNENSE* AND *A. TRISTE* IN SOUTH AMERICA, THE MAIN VECTORS OF SPOTTED FEVERS TO HUMANS.

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Tick-borne pathogens affecting human health are a serious threat in many regions of the World. Species of *Rickettsia* are distributed worldwide, and are transmitted by several species of ticks. These diseases are linked to different geographical regions, as well as reservoirs and environmental traits, like climate, reservoir hosts, and landscape patterns. In parts of Brazil, Argentina and Uruguay, Spotted Fever is a serious problem affecting humans, produced by both *R. ricketsii* and *R. parkeri*, and transmitted by different tick species. To characterize the areas under risk by those diseases, it is necessary to understand the factors regulating the distribution of the manin vector ticks, namely *Amblyomma cajennense* and *A. triste*.

This study is devoted to the use of several environmental data layers, providing an adequate framework to explain the different factors regulating the distribution f these two tick species, to map the most probably distribution of the ticks. Several discriminatory variables have been used, namely, a set of monthly temperature and rainfall variables, and a set of NDVI (a measure of vegetation stress) together with monthly temperature at the same resolution. Most important to this study is the use of clustering techniques to delineate different populations of the tick vectors, aimed to increase the discriminatory power of the models. Clustering of original records describes the climatic borders of the different populations of the ticks, thus providing a better understanding of regulatory variables. For both tick species, best models are obtained by the joint use of both monthly NDVI and temperature. Because the very restricted distribution of *A. triste*, clustering techniques did not improved the models. The ecological and systematic significance of the very different populations of *A. cajennense* is discussed, under the light of the significantly different regulatory variables displayed by clustering algorithms.

Parts of this research were facilitated through the Integrated Consortium on Ticks and Tick-borne Diseases (ICTTD-3), financed by the International Cooperation Program of the European Union, coordination action project No. 510561.

P31: TICKS AND TICK-BORNE ZOONOSES IN THE (SUB-)TROPICS: THE USE OF AN INTEGRATED DATABASE AS A TOOL FOR RISK ASSESSMENT.

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Tick-borne zoonoses (TBZ) pose a serious public health threat in both Southern and Northern hemispheres. Over the last decades, TBZ causing serious illnesses and deaths in humans (e.g. Crimean-Congo haemorrhagic fever and Spotted fever-SFG rickettsiosis) have been increasing.

A geographical database on TBZ in the (sub-) tropics was developed with the aim of producing updated TBZ distribution maps, constructing ecological niches models for vectors/zoonoses and drawing risk

maps. A list of TBZ pathogens relevant for the (sub-) tropics has been compiled. The database includes data on TBZ within the tropical and sub-tropical belt (35°S-35°N).
Data/records are being collected by a multidisciplinary team (veterinarians, biologists, medical doctors, etc.) through an extensive bibliographic search of scientific papers, conference proceedings, other publications on TBZ; unpublished material, reports from national/international institutions and info from relevant experts in countries of interest are also included.
In addition to data on pathogens, information on animal and human hosts and vectors are recorded. When available, information about the type of study (e.g. prevalence survey or case reporting), method of pathogen detection are also included. As spatial information is the core component of the database, each record is geo-referenced: geographical coordinates, when not provided by the authors, were extracted from web gazetteers. Accuracy of such records varies depending on the administrative level of the location cited (e.g. village or district). As coordinate precision level is a key issue for modelling purposes, the database accounts for such uncertainties. A quantitative estimate of the mean error of village-level coordinates from gazetteer was carried out by comparing some hundred records of coordinates provided by the authors to those of the same locations obtained from gazetteer, thus resulting about 10 km.
From a total of 524 relevant publications reviewed and entered in the database, 705 geo-referenced TBZ records (out of 1,446) have been extracted. About 347 records are geo-referenced at village level and 43 have coordinates provided by authors.
Thanks to the participation of key collaborators in Latin America, the richest dataset is about SFG *Rickettsia* species in the Neotropics. Ecological niche characterization of SFG outbreaks in Latin America is being finalized and can be used as model to develop preliminary risk maps for TBZ.
This research was facilitated through the Integrated Consortium on Ticks and Tick-borne Diseases (ICTTD-3), financed by the International Cooperation Program of the European Union, coordination action project No. 510561 (www.icttd.nl). Thanks to all collaborators who provided info and records.

P32: A DATA SET OF LANDSCAPE, CLIMATE AND HOST FEATURES TO MAP DISTRIBUTION OF TICK SPECIES.

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The Database (THPbase/GIS working group of the Integrated Consortium on Ticks and Tick-Borne Diseases (ICTTD-3) has been developing a large dataset of tick records throughout the Mediterranean region, Africa, and the Neotropics. The database is aimed to provide baseline data for subsequent development ecological studies on ticks and tick-borne diseases. The working group aimed to create a fully integrated framework including original data into a single product including each of the three target regions. The database includes known distribution data for the predominant tick species, livestock animal density data, monthly temperature, rainfall and NDVI data.
The total data set includes more than 50,000 tick records from the Mediterranean region, Africa and the Neotropics. Geographical coordinates have been assigned to each record with an error of approximately 5 km. Moreover, different layers of ecological information are linked to the tick records and provide users with important information regarding the relationships of various tick species with changes in their environment. The whole data set is developed around a common open format, readable by common GIS software and free packages, allowing its use under different operating systems and without the need to obtain additional software packages. The database is a useful tool for studies concerning climate change and for subsequent development of predictive distribution maps of a number of key tick species affecting human and animal health.
Acknowledgments: This research was facilitated through the Integrated Consortium on Ticks and Tick-borne Diseases (ICTTD-3), financed by the International Cooperation Program of the European Union, coordination action project No. 510561.

P33: DELINEATION OF A NEW SPECIES IN *BORRELIA BURGDORFERI* SENSU LATO COMPLEX FROM ATYPICAL AMERICAN STRAINS.

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From 14 *Borrelia* species recognized today around the world 9 were identified in and strictly associated with Eurasia (*B. afzelii*, *B. garinii*, *B. japonica*, *B. lusitaniae, B. spielmanii*, *B. sinica, B. tanukii*, *B. turdi*, *B. valaisiana*), while another 3 (*B. andersonii*, *B. californiensis* and *B. carolinensis*) were identified only in the USA. Recently published investigations confirmed that remaining two species, *B. burgdorferi* sensu stricto and *B. bissettii,* shares the distinction of being present in both the Old and the New World. In addition to the well established populations of the above named species two new North American *B. burgdorferi* sensu lato species were delineated from a group of atypical American strains and were proposed to keep as genomospecies 1 and genomospecies 2.
Analysis of a group of 118 isolates from the southeastern United States revealed the presence of well established populations of *Borrelia burgdorferi* sensu stricto, *Borrelia bissettii*, *Borrelia carolinensis* and *Borrelia* sp. nov. Seven samples representing *Borrelia* sp. nov. were isolated from nymphs of *Ixodes minor* collected from birds from South Carolina, USA. Multilocus sequence analysis of five genomic loci from seven *Borrelia* sp. nov. isolates showed that they were closely related to California strains known as genomospecies 1. One nucleotide difference in the size of 5S-23S intergenic spacer region, 1 substitution in 16S rRNA gene signature nucleotides and silent nucleotide substitutions in sequences of flagellin and p66 genes that do not affect the proteins clearly separate *Borrelia* sp. nov. isolates from South Carolina into two subgroups. The sequences of isolates of each subgroup share the same RFLP patterns of 5S-23S intergenic spacer region and contain unique signature nucleotides in the 16S rRNA gene. The analysis of *Borrelia* sp.nov. and genomospecies 1 revealed the close association between themselves regardless of which of the five loci targeted, and the distance from any other known species of *Borrelia burgdorferi* sensu lato complex. We propose that seven *Borrelia* sp. nov. isolates from South Carolina and two California isolates (CA8 and CA29) previously designated as genomospecies 1 comprise a single species. The currently recognized geographic distribution of *Borrelia* sp. nov. is South Carolina and California, all strains are associated with *Ixodes pacificus* and *Ixodes minor* and their rodent and bird hosts.
This research has been facilitated through *The Integrated Consortium on Ticks and Tick-borne Diseases* (ICTTD-3) financed by the International Cooperation Programme of the European Union through Coordination Action Project no. 510561.

P34: IMMUNE PROTEINS OF *IXODES RICINUS*.

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Castor bean/sheep tick *Ixodes ricinus* is the vector of Lyme disease and tick-borne encephalitis, two first-line arthropod-borne infectious diseases of humans and animals in Central Europe. While taking a blood meal ticks encounter a large diversity of pathogens from hosts. In the complex response of the gene expression towards the presence of the
pathogenic agent ticks produce the proteins that represent the molecular factors of innate immunity either involved in defense reactions or pathogen pattern recognition. One of the facets of the tick defense is a rapid and transient synthesis of a set of potent antibacterial peptides (AMPs) following infection or trauma. The naturally occurring AMPs form the first line of tick defense. Due to extreme diversity they are active against wide variety of pathogens. With the growing problem of pathogens that resist

conventional antibiotics, ticks are becoming fruitful sources to find novel pharmaceutical substances to treat infections.
Recently, our investigation of the genes of *I. ricinus* that are involved in mechanism of vector-pathogen interaction resulted in isolation of the genes encoding tick immune proteins involved in tick digestion, transmission of *B. burgdorferi*, antioxidant defense (ROS), pathogen recognition and defense. They include: ML-domain containing protein, allergen-like protein, two different thioredoxin peroxidase (peroxiredoxin) genes, SALP 15 gene, gene encoding tick receptor for OspA (TROSPA), two isoforms of defensin, novel gene encoding histidin-rich defense protein ricinusin and other.
The finding that *I. ricinus* has a set of defense/antimicrobial peptides, but does not capable of elimination of *B. burgdorferi* implicates other factors in *I. ricinus* antimicrobial defense. During transmission of Lyme disease spirochete, *I. ricinus* is able to eliminate about 90% of *Borrelia*, while non-vector tick *Dermacentor variabilis* eliminates spirochetes totally. Tick-pathogen compatibility/incompatibility is a major factor that influences the establishment, development, and transmission of a parasite, a factor that is inadequately understood. Vector control programs relying on the genetic manipulation of vectors require an understanding of basic physiological and biochemical processes that regulate tick-pathogen interaction, including a detailed understanding of innate defense mechanisms already used by ticks.
This research has been facilitated through *The Integrated Consortium on Ticks and Tick-borne Diseases* (ICTTD-3) financed by the International Cooperation Programme of the European Union through Coordination Action Project no. 510561.

P35: MIXED INFECTION OF BENIGN *THEILERIA* SP. IN SAMBAR DEER *(CERVUS UNICOLOR)*

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Theileriosis is a tick-borne disease caused by different species of *Theileria*. Some of the members of this group are pathogenic while others are considered to be less pathogenic or benign. Microscopic examination of a thin blood smear of a clinically healthy Sambar deer *(Cervus unicolor)* revealed the presence of small piroplasms. DNA was extracted from a blood sample; the V4 variable region of the 18S rRNA gene was amplified, subcloned and sequenced. The sequences were aligned with published sequences of related genera. Two unique sequences were found which showed the highest similarity to *Theileria* reported in roe deer *(Capreolus capreolus)* as well as red deer *(Cervus elaphus)* and sika deer *(Cervus nippon centralis)*. Phylogenetic analysis of both sequences was also confirmed that *Theileria* detected from this sambar deer comprises a clade that is clearly distinct from the clade comprised of *Theileria* from cattle and sheep. Clinical normalcy of the Sambar deer along with the findings confirms the benign state of isolate.

P36: PHYLOGENETIC ANALYSIS OF *EHRLICHIA SP.* FROM BRAZILIAN WILD FELIDS BASED ON 16S RRNA AND OMP-1 GENES

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Brazilian wild felids are currently endangered. Ehrlichiosis, an emergent tick-borne disease that affects both humans and animals, may represent a real threat to the survival and preservation of these species of animals. Few reports have studied ehrlichiosis in Brazilian felids. In this study we performed sequence alignment to establish the identity of the parasite species infecting these animals using 16S rRNA and

omp-1 genes. Each sample of extracted DNA from 78 Brazilian wild felids was used in nested PCRs assays for *Ehrlichia canis*, *E. chaffeensis*, *E. ewingii, Anaplasma phagocytophilum*, *A. platys* and *Neorickettsia risticci* using 16S rRNA gene described elsewhere. Additionally, a nested PCR for *Ehrlichia spp.* using omp-1 multigene family was used for those samples positive at 16S rRNA PCR. After sequencing of positive amplicons, the CLUSTAL W and MEGA programs were used for alignment and phylogenetic analysis (with distance Neighbor-Joining method, Kimura-2-parameter model and bootstrap test with 1000 replications), respectively. Eleven samples were positive for nested PCR *E. canis* based on 16S rRNA: 5 ocelots, 1 jaguarundi, 2 little spotted cats, 1 puma and 2 jaguars. Of these, only 4 samples were also positive to omp-1 nested PCR. After sequencing the isolates, the percentage of identity among sequences ranged between 98% and 100%. DNA sequencing using 16S rRNA gene showed that the *Ehrlichia sp.* DNA obtained from these neotropical felids are closely related (98.0% identity) to *E. canis* from dogs in Mexico, Brazil, Portugal, Thailand, and Greece, and from cats in Taiwan. The *Ehrlichia sp.* isolates from Brazilian wild felids clustered together with *E. canis* obtained from dogs and cats from Thailand, Brazil, China, and Taiwan and with *E. canis* obtained from a single individual (human) in Venezuela. As well, when omp-1 multigene family was used, the *Ehrlichia sp.* DNA from Brazilian felids were closed related (97.0% identity) to *E. canis* strain Jaboticabal, strain Jake, and *E. canis* strain São Paulo. On the other hand, when phylogenetic analysis was performed based on omp-1 sequences, the four *Ehrlichia sp.* isolates from Brazilian wild felids formed a cluster distinct from other *E. canis* isolates found in Brazil and the USA. The *Ehrlichia sp.* found in these felids possibly may be novel specie of *Ehrlichia sp.* Further studies are necessary to clarify the real identity of this organism.

P37: SEROEPIDEMIOLOGY OF *EHRLICHIA CHAFFEENSIS* AND *ANAPLASMA PHAGOCYTOPHILUM* IN BRAZILIAN MARSH DEER (*BLASTOCERUS DICHOTOMUS*) FROM PORTO PRIMAVERA HYDROELETRIC POWER STATION, PARANA RIVER, BRAZIL

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Members of Anaplasmataceae Family (Order Rickettsiales) form a group of obligately intracellular gram-negative bacteria that can infect both animals and humans, whose vectors are ticks. Human monocytotropic and granulocytotropic ehrlichiosis, caused by *Ehrlichia chaffeensis* and *Anaplasma phagocytophilum*, respectively, has been reported in several regions of the world. In USA, the white-tailed-deer (*Odocoileus virginianus*) is considered the natural reservoir of the *E. chaffeensis,* and cervids and other animals are incriminated in the transmission of *A. phagocytophilum*. In Brazil, antibodies to *E. chaffeensis* were detected in dogs and humans, in Minas Gerais State. Moreover, the *E. chaffensis* DNA was detected in blood samples of Brazilian marsh deer (*Blastocerus dichotomus*) in a region located between São Paulo and Mato Grosso do Sul States, the Porto Primavera Hydroeletric Power Station, in Paraná River, that suggest the animals are reservoirs of the agent. The present work aimed to detect antibodies to *E. chaffeensis* and to *A. phagocytophilum* in 143 sera samples of Brazilian marsh deer (*B. dichotomus*), from Porto Primavera region, by using the Indirect Immunofluorescense Assay (IFA). The animals were captured between 1998 and 2002 and the sera samples were stored at -20°C. Antigenic substrates constituted by DH82 cells with *Ehrlichia chaffeensis* morulae and HL-60 cells infected with *A. phagocytophilum* (Focus Diagnostics®, USA) and conjugate (rabbit IgG anti-IgG deer, labeled with fluorescein isotycianate) at dilution of 1:10 (KPL) were used in the IFA test. Sera initial dilution was 1:40 and fluorescent morulae of *E. chaffeensis* and *A. phagocytophilum* were detected in 116 (81,12%) and 24 (16,78%) of the 143 sampled animals, respectively. The results suggest that the Brazilian marsh deer has been exposed to *E. chaffeensis* and *A. phagocytophilum*. Additionally, further studies should be done about ehrlichial infection epidemiology among Brazilian wildlife, vectors (ixodides) and the possibility of these agents to cause disease in humans.

P38: USE OF MTT COLORIMETRIC ASSAY TO MEASURE THE VIRULENCE OF FIVE LOCAL STRAINS OF *THEILERIA ANNULATA*

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Theileria macroschizonts divide within host leucocytes, and thus growth rate and virulence characteristics must be a product of both parasite and host factors. Growth rates of *Theileria* parasites in vivo are very hard to measure due to their tissue tropisms. Therefore this study was undertaken to compare the proliferative index of macroschizont-infected lymphoblastoid cells of five strains of *Theileria annulata* by an in vitro MTT [3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide], colorimetric assay . The results of this study confirmed the MTT assay to be a fast, simple, cheap, and accurate method for assessing the cell activation rate and growth. In particular, the MTT method proved to be useful to estimate the virulence of *Theileria annulata* strain. As predicted our study reconfirmed positive associations between parasite growth rate and virulence to the host for clones of *Theileria annulata* in cattle.

P39: THE FIRST FINDING OF A NATURAL INFECTION OF *THEILERIA EQUI* IN AN EWE: AN UNUSUAL HOST

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Some species of *Babesia* or *Theileria* can occasionally infect species other than their usual host, including humans. In the equine , *Babesia equi* which has now been redescribed as *Theileria equi*, is a major pathogen.
Here, we describe an 3-year-old ewe infected with *Theileria equi* had a clinical disease characterized by fever , icterus (jaundice) , hemolytic anemia, and hemoglobinuria .The diagnosis was made by microscopic examination and sequencing studies .Classification of the organism as *Theileria equi* was based on DNA sequencing and comparison to sequences for *Theileria equi* obtained from GenBank.The described case is to the best of our knowledge the first presentation of a naturally acquired *Theileria equi* infection in non-equine animals with sever clinical signs resulted in the death. However, more well-designed studies are needed to confirm the *Theileria equi* as causative agent for ovine piroplasmosis.

PS1: COMPARATIVE STUDY OF PCR AND GIEMSA STAINING ANALYSIS FOR DETECTION OF *ANAPLASMA SPP.* IN RESERVOIR CATTLE

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Anaplasmosis belong to the complex of several tick-borne diseases with different etiological agents such as protozoa, rickettsia, bacteria and viruses. The only common feature between these diseases is that they can all be transmitted by ticks. Anaplasmosis can cause diseases in the livestock with high economical losses. Cattle that recover from acute infection become carriers and the parasite can persist most probably for the life-time in the blood. The Giemsa staining of blood smear is the common traditional method for the identification and characterization of anaplasmosis in Iran, which especially in the case of carrier states accompanied with some technical problems causing false morphological diagnosis. The most

specific method for the differential diagnosis of anaplasmosis is the method of polymerase chain reaction. Hundred and fifty blood samples and corresponding blood smears of cattle without any signs of diseases were prepared from a region in Isfahan / Iran with the previous history of acute anaplasmosis. The blood smears were first analyzed by Giemsa staining and the extracted DNA from blood cells were analyzed by *Anaplasma marginale* specific nested PCR and PCR-RFLP using primers derived from 16S rRNA gene and restriction endonucleasis Bst1107 I. *Anaplasma* like structures could be identified in the limited amount of erythrocytes of 75 blood smears. In these samples, the percentage of erythrocytes harboring *Anaplasma* like structures varied from 10^{-3}% to 10^{-2}%. Nested-PCR and PCR-RFLP analysis showed 58 *A. marginale* positive cases within 75 Anaplasma suspected blood samples. In 150 total blood samples, 50% were *A. marinale* positive. Our results revealed that the traditional Giemsa staining method is not applicable for the determination of the persistently infected cattle. In addition, the results showed that the carrier animals must be wide-spread in the *Anaplasma* endemic areas in Iran.

PS2: ESTABLISHMENT AND MAINTENANCE OF A GLOBAL TICK CELL LINE COLLECTION

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Tick cell lines are becoming increasingly important as tools in research on ticks and tick-borne pathogens. We have secured funding from the Wellcome Trust to set up a global tick cell line biobank at Edinburgh University's Roslin Institute. The panel of 26 tick cell lines established over the past 30 years at Edinburgh University will be joined by additional lines provided by Timothy Kurtti and Ulrike Munderloh (University of Minnesota) and Patricia Holman (Texas A&M University). As well as supplying tick cell lines on request to the international tick and tick-borne disease research community, we will provide recipient scientists with training in their care and maintenance. We will continue to establish new tick cell lines, characterise the existing and new cell lines, develop methods to clone tick cells, and screen them for symbiotic microorganisms.

PS4: EFFECTS OF THE TICK-DERIVED ANTIGEN 64P ON TRANSMISSION OF *BORRELIA AFZELII* IN LABORATORY C3HN MICE

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Vaccines against blood-feeding disease vectors have a potential to protect against diseases caused by vector-borne pathogens, as demonstrated by the ability of an anti-tick vaccine candidate derived from the cement protein (64P) of *Rhipicephalus appendiculatus* to protect mice against tick-borne encephalitis virus transmitted by infected *Ixodes ricinus* ticks. Therefore it would be reasonable to expect a reduction in transmission of other pathogens after immunization against this tick antigen.
We immunized Lyme disease susceptible C3H/N mice with truncated constructs (64TRPs) of 64P and subsequently challenged with *Borrelia afzelii*- infected *I. ricinus* nymphs that co-fed with pathogen-free larvae. Parameters measured were: percentage of larval ticks that became infected when co-fed with infected nymphs, percentage of mice that supported spirochete transmission, and percentage of mice that acquired infection by infected tick bite. To evaluate spirochete transmission in the presence of disseminated infection, five and nine weeks after the first tick infestation, mice were repeatedly challenged with pathogen-free *I. ricinus* larvae and the percentage of ticks acquiring infection was determined. *Borrelia* prevalence was detected in detached ticks after their moulting by nested PCR and

subsequent genotyping by Reverse Line Blot (RLB). Infection in mice was detected in ear skin biopsies by PCR and RLB, and in sera by ELISA.
Immunisation of laboratory mice with 64TRPs did not impair establishment of the pathogen in the host. Five and nine weeks post-infection, sera of almost all experimental mice showed increased antibody levels to *B. afzelii,* which corresponded with positive PCR results for skin biopsies. In contrast to viruses, co-feeding transmission rate of *B. burgdorferi* s.l. spirochetes among *I. ricinus* ticks is lower and transmission increases during prolonged tick feeding and establishment of the pathogen in the host skin. Co-feeding transmission of *B. afzelii* by *I. ricinus* was 0% (GST control) and 12.3% (unimmunized control), whereas in mice immunized with 64TRPs it was 1.2% (TRP5+6) and 6.5% (TRP2, TRP5). Percentage of infection in ticks fed on infected mice five and nine weeks post-infection was generally higher: 30% (TRP5+6) and 66% (control) during the second infestation and 5% (TRP5+6) and 32% (TRP5) during the third infestation. In comparison with controls (33%), fewer 64TRP-immunized mice (6-25%) supported co-feeding transmission. The highest level of protection against co-feeding transmission of the spirochete was provided by the TRP5+6 cocktail; however, GST alone appeared to impair pathogen transmission completely (0%).
Further studies are needed to tease out the effects of GST, type of pathogen, and mouse strain, when GST fusion proteins are used as candidate vaccine antigens.
Acknowledgement. The work was supported by ICTTD-3 and project APVV-51-004505. We also thank A.R. Trimnell for her help and support.

PS5: DEVELOPMENT OF A LOOP-MEDIATED ISOTHERMAL AMPLIFICATION METHOD FOR DETECTION OF *THEILERIA LESTOQUARDI*

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A loop-mediated isothermal amplification (LAMP) was developed for diagnosis of *T. lestoquardi*. The primers were designed based on the clone-5 sequence of *Theileria lestoquardi*. The specificity and sensitivity of the assay were established. The specificity experiment showed that LAMP primers amplified DNA *T. lestoquardi* successfully, while no amplification was seen with *Theileria annulata*, *Theileria ovis, Babesia ovis, Anaplasma ovis* or ovine genomic DNA and water control. The sensitivity of the LAMP assay was analyzed with a detection limit of 10 fg/µl of plasmid DNA using a plasmid construct containing the target sequence. The LAMP product was confirmed by restriction digestion. To simplify the handling of the established LAMP assay, cells from a cell culturel suspension were boiled and used as templates along with the extracted DNA template. The detection limit using boiled cells required 10 cells per tube. These results introduces LAMP as an alternative molecular diagnostic tool, which needs to be validated using field samples.
Supported in part by EU CA project ICTTD-3 Asian component (no. 510561).

PS6: COMPARISON BETWEEN REVERSE LINE BLOT AND ENZYME-LINKED IMMUNOSORBENT ASSAY IN DIAGNOSIS OF MAJOR TICK-BORNE DISEASES OF CATTLE IN SOUTHERN SUDAN

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The performance of reverse line blot (RLB) in detecting DNA of *Theileria parva*, *Theileria mutans* and *Babesia bigemina* was assessed in comparison with specific antibody detection using indirect Enzyme-

linked Immunosorbent assays (ELISA) for the same parasites. Among 90 field samples from Equatoria state, Southern Sudan, ELISA reported more positive samples than RLB did. The two tests positively recognized 53 out of the 90 samples as having *T. parva* antibodies and DNA, simultaneously while 7 samples were negative for *T. parva* infection using the two techniques. The concordance of RLB showed 66.7% (60/90) relative to the results of ELISA. With regards to *T. mutans*, there were 71 positive samples in RLB among 80 positive samples in ELISA, while 2 samples were negative in RLB out of 10 negative in ELISA. Subsequently, the concordances of RLB showed 81.1% (73/90) relative to the results of ELISA. Among the 90 field samples, there were 46 samples that reported positive by ELISA for *B. bigemina* antibodies, while RLB did not detect any of these 90 samples as positive showing concordance of 48.9% (44/90) relative to the results of ELISA. It has to be borne in mind that the results of ELISA might represent previous infections, while that of RLB would not only reflect an active infection, but also a carrier status. Therefore, the selection of the test would depend on the specific aims of the study.
Supported in part by EU CA project ICTTD-3 Asian component (no. 510561).

PS7: IDENTIFICATION OF *THEILERIA UILENBERGI* IMMUNODOMINANT PROTEIN FOR DEVELOPMENT OF AN INDIRECT ELISA

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Theileriosis of small ruminants in the northwest of China is a protozoan disease restrictive for the development of the livestock industry and caused by infection with *Theileria uilenbergi* and *T. luwenshuni*, both of which are transmitted by ixodid *Heamaphysalis* ticks. The development of serological tools as a means for integrated prevention and control of the disease is an urgent and important requirement. Here we describe the identification and recombinant expression of a *T. uilenbergi* immunodominant protein (TuIP), which was identified by immunoscreening of a merozoite cDNA library. Based on the recombinant TuIP, a novel indirect ELISA was established using 329 negative serum samples to determine the cut-off value. The internal quality control revealed satisfactory stability and repeatability of the assay. Preliminary validation using 128 positive and 48 negative reference samples demonstrated that the rTuIP ELISA is able to detect infection with *T. uilengergi* with high sensitivity and specificity. No cross reactivity was found in serum from animals infected with *T. lestoquardi*, *Babesia* sp China or *Anaplasma ovis*. Furthermore, circulating antibodies were detected in sera collected from endemic regions in China. Analysis of the antibody response of animals experimentally infected with *T. uilenbergi* demonstrated that tick infestation resulted in a sharply rising and stronger production of specific antibodies against TuIP while blood infection induced an earlier production of TuIP specific antibodies. The persistence of the TuIP specific antibodies lasted more than 100 days post infection. These data indicate a potential usefulness of the TuIP antigen for development of diagnostic methods and as a candidate for vaccine design.
Supported in part by EU CA project ICTTD-3 Asian component (no. 510561). ZL is the recipient of a DAAD scholarship.

PS8: VALIDATION OF A COMPETITIVE ELISA FOR DETECTION OF *THEILERIA ANNULATA* INFECTION

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Theileria annulata, the tick-transmitted pathogenic agent of bovine tropical theileriosis causes morbidity and loss in productivity in indigenous cattle, and in imported high-grade cattle theileriosis is a severe and often lethal disease.
Theileria annulata surface protein (TaSP) was identified as an immunodominant antigen and successfully used to develop and validate a recombinant-protein-based indirect ELISA for the detection of circulating antibodies in serum of *T. annulata*-infected animals. Because of numerous cross-reactivity reactions especially with *Theileria parva*, which is the causative agent of East Coast Fever, a competitive ELISA (cELISA) to increase specificity was developed using the same antigen and the monoclonal antibody 1C7 which was found to bind to TaSP. The cELISA accurately differentiated *T. annulata*-infected from uninfected animals without observing any cross-reactivity reactions.
To get information about the suitability of the cELISA in the field, 270 sera with unknown status from Iraq were compared in indirect and competitive ELISA. There was a significant ($p<0.000$) correlation ($r=0.407$) between both tests. The cELISA determined more sera as negative (44/270) compared to the indirect ELISA (27/270), which can be attributed to its higher specificity. Accordingly, less sera were identified to be positive in competitive (226/270) than in indirect ELISA (243/270).
Taken together, the cELISA proved its suitability for field application and is qualified for use in serological surveys to monitor the prevalence of the disease or to identify carrier animals with high specificity without cross reactivity with the related *T. parva.* This is especially true for regions of the world where both *Theileria annulata* and *T. parva* coexist, e.g. in the South of Sudan.
Supported in part by EU CA project ICTTD-3 Asian component (no. 510561).

PS9: DEVELOPMENT OF A RECOMBINANT INDIRECT ELISA FOR THE DIAGNOSIS OF *THEILERIA UILENBERGI* INFECTION IN SMALL RUMINANTS

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In China, two pathogenic *Theileria* species, *T. luwenshuni* and *T. uilenbergi*, have been described to infect small ruminants. For the improvement of the diagnosis, efforts have been made to develop ELISA and PCR-based tools, resulting in the development of an ELISA based on crude antigen and on a recombinantly expressed *T. lestoquardi* protein. Although both ELISAs were effective in detecting antibodies against *T. uilenbergi* in serum of infected sheep, both bear the potential of cross reactivitiy and there is still a need for an ELISA based on a recombinant protein of the parasite itself. To identify immunodominant proteins of *T. uilenbergi*, a merozoite cDNA library was screened by random sequencing. Three highly conserved cDNA sequences, namely clone 2, clone 9 and clone 26 (a deduced gene family) were obtained. Clone 9 was recombinantly expressed in *E. coli* and tested for specific reactivity with positive serum of *T. uilenbergi.* Positive results led us to establish an ELISA assay based on this recombinantly expressed protein. The cut-off was calculated at 44.4 % percent positivity using 25 serum samples from uninfected animals. The ELISA was applied to investigate the antibody response of sheep experimentally infected by tick infestation or inoculated by parasite containing blood. Generally, it was observed that animals infected by tick infestation produced higher titers of specific anti-clone-9 antibody than their counterpart blood inoculation ones. A total of 101 field samples collected from an endemic area in China were used to evaluate the clone 9 ELISA.
Supported in part by EU CA project ICTTD-3 Asian component (no. 510561).

PS 10: IDENTIFICATION OF BABESIPAIN-1, A CYSTEINE PROTEASE FROM THE BOVINE PIROPLASM *BABESIA BIGEMINA*

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The tick-transmitted pathogen, *Babesia* is an intracellular haemoprotozoan parasite causing a malaria-like disease, called babesiosis, responsible for a considerable worldwide economic, medical, and veterinary impact. Cysteine proteases play numerous indispensable roles in the biology of parasitic organisms. Their functional diversity is in turn derived from their unique nucleophilicity, adaptability to different substrates, and stability in different biological environments.Parasite cysteine proteases are unusually immunogenic and have been exploited as serodiagnostic markers and vaccine targets. Five genes were identified by sequence similarity search to be homologous to the cysteine protease family in the ongoing *Babesia bigemina* genome sequencing project database and were compared with the annotated genes from the complete bovine piroplasms genomes of *B. bovis*, *Theileria annulata*, and *T. parva*. Babesipain-1, one of the newly identified cysteine protease genes in the *B. bigemina* genome was expressed, and shows activity against peptidic substrates.

PS11: APPLICATION OF POLYMERASE CHAIN REACTION (PCR) FOR THE DETECTION OF *THEILERI ANNULATA* AMONG CATTLE IN KURDISTAN REGION / IRAQ

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The current study was conducted to determine the infection rate of *Theileria annulata* among cattle in three governorates (Erbil, Duhok and Sulaimanyia) of Kurdistan region in Iraq using PCR and nested PCR PCR techniques. 205 (68.6%) were found positive for *T. annulata* distributed among cattle in the three governorates of Kurdistan region of Iraq. The infection rate of theileriosis among cattle for each governorate was 62.6% in Erbil, 69% in Duhok and 74% in Sulaimanyia. The current study also investigated the differences of sensitivity and accuracy between the single PCR and nested PCR for the detection of *T. annulata*. 191 DNA samples were found to be negative for *T. annulata* by a single PCR assay using Tams1 and Tams4 primers, 97 (50.7%) of these were found to be positive by applying furthre nested PCR assay using Tams2 and Tams3 primers. The sequences of positive DNA samples were recorded in the Genbank/USA under the name of *T. annulata* isolate of Kurdistan/Iraq, meroziote - piroplasm surface antigen Tams 1 gene under the accession number (FJ159695). In conclusion, bovine theileriosis was highly prevalent (68.6%) among cattle in three governorates of Kurdistan Region / Iraq. The nested PCR technique proved to be highly sensitive when compared with single PCR technique for detection of *Theileria annulata*.
Supported in part by EU CA project ICTTD-3 Asian component (no. 510561).

NEMATODIASIS

P40: ESTABLISHMENT OF THE RNA INTERFERENCE TECHNIQUE TO A NEMATODE PARASITE, MEMBER OF THE ANCYLOSTOMATIDAE FAMILY

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The hookworms are a class of blood ingesting parasites that cause disabilities to the humans and animals around the underdeveloped countries in the world. Several studies have been currently developed aiming to find an efficient vaccine to members of the Ancylostomatidae family. One vaccine candidate is the APR-1 protein, an aspartic protease that has been showed to take part in the cascade of haemoglobin digestion. In this work we used the dog parasite *Ancylostoma ceylanicum* hookworm as a model to establish the RNA interference technique to the APR-1 gene. It was shown that the APR-1 RNA expression decreased by 87.36%, ± 10.39 (n=8) in the test group in comparison to the control group. Furthermore, a lower level of the APR-1 protein was also detected by western blot using an antibody raised against a recombinant APR-1 protein. This experiment shows for the first time the effectiveness of RNAi technique to hookworms, opening the possibility of elucidating phenotypes of important genes, as the family of Ancylostoma Secreted Proteins (ASPs), that in despite of have been well characterized and known to be important to the parasitism course, have not had their precise function established in the parasite.

P41: INTERFERON γ HAPLOTYPE B GENE ASSOCIATED TO A *HAEMONCHUS CONTORTUS* INFECTION ON PELIBUEY SHEEP

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The aim of this study was to determine the biological role of *inf-γ* haplotype B on the resistance or susceptibility of Pelibuey-breed sheep to an infection with the blood-feeder nematode *Haemonchus contortus* on a tropical region. Fifty-nine Pelibuey-breed lambs from four to five months-old were used in this study. Animals were experimentally infected with *H. contortus* L_3 and maintained in a tropical experimental station for three months. Blood samples were taken to analyze the allelic variations considering the haplotype B of *inf*-γ, which was also associated with faecal egg per gram (*epg*) and packed cell volume (*pvc*) traits. The *inf*-γ, haplotype B variation was determined by Real-Time PCR (RT-PCR) amplification followed by the fluorescence-labelled probe hybridization and melting curve analysis by the LightCycler single-tube technique. Data were analyzed by a GLM procedure using the SAS statistical Program to determine the role of B mutation on susceptible or resistance immune response against hemonchosis disease. In addition, data were correlated with *epg* and *pvc* phenotype traits showing 53.8% of Pelibuey-lambs with *inf*-γ, B haplotype mutation related to hemonchosis resistance and 38.46% lambs conserved the wild *inf*-γ, B allele highly susceptible to the nematode infection. A small group of 7.69% showed both alleles the wild *inf*-γ haplotype B. We concluded that the assayed mutant allele was mainly correlated with resistance against haemonchosis in Pelibuey sheep.
This project was financially supported by CONACYT- México, SAGARPA 067

Session 12 Poster session II

PROTOZOAN DISEASES

P43: THE EFFECTIVENESS OF A TARGETED RE-TREATMENT INTERVENTION PROGRAMME IN REDUCING THE INCIDENCE OF TRYPANOSOMIASIS IN CATTLE IN UGANDA.

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The "Stamp Out Sleeping Sickness" programme, which was launched in 2006, aims to halt northward spread of *Trypanosoma brucei rhodesiense* sleeping sickness in Uganda by mass trypanocidal treatment of the cattle reservoir. Phase 1 targeted the most northerly of the districts newly affected by this parasite. Post treatment monitoring revealed a cluster of villages in which *T. b. rhodesiense* remained present in the cattle reservoir. They were located close to one another and within parishes that continued to report human sleeping sickness cases, indicating the transmission cycle may not have been properly interrupted in these areas. As a result, re-treatment of these high risk areas was proposed.

This study aims to assess the impact of the SOS re-treatment intervention on the prevalence of *T. vivax*, *T. b. brucei* and *T. b. rhodesiense* by analyzing cattle blood samples from 20 randomly selected villages within the re-treatment area, 10 of which are to be human sleeping sickness case villages and 10 of which have no reported human sleeping sickness cases. Samples have been taken immediately before and 6 months post re-treatment, and will be subjected to PCR based methods for the detection of parasite DNA.

This poster will review the results of the SOS re-treatment surveillance samples, and their implications both for the SOS programme and for the epidemiology of trypanosomiasis in Uganda as a whole. The case control aspect of the follow up study allows for the comparison of trypanosome prevalence in villages known to have reported human sleeping sickness cases between January and July 2007 and those that did not.

P45: *IN VIVO* ACTIVITY OF (8-HYDROXYMETHYLEN)-TRIEICOSANYL ACETATE AGAINST *TRYPANOSOMA CRUZI* DURING ACUTE PHASE OF THE INFECTION.

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The antiprotozoal activity against *Trypanosoma cruzi* (*T. cruzi*) of the compound (8-hydroxymethylen)-trieicosanyl acetate isolated from *Senna villosa* was evaluated in BALB/c mice during the acute phase of the American trypanosomiasis disease (15 days after infection). Animals were treated during 15 days at doses of 16.8 and 33.6 µg/g, reduced parasitemia level of 77.6 and 64.1% was observed respectively, in comparison with mice from positive control group (allopurinol 8.5 µg/g), showing a 29.7% of parasitemia reduction. The number of amastigote nest in cardiac tissue was significant reduced in treated mice, counting 53 and 82 nests in the cardiac tissue from mice treated at doses of 16.8 and 33.6 µg/g respectively in comparison with untreated and allopurinol treated mice (394 and 356 amastigote nests

respectively). The regression of effect induced by the compound was evaluated, animals were infected (with $5x10^4$ trypomastigotes of *T. cruzi*) and simultaneously began the treatment with the (8-hydroxymethylen)-trieicosanyl acetate. The treatment was given during 20 days (16.8 and 33.6 µg/g), and mice were monitored after the end of administration of the compound for one more week. Efficient antitrypanosomal response was observed (66.1 and 68.9% less than untreated mice). Even 8 days after suspension of treatment, a reduction of 58.6 and 56.29 % of trypomastigotes in treated animals was observed. The number of amastigote nest was 2.7 minor in mice treated with the highest dose evaluated (33.6 µg/g) than untreated animals. The antiprotozoal activity of (8-hydroxymethylen)-trieicosanyl acetate against *T. cruzi* has been demonstrated in this work. Even when the treatment began 15 days after infection, a reduction on trypomastigotes and amastigotes forms of the parasite was observed. It is necessary to evaluate the effectiveness during longer periods of time and during the chronic phase of the disease.

P46: CARDIAC LESIONS IN DOGS NATURALLY INFECTED WITH *TRYPANOSOMA CRUZI*.

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The pathogenesis of Chagasic cardiopathy is not fully understood. There are degenerative and progressive clinical events associated with the continuing presence of *Trypanosoma cruzi* or their antigenic components (DNA) in the cardiac tissue, particularly in the cardiac septum. Consequently, an abnormal response of the immune system is unable to control the infection and the immune response acts as cellular damage mediator, inducing to a cardiac lesions. The aim of this study was to determinate the frequency and type of cardiac lesions associated with positive titters against *T. cruzi* in naturally infected dogs. Ninety one hearts, complete blood and serum samples were obtained from dogs captured and euthanized by the dog pound of Merida, Yucatan, Mexico. During necropsy, macroscopic cardiac lesions were identified. DNA was obtained to identify the presence of *T. cruzi* genome by Polymerase Chain Reaction (PCR). Serum samples were evaluated by Indirect Immunofluorescence (IFI) to detect IgG antibody titers against *T. cruzi* and latter confirmed by Western Blot (WB). Septum samples from hearths were obtained, fixed in paraffin and stained with Hematoxilin-Eosin. The frequency of microscopic cardiac lesions was determinate and classified. Additionally, seropositive dogs were evaluated by PCR using as template DNA from the cardiac tissue. Twelve percent (13/91) of dogs were *T. cruzi* seropositive (IFI+WB) and 6.5% were PCR-blood positive. The most common macroscopic cardiac lesions were Right ventricular dilatation (70.5%), Endocardosis grade I (70%), II (30.5%) and III (17.6%). No statistical differences between seropositive dogs with macroscopic cardiac lesions were found. All *T. cruzi* seropositive dogs showed microscopic lesions ($P<0.05$) characterized by a severe inflammatory interstitial infiltrate around the cardiac fibers with predominance of lymphocytes and plasmocytes. Also, several zones with fibrosis around degenerated cardiac fibers were also present. No amastigote nests were observed. Seronegative dogs showed normal cardiac cells. From the IFI positive dogs only 2 were PCR-blood positive; all IFI positive dogs were PCR-cardiac tissue positive. The presence of parasite in heart is determinant to induce the development of the microscopic lesions. Four PCR positive dogs and IFA negative were found without cardiac lesions which may indicate an early phase of infection. It is concluded that naturally infected dogs with *T. cruzi* do not show a specific macroscopic lesion in the hearth but develop a high frequency of microscopic cardiac lesions characterized by lymphocytoplasmatic myocarditis and myocardial fibrosis. Although amastigotes are not seen, presence of the genome in cardiac tissue indicates a positive colonization of the hearth septum.

P47: SEROLOGICAL SURVEY OF AMERICAN TRIPANOSOMIASIS IN DOGS AND THEIR OWNERS FROM AN URBAN AREA OF MERIDA YUCATAN, MEXICO.

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American tripanosomiasis (also known as Chagas´ disease) is an important protozoal disease which affects more than 15 million persons in America causing 12,500 death/year. In America, 28 million of persons are at risk to become infected in 21 countries. In Mexico Chagas´ disease is widely distributed along the country mostly in states with tropical weather conditions. Yucatan State is located in the southeast of Mexico and the etiological agent *Trypanosoma cruzi* and their vector has been well characterized and described. In Merida (capital city of Yucatan) the prevalence of Chagas´ disease in stray dogs is 17 % evidencing the well establishment of the vector to urban areas. The objective of this study was to determine the frequency of IgG antibodies against Chagas´ disease in dogs and their household owners from the south area from Merida which characterized by a lower socio-economical status compared to other areas of the city. Thirty five dog from 75 household owners were sampled to obtain serum and to detect IgG antibodies against *T. cruzi* by the ELISA test latter confirmed with IFI and Western blot. Prevalence was determined and the risk factors to become infected in both populations were evaluated using a 2 x 2 contingency table. The overall percentage of seropositivity was 34 % in dogs and 8.0% for sampled owners. Some owners brought 8 samples of insect vectors found in their household and were evaluated by PCR using BALB/c mice as source of food for donated insects. All tested insect were positive to *T.cruzi* after BALB/C mice were in contact with these vectors, and mice resulted IFI+WB positive after 45 days. Owner's occupation (bricklayer) and presence of dogs into the house during the night was an important risk factor for humans to become infected. Seroprevalence of Chagas´ disease in owned dogs agrees with the reported seroprevalence from the stray dog population in the same area. There is a high risk of exposition to the vector even in dogs living in households from the southern area of the city characterized by brick walls with cracks and timber-cardboard ceilings that vectors may colonize. Bricklayers are at risk to become infected when sleeping in construction areas where vectors may be adapted. The presence of seropositive dogs in households where vectors are well adapted represent a high risk for humans to become infected too when bitten by a vector infected by a positive dog.

P48: SEROLOGICAL SURVEY OF TOXOPLASMOSIS IN DOMESTIC CATS FROM MERIDA, YUCATAN, MEXICO.

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The *felidae* family is an important host for the *Toxoplasma gondii* (*T. gondii*) life cycle. In these species an enterosexual cycle of the parasite occurs and is able to produce and eliminate infective oocysts to the environment. Toxoplasmosis is a common infectious disease found in domestic cats throughout the world. The prevalence of *T gondii* infection may vary from between countries and socio-cultural situations. A total of 224 domestic cats from Merida, Yucatan Mexico, were examined by IgG Indirect–ELISA to determine the seroprevalence of *T. gondii*. An overall prevalence of 92.30% was found. This percentage

is higher than other surveys recently reported in Mexico such as Durango (21%) (Alvarado-Esquivel et al., 2007), in Colima (28.8%) (Garcia-Marquez et al., 2007) and in Mexico City (21.8%) (Besne-Merida et al., 2008). Probably the weather conditions from the surveyed places (dry-hot or cold), in contrast to the tropical weather conditions from Merida Yucatan may predispose cats to become in contact with the agent. Tropical conditions may enhance esporulation of oocyts and more infected preys (lizards, mice, birds, etc.) may be available for cats.

It is important to notice, an overpopulation of cat in the city. These cat populations may result in a high contamination of the environment with infective oocysts. Sero-positive cats were found widely distributed along the metropolitan area from the city. It is important promotes the prevention of toxoplasmosis, involving limit contact with known routes of transmission. Since the most common mode of transmission in humans is ingestion of tissue cysts in undercooked meat, meat should be heated to 66°C and hands should be washed thoroughly after it is handled. Also, gloves should be worn during contact with soil that is possibly contaminated with cat feces. Cat litter pans should be emptied daily, since oocysts require 1 to 5 days to become infective. If this is done, direct contact with cat feces during the cleaning of a litter box is an unlikely mode of transmission. However, pregnant women or immunosuppressed individuals should avoid changing cat litter.

P49: PRESENCE OF IGM AND IGG ANTIBODIES AGAINST *TOXOPLASMA GONDII* IN PIG FARMS FROM YUCATAN, MEXICO.

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Toxoplasmosis is protozoal zoonotic disease widely distributed around the world. Consequences of the infection in humans may be severe during pregnancy because can lead to serious or fatal consequences in the fetus or newborn. Also, the infection can implicate the reactivation of tissue cysts after immunosuppression (i.e. AIDS or transplantations) leading to a fatal encephalitis and/or reactivation of cysts in the retina. The disease can be contracted by the oral ingestion of oocysts present in cat feces and the environment, or tissue cysts present in the meat of infected animals. In humans, pork meat has been considered as an important source of infection. In Mexico, reports of toxoplasmosis in pigs show seroprevalences of 8.9 % in the State of Morelos. In Yucatan a preliminary report from 1978 showed seroprevalences of 50% in sows. A cross-sectional study was performed to determine the seroprevalence of ELISA IgG and IgM antibodies against *T. gondii* from 429 fattening pigs designated to human consumption from 39 fattening pig farms in Yucatan Mexico. Results revealed that 96.7% of pigs (415/429) showed specific IgM antibodies (IC 95% 94.93-98.53) and 90.4% (388/429, IC 95% 87.54-93.34) of IgG antibodies against *T. gondii*. From sampled farms, 35 from them 100% of pigs were seropositive to IgG, two showed prevalences of 30% and 95% respectively and one was negative. Results indicate a high exposition and active infection in most of the pig farms of Yucatan designate for human consumption. Variations in the IgG seroprevalence of the studied farms may vary according to the production system and control of predisposing factors such as presence of cats and control of rodents. The consumption of these undercook or incompletely cooked meat represents a high risk for the transmission of the parasite to humans. People in contact with raw meat should be informed of the risk of contract the disease and improve preventive practices. Considering that Yucatan is the 4th consumer of pork meat in Mexico, it is necessary to study factors that predispose the contamination of pigs and improve the farm conditions for the control of the disease in the production systems of the local pork meat industry.

P50: THEORETICAL MODEL OF INHIBITION OF *LEISHMANIA* SPP. TRYPANOTHIONE REDUCTASE

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The present work reports the development of a theoretical model for the inhibition of trypanothione reductase from *Leishmania spp* by seven monosubstituted *NN´*-diphenilbenzamidines (H, NO_2, CH_3, OCH_3, CN, Br e Cl). Compounds were synthesized through a specific route and had their biological activity evaluated for some species of microorganisms, particularly trypanosomatids, showing interesting results. The methoxy-derived compound seemed to be very promising, due to its high activity against parasite plus its low citotoxicity for the host. Studies of enzymatic inhibition, performed in the Laboratory of Biochemistry of Trypanosomatids at Fundação Oswaldo Cruz (RJ) showed that this class of amidines has inhibitory activity for trypanothione-reductase, essential enzyme for the parasite defense against host immune system cells. Records in literature indicate this enzyme to be a molecular target for the development of drugs against trypanosomatids. From experimental data of biological activity and structural models obtained from x-ray crystallography and molecular modeling, two hypotheses were set up in order to elucidate the mechanism of enzyme inhibition by these compounds. The first one involved the possibility of a nucleophilic attack to the amidinic carbon by a sulfide group of Cis 52 presented in the enzyme active site. Nevertheless, stericals and thermodynamical disabilities showed that this is an improbable hypothesis. Considering that, in biological systems, aromatic compounds are usually submitted to epoxidation by enzymes such as, for example, cytochrome P_{450} epoxidase, we studied the possibility of an epoxided derivative of the original molecule exposed to parasite be the one involved in the formation of a covalently bounded complex to the enzyme. Data obtained from molecular modeling using semi-empirical method PM3 showed results that support this hypothesis indicating which amino acids are involved in the formation of enzyme-inhibitor complex for the developed models, additionally it was detected that the formation of covalent bound showed a favorable enthalpy of nearly -20 $kcal.mol^{-1}$ for the studied epoxided amidines.

VIRAL AND BACTERIAL DISEASES

P51: RESISTANCE PATTERN OF *STAPHYLOCOCCUS* SPP FROM BOVINE MASTITIS IN RIO DE JANEIRO, BRAZIL

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The increased resistance of *Staphylococci* to several antimicrobial agents in dairy cattle intramammary infections has been reported worldwide. It impacts the effectiveness of therapy, once elimination of this organism from dairy herds requires treatment of infected mammary glands with specific antimicrobial agents. The determination of antimicrobial susceptibility of clinical isolates is required not only for therapy but also for monitoring the spread of resistant strains throughout the populations. ß-lactamic antibiotics are the most frequently used in intramammary infusion therapy. Bacterial resistance mechanisms to this class of antibiotics include production of ß-lactamases and low-affinity penicillin-

binding protein 2a (PBP2a) determined by the presence of the chromosomal *mecA* gene. Designated for methicillin resistance, this gene precludes therapy with any of the currently available ß-lactam antibiotics and may predict resistance to several classes of antibiotics. The aim of the present study was to characterize pheno and genotipically the resistance pattern of *Staphylococcus aureus* strains isolated from subclinical mastitis in Rio de Janeiro, Brazil. From 228 milk samples, 150 were identified as coagulase-positive *Staphylococcus* spp according to the results of phenotypical assays. Fifty strains (33,3%) were confirmed by PCR amplification of the 23s DNA specific to *S. aureus* and thirty-four (22%) were positive for *nuc* gene being identified as *S. intermedius*. Antibiotic susceptibility was determined by the standardized agar diffusion test on Müller-Hinton agar (MERCK). *S. aureus* ATCC 25923 was used as reference strain. Resistance to methicillin was determined according to the CLSI recommendations. PCR for *mecA* gene was carried out as described in literature. Antibiotic resistance assays revealed that 45 isolates (30%) were susceptible to all antimicrobials tested. Among the twenty different antibiotic patterns observed, predominant ampicillin/penicillin resistance pattern was observed in 31 isolates (20,6%). It was also detected 43 strains (28,6%) resistant to oxacillin and 36 strains (24%) resistant to vancomicin. A total of 95 strains (63,3%) investigated exhibited resistance to penicillin. Probably this resistance is an a consequence of the widespread application of ß-lactamic antibiotics frequently used in intramammary infections in Brazil because overuse and misuse of antibacterial agent have been incriminated as the major selective forces encouraging the development of resistance in bacteria. PCR for *mecA* gene was positive for 106 strains (70,7%). All oxacillin-resistant isolates were *mecA*-positive. These findings might be the base for preventive strategies to reduce the spread of resistance and to warn that staphylococcal mastitis control programs need to be more efficient.

P52: *STAPHYLOCOCCUS AUREUS* VIRULENCE FACTORS FROM BOVINE MASTITIS AT RIO DE JANEIRO, BRAZIL

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Staphylococcus aureus is recognized as a major pathogen causing subclinical intramammary infections in dairy cows leading to severe economic losses worldwide. In Brazil, *S. aureus* isolates are commonly recovered from mastitic milk samples. Nevertheless, little information is available about the genetic diversity of *S. aureus* isolated from cows with mastitis in Brazil. This bacterium produces a variety of exoproteins that contribute to its ability to colonize mammary gland, such as hemolysins, coagulase, slime and protein A. Beta-hemolysin is a Mg^{2+}-dependent sphingomyelinase C which degrades sphingomyelin in the outer phospholipid layer of the membrane. Coagulase protein has the ability to turn fibrinogen into fibrin threads by a mechanism different from natural clotting. The presence of this protein is a remarkable feature of *S. aureus*. The slime is a polysaccharidic extracellular component that mediates adhesion enabling bacterial cells to agglomerate in multilayered biofilms making bacteria less accessible to the host defense system. Protein A is a membrane-bound exoprotein characterized and well known for its ability to bind to the Fc region of immunoglobulins. The present study was conducted to characterize pheno-genotypically these virulence factors in 50 *Staphylococcus aureus* isolates from 228 milk samples of cows with subclinical mastitis located in dairy cattle farms located at Rio de Janeiro, Brazil. Samples were sent to the Laboratory of Bacteriology at UFRRJ for bacterial isolation and identification. Polymerase chain reaction analysis of the *rDNA*, *coa, spaA, icaA, icaD, hla, hlb* and *agr* genes were carried out using the primers and respective amplifications program available in literature. All 50 isolates were confirmed by PCR amplification of the 23s DNA specific to *S. aureus*. PCR for *coa*-gene were positive for all isolates and displayed three different size polymorphisms with approximately 520bp, 800 bp and 900 bp. The amplification of region X from *spaA* yielded a single amplicon for each isolate with the prevalent amplicon size being of 315bp for 32 strains (64%). Multiplex PCR detected that 24% (n= 12) and 16%

(n=8) of tested strains were positive for *hla* and *hlb* genes and 18% (n=9) for *icaA* and *icaD*. Amplification of *agr* gene yielded an amplicon size of 350bp and 550bp in 74% of the strains. These findings are valuable to the comprehension of the profile of *S. aureus* strains distribution in Rio de Janeiro.

P53: POTENTIAL PATHOGENICAL BACTERIA ISOLATED FROM BIVALVE MOLLUSKS AT THE ARCHIPELAGUS OF SANTANA – MACAÉ.

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Mussels are bivalve mollusks that developed a filtration system enabling them to uptake nutrients from water. This is a not selective mechanism so the microbiological analysis of these mussels can show their aquatic environmental quality. The present work aimed to isolate and identify bacterial microbiota from bivalve mollusks incrustation into the rocky coast of the South Little Island at the Archipelagus of Santana, Macaé, Rio de Janeiro. The antimicrobial resistance pattern of the isolated microorganisms was also analyzed. Surrounding water was evaluated in order to establish its microbiological quality and the possible contamination sources from fishing and subaquatic activities in the studied region. Mussels were harvested four times in the period from May 2007 through May 2008. In each harvest, 2 samples of 25units were sent to the Laboratory of Bacteriology at Universidade Federal Rural do Rio de Janeiro. Protocols of isolation and identification were performed following literature reports for each of the isolated species. A total of 51 isolates of Vibrio spp. was obtained from the mussel's bacteriological analysis. V. damsela (n=15) was the prevalent specie, followed by V. harveyi (n=13) and V. alginolyticus (n=07). It was also obtained a total of 20 isolates of Enterobacteriaceae species. Escherichia coli (n=06) was the prevalent one, followed by Proteus vulgaris (n=04). Antimicrobial susceptibility pattern was evaluated for the species of Vibrio. It was considered the public healthy impact of these bacteria and the potential risk of cutaneous injuries to fishers and others professionals working in subaquatic activities in the studied area. Vibrio spp. isolates presented 100% of sensitivity to tested antimicrobials, except for ampicillin with no detected sensitivity corroborating to literature. For enterobacteria, it was detected a high percentile of sensitivity to all tested antimicrobials. In the six samples of ocean water analyzed it was not possible to detect total or fecal coliforms. The low percentile of isolated microorganisms from mussels at Archipelagus of Santana can be justified for its location at the open sea, far away from the coast and influenced by sea currents, in a environment not yet altered by human action.

P54: BOVINE TUBERCULOSIS IN THE DEVELOPING WORLD

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This is a multidisciplinary-Wellcome Trust-funded project that was initiated in April 2005. The project goal is to make the first comprehensive assessment of host and parasite prevalence, genotypes, economic impact of m. bovis and potential for disease control using novel vaccination strategies for bovine tuberculosis's in a developing country. The country focus of the project is Ethiopia. The project represents a collaboration between institutions located in Ethiopia, Kenya, Switzerland and the UK.
The seven interlinked work packages address four major objectives:
(1) A series of studies are screening for bovine TB in different parts of Ethiopia. A prevalence of 48% infection was found at a farm in Holeta where Holstein Freisian cows are reared under intensive conditions as part of a government breeding programme (WP7)

(2) The association between breed and susceptibility is being explored in detail using a SNP-based genome screen for admixture mapping of *B. indicus* and *B. taurus* in infected and uninfected animals (WP4).Differences in immune response to Mycobacteria between *B. indicus* and *B. taurus* are being studied in an experimental challenge model (WP5). BCG vaccination experiments demonstrate higher T cell reactivity as assessed by interferon gamma production in *B. taurus* animals relative to *Bos indicus*:
(3) To map the population structure of *M. bovis* and assess transmission to humans.

- Mycobacteria are cultured from infected cattle tissues identified in abattoirs at five sites (WP1), and from fine needle aspirates (FNA) collected as diagnostic samples for human lymph node TB in clinics in the same areas (WP2). Human lymph node TB is caused by M. tuberculosis; there is no evidence of any contribution from *M. bovis*
- The population structure of *M. tuberculosis* in Ethiopia comprises two major lineages

(4) To assess the feasibility of interventions for control of bovine TB by vaccination
Overall Project Coordinator; Professor Douglas Young; Imperial College, London University, UK.
Work package leaders; WP1 *M. bovis* genotypes in livestock in Ethiopia. Steven Gordon, Veterinary Laboratories Association (VLA) UK; WP2 Human M. bovis prevalence and genotype in lymph nodes. Abraham Aseffa AHRI; WP 3; Prevalence of *M. bovis* in livestock, Martin Vordermeier, VLA; WP4 Association of *M. bovis* prevalence with host cattle genotype. Dan Bradley Trinity College Dublin; WP5 Experimental infection of *M. bovis* in *Bos indicus* and *Bos Taurus* cattle. Richard Bishop ILRI Kenya; WP6 Societal and economic impact of *M. bovis*; Jacob Zinnstag Swiss Tropical Institute; WP7 Experimental vaccination of calves using BCG priming and recombinant MVA boosting. Glyn Hewinson VLA.

P55: EMERGENCE OF BLUETONGUE VIRUS IN NORTHERN EUROPE AND ITS CURRENT SITUATION IN GREAT BRITAIN.

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Since the end of the last decade the bluetongue virus (BTV), which is transmitted by the biting midge *Culicoides* spp., has been spreading northwards in Europe. Several authors have explained that climatic changes along with changes in wind patterns that could cause drift of the midge population, may have favoured this phenomenon. In Great Britain in 2007, one of the wettest springs on record followed both, a very mild winter and one of the hottest summers and autumns in the previous year. BTV causes bluetongue, a disease of ruminants where cattle normally act as reservoirs; whereas for sheep, the disease can cause serious economic loses. Molecular genetics analyses identified the viral strain as serotype 8 (BTV-8). As a consequence the Department of Food and Rural Affairs (DEFRA) of the United Kingdom not only introduced a ban on the movement of imported animals from areas where the disease was widespread, but also introduced a vaccination program against BTV-8. Alongside, they declared and divided the country into protection zones. All of these measures are part of a contingency plan put into action in 2007. In this paper we revised the emergence of BTV in northern Europe and the current situation of bluetongue in Great Britain, two years after it was first ever reported. Sheep and cattle trade has been under constant surveillance and information on restriction zones of countries on the near continent has been made available to farmers. Treatment of animals and animal housing with synthetic insecticide products such as pyrethroids, along with the use of insect-proof animal quarters and good farming practice is proving an effective control method against the midges. In 2009 the UK government proposed a voluntary vaccination program in England and Wales, whereas for Scotland it is still compulsory. Where vaccination is not compulsory farmers are now responsible to obtain the vaccines from commercial laboratories. Nevertheless, in 2008, according to DEFRA, only 8 cases of bluetongue were confirmed, all of them as a result of post-importation of animals from continental Europe.

P56: SCREENING FOR *LEPTOSPIRA SPP.* IN DIFFERENT ANIMAL SPECIES IN SICILIAN ISLAND, ITALY.

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Leptospirosis, is considered an important re-emerging infectious disease worldwide since spirochetes have the ability to survive in a wide range of environmental conditions and several mammals species can be infected. In Italy the majority of leptospirosis cases in humans as in animals are reported in the northern part of the Country. Sicilian climate used to be quite dry but , it is now changing with warmer temperature all year around and episodes of sudden thunderstorm that are becoming more and more frequent in summer. A molecular epidemiological survey for animal leptospirosis in Sicily have been started six years ago to have more informative epidemiological data and establish risk assessment for the disease in the island. A continuous observation is performed in dogs from municipal kennels, in an autochthon swine breed, in bovine herd and in a horse farm in which a leptospirosis outbreak causing abortion has been detected.
Samples collection: Kidneys, urethral swabs, urine, aborted foetus form different animal species, swine, dogs, horses, bovine. DNA was extracted from kidney samples blood samples and urethral swabs using Fast DNA Kit from Q BIO GENE and Rybolyser Homogenization according to manufacture's instruction. PCR analysis had been performed using 16S rRNA gene as PCR target (primers: LEPTO E1 GGGAAAAATAAGCAGCGATGTG and LEPTO E2-ATTCCACTCCATGTCAAGCC amplicon 571 bp). This PCR is specific for pathogen leptospira only. The overall results showed a prevalence of leptospira in animal species starting from 4 % up to 24% . The highest prevalence were detected from the animals living free in natural environment like the autochthon swine breed "black pig of Nebrodi" **.** They are freely living in area of woods and water reservoirs and are restricted in a small area for a short period only before slaughtering. Analysis on urethral swabs from dogs located in 4 kennels of western Sicily showed a prevalence of 8% . In bovine population 7 % positive results were detected with no difference among different breed and herds management conditions. Forty nine aborted fetus from sheep (30), cows(10), equine (6) dogs(9) were also analyzed with the following positive results only for 1/ 9 in dogs and 1/ 6 equine.
Acknowledgement: This work had been supported by a grant of Italian Ministry of Health to Maria Vitale RC 2001. Many thanks. M. La Giglia and M. Piazza for technical assistance.

P57: GENETIC CHARACTERIZATION OF DAIRY MASTITIS *ESCHERICHIA COLI* STRAINS FROM RIO DE JANEIRO SATE, BRAZIL

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Regarding dairy mastitis, one of the most important environmental etiologic agent is *Escherichia coli*. Moreover, epidemiological studies relate the human diseases caused by *E. coli* strains with the consumption of bovine milk and its derivates, submitted or not to thermal treatment. Among its strains, the Shiga toxigenic (STEC) stands out by the possible of infection in consuming raw meat or milk. Even though it is an agent with a great pathogenic potential. In Brazil the studies are limited, specially broaching genetic diversity aspects of *E. coli* strains isolated from bovine mastitis.
This study aimed to make a genotypic characterization of *E. coli* samples identified from dairy mastitis cases in municipalities of Rio de Janeiro state, Brazil. As well as to detect genes that produces the referred toxin among this samples.

Ten dairy farms were visited in Barra Mansa and Resende municipalities. On these farms, all cows were evaluated by the California Mastitis Test (CMT). Positive samples were placed on sterile tubes and sent to the Laboratory of Bacteriology at UFRRJ for bacterial isolation and identification. *E. coli* samples were sent to the Laboratory of Molecular Epidemiology of FIOCRUZ for ***Random Amplification of Polymorphic DNA*** (RAPD-PCR) as a genetic diversity evaluation tool. Also PCR was performed in order to detect Shiga toxin gene (*stx*).
The prevalence of mastitis on the visited dairy farms was 20,63% considering 273 evaluated cows. Fourteen *E. coli* samples (15,22%) were evaluated, 13 isolated from subclinical and one from clinical mastitis. Genotypic evaluation demonstrated ***clonal*** varieties on *E. coli* subpopulations of different mammary quarters of the same cow or between animals from the same farm. Also, it was detected that 14,28% of the samples were positive for Shiga toxin gene (*stx*). These findings may contribute to the bio-genetic-epidemiological knowledge of the studied pathogen and it is important to the establishment of monitoring and controlling actions in dairy cattle.

P58: ENTEROBACTERIAL MICROBIOTA FROM *STOMOXYS CALCITRANS* (LINNAEUS, 1758) SEGMENTS

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Synanthropic flies from the family Muscidae are considered as potential pathogen carriers. The stable fly, *Stomoxys calcitrans,* member of this family, has among its parasitic characteristics the hematophagism, host expoliation, and a high capacity of transmission of pathogenic agents due to its feeding behavior. Among those agents, bacteria from the family Enterobacteriaceae deserve a major focus, especially because this fly uses fecal matter for feeding and reproduction.
The objective of this study was to evaluate the enterobacterial microbiota from three segments of the stable fly, assessing its distribution on external surface, buccal apparatus and abdominal digestive tract, as well the enterobacterial infection rate in these flies.
Ten dairy properties from the municipalities of Barra Mansa e Resende, State of Rio de Janeiro, Brazil, were visited. Twenty *S. calcitrans* specimens were individually collected per farm and taken to the Laboratory of Bacteriology of UFRRJ for enterobacterial isolation and identification.
After colony isolation and identification, a total of 159 agents were verified. Among these, 73 (45.91%) were isolated from external surface, 46(28.93%) from buccal apparatus and 40 (25.93%) from abdominal digestive tract, confirming that external surface is the most prevalent segment as stated in the literature. Besides, a significant difference was noted when comparing enterobacterial prevalence among the studied segments ($p<0.01$). In the present study, an enterobacterial infection rate of 56% was observed, where 112 flies have presented agents from this group in at least one of the evaluated segments. Among the identified microbiota, some species are highlighted because of their high pathogenic potential, such as *Escherichia coli*, *Salmonella* spp. and *Shigella* spp.
In accordance to the results, *S. calcitrans* has the capacity to carry enterobacteria in all evaluated segments, being the external surface the one presenting higher prevalence. Further studies should be performed aiming evaluate the capacity of transmission of those agents to its hosts. Moreover, there is a necessity to evaluate the pathogenic and entomopathogenic potential of these bacteria in order to reinforce the epidemiological surveillance on *S. calcitrans* in Brazilian herds.

P59: OCURRENCE OF SHIGA-TOXIGENIC *ESCHERICHIA COLI* IN *STOMOXYS CALCITRANS* (LINNAEUS, 1758)

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The stable fly, *Stomoxys calcitrans,* has major importance in the production-animal safety relationship, due to its expoliative potential, as well its role in the vehiculation of several pathogens. This fact is highlighted by its interrupted feeding behaviour, which favors microorganism dissemination among hosts. One of the most studied stable-fly-carried agents is the enterobacteria group, with focus on *Escherichia coli*. Despite this species is included in the indigenous microbiota of all mammals' intestinal tract, some strains such as Shiga-toxigenic *E. coli* (STEC) are associated to diarrhea in men and animals. The STEC natural reservoirs are domestic and wild ruminants, especially cattle, which disperse the agent through the environment via feces. Since this muscid uses feces from those animals for feeding and oviposition during its immature and parasitic phases, the aim of this study was to report the occurence of *E. coli* in three stable fly segments and, thus, detect Shiga-toxin production genes among the identified samples.
Ten dairy properties from the municipalities of Barra Mansa e Resende, State of Rio de Janeiro, Brazil, were visited. Twenty *S. calcitrans* flies were individually collected from each farm and taken to the Laboratory of Bacteriology of UFRRJ for bacterial isolation and identification from the external surface, mouth apparatus and abdominal digestive tract of the stable flies. Afterwards, the identified *E. coli* samples were taken to the Laboratory of Molecular Epidemiology of FIOCRUZ in order to perform the genetic assay using Polymerase Chain Reaction test – Multiplex (Multiplex-PCR) for detection of Shiga-toxin producers genes *stx1*, *stx2* and *eae*.
According to the results, 159 colonies were isolated from flies' segments. From those, 44 (27.67%) were identified as *E. coli*. Comparing the analysed segments, the external surface harbors more isolates (23), followed by mouth apparatus (14) and digestive tract (7). STEC characteristic genes were identified in six (13.63%) out these 44 *E. coli* isolates.
This study evinces that *S. calcitrans* potentially vehiculates agents of highly pathogenic concern. Therefore, it suggests that stable fly control methods should be implemented, in order to reduce the possible transmission of diarrheagenic *E. coli* strains within populations of poor sanitary conditions, such as the visited ones.

P60: IDENTIFICATION, ANTIMICROBIAL SUSCEPTIBILITY AND VIRULENCE GENES OF *ENTEROCOCCUS* ISOLATED FROM CAMELS.

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Enterococcus has emerged as a major cause of nosocomial infections in human and veterinary medicine. Clinical strains have become increasingly resistant to a broad range of antimicrobial agents, including aminoglycosides, penicillins and glycopeptides. Reservoirs for antibiotic-resistant enterococci have not been completely determined. Animals, human, food and environment have been suspected as sources for some resistant clinical isolates. We have investigated the resistance of *Enterococcus* isolated from camel faeces to antibiotics commonly used as therapy of enterococcal infections. Identification was made by the method of Facklam and Collins and by Polymerase Chain Reaction. Minimal Inhibitory Concentrations of penicillin, ampicillin, gentamicin and streptomycin were determined. Antimicrobial susceptibility to 11 antibiotics was determined using disk diffusion method. Genes codifying resistance to vancomycin, tetracycline and erythromycin were determined by PCR. Sixty six *Enterococcus* strains were isolated and distribution by species using PCR was: 48.5% *E. hirae*, 31.8% *E. faecium*, 6% *E. malodoratus*, 4.6% *E. faecalis*, 4.6% *E.*

mundtii, 3% *E. casseliflavus* and 1.5% *E. durans*. None of the strains was resistant to VAN, TEC, P, AM or HLAR. The highest percentage of resistance found was 42.42% strains resistant to rifampicin followed by 33.33% resistant to trimethoprim – sulfamethoxazole. The gene Tet M was detected in three strains and all of them were resistant to tetracycline. The gene Van C2-C3 was found in two strains, both of them identified as *E. casseliflavus*, a species of *Enterococcus* that intrinsically harbour this gene. Erm A, Erm B, Erm C, Tet L, Van A, Van B and Van C1 genes were not detected in any of the strains. The resistance rates among *Enterococcus* strains isolated from camel were lower than those found among human strains isolated from hospital patients in recent Canary studies. To evaluate the pathogenic ability of isolates, the distribution of virulence genes (*cyl*A and *cyl*B) was investigated. These genes were not detected in any of the strains. We would like to remark the importance of monitoring the presence of resistant enterococci in animals including the ones used for recreational purposes. In addition to this, faecal enterococci could contribute to horizontal spread of virulence and resistance genes to other clinically important bacteria. Epidemiological studies in different animals should be continued to monitor the presence and dissemination of resistance and virulence genes of enterococci in order to establish public health measures.

P62: IDENTIFICATION OF *LEPTOSPIRA* SPECIES BY *FLAB*-POLYMERASE CHAIN REACTION-BASED TECHNIQUES

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Leptospirosis is a worldwide zoonosis caused by pathogenic species of *Leptospira* that are invasive for a wide range of mammalian hosts. Historically, several hundred serovars of *Leptospira* were classified into two species, the pathogenic strains (*L. interrogans*) and saprophytic strains (*L.biflexa*). The direct methods of diagnosis are important but limited. The serological is time consuming and laborious and DNA-DNA relatedness cannot be easily performed. Therefore the goal of this study was to examine the polymerase chain reaction (PCR)-based strategies for identification of leptospires. PCR-sequencing and PCR-restriction fragment length polymorphism (RFLP) were designed to examine. Representative strains from each of the 23 pathogenic and one saprophytic leptospires were used to analyze. Using two sets of primers, *rrs* (as an internal control) and *flaB*, amplification of 331 bp of *rrs* was observed in all 24 strains tested and amplification of 793 bp of *flaB* was observed in 21 pathogenic strains tested. The *flaB* primers could amplify genomic DNA from pathogenic leptospires strains tested but not an intermediate pathogenic (*L. meyeri* serovar ranarum strain ICF) and saprophytic *L. biflexa* serovar patoc strain PatocI. On the other hand one strain of pathogenic species *L. borgpetersenii* was not amplified with primer sets of *flaB* gene. The nucleotide sequence of *flaB* from 21 reference strains tested and 37 strains provided data from Genbank were used to construct the phylogenetic tree using Mega4 program version 4028. *flaB* RFLP has been demonstrated by using pDRAW32 version 1.1.100. The PCR-based strategies results were similar to the previous report that used DNA-DNA relatedness. They could be used to characterize and differentiate between pathogenic and non-pathogenic leptospires. In addition, they could be used to confirm the high level of divergence among the species of *Leptospira*. The *flaB* molecular typing scheme has clear methodological advantages over conventional techniques and, with the widespread availability of PCR-based techniques, as the epidemiological tool that could foreseeable replace conventional techniques. Furthermore, these techniques could be applied to clinical diagnosis without leptospiral isolation.

P63: RESTRUCTURING POLICY FOR SMALL SCALE POULTRY PRODUCER TO CONTROL HPAI IN INDONESIA

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Many developing and more developed countries in SE Asia such as Indonesia, China, Thailand, Vietnam and Cambodia had proved to be the origin of HPAI outbreak. In the last five years HPAI has repeatedly re-occurred or becomes endemic in some of these countries. One reason for this might be the fact that 30-60 percent of livestock production in these countries are operated by small scale poultry producers. Low education level, low economic capacity, are the main characteristics that make those farmers unable to implement appropriate HPAI control measures. However, small scale producers are in particular affected due to HPAI in those countries.

Even though the government of Indonesia had initiated various policies on HPAI control measures within the five last years, HPAI remains even endemic in many parts of the country with continuously fatal human cases.

From experiences it can be assumed that government's policies implemented on HPAI control in Indonesia are not effective, unless they support also restructuring policies directed to the huge small scale producer sector in the country. These policies should consider in particular livelihood of small scale enterprises as well as increase consumer awareness to exclude transmission of HPAI to human.

Restructuring means here to improve ownership of small scale enterprises by: (i) improved on-farm management, (ii) limited transportation of inputs and outputs and (iii) modification of post harvest systems (e.g. change marketing from live to processed poultry).

In addition it is foreseen that small scale poultry enterprises should be discarded from densely populated villages. This may also contribute to the idea of eco-health by reducing the side effects of highly density poultry areas (e.g. manure management) and the public health risk as well as. Recent research results from Indonesia, China, Vietnam and Thailand have also shown that small scale farmers often ignore HPAI as serious constraints due to limited funds for control or no recent history of experienced HPAI outbreaks .

This paper proposes ideas on restructuring of small scale poultry enterprises as an effective HPAI control measures.

P65: NEGATIVE REGULATION IN THE IMMUNE RESPONSE BY EXOGENOUS INTERFERON GAMMA APPLICATION IN NEWBORN CALVES VACCINATED WITH THE CULTURE FILTRATE PROTEIN OF *MYCOBACTERIUM BOVIS*

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The live tuberculosis vaccine are more efficient in promote the resistance which indicates indirectly that secreted antigens present in the culture filtrate protein extract of *M bovis* (CFPE) are essential for this purpose. Alternatively, studies in mice show that administration of exogenous IFNγ at the time of immunization increases the cellular immune response involved in the elimination of intracellular bacteria. Thus, the aim of this study was to evaluate the effectiveness of *M bovis* CFPE as immunogen for the prevention of bovine tuberculosis and determine the immunomodulator effect of IFNγ when administered prior to immunization. 18 Holstein calves 8 months old negative to the diagnosis of tuberculosis were used for the study. The calves were divided into three groups of six animals. The first group was inoculated subcutaneously with 300 µg of CFPE, the second group was inoculated with the same dose of CFPE + IFNγ recombinant bovine (200ng) and the third group was the control. The calves received a second immunization three weeks later using the corresponding treatment. Five months after the first immunization, they were intratracheally challenged with 10^5 UFC of a field strain of *M bovis*, and after six

months, they were slaughtered and the presence of lesions was evaluated. The immune response was evaluated by ELISA and IFNγ production in cultures of PBMC (peripheral blood mononuclear cells) stimulated with PPD and with the antigens of 19 and 47 kDa of *M bovis*. RNA from stimulated cells was extracted to determine the expression of cytokines by RT-PCR. The results of production of IFNγ no were significant for the groups immunized with any of the treatments assayed before challenge. However, antibody levels were significant for the group treated with recombinant IFN-g, which correlated with increased expression of IL-4 in cultures stimulated with different antigens. To challenge the production and expression of IFNγ was significant for the group with CFPE and control group, whereas in the group treated with IFNγ was notable an expression of IFNγ and IL-10. The assessment of the extension and degree of lesions in different groups showed a minor protective effect in that received the IFNγ, although these were less severe than those observed in the control group. Thus, the treatment prior to immunization with IFNγ showed an adverse effect on the establishment of protective immunity due to a negative modulation in the Th1 immune response required for resistance to disease to a Th2 type response.

P66: PRESENCE OF GLYCOPROTEINS IN MANNHEIMIA HAEMOLYTICA AND PASTEURELLA MULTOCIDA

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The presence of glycoproteins may be related to the mechanisms of adhesion and colonization in bacteria, according to studies in *Pseudomonas aeruginosa* and *Escherichia coli*, in which it was established that these processes are affected by structural changes in their glycoconjugates. In the case of *Manheimia haemolytica* and *Pasteurella multocida* bacteria causing pneumonia in ruminants there is no information concerning the presence of such components, but its existence was suspected by the identification of proteins related to sugar residues of N-acetylated in saline extracts of them. Hence, the purpose of this study was to identify and carry out a partial characterization of glycoconjugates present in saline extracts of the bacteria mentioned. For that, saline extracts were obtained from different isolates of these bacteria. They were cultured on BHI agar for 18 h at 37°C. The bacterial mass were harvested and suspended in phosphate buffered saline (PBS ph 7.2). Subsequently, they were warmed a 56°C for one hour in a shaking water bath. Bacteria were removed by centrifugation, and to the supernatants were added ammonium sulfate to a saturation of 70% to precipitate proteins. The precipitates were recovered by centrifugation at 12000 *g* and eliminated the excess salts by dialysis. The composition of sugars in the extracts was determined by chromatography. Proteins were separated by sodium dodecyl sulfate-polyacrylamide gels electrophoresis (SDS-PAGE, 12%) and transferred to nitrocellulose membranes. The membranes were immersed in sodium periodate solution to oxidize residues carbohydrates, and then adding biotin-hydrazide to label the residues and their presence was finally revealed using streptavidin-alkaline phosphatase. Thus, it was possible to determine the presence of three glycoproteins in extracts of *M. haemolytica* with molecular weights of 70, 42 and 35 kDa, whereas those of *P. multocida* were identified as glycoproteins with weights of 70, 45 and 30 kDa. The isolation and analysis of the 70 kDa glycoprotein of *M. haemolytica* was carried out by affinity chromatography and by isoelectric focusing, its nature glycosidic was confirmed in polyacrylamide gels treated with periodic acid and alcian blue stain. The analysis shows the presence of glycoproteins in extracts of the bacteria analyzed, and represents the first report on the existence of such conjugates in the genera *Pasteurella* and *Mannheimia*. It is also possible that some of these proteins may be involved in the mechanism of adhesion, as has been revealed in the specific case of the 70kDa protein of *M. haemolytica* in previous studies.

P67: INTER TRANSMISSION OF LEPTOSPIROSIS IN BOVINE, CANINE, GOATS, HORSES AND SHEEP IN ONE FARM WITH LOSSES IN REPRODUCTIVE EFFICIENCY

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Leptospirosis is a worldwide zoonosis that affects domestic animals, wildlife and humans, becoming a major public health problem. It causes economic losses, mainly caused by abortion, birth of weak and premature fetuses. Outbreaks of the disease usually occur with abortions and death of cattle, sheep and goats, showing significant impact on reproductive efficiency of ruminants. The dog has an important role in the transmission of leptospirosis, by it maintain the agent causative during a long time in the kidneys, being able to eliminate him in the urine. In horses, young foals and pregnant females are particularly susceptible to the disease. Considering the epidemiological importance of the creation of different promiscuous species, this research aimed to identify the possibility of transmission the disease among sheep, goats, cattle, horses and dogs in a farm with reports of reproductive problems in the sheep, cattle and goat herds. The Microscopic Agglutination Test (MAT) was used to identify the prevalence of *Leptospira interrogans* and the most common serovar in each species. In October of 2007 was collected blood from all animals used in this study. In cattle,11(68.75%) out of 16 tested were seropositive for *Leptospira. Interrogans*, the most frequent serovars were: *wolffi, hardjo* and *tarassovi* (25%), *icterohaemorrhagiae* and *autumnalis* (18.75%), *grippotyphosa* and *hebdomadis* (12.5%), *bratislava* and *canicola* (6.25%). The 27 dogs tested, 16 (59.25%) were seropositive, in decreasing order the serovars were: 25% *hardjo*, *icterohaemorrhagiae* and *pomona*; (18.75%) *bratislava*, *canicola* and *wolffi*, and 6.25% for autumnalis and *australis*. In the 17 goats tested, had 3 (17.64%) positive animals, the serovars detected were: *autumnalis* (17.64%), *wolffi* and *hardjo* (5.88%). In horses, 8(72.72%) out of 11 tested were seropositive, with higher prevalence of serovar *tarassovi* (45.45%), followed by *icterohaemorrhagiae* (36.36%), *hardjo* and *autumnalis*, (27.27%), *bratislava,* (18.18%) and sentot (9.09%). Testing sheep, 23 (22.54%) out of 98 were seropositive and the most frequent serovars were: *bratislava* (52.17%), *grippotiphosa* (26.08%), *wolffi* and *hardjo* (8.69%) followed by *djasiman*, *panama*, *australis* and *icterohaemorrhagiae* (both 4.34%). In the five species studied, all showed the serovars *hardjo* and *wolffi*. Therefore there is the possibility of trasnmission of leptospirosis among the five species examined in this study.

Financial Support: CNPq and MAPA.

P68: TRANSMISSIBILITY OF *LEPTOSPIRA INTERROGANS* AMONG CATTLE, HORSES AND SWINE

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Leptospirosis is a zoonosis distributed worldwide, caused by *Leptospira interrogans*, which affects domestic animals, wildlife and humans. The clinical signs commonly seen in cattle and pigs are reproductive changes, such as abortion, retention of placenta, infertility, and reduction in production rates. The equine leptospirosis manifests itself mainly as recurrent uveitis. Due the fact that different animal species live in the same environment, even in intensive systems of production, the objective of this study was verifying the occurrence of the disease and the most frequent serovars among the species examined. The Microscopic Agglutination Test (MAT) was used to identify seropositive animals, using 12 serovars

of *L. interrogans*. The samples were obtained from animals of different age groups and both sex from a dairy farm located in Uberlandia, Minas Gerais. All 25 cattle tested were positive *for L. interrogans*. The most frequent serovars in decreasing order were: *wolffi*, *tarassovi*, *icterohaemorrhagiae* and *hardjo*. The three horses tested all had positive serology (100%), in decreasing order the serovars were: *icterohaemorrhagiae, tarassovi, wolffi* and *autumnalis*. The three pigs submitted to testing, all were positive, with higher prevalence of serovar i*cterohaemorrhagiae*, followed by *tarassovi* and *autumnalis*. The two dogs tested were seronegative for leptospirosis. Of the 4 serovars present in the bovine species, 3 also occurred in horses and 2 in swine, antibodies to serovar *icterohaemorrhagiae* were present in all of them. Furthermore, was observed the occurrence of serovar *autumnalis* common in equine and swine. The clinical and epidemiological evidences observed in this study demonstrate that the disease occurred in all cattle tested, and suggest that the presence of cattle seropositive for *Leptospira interrogans* may be associated with infection of horses and swine. Based on these observations is suggested the possible transmissibility of leptospirosis among cattle, horses and swine.
Financial Support: CNPq and MAPA.

P69: SHEEP CARCASSES COULD BE CONTAMINATED DURING SLAUGHTERING PRACTICE

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Background: *Escherichia coli* O157:H7 is a highly virulent food-borne pathogen and a threat for Public Health. It's firmly associated with severe human illnesses like bloody diarrhea and hemolytic uremic syndrome (HUS). It rarely causes disease in animals, and lives in the intestines of healthy ruminants. Sheep faces may be an important source of Verotoxin-producing *Escherichia coli* (VTEC). Verotoxins (VT) 1, 2 and eaeA genes were tested for this propose.
Material and Methods: From September 2005 to August 2006, swab surfaces samples from sheep were collected at the major slaughterhouses of Shiraz-Iran.
Results: The study (remove: were) revealed the occurrence and characterization of *E. coli* O157 strains in Iranian domestic sheep and lamb. The monthly prevalence of *E. coli* O157 in sheep was obtained and ranged from 3.92 to 0.2% and was at its highest level in spring and late summer. Six (3.92%) carcasses of sheep were contaminated by *E. coli* O157:H7. Five (3.34%) out of 115 and 1(2.63%) out of 38 samples were from ewes and lambs carcasses respectively. Only six samples were positive to specific PCR for the VT2 gene and produced verocytotoxin VT2, whereas all isolates were negative for the presence of VT1 and eaeA virulence genes (remove: considered).
Conclusion: Geographical variations and season may influence the prevalence of the pathogen. Distribution of VTEC in sheep and lamb in (remove: subject) target area is wide. The composition of the gastrointestinal flora may be changed by different diet and, therefore O157 VTEC rate in sheep and lamb was different. Iranian sheep have shown to be (remove: indicated as a) natural host of *E. coli* O157 strains (remove: therefore may be) and potentially pathogenic for humans. This is the first report of *E. coli* O157 detection from sheep in Iran.

P70: O157 AND NON-O157 STEC MAJOR GENE FROM CATTLE IN SOUTHERN IRAN

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There is limited information about Shiga toxin-producing *Escherichia coli* (STEC) strains in the cattle gastrointestinal tract in Iran. The aim of this study was to investigate the presence of dominant genes in O157 and non-O157 STEC isolated from cattle.

In the period from September to November 2007 and 2008, carcass surfaces and recto anal mucosal swab samples from Shiraz cattle were collected at the main slaughterhouses of the Shiraz southern of Iran. The samples were analyzed by conventional plating and then compared with PCR. 440 samples from 220 cattle were collected.
STEC were detected from 86 of 220 cattle. Most of the isolates belonged to *E. coli* serotypes other than O157, suggesting a low prevalence of strains of this serotype. *E. coli* O157:H7 was present in 4.54% of all samples and was isolated from 10 cattle (10/220) belonging to different herds. Six (2.72%) of them were from carcass surfaces and four (1.81%) from recto anal samples. With the exception of four isolates from adult cattle which appeared to be negative for *stx* genes, all *E. coli O157* isolates were positive for both *stx1* and *stx2*, and *E. coli* attaching-and-effacing gene sequences (eaeA), and therefore, they were regarded as potential human pathogens. The presence of stx1, stsx2, eaeA and O157 genes in 15.47, 31.19, 19.04 and 2.61% strains of *E. coli* isolates was investigated. Fifty four carcasses were contaminated with *E. coli* those have at least one gene (stx1, stx2) versus 47 recto anal samples, and this results indicated that most carcasses might be cross contaminated. Ten strains were isolated by conventional plating while 14 *E. coli* O157 was diagnosed by PCR. So PCR appeared to be significantly more sensitive than conventional plating method for detection of the organism in feces and from carcass surfaces.
Variation in isolates according to stx1, stx2, eaeA and O157 genes would appear to be the causative agents of different STEC. Keeping animals together in pens, which enhances faecal-oral contact and close contact in slaughter lines, was suggested as a possible explanation for the differences in stx occurrence in rectum and carcass of the same cattle. We were further interested in the role of cattle as a reservoir for STEC in southern of Iran. Our report demonstrates that domestic cattle could represent an important natural reservoir for these organisms in this country and could cause a life-threatening.

P71: *ESCHERICHIA COLI* O157:H7 STRAINS ASSOCIATED WITH DIARRHEA AND HEMOLYTIC UREMIC SYNDROME BUT DO NOT PRODUCE SHIGA TOXIN

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Background: *Escherichia coli* O157 has been linked to a spectrum of disorders, including watery diarrhea, bloody diarrhea (hemorrhagic colitis), and HUS. It has ability to produce shiga toxin (stx). Detection of *E.coli* O157 that do not produce stx is the objective of this study.

Material and Methods: A total of 225 recto-anal mucosal swabs from healthy cattle in Fars province were screened in 2007-2008. Detection were performed on TSB and containing on SMAC agar plates. PCR was conducted with sorbitol positive and negative bacterial colony.
Results: In addition, from 225 stool specimens, 10 samples from 10 different cattle were positive with O157 antiserum. Multiplex PCR assay showed that four *E.coli* O157 did not carry the gene for either stxs, whereas two *E.coli* O157 with mines stx harbored a chromosomal *eae* gene encoding intimin regards as a human pathogen, as well as the rfb O157 genes.
Conclusion: We are unable to state with certainty that stx had no role in the diseases. The previous data support the role of stx as a cause of bloody diarrhea, in contrast to non-bloody diarrhea. Stx does not appear to be necessary for all manifestations of the *E.coli* O157:H7 diseases. HUS might result from non-stx factors produced by *E.coli* O157:H7. Hypothesis raise the possibility that other properties of *E.coli* O157:H7 might also be sufficient to produce HUS. Therefore to identified stx-deficient *E.coli* O157 in microbiologic evaluations, need using stx-independent recovery techniques. And detection systems should be complemented by additional methods.

P72: PREVALENCE OF THE STX2 GENE IN COLIFORM POPULATIONS

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The major virulence characteristics of STEC strains are the production of two potent phage-encoded cytotoxins called Shiga toxins with several Stx2 variants(c, d, e, and f).They ability to colonize the bowel through the adhesin protein intimin, encoded by eae gene. There is limited information about the lysogenic strains and bacteria present in GI tract that are susceptible to phage infection. This study reports the prevalence and distribution of the stx2 gene in STEC strains in samples of different origins.

During one year period 2006-2007, 420 cattle were examined to assess seasonal changes in STEC shedding the population of stx2 gene-carrying bacteria was also monitored. The samples were transferred to laboratory and incubated in tryticase soy broth overnight. Plated onto CT-SMAC and biochemical test was carried out. Used specific primers, stx1, stx2 and eae for PCR procedures after DNA extraction. PCR products samples have sent for sequencing.

A total of 129(30.71%) STEC strains carrying the stx2 gene were isolated. Both stx2 and stx1 genes were detected in 51(12.14%) strains. In 21(5%) strains, were found to be positive for stx1,stx2 and eaeA genes,19 (70.3%)strains for both stx2 and eaeA These distributions explain by 63(15%) stx1 and 80(19%) intimin protein encoded by the chromosomal gene *eae.*

This study shows that most *E.coli* strains are found to be toxigenic and stx2 is the dominant genotype. According to sequence analysis data reviled that stx2d is more separated than others. Epidemiological studies have shown Stx2 is more associated with severe human disease than Stx1.It has been reported that O157:H7 that express Stx_2 alone are more likely to be associated with progression to HUS than are strains producing Stx1 alone or, curiously, both Stx1 and Stx2.Cattle seem also to be a reservoir of O157:H7 strains, but most of the isolates belonged to *E.coli* serotypes other than O157,suggesting a low prevalence of strains of this serotype carrying the *stx*2 gene. Therefore most of the isolates belonged to toxigenic *E.coli* serotypes are potentially pathogenic for humans.

P73: EVALUATION OF HUMAN CYTOMEGALOVIRUS (HCMV) VIRAL LOAD USING REAL-TIME PCR IN COMPARISON WITH HCMV ELISA IN RENAL TRANSPLANT PATIENTS IN KHARTOUM STATE-SUDAN

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This study was carried out to detect human Cytomegalovirus (HCMV) IgG and IgM antibodies in renal transplant patients in Khartoum state, Sudan and to improve the diagnosis of HCMV through the introduction of Real-time PCR. A total of 98 plasma samples were collected at random from renal transplant patients at Ibin Sina hospital and Salma Centre for transplantation and haemodialysis from August to September 2006. Among the 98 renal transplant patients, 65 were males and 33 females. The results revealed that, ELISA detected HCMV IgG in all patients' plasma 98/98 (100%), while only 6/98 (6.1%) have IgM antibodies in their plasma. The results of IgG ELISA may suggest a high incidence of previous infection in all group tested, while the finding of IgM may reflect a recent infection and reactivation. The load of HCMV DNA was detected in 32 patients 32/98 (32.7%) with real-time PCR. HCMV detection with real-time PCR in the present study indicated high prevalence among renal transplant patients in Khartoum. In conclusion, the incidence and existence of HCMV in Khartoum State was documented through the detection of HCMV specific antibodies. Further research work should be carried out to characterize HCMV at molecular level.

PROTOZOAN DISEASES

P78: COMPARISON BETWEEN THE PATHOGENESIS OF LOW AND HIGH PASSAGE OF *NEOSPORA CANINUM* TACHYZOITES IN CHICKEN EMBRYONATED EGGS

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Bovine neosporosis is a common cattle production problem worldwide and caused by the protozoan parasite, *Neospora caninum.* Previous study has shown that chicken embryonated eggs could be a useful model to study the parasite's biology. However, it is not known whether this model is suitable for testing the pathogenesis of different passage of *N. caninum* tachyzoites. Therefore *N. caninum* tachyzoites were passaged for different lengths of time in vitro and compared for their ability to pathologic effects in cause disease in chicken embryonated eggs. A lethal challenge model was developed and the LD50 was determined to be 10 [3] *N. caninum* tachyzoites/embryonated egg, delivered by the allantoic cavity route. Groups of 8-day-old embryonated eggs were inoculated by the allantoic cavity route with 1 x 10(2) or 1 x 10(3) of low-passage or high-passage *N. caninum* tachyzoites. The embryonated eggs inoculated with the high-passage tachyzoites have lower mortality rate and showed fewer pathologic lesions of neosporosis, compared to those receiving the low-passage tachyzoites. This study showed that increase in passage could decrease in *Neospora caninum* virulence; also these results confirm that the chicken embryonated eggs can be a valuable alternative approach for in vivo testing of live attenuated potential vaccine.

P79: HISTOPATHOLOGICAL AND MOLECULAR INVESTIGATIONS IN *NEOSPORA CANINUM* EXPERIMENTALLY INFECTED EMBRYONATED QUAIL EGGS

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Neospora caninum is a coccidian parasite of animals. It is a major pathogen for cattle and dogs. As intermediate hosts, many species like chickens are reported. It seems that other bird species might perform this role in wildlife as well. Understanding the sylvatic life cycle of *N. caninum* is needful and effective to reveal the exact pathogenesis of this parasite. In that sense, this study aimed to observe tissue distribution and histopathological lesions in quail embryos experimentally infected with *N. caninum* and after they have been hatched. Experimental infections were conducted in embryonated eggs. Sixty embryonated quail eggs were inoculated by the allantoic cavity route in 6 groups of 10, 10^2, 10^3, 10^4, 10^5, 10^6 tachyzoites and ten eggs without inoculation as control group. Infected eggs demonstrated susceptibility to infection, with mortality rates dependent of the inoculum dose. Suitable samples from different tissues of dead embryos were collected for histopathological and molecular study and stored respectively in 10% buffered formalin and -70 ^{oc} refrigerator. Histopathologic lesions of acute neosporosis were detected in the liver, heart and brain. The presence of the parasite in these tissues was proved with PCR method too. The lesions implied that embryonated quail eggs can be a suitable model of acute neosporosis.

P80: COMPARISON BETWEEN *NEOSPORA CANINUM* INFECTION IN BROILER AND EGG-LAYING CHICKEN EMBRYONATED EGGS

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Neospora caninum is one of the most efficiently transplacentally-transmitted coccidian parasites that frequently diagnosed in bovine abortions worldwide. Moreover, the presence of birds in cattle raising farms represents increased risk factors for abortion storms related to *N. caninum*. Although *Neospora caninum* has been widely described in mammals, there is a limited investigation that has been performed about birds, especially broiler chickens. Therefore, this work aimed to comparison the infection by *N. caninum* in broiler and egg-laying chicken's embryonated egg. Experimental infections were conducted in 70 broiler and egg-laying chicken's embryonated eggs that were divided into seven groups. Six group were inoculated with 10, 10^2, 10^3, 10^4, 10^5, 10^6 tachyzoites/ embryonated egg. The other group considered as control group without inoculation. The embryonated eggs were maintained at an incubator with controlled temperature, humidity and rotation. In contrast to the egg-laying chicken, the mortality rate in broiler chickens was dependent of the inoculum dose. Suitable samples from different tissues of dead embryos in each group were collected for histopathological and molecular study and stored respectively in 10% buffered formalin and -70 ^{oc} refrigerator. Histopathologic lesions of acute neosporosis were detected in the liver, heart and brain. The presence of the parasite in these tissues also was proved with PCR. After hatch there was a little neurologic signs in broiler chickens but most of egg-laying chickens in groups with inoculum dose more than 10^2 tachyzoites/ embryonated egg had neurologic signs like ataxia, lack of coordination, pedalling movements or hind limb paralysis and circular walking patterns. These results reinforce that there is genetic susceptibility to *N. caninum* in chickens like mice and provide new insights to reach an inexpensive and available animal model for *N. caninum* infection.

P81: PREVALENCE OF CRYPTOSPORIDIUM INFECTION IN CALF IN KAZEROON SOUTHWESTERN IRAN

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Cryptosporidium is a zoonotic protozoan pathogen of the Phylum Apicomplexa and causes a diarrheal illness called cryptosporidiosis. Other apicomplexan pathogens include the malaria parasite Plasmodium, and Toxoplasma, the causative agent of toxoplasmosis. Unlike Plasmodium, which transmits via a mosquito vector, Cryptosporidium does not utilize an insect vector and is capable of completing its life cycle within a single host, resulting in cyst stages which are excreted in faeces and are capable of transmission to a new host. A number of Cryptosporidium infect mammals. In humans, the main causes of disease are *C. parvum* and *C. hominis* (previously C. parvum genotype 1). C. *canis, C. felis, C. meleagridis*, and *C. muris* can also cause disease in humans.Cryptosporidiosis is typically an acute short-term infection but can become severe and non-resolving in children and immunocompromised individuals. In humans, it remains in the lower intestine and may remain for up to five weeks. The parasite is transmitted by environmentally hardy cysts (oocysts) that, once ingested, excyst in the small intestine and result in an infection of intestinal epithelial tissue.The various symptoms of cryptosporidiosis include, Frequent, watery, diarrhea, Nausea, Vomiting, Abdominal cramps, Low-grade fever, Debilitating cholera-like diarrhea (up to 20 liters/day), Severe abdominal cramps, Malaise, Weight loss and Anorexia.
Fecal samples, collected from 150 calf during March 2006 to Jun 2007 randomly selected from 10 regions in Kazeroon, Iran, were examined to investigate the prevalence of Cryptosporidium infection. Cryptosporidium oocysts were identified by using sheather's concentration and the Ziehl–Neelsen

modified staining technique. We identified 18.0 % of the positive.sexes of cattle were infected with Cryptosporidium parasites, but prevalences were higher in diarrheic than in non-diarrheic calf. The study for age infection, 51.0% 1-15 daily, 31.0 % 15-25 daily and 18.0% 25-45 daily were seen.
Cryptosporidium is a zoonotic disease , found in soil, food, water, or surfaces that have been contaminated with infected human or animal faeces. Transmission occurs through animal-to-human or human-to-human contact. People may also be infected by consuming contaminated water or food, or by swimming in contaminated water (for example in lakes or rivers). Infection is frequently associated with foreign travel. The Health Protection Agency provides advice on controlling outbreaks of cryptosporidiosis. We monitor any outbreaks to try to find the source of the infection, so that we can help to prevent other people from becoming infected.

NEMATODIASIS

P82: THE SURVEY OF *DIROFILARIA IMMITIS* INFECTION RATE OF STRAY DOGS IN KAZEROON , SOUTHWESTERN IRAN

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Dirofilaria immitis, the canine heartworm, is one of the most important filaroids parasite in carnivores. The dog and its close relative are natural hosts, but infection also occurs in cat, wild carnivores and man. Adult worms reside in the right ventricle and pulmonary artery of the dog while the unsheathed microfilariae circulate in the blood. Once ingested by the appropriate mosquito vector the microfilariae undergo development into infective larvae which can be transmitted to both dogs and humans. Development of the larvae into adult worms takes about 180 days in dogs. The worms never reach full maturity in humans. Most humans infected with *D. immitis* are asymptomatic. Coin sized lesions can be found in the lungs during radiological examinations. These nodules result from an immune response to the dead or dying worms which are either necrotic or in the processes of being calcified. Knott method is the most common test for its diagnosis in many regions including Iran.
In the present study Survey of microfilaria *Dirofilaria immitis* , in kazeroon`stray dogs , within 6 months ,50 dogs hourly blood sampling via the saphenous and cephalic veins and was tested by modified Knott's technique. Totally 5 cases infected by microfilariae , 2 cases (4.0%) infected by *Dirofilaria immitis* and 3 cases (6.0%) infected by *Diptalonema recoditum* were isolated.
Helminthologically different filarial infections in Iran are from reptiles, birds, rodents, camels, cows, dogs, foxes, and jackals. *D. immitis* is a very common filaria of stray dogs and sheep dogs in the right ventricle of the heart and causes disabilities in dogs . Infected dogs are unable to follow the sheep kept in the yards. Microfilariae in the blood of sick dogs are transmitted through mosquito biting. In Iran, reports of human dirofilariasis in frontal subcutaneous area , small nodule on the 5th finger and the other in the wrist , eye , testis area , subcutaneous nodule and ubconjuctival tissue or peri ocular tissue are too. Because of high prevalence of *Dirofilaria immitis* in dogs and the risk of human infection, a control program must be carried out for controlling of this worm and its intermediate host.

VIRAL AND BACTERIAL DISEASES

P83: DETECTION OF ARCOBACTERS IN FAECAL SAMPLE OF HEALTHY CHICKENS IN OSOGBO, NIGERIA

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Statement: Arcobacters are commonly present on food of animal origin with the highest prevalence in poultry.
Method: In order to determine the occurrence of Arcobacter in stool sample of chicken 150 from chicken were examined for the presence of Arcobacter by enriching all samples in Arcobacter broth with selective supplement- CAT (Cefoperazone, Amphotericin B and Teicoplanin), filtration and plating on selective medium. Real time PCR designed for detection of Arcobacter species in one step reaction. PCR product of Arcobacter butzleri and A. cryaerophilus from chicken were evaluated by means of sequencing to confirm their identity with existing genomic data in Genome bank.
Result: Out of 150 feacal samples from chicken only 2(1.3%) yielded Arcobacter species. The results in this study confirmed that Arcobacters are not likely to be part of the intestinal flora of poultry despite its presence in the poultry meat and that the Arcobacters strains from Nigeria isolated had a common origin with strains from other parts of the world.
Conclusion: The presence of Arcobacter in poultry carcass appears to be as a result of environmental contamination during meat processing and not from faecal material

P84: ISOLATION AND IDENTIFICATION OF *SALMONELLA* SPP. IN CHICKEN EGGS

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Foodborne illness is the major public health concern for both the meat and the poultry industries in the world specially in developing countries. The present studies, therefore, investigated the occurrence of *Salmonella typhimorium* in cracked eggs available in the some markets. *Salmonella typhimorium* is a major agent responsible for the prevalent and severe paratyphoid fever, and the effect of some heat treatments on *Salmonella typhimorium* in cracked eggs. A representative sample of 500 cracked eggs was collected as five batches from 5 diferent egg markets. PCR test was carried out to identify the *Salmonella typhimorium* strains in the samples. Another set of 100 cracked eggs was stored at ambient temperature from day zero to four to determine the changes in the *Salmonella typhimorium* counts. The first portion was subjected to hard-boiling for fifteen minutes, while the second was mildly fried in vegetable oil prior to microbiological analysis. Table 1 shows the results of *Salmonella typhimorium* counts in the market-cracked eggs and in the whole eggs that were subjected to laboratory cracking, changes in *Salmonella typhimorium* counts during storage, and the effect of boiling and frying on the *Salmonella typhimorium* counts. The results revealed that 450 out 500 cracked eggs (90%) were free from *Salmonella typhimorium,* which indicated about 10% contamination. This is comparable with the *Salmonella typhimorium* counts in the market-purchased cracked eggs where noticeable increase was recorded. This could be due to heavy

pore penetration of *Salmonella typhimorium* from the environment before purchasing. Boiling brought about reduction in the *Salmonella typhimorium* counts from day three to day four of storage with counts ranging from $0.20x10^2$cfu/g to $1.70x10^2$cfu/g. That was in contrast to the higher *S. typhimorium* counts of $1.10x10^2$cfu/g from day two to $5.80x10^3$cfu/g on day four of storage when the cracked eggs were fried. This suggests that boiling is more potent than frying. All these results suggest that cracked eggs are not safe for human consumption.

P85: COMPARISON OF THE EFFECTS OF ULTRASONIC WAVES, FORMALDEHYDE AND B-PROPIOLACTONE ON INACTIVATING OF NEWCASTLE DISEASE VIRUS

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In this research three different inactivation methods were compared with each other in Newcastle disease virus (NDV) inactivation: 1- Sonication method 2- Formaldehyde method and 3- β-propiolactonee method.

NDV- lasota strain was obtained from Razi Institute, Hesarak, Karaj. All NDV or inactivated samples in each stage were inoculated in SPF embryonated eggs. According to the sonication method, ultrasonic waves irradiated to the standard concentration of the NDV under the constant wave length and frequency but 3 different temperatures at 10^{oc}, 20^{oc} and 30^{oc}. Sampling was carried out in 30 minutes intervals and sonication was terminated after 120 minutes irradiation.

Also formaldehyde 0.5/1000 and 1/1000 as final concentrations and β-propiolactone in final concentrations 1/1000 and 2/1000 also tested for the virus inactivation. HA and IT tests were done to confirm the inactivation. Each inactivated samples were passed the passage 5 times in SPF embryonated eggs to find any left live NDV and each time the amnioallantoic fluid tested with HA and IT. On the basis of the results sonication method could inactivate the NDV at 10^{oc} and 20^{oc} temperature after 90 minutes without any decrease in the primary HA titer of the virus (NDV were titrated by HA test before and after each stage of inactivation. On the other hand in spite of formaldehyde and β-propiolactone, the sonication method causes an increase in HA level compared with the primary HA level of the NDV solution from 512 to 1024.

Also sonication is useful for small amount of the NDV solution because preparing small volume of NDV inactivated solution with formaldehyde (0.5/1000 or 1/1000) and β-propiolactone (1/1000 or 2/1000) and the need for continuous shaking at 37^{oc} to some extend is difficult. The inactivation time for formaldehyde in the mentioned concentrations were longer than 2 other methods and were about 18 to 24 hour under continuous shaking at 37^{oc} and the inactivation time were the same for β-propiolactone and sonication method (90 minutes). As an important finding the sonication procedure is fully advisable when NDV inactivated solution must be without any harmful materials such as formaldehyde or β-propiolactone.

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