BRUCELLOSIS
New Delhi 2016
International Research Conference
National Agricultural Science Center (NASC), New Delhi, India
November 17-19, 2016

ABSTRACT BOOK

www.dbtbrucellosis.in/brucellosis2016
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Local Organising Committee  
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Welcome Message

The local Organising Committee welcomes all of you and express a sincere appreciation to the International Brucellosis Society (IBS) for choosing New Delhi “The Capital of India” as the place for 69th Brucellosis Meeting and International Conference on Brucellosis 2016. This meeting will be a gathering of international experts, researchers, policy makers and students all over the world to share and discuss their finding regarding the broad and interdisciplinary field of “One Health” concept revolving around Brucella Pathogenesis & Host-pathogen interaction; Human Brucellosis; Epidemiology and Control; Brucella research in India; Canine and Wildlife Brucellosis; Diagnostic methods; and Vaccines & Immunology. For this three days meeting, we have chosen National Agricultural Science Centre Complex so as to provide an academic ecosystem where all the participants shall have opportunities to exchange ideas and networking for establishing future research collaborations.

We look forward to warmly welcoming and hosting you in New Delhi, one of the most happening cities in historical, political and cultural terms in India, and hope this meeting shall be a memorable moment.

On behalf of the local Organizing Committee

Dr. H. Rahman
Chairman

Dr. S. R. Rao
Organizing Secretary
Local Organizing Committee

Chief Patron
Dr. K Vijay Raghavan
Secretary, Department of Biotechnology

Patron
Dr. Trilochan Mohapatra
DG- ICAR and Secretary, DARE

Chairman
Dr. H. Rahman
DDG, ICAR

Organizing Secretary
Dr. S.R. Rao
Advisor, Department of Biotechnology

Office Secretary
Dr. Padma Singh
Scientist ‘D’, Department of Biotechnology

Members
Dr. Suresh Hannapogol, Commissioner, Animal Husbandry, DADF
R. K. Singh, Director, ICAR-IVRI, Izatnagar
Dr. A. J. V. Prasad, Joint Secretary (LH), DADF
Dr. Ashok Kumar, ADG, ICAR
Dr. A.K. Rawat, Scientist ‘F’, DBT
Dr. (Mrs.) Rajeswari Shome, Principal Scientist, ICAR-NIVEDI, Bengaluru
Dr. Jyoti Misri, Principal Scientist (AH), Division of Animal Science, New Delhi
Dr. Lipi K. Sairiwal, Livestock Officer, (AH), DADF, New Delhi
Dr. Rathnagiri Polavarapu, President &CEO, Genomix Biotech Inc., Hyderabad
Scientific Committee

Nammalwar Sriranganathan, BVSc, MVSc, PhD, Dip. ACVM (Co-Chair)
Professor of Bacteriology
Department of Biomedical Sciences & Pathobiology
Director, Center for Molecular Medicine & Infectious Diseases
Virginia Maryland College of Veterinary Medicine
Virginia Tech, Blacksburg, VA 24061
Email: nathans@vt.edu

Ramesh Vemulapalli, BVSc, MVSc, PhD (Co-Chair)
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Professor - Veterinary Immunology, Veterinary Science
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Prof. Dr. med. Sascha Al Dahouk, M.Sc.
BundesinstitutfürRisikobewertung
Produkthygiene und Desinfektionsstrategien
AbteilungBiologischeSicherheit
Federal Institute for Risk Assessment
Product Hygiene and Disinfection Strategies
Department of Biological Safety
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E-mail: Jacques.godfroid@uit.no

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Universidade Federal de Minas Gerais
Escola de Veterinária
Departamento de Clínica e CirurgiaVeternárias
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Dr. B R Shome
Principal Scientist
Microbial Pathogenesis and Pathogen Diversity Laboratory
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Email: brshome@gmail.com

Dr. J. Rajendhran
Assistant Professor
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School of Biological Sciences
Madurai Kamaraj University
Madurai - 625 021, India
Email: jrajendhran@gmail.com

Dr. G. B. Manjunath Reddy
Scientist In charge, PMU,
ICAR-NIVEDI,
Bengaluru- 560064, India
Email: Gbm.Reddy@icar.gov.in
Scientific Programme
### Day 1: Thursday, 17 November, 2016

**9:00 a.m. - 6:00 p.m.**  
A P Shinde Hall

**9:00 a.m. - 3:00 p.m.**  
Registration

**10:00 a.m. - 10:52 a.m.**  
Opening Ceremony

- **10:00 a.m.-10:05 a.m.**  
  Lighting of Lamp and Invocation

- **10:05 a.m.-10:08 a.m.**  
  Welcome address by Dr. S R Rao, Organizing Secretary and Advisor, DBT

- **10:08 a.m.-10:11 a.m.**  
  Address by Dr. H. Rahman, Chairman and DDG, Animal Sciences, ICAR

**10:11 a.m.-10:52 a.m.**  
Opening Remarks

- **10:11 a.m.-10:14 a.m.**  
  Prof. K. Vijay Raghavan, Chief Patron & Secretary, DBT

- **10:14 a.m.-10:27 a.m.**  
  Releases of Diagnostic Kits and Publications

- **10:27 a.m.-10:30 a.m.**  
  Remarks by Dr. Sriranganathan Nammalwar, Chairman, International Brucellosis Society

- **10:30 a.m.-10:35 a.m.**  
  Presidential address by Sh. Sudarshan Bhagat, Hon’ble Minister of State for Agriculture and Farmers welfare

- **10:35 a.m.-10:42 a.m.**  
  Inaugural address by Y. S. Chowdary Hon’ble Minister of State for Science & Technology and Earth Sciences

- **10:42 a.m.-10:45 a.m.**  
  Vote of Thanks by Dr. Padma Singh, Office secretary & Scientist D, DBT

- **10:45 a.m.-11:30 a.m.**  
  National Anthem

**10:45 a.m.-11:30 a.m.**  
Coffee Break

### Session 1: Brucella Pathogenesis & Host-pathogen interaction

**Chair**  
Dr R K Singh, Director IVRI

**Co-Chair**  
Dr. Puran Chand, Professor (Retired), LUVAS, Hisar, Haryana

**Rapporteur**  
Dr. J Rajendhran, Assistant Professor, Madurai Kamaraj University, Madurai, Tamil Nadu

**L-1**  
11:30 a.m. – 12:30 p.m.  
**Keynote Lecture:** Dr. Renee Tsolis, Professor, Department of Medical Microbiology and Immunology, University of California at Davis, Davis, California, USA

“`The Janus-faced nature of Brucella-host interactions`”
<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>12:30 p.m. – 2:00 p.m.</td>
<td>Poster Session-1 (PS1-1 to PS1-16, Human brucellosis) Lunch Break</td>
</tr>
<tr>
<td>2:00 p.m. – 3:45 p.m.</td>
<td>Oral Presentations (7 Presenters)</td>
</tr>
</tbody>
</table>

**OS1 - 1** 2:00-2:15

Characterization of an atypical *Brucella* spp. isolate from a Pac-Man Frog (*Ceratophrys ornata*) reveals characteristics departing from Classical Brucellae.

Pedro F. Soler-Llorens\(^1,2\), Chris R. Quance\(^3\), Sara D. Lawhon\(^4\), Tod P. Stuber\(^3\), John F. Edwards\(^5\), Thomas A. Ficht\(^6\), Suelle Robbe-Austerman\(^3\), David O’Callaghan\(^1,2\) and Anne Keriel\(^1,2\),

\(^1\)Inserm, U1047, UFR de Médecine, Nîmes, France; \(^2\)Université de Montpellier, U1047, Nîmes, France; \(^3\)Mycobacteria and Brucella Section, National Veterinary Services Laboratories, USDA-APHIS, Ames, IA, USA; \(^4\)Clinical Microbiology Department of Veterinary Pathobiology, College of Veterinary Medicine and Biomedical Science, Texas A&M University, College Station, TX, USA; \(^5\)Department of Veterinary Pathobiology, Texas A&M University, College Station, TX, USA.

**OS1 - 2** 2:15-2:30

Cloning, expression and characterization of the immunoreactive *Brucella suis* 18 kDa cytoplasmic protein encoded by *Brucella* Lumazine Synthase gene

Leena, G\(^1\), Rajeshwari Shome\(^2\), Isloor, S\(^3\), Nagalingam, M\(^4\), Balamurugan, V\(^5\), Reddy, G. R\(^6\), Rathnamma, D\(^7\), M.R. Jayashankar\(^8\), Veeregowda, B.M\(^9\) and H. Rahman\(^10\),

\(^1\)Associate Professor and Head, Dept of Veterinary Public Health and Epidemiology, Veterinary College, Hebbal, Bengaluru, India; \(^2\)Veterinary College, Hebbal, Bengaluru, India; \(^3\)IVRI, Hebbal, Bengaluru, India; \(^4\)NIVEDI-ICAR, Bengaluru, India; \(^5\)DDG, ICAR, New Delhi, India.

**OS1 - 3** 2:30-2:45

How the transcriptional response of the two component regulatory system BvrR/BvrS from *Brucella abortus* is affected by changes in pH.

Ruiz-Villalobos Nazareth\(^1,2\), Chaves-Olarte Esteban\(^1,2\), Moreno Edgardo\(^1,3\) and Guzman-Verr Caterina\(^1,2\),

\(^1\)Programa de Investigación en Enfermedades Tropicales, Escuela de Medicina Veterinaria, Universidad Nacional, Heredia, Costa Rica; \(^2\)Centro de Investigación en Enfermedades Tropicales, Facultad de Microbiología, Universidad de Costa Rica, San José, Costa Rica; \(^3\)Instituto Clodomiro Picado, Facultad de Microbiología, Universidad de Costa Rica, San José, Costa Rica.

**OS1 - 4** 2:45-3:00

Comparison of potential protection conferred by three immunization strategies (Protein/Protein, DNA/DNA, and DNA/Protein) against *Brucella* infection using truncated Omp2b protein in BALB/c Mice

Maryam Golshani and Saeid Bouzari

Department of Molecular Biology, Pasteur Institute of Iran, Tehran, Iran.
<table>
<thead>
<tr>
<th>Session 1: Brucellosis in India</th>
<th>Time</th>
<th>Details</th>
</tr>
</thead>
</table>
| OS1 - 5                          | 3:00-3:15 | Molecular mechanism of TIR domain-containing protein from *Brucella* (TcpB)-mediated suppression of Toll-like Receptor-4-signaling. Padmaja Jakka¹ and Girish Radhakrishnan²  
   ¹Division of Infectious Diseases, National Institute of Animal Biotechnology, Hyderabad, India; ²Division of Infectious Diseases, National Institute of Animal Biotechnology, Hyderabad, India. |
| OS1 - 6                          | 3:15-3:30 | Genome-scale reconstruction of transcriptional regulatory networks of *Brucella*  
   Udayakumar S. Vishnu¹, Jagadesan Sankarasubramanian¹, Paramasamy Gunasekaran² and Jeyaprakash Rajendhran¹  
   ¹Department of Genetics, School of Biological Sciences, Madurai Kamaraj University, Madurai – 625 021, Tamil Nadu, India; ²Pro Vice-chancellor, VIT University, Chennai-600 127, Tamil Nadu, India |
| OS1 - 7                          | 3:30-3:45 | Comparative transcriptome profile of *Brucella abortus* S19Δper infected mice  
   Khushal Singh Solanki¹, Amit Ranjan Sahu¹, Sajad Ahmad Wani¹, Ravi Kumar Gandham¹ and Pallab Chaudhuri²  
   ¹Division of Veterinary Biotechnology, ICAR-Indian Veterinary Research Institute, Izatnagar-243122, Bareilly (UP), INDIA; ²Division of Bacteriology and Mycology, ICAR-Indian Veterinary Research Institute, Izatnagar-243122, Bareilly (UP), INDIA |
|                                | 3:45 p.m. – 4:30 p.m. | Poster Session 2 (PS2-1 to PS2-6 Host-Pathogen Interactions)  
   Tea Break |

**Session 2: Human Brucellosis**

<table>
<thead>
<tr>
<th>Time</th>
<th>Details</th>
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<tbody>
<tr>
<td>4:30 p.m. – 6:00 p.m.</td>
<td>Oral Presentations (6 Presenters)</td>
</tr>
</tbody>
</table>
| OS2 - 1 | 4:30-4:45 | Human brucellosis in India: Systematised review and Meta analysis.  
   S. B. Barbuddhe¹*, Ajay D. Pathak³, Abhay V. Raorane¹, Lata Jain¹, Mamta Tigga¹, Nitin V. Kurkure², and Sandeep P. Chaudhari²  
   ¹ICAR-National Institute of Biotic Stress Management, Baronda, Raipur, 493 225 India; ²Nagpur Veterinary College, Maharashtra Animal and Fishery Sciences University, Nagpur 440006, India |
| OS2 - 2 | 4:45-5:00 | Human brucellosis: sero-prevalence and associated risk factors in Punjab  
   JPS Gill¹, JS Bedi¹, Simranpreet Kaur¹, Randhir Singh¹, Shweta¹ and DS Pooni²  
   ¹School of Public Health and Zoonoses, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141004, Punjab, India; ²Medical Superintendent, Punjab Agricultural University Hospital, Ludhiana-141004, Punjab, India |
<table>
<thead>
<tr>
<th>Session</th>
<th>Title</th>
<th>Author Details</th>
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</thead>
<tbody>
<tr>
<td>OS2 - 3</td>
<td>Surveillance of human brucellosis in France from 2005 to 2015 highlights evolution of contamination origins.</td>
<td>Ponsart C.1, Septfons A.2, Jaÿ M.1, Sotto A.3, O’Callaghan D.4, DeValk H.2, Vaillant V.2; Maurin M.5, Garin-Bastuji B.1, Mick V.1, Lavigne J.P.3,4 Mailles A.2 1ANSES, Maisons-Alfort, France; 2Agence Nationale de Sante Publique, Saint Maurice, France; 3CHU CAREMEAUX, Nimes, France; 4 INSERM U1047, Universite de Montpellier, UFR Medecine, Nimes, France; 5 CHU Grenoble Alpes, La Tronche, France.</td>
</tr>
<tr>
<td>OS2 - 4</td>
<td>Neurobrucellosis: Clinical, laboratory and epidemiological aspects</td>
<td>Smita Mangalgi and Annapurna Sajjan</td>
</tr>
<tr>
<td>OS2 - 5</td>
<td>Is brucellosis a potential cause of fever of unknown origin in Madagascar?</td>
<td>Ides Boone1, Klaus Henning2, Angela Hilbert2, Heinrich Neubauer2, Vera von Kalckreuth3, Denise Dekker1, Norbert Georg Schwarz4, GiDeok Pak1, Andreas Krüger5, Ralf Hagen5, Hagen Frickmann5, Jean Noël Heriniaina1, Raphael Rakotozandraindrainy1, Jean Philibert Rakotondrainiarivelo3, Tisry Razafindraibe2, Benedikt Hogan4, Jürgen May4, Florian Marks3, Sven Poppert4,8 and Sascha Al Dahouk1,9</td>
</tr>
<tr>
<td>OS2 - 6</td>
<td>Zoonotic impact and molecular characterization of Brucella species among human-animal interface in Cameroon</td>
<td>Abel Wade1, Maurice Boda2, Abdoukadir Souley1, Wilfred Mbacham2 1National Veterinary Laboratory (LANAVET) Annex Yaounde, Cameroon; 2University of Yaounde 1, Yaounde, Cameroon; 3 LANAVET Garoua, Cameroon</td>
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</tbody>
</table>

7.00 P.M. Dinner
# Day 2: Friday, 18 November, 2016

<table>
<thead>
<tr>
<th>Time</th>
<th>Location</th>
<th>Session 3: Epidemiology and Control</th>
</tr>
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<tbody>
<tr>
<td>9:00 a.m. - 5:30 p.m.</td>
<td>A P Shinde Hall</td>
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**Chair**

Dr. H. Rahman, Deputy Director General (Animal Health)
ICAR, Krishi Bhawan, New Delhi

**Co-Chair**

Dr. R. Shome, Principal Scientist, NIVEDI, Bengaluru

**Rapporteur**

Dr. G. K. Saikia, Professor, AAU, Khanapara, Guwahati

<table>
<thead>
<tr>
<th>L-2</th>
<th>9:00 a.m. - 10:00 a.m.</th>
<th>Keynote Lecture: Dr. José Soares Ferreira Neto, Professor, Faculty of Veterinary Medicine and Animal Husbandry, University of São Paulo, São Paulo, Brazil</th>
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<tr>
<td></td>
<td>“Epidemiological status of bovine Brucellosis and Tuberculosis in South America”</td>
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<tr>
<th>Time</th>
<th>Event</th>
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<tr>
<td>10:00 a.m. - 10:30 a.m.</td>
<td>Poster Session 3 (PS3-1 to PS3-12 Epidemiology)</td>
</tr>
<tr>
<td>10:45 a.m. - 12:30 p.m.</td>
<td>Oral Presentations (7 Presenters)</td>
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**OS3 - 1**

10:45 - 11:00

Meta-analysis reveals increased sero-prevalence rate of bovine brucellosis in India.

Sneha Saha¹, Sandip Santra², S.S. Patil³, Rajeshwari Shome⁴ and K.P. Suresh⁵

ICAR-National institute of Veterinary Epidemiology and Disease Informatics (NIVEDI) Yelahanka, Bengaluru-560064

**OS3 - 2**

11:00 - 11:15

Cost-benefit analysis of vaccination strategies for brucellosis in small ruminants in Jordan

Imadidden Musallam¹, Mahmoud Abo-Shehada² and Javier Guitian²

¹ Jordan food and drug administration (JFDA), Amman, Jordan; ² Veterinary Epidemiology, Economics and Public Health Group, Department of Production and Population Health, The Royal Veterinary College, University of London, North Mymms, Hertfordshire AL9 7TA, United Kingdom

**OS3 - 3**

11:15 - 11:30

Brucellosis Prevention & Control: Institutional Barriers & Facilitators.

Manmeet Kaur¹, Pauline Allen², Hannah Rebecca Holt³, Punam Mangtani², Satinder Bharati¹, Vivek Sagar¹, Amit Kulashri¹, Javier Guitian³ and Rajesh Kumar¹

¹ School of Public Health, Post Graduate Institute of Medical Education and Research, Chandigarh, India; ² London School of Hygiene and Tropical Medicine, London, UK; ³ Royal Veterinary College, Hertfordshire, UK

**OS3 - 4**

11:30 - 11:45

Seroprevalence and risk factors for human brucellosis in Ludhiana district in Punjab, India

Punam Mangtani¹, Wendy Beauvais¹, Hannah Holt², Amit Kulashri³, Satinder Bharti³, Vivek Sagar³, Jasbir Bedi⁴, Manmeet Kaur³, Javier Guitian², Gagndeep Singh Grover⁵ and Rajesh Kumar³

¹ London School of Hygiene and Tropical Medicine, London, UK; ² Royal Veterinary College, London, UK; ³ School of Public Health, Postgraduate Institute of Medical Education and Research, Chandigarh, India; ⁴ Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India; ⁵ Directorate of Health & Family Welfare, Govt. of Punjab, Chandigarh
<table>
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<tr>
<th>Time</th>
<th>Event Description</th>
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<tr>
<td>11:45-12:00</td>
<td><strong>OS3 - 5</strong>&lt;br&gt;Estimation of economic losses due to brucellosis in small ruminant of Uttar Pradesh&lt;br&gt;D.K. Sinha¹, D.K. Singh², S. Quereshi³, M. R. Verma⁴, Vinodh Kumar O.R. ¹, and B.R. Singh¹&lt;br&gt;¹Division of Epidemiology; ²Division of Veterinary Public Health; ³Division of Biological Standardization; ⁴Division of Livestock Economics and Statistics ICAR-Indian Veterinary Research Institute Izatnagar, Uttar Pradesh, India</td>
</tr>
<tr>
<td>12:00-12:15</td>
<td><strong>OS3 - 6</strong>&lt;br&gt;Serological Investigation Indicates Incidence of Brucellosis in Livestock along the Livestock Movement Routes in Meghalaya.&lt;br&gt;I. Shakuntala¹, S. Ghatak¹, R. Sanjukta¹, K. Puro¹, S. Das¹, A. Karam¹, K. Kakoty¹, A. Dutta¹ and A. Sen¹&lt;br&gt;¹Division of Animal Health, Indian Council of Agricultural Research (ICAR) Research Complex for Northeastern Hill Region, Ribhoi, Meghalaya, India</td>
</tr>
<tr>
<td>12:15-12:30</td>
<td><strong>OS3 - 7</strong>&lt;br&gt;Seroprevalence studies on brucellosis in cattle and buffaloes in Ludhiana distt. of Punjab, India.&lt;br&gt;Paviter Kaur¹, H. R. Holt², N. S Sharma³, J.S Bedi⁴, J. McGiven⁵, A. K Arora⁶, M. Chandra⁶, J P S Gill⁷ and J. Guitian⁹&lt;br&gt;¹,³,⁶,⁷,⁹ Department of Veterinary Microbiology, GADVSAU, Ludhiana; ²,⁸School of Public Health and Zoonosis, GADVASU, Ludhiana; ⁵ Royal Veterinary College, London; ⁷ Animal and Plant Health agency, London</td>
</tr>
<tr>
<td>12:30 p.m. – 2:00 p.m.</td>
<td><strong>Poster Session 4 (PS4-1 to PS4-21 Diagnostics)</strong>&lt;br&gt;Lunch Break</td>
</tr>
<tr>
<td>2:00 p.m. – 3:00 p.m.</td>
<td><strong>L-3</strong>&lt;br&gt;Keynote Lecture: Dr. Javier Guitian, Professor, The Royal Veterinary College, University of London, Hatfield, UK&lt;br&gt;“Barriers towards sustainable brucellosis control in endemic settings in West Africa, Middle East and South Asia”</td>
</tr>
<tr>
<td>3:00 p.m. – 4:00 p.m.</td>
<td><strong>AgResults - US$30 million Prize Competition - New Vaccine for Brucellosis (<a href="http://www.agresults.org">www.agresults.org</a> )</strong></td>
</tr>
<tr>
<td>4:00 p.m. – 4:30 p.m.</td>
<td><strong>Poster Session 5 (PS5-1 to PS5-10 Control and Eradication)</strong>&lt;br&gt;Tea Break</td>
</tr>
<tr>
<td>4:30 p.m. – 5:30 p.m.</td>
<td><strong>Panel Discussion – Control and Eradication of Animal Brucellosis</strong>&lt;br&gt;Members: Dr. Ramesh Vemulapalli, Dr. Javier Guitian, Dr. Jacques Godfroid, Dr. H Rahman, Dr S R Rao, Dr Siddiquir Rahman (Bangladesh) and Dr. Gayani Weerasooriya (Srilanka)</td>
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<tr>
<td>5:30 p.m. – 6:30 p.m.</td>
<td><strong>International Brucellosis Society Business Meeting</strong></td>
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<td>7:00 p.m.</td>
<td><strong>Gala Dinner &amp; Cultural Program</strong></td>
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### Day 3: Saturday, 19 November, 2016

<table>
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<th>Time</th>
<th>Session 4: Brucella Research in India</th>
<th>Session 5: Canine and Wildlife Brucellosis</th>
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<tbody>
<tr>
<td>9:00 a.m. - 5:30 p.m.</td>
<td>A P Shinde Hall</td>
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<tr>
<td><strong>Session 4: Brucella Research in India</strong></td>
<td></td>
<td><strong>Session 5: Canine and Wildlife Brucellosis</strong></td>
</tr>
<tr>
<td><strong>Chair</strong></td>
<td>Dr. S K Gupta, Emeritus Scientist, NII, New Delhi</td>
<td></td>
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<tr>
<td><strong>Co-Chair</strong></td>
<td>Dr. B S Chandel, Professor, Sardarkrushinagar Dantiwada Agricultural University, Gujarat</td>
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<tr>
<td><strong>Rapporteur</strong></td>
<td>Dr. G B Manjunatha Reddy, Scientist, NIVEDI, Bangalore</td>
<td></td>
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<tr>
<td><strong>9.00 am - 10.00 am</strong></td>
<td>Oral Presentations (5 Presenters)</td>
<td><strong>10:45 a.m. – 11:15 a.m.</strong></td>
</tr>
<tr>
<td><strong>OS4 - 1</strong></td>
<td>Brucellosis Research in India</td>
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<tr>
<td>9:00-9:15</td>
<td>H. Rahman, DDG Animal Sciences ICAR</td>
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<tr>
<td><strong>OS4 - 2</strong></td>
<td>Brucellosis epidemiology in India</td>
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<tr>
<td>9:15-9:30</td>
<td>Rajeshwari Shome, NIVEDI, Bangalore</td>
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<tr>
<td><strong>OS4 - 3</strong></td>
<td>Genomic insights into the host specificity of <em>Brucella</em>.</td>
<td></td>
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<tr>
<td>9:30-9:45</td>
<td>Jeyaprakash Rajendhran, MKU, Madurai, Tamil Nadu</td>
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<tr>
<td><strong>OS4 - 4</strong></td>
<td>Initiatives in vaccine development and control of bovine brucellosis in India scenario</td>
<td></td>
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<tr>
<td>9:45-10:00</td>
<td>Pallab Chaudhari, IVRI, Izatnagar, Bareilly, Uttar Pradesh</td>
<td></td>
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<tr>
<td><strong>OS4 - 5</strong></td>
<td>Point of care diagnostics for resource limited Areas: Shielding the livestock from Brucellosis</td>
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<tr>
<td>10:00 a.m. – 10:15</td>
<td>Rathnagiri Polavarapu, Genomix Molecular Diagnostic Pvt. Ltd, Hyderabad</td>
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<tr>
<td><strong>10:15 a.m. – 10:45 a.m.</strong></td>
<td>Poster Session 6 (PS6-1 to PS6-19 Brucellosis in India)</td>
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<td>Session 6: Diagnostic methods</td>
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<tr>
<td><strong>Chair</strong></td>
<td><strong>Dr. Gaya Prasad, Vice Chancellor, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut</strong></td>
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<tr>
<td><strong>Co-Chair</strong></td>
<td><strong>Dr. G Dhinakar Raj, Director, TRPVB, Chennai</strong></td>
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<tr>
<td><strong>Rapporteur</strong></td>
<td><strong>Dr. Tirumurugaan K G, Scientist, TRPVB, Chennai</strong></td>
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<tr>
<td><strong>11:30 a.m. – 12:45 p.m.</strong></td>
<td><strong>Oral Presentations (2 Presenters)</strong></td>
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<tr>
<th>OS6 - 1</th>
<th><strong>Conjugated O-polysaccharide antigen is more effective in iELISA at differentiating between sera from <em>B. abortus</em> S19 vaccinated and field infected cattle than sLPS or synthetic disaccharide antigens</strong></th>
</tr>
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<tr>
<td>11:30-11:45</td>
<td><strong>Erdelenig, L. Duncombe², L. Howells², O. Keskin¹, O.Y.Tel¹, J. McGiven²</strong></td>
</tr>
<tr>
<td></td>
<td>¹Harran University Faculty of Veterinary Medicine, Microbiology Department, Eyyubiye Campus, 63200 Sanliurfa, TURKEY; ²FAO/WHO Collaborating Centre for Brucellosis, OIE Brucellosis Reference Centre, Department of Bacteriology, Animal and Plant Health Agency (APHA), Addlestone, Surrey, KT15 3NB, UK</td>
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<tr>
<th>OS6 - 2</th>
<th><strong>Evaluation of various blood components for the molecular detection of <em>Brucella spp</em> from human.</strong></th>
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<td>11:45-12:00</td>
<td><strong>Urmita Chakraborty¹ and Satadal Das¹</strong></td>
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<td><strong>Peerless Hospital &amp; B.K. Roy Research Center, Kolkata, India.</strong></td>
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<td><strong>12:00 p.m. – 1:45 p.m.</strong></td>
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<td><strong>Poster Session 7 (PS7-1 to PS7-8 Genomics, Bioinformatics and Proteomics)</strong></td>
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<td><strong>Lunch Break</strong></td>
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<tr>
<th>Session 7: Vaccines &amp; Immunology</th>
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<tr>
<td><strong>Chair</strong></td>
<td><strong>Prof. Ramesh Vemulapalli</strong></td>
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<td><strong>Co-Chair</strong></td>
<td><strong>Dr. Rakesh Bhatnagar, JNU, New Delhi</strong></td>
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<tr>
<td><strong>Rapporteur</strong></td>
<td><strong>Dr. Pallab Chaudhari, IVRI, Izatnagar, Bareilly, Uttar Pradesh</strong></td>
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<tr>
<td>Time</td>
<td>Oral Presentations (8 Presenters)</td>
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| 1:45 p.m. – 3:30 p.m. | **Meta-analysis and advancement of brucellosis vaccinology**  
Tatiane F. Carvalho¹, João Paulo A. Haddad¹, Tatiane A. Paixão², Renato L. Santos¹  
¹Escola de Veterinaria – Universidade Federal de Minas Gerais, Belo Horizonte, Brazil; ²Instituto de Ciencias Biologicas – Universidade Federal de Minas Gerais, Belo Horizonte, Brazil. |
| OS7 - 1      | 1:45-2:00                                                                                   |
| OS7 - 2      | **Attenuated *Brucella neotomae* as a vaccine against *B. suis* challenge**  
Jain-Gupta N.¹, Tenpenny N.M.¹, Waldrop S.G.¹, Witonsky S.G.², Boyle S.M.¹ and Sriranganathan N.¹  
¹Department of Biomedical Sciences and Pathobiology, Center for Molecular Medicine and Infectious Diseases, Virginia-Maryland College of Veterinary Medicine, Virginia Tech, Blacksburg, VA 24061-0342, United States; ²Department of Large Animal Clinical Sciences, Center for Molecular Medicine and Infectious Diseases, Virginia-Maryland College of Veterinary Medicine, Virginia Tech, Blacksburg, VA 24061-0342, United States |
| OS7 - 3      | 2:00-2:15                                                                                   |
| OS7 - 4      | **Pharmacologic effects of the macrolide antibiotic tulathromycin on *Brucella melitensis* infection in open and pregnant goats.**  
S.C. Olsen¹*, P Boggiatto¹ and T. Rowan²  
¹USDA/ARS, National Animal Disease Center, Ames, IA, USA; ²Global Alliance for Livestock Veterinary Medicine, Edinburgh, Scotland |
| OS7 - 5      | 2:15-2:30                                                                                   |
| OS7 - 5      | **Immune response of calves vaccinated with *Brucella abortus* S19 or RB51 and revaccinated with RB51**  
Elaine Dorneles¹ and Andrey P. Lage²  
¹Universidade Federal de Lavras, Lavras, Brazil; ²Universidade Federal de Minas Gerais, Belo Horizonte, Brazil. |
| OS7 - 5      | 2:30-2:45                                                                                   |
| OS7 - 5      | **Immunogenicity and efficacy studies of a Glyco-conjugate vaccine developed against Bovine Brucellosis**  
K.Nagmani¹, F.Mukherjee², A. Prasad², V.S. Bahekar², B. Ramalakshmi¹, K.S.N.L. Surendra², T. Mythili³, L. Rajendra⁴, S.K. Rana³, G.K. Sharma⁵ and V.A. Srinivasan⁶  
¹Research and Development Centre, Indian Immunologicals Limited, Hyderabad 500032, Telangana, India; ²National Dairy Development Board Research and Development Laboratory, IIL Campus, Hyderabad 500032, Telangana, India; ³38 Callander Street, Thomson East Geelong, Victoria, Australia-3219; ⁴Plot No.68. Flat No. 4A, Challa Pushpa Doyen Dacha Apartments, Sri Nagar Colony, Hyderabad 500032, Telangana, India; ⁵National Dairy Development Board, Anand 388001, Gujarat, India; ⁶House No. 33, Telecom Nagar, Gachibowli, Hyderabad 500032, Telangana, India |
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<th>Session Details</th>
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| 3:00-3:15  | **OS7 - 6**  
Evaluation of the immune response against graded doses of *Brucella abortus* S19 (calfhood) vaccine in buffaloes, India  
1ICAR-National Institute of Veterinary Epidemiology and Disease Informatics, Ramagondanahalli, Yelahanka, Bengaluru-560 064; 2Intervet India Pvt. Ltd. Briahnagar, Waghli, Pune – 412207; 3Division of Animal Science, Indian Council of Agricultural Research Krishi Bhawan, New Delhi- 110 001 |
| 3:15-3:30  | **OS7 - 7**  
Characterization of the NOD-scid IL2rγnull Mouse Model to Study the Safety of *B. abortus* S19 ΔvjbR Vaccine Candidate in *Brucella*-induced Osteoarticular Disease  
Khalaf O1, Garcia D1, Chaki SP1, Ficht TA1, Arenas-Gamboa AM1  
Dept. of Veterinary Pathobiology, College of Veterinary Medicine & Biomedical Sciences, Texas A&M University, College Station, Texas, USA |
| 3:30-3:45  | **OS7 - 8**  
The application of synthetic oligosaccharides to develop a DIVA vaccine and diagnostic partnership based on the induction and detection of epitope specific anti-Brucella OPS antibodies  
J. McGiven1, L. Duncombe1, L. Howells1, N. V. Ganesh2, S. S. Mandal2, S. Sarkar2, J. M. Sadowska2, D. Bundle2  
1FAO/WHO Collaborating Centre for Brucellosis, OIE Brucellosis Reference Centre, Department of Bacteriology, Animal and Plant Health Agency (APHA), Addlestone, Surrey, KT15 3NB, UK 2 Department of Chemistry, University of Alberta, 11227 Saskatchewan Dr NW, Edmonton, Alberta T6G 2G2, Canada |
| 3:45 p.m. – 4:30 p.m. | **Poster Session 8 (PS8-1 to PS8-9 Wildlife brucellosis and World brucellosis)**  
Tea Break |
| 4:30 p.m. – 5:30 p.m. | **Student Awards & Closing Ceremony** |
Poster Presentation Schedule
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<td><strong>Poster No.</strong></td>
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| PS1-1 | Seroprevalence of human brucellosis and their work practice analysis among veterinarians in Gujarat  
Barfal Pankaj¹, Yadav Suresh², Kanani Amit³, Vyas Poonam¹, Modi HA⁴  
¹National Institute of Occupational Health, Microbiology Division, Ahmadabad 380016 Gujarat, India; ²National Institute of Occupational Health, Environmental Studies, Ahmadabad 380016 Gujarat, India; ³Animal Disease Investigation Laboratory, Polytechnic campus, Ambawadi, Ahmedabad 380015, Gujarat, India; ⁴Department of Life Sciences, Gujarat University, Ahmadabad 380009 Gujarat, India. |
| PS1-2 | Occupational risk factors associated with Human brucellosis among dairy farm workers in Gujarat  
Barfal Pankaj¹, Yadav Suresh², Kanani Amit³, Derasari Anuradha¹, Vyas Poonam¹, Modi HA⁴  
¹National Institute of Occupational Health, Microbiology Division, Ahmadabad 380016 Gujarat, India; ²National Institute of Occupational Health, Environmental Studies, Ahmadabad 380016 Gujarat, India; ³Animal Disease Investigation Laboratory, Polytechnic campus, Ambawadi, Ahmedabad 380015, Gujarat, India; ⁴Department of Life Sciences, Gujarat University, Ahmadabad 380009 Gujarat, India. |
| PS1-3 | Development of novel immunodiagnostic test for screening of human brucellosis cases using the whole cell antigens of Brucella abortus S19  
Nidhi M. Bhartiya, Ali A. Hussain, Hatim F. Daginawala, Lokendra R. Singh and Rajpal S. Kashyap  
Biochemistry Research Laboratory, Dr. G.M. Taori Central India Institute of Medical Sciences, Nagpur - 440010, India |
| PS1-4 | Seroprevalence of human brucellosis in Telangana and Andhra Pradesh  
A. Vijaya Kumar¹, N.Krishnaiah², L.Venkateswar Rao³, Y.Narasimha Reddy² and K.Kondal Reddy²  
¹College of Veterinary Science, Korutla, Karimnagar, Telangana, India; ²College of Veterinary Science, Rajendranagar, Hyderabad, Telangana, India; ³College of Veterinary Science, Proddatur, Andhra Pradesh, India |
| PS1-5 | Occupational Health Risk and Zoonotic hazards among Veterinarians  
Rajendra Palkhade¹ and Mishra SD²  
¹Laboratory Animal Facility; ²Bio-Statistics Division National Institute of Occupational Health, Indian Council of Medical Research (ICMR) Meghani Nagar, Ahmedabad-380016 |
| PS1-6 | Human brucellosis: Review of 61 cases from a tertiary care hospital of southern India  
Sudipta Patra¹, Vandana K E¹, Chaitanya T A K² and Chiranjay Mukhopadhyay¹  
¹Kasturba Medical College, Manipal, India; ²Manipal Centre for Virus Research, Manipal, India |
| PS1-7 | **Human brucellosis sero-surveillance using monoclonal antibody based blocking ELISA in high risk groups**  
G. Dhinakar Raj¹, S. Thiyagarajan¹, Maroudam V¹, Girish Radhakrishnan² and Raman M¹  
¹Translational Research Platform for Veterinary Biologicals, TANUVAS, Chennai, India; ²National Institute of Animal Biotechnology, Hyderabad, India. |
| PS1-8 | **Evaluation of Fluorescence Polarization Assay Technology as a Human Brucellosis Diagnostic in Georgia**  
Marine Ramishvili¹, Stella Avdalova¹, Ryan Arner², Nino Trapaidze¹, Mikeljon P. Nikolich³ and Ekaterine Adeishvili¹  
¹National Center for Disease Control and Public Health, Tbilisi, Georgia; ²Metabiota, Inc., Washington DC, USA; ³Walter Reed Army Institute of Research, Silver Spring, MD, USA |
| PS1-9 | **Ophthalmic brucellosis, a case report from India.**  
Anindita Sen¹, Manas Pal² and Satadal Das³  
¹MGM Medical College & LSK Hospital, Kishanganj, India; ²Regional Institute of Ophthalmology, Medical College, Kolkata, India; ³Brucella Research Lab, Peerless Hospital & B K Roy Research Centre, Kolkata, West Bengal, India. |
| PS1-10 | **Anti-lipopolysaccharide antibodies against brucellosis among risk groups in Central India**  
Chhaya Sonekar, S. P. Chaudhari, Rajpal Singh Kashyap, N. V. Kurkure, S. P. Awandkar, W. A. Khan, Vishvas Sherkhane, Amol Sahare, Smita Bhyor, Neha Paliwal and Shipshri Shinde  
Center for Zoooses, Nagpur Veterinary College, Maharashtra Animal & Fishery Sciences, Nagpur 440006 India |
| PS1-11 | **Human brucellosis: A study on seroprevalence and potential risk factors among the occupational high risk groups**  
Shome, R.¹, Suresh, K. P.¹, Krithiga, N.¹, Padmashree, B. S.¹, Reshma, K.¹, M.¹, Aradhya, Y¹, Kumar, C², Ranjitha, S., Shome, B. R.¹ and Rahman, H.³  
¹ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (ICAR-NIVEDI) Bengaluru, India; ²Central Coastal Agricultural Research Institute (CCARI), Goa, India; ³Indian Council for Agricultural Research (ICAR), New Delhi, India |
| PS1-12 | **Prevalence of Neurobrucellosis from Central India: A Hospital based study**  
Pallavi A. Tembhurne, Ajaz Ali, Hatim F. Daginawala, Lokendra R. Singh, Rajpal S. Kashyap  
Biochemistry Research Laboratory, Dr. G. M. Taori Central India Institute of Medical Research, Nagpur, India |
| PS1-13 | **Rapid diagnosis and treatment follow up of human brucellosis by SYBR green based quantitative real-time polymerase chain reaction (qRT-PCR)**  
Shalini Thakur¹, JS Bedi², Randhir Singh¹, JPS Gill¹, AK Arora² and Neeraj Kashyap³  
¹School of Public Health and Zoonoses, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141004, Punjab, India; ²Department of Veterinary Microbiology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141004, Punjab, India; ³Department of Animal Breeding and Genetics, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141004, Punjab, India. |
| PS1-14 | **Human Brucellosis: Seroprevalence, diagnosis and treatment.**  
Chandel B S¹, Chauhan H C¹, Patel K B¹, Patel B K¹, Patel A C¹, Patel S S¹, Shrimali M D¹ and Shah Mayank²  
¹Department of Animal biotechnology and Microbiology, COVSc&AH, SDAU, Sardarkrushinagar-Dantiwada, Gujarat, India. ²MD(Medicine), Shah’s Medical and Cardiac Hospital, Palanpur-385001 - Gujarat - India |
| PS1-15 | **Discussion of the rationale for urgent need of a national study for accurate nationwide estimates of human brucellosis in India**  
S. Yadav¹, Sanjeev Gupta¹ and Karuna Yadav²  
¹National Institute of Occupational Health (ICMR), Meghani Nagar, Ahmedabad, Gujarat, India; ²Amity Institute of Virology and Immunology, Amity University, Noida, UP, India |
| PS1-16 | **Neurobrucellosis – Microbiological and clinical evaluation**  
Nagarathna S¹, Sayani Maji¹, Veenakumari HB¹, Netravathi M², Rajeshwari Shome³, Ravikumar R¹ and Satish Chandra P²  
¹Department of Neuromicrobiology NIMHANS, Bangalore, Karnataka, India; ²Department of Neurology NIMHANS, Bangalore, Karnataka, India; ³NIVEDI, Senior Scientist, Bangalore, Karnataka, India |
### Session 2: Host-Pathogen Interactions (6 Presentations)

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<td>PS2-1</td>
<td><strong>Patho-physiological response of experimentally infected mice with <em>Brucella abortus</em> S19 vaccine and S19Δper mutant strain</strong>&lt;br&gt;Khushal Singh Solanki¹, Bipin Kumar², Pawan Kumar³, Dheeraj Pal⁴, Monalisa Sahoo³, U K De², K P Singh³, T K Goswami⁴ and Pallab Chaudhuri⁵&lt;br&gt;&lt;sup&gt;¹Division of Veterinary Biotechnology; ²Division of Veterinary Medicine; ³Division of Veterinary Pathology; ⁴Immunology Section; ⁵Division of Bacteriology and Mycology, ICAR-Indian Veterinary Research Institute, Izatnagar-243122, Bareilly (UP), INDIA**</td>
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<tr>
<td>PS2-2</td>
<td><strong>Infection of Human Placental Trophoblast by <em>Brucella papionis</em>.</strong>&lt;br&gt;Karellen B. García-Méndez¹, Vilma Arce-Gorvel², Jean-Pierre Gorvel², David O ‘Callaghan D¹ and Anne Keriel¹.&lt;br&gt;&lt;sup&gt;¹Inserm U1047, Université de Montpellier, Nîmes France; ²Centre d’Immunologie de Marseille-Luminy, Aix-Marseille Université UM2**</td>
</tr>
<tr>
<td>PS2-3</td>
<td><strong>Omp31 plays an important role on outer membrane properties and intracellular survival of <em>Brucella melitensis</em> in murine macrophages and HeLa cells</strong>&lt;br&gt;Verdiguel-Fernández, L¹, Oropeza-Navarro R², Castañeda-Ramírez A³ and Verdugo-Rodríguez A¹.&lt;br&gt;&lt;sup&gt;¹Laboratorio de Microbiología Molecular. Departamento de Microbiología e Inmunología, Facultad de Medicina Veterinaria y Zootecnia, UNAM, Ciudad de México; ²Departamento de Microbiología Molecular, Instituto de Biotecnología, UNAM, Cuernavaca Morelos; ³Departamento de Zootecnia, Universidad Autónoma de Chapingo, Estado de México**</td>
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<tr>
<td>PS2-4</td>
<td><strong>Chronic low grade Th1 inflammation generated by <em>Brucella</em> infection induces selective alterations of marginal zone macrophages in spleen</strong>&lt;br&gt;Jean-Jacques Letesson¹, Arnaud Machelart¹, Abir Khadrawi¹ and Eric Muraille².&lt;br&gt;&lt;sup&gt;¹Unité de Recherche en Biologie des Microorganismes, Laboratoire d’Immunologie et de Microbiologie, Université de Namur. Namur, Belgium; ²Laboratoire de Parasitologie, Université Libre de Bruxelles, Campus Erasme, Bruxelles. Belgique**</td>
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<tr>
<td>PS2-5</td>
<td><strong>SytV gene silencing and its effect on early stages in phagocytosis in human macrophages infected by <em>Brucella melitensis</em></strong>&lt;br&gt;Ordoñez-López L¹, Oropeza-Navarro R², Castañeda-Ramírez A³,González-Noriega A⁴, Verdugo-Rodríguez A¹.&lt;br&gt;&lt;sup&gt;¹Departamento de Microbiología e Inmunología, Laboratorio de Microbiología Molecular, Facultad de Medicina Veterinaria y Zootecnia, UNAM.Ciudad de México; ²Departamento de Microbiología Molecular, Instituto de Biotecnología, UNAM.Cuernavaca, Morelos; ³Departamento de Zootecnia, Universidad Autónoma de Chapingo, Estado de México; ⁴Departamento de Biología Celular y Fisiología, Instituto de Investigaciones Biomédicas, UNAM. Ciudad de México.**</td>
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MbfA, an Iron Export System in Brucella Providing a Novel Iron-mediated Mechanism for Resistance Against the Immune System


1School of Biological Sciences, or 4Food & Nutritional Sciences, University of Reading, Whiteknights, Reading, RG6 6AJ, UK; 2Department of Biomedical Sciences, Defence Science and Technology Laboratory, Porton Down, Salisbury SP4 0JQ, UK; 3Department of Chemistry, University of Durham, Durham, DH1 3LE; 5Centre for Biological Sciences, University of Southampton, Southampton SO17 1BJ, UK.
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| PS3-1 | **Assessment of cattle brucellosis surveillance from 2011-2013 in the Republic of Armenia**  
Hovik S. Batikyan  
The “Food Safety State Service” of the Ministry of Agriculture of the Republic of Armenia, Yerevan, Republic of Armenia |
| PS3-2 | **Herd and Individual Prevalence of Brucellosis in Georgia**  
Tengiz Chaligava, Otar Parkadze, Lasha Avaliani  
National Food Agency of the Ministry of Agriculture of Georgia, Tbilisi, Georgia |
| PS3-3 | **Determination of the Geographical Distribution of Brucellosis in Georgia Using Geographic Information Systems**  
S. Chubinidze, M. Grdzelidze, M. Ramishvili, Sh. Tsanava, P. Imnadze  
National Center for Disease Control and Public Health, Tbilisi, Georgia |
| PS3-4 | **Sero-prevalence of Brucellosis in Mithun**  
Sakshi Dubey¹, Bhoj R Singh¹, Vidya Singh², DK Sinha¹ and Vinodh Kumar OR¹  
¹Division of Epidemiology, and ²Division of Pathology, Indian Veterinary Research Institute, Izatnagar, Bareilly-243122, India |
| PS3-5 | **Unusual productive and reproductive behaviour in cows affected with brucellosis – a field study in Odisha, India**  
Sourabh Ranjan Hota¹, Niranjana Sahoo¹ and Sarangadhar Satpathy²  
¹Department of Epidemiology and Preventive Medicine, College of Veterinary Science and Animal Husbandry Orissa University of Agriculture and Technology Bhubaneswar-751003, Odisha, INDIA, ²Additional District Veterinary Officer, Koraput, Odisha, INDIA |
| PS3-6 | **Epidemiological investigation and molecular detection of *Brucella* spp. in cattle at Mymensingh district of Bangladesh**  
Md. Ariful Islam, Ismail Hossain, Mst Minara Khatun, Sukumar Saha  
Department of Microbiology & Hygiene, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh |
| PS3-7 | **Seroprevalence of brucellosis in goats at the selected areas of Mymensingh district**  
Department of Microbiology & Hygiene, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh |
| PS3-8 | **Risk analysis of concurrent occurrence of Brucellosis and Infectious Bovine Rhinotracheitis in organized dairy farms**  
P. Krishnamoorthy, Rajeswari Shome, S.S. Patil, G. Govindaraj, B.R. Shome  
ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), Bengaluru, India |
| PS3-9 | Molecular Epidemiology of *Brucella abortus* isolated from cattle in Brazil, 2009 – 2013  
Mayra Silva Oliveira¹, Elaine Maria Seles Dorneles¹,², Paulo Martins Soares Filho³,  
Antônio Augusto Fonseca Junior³, Lívia Orzil³, Patrícia Gomes de Souza³, Andrey Pereira Lage¹  
¹Universidade Federal de Minas Gerais, Belo Horizonte, Brazil; ²Universidade Federal de Lavras, Lavras, Brazil; ³Laboratório Nacional Agropecuário, Pedro Leopoldo, Brazil |
| PS3-10 | Clinico and Molecular Epidemiological Characteristics study of Brucellosis in Animals.  
Kirit B Patel¹, Chauhan H C², Patel B K², Patel S S², Shrimali M D², Kala J k², Patel Maulik G², Rajgor M², Shome Rajeshwari³ and Chandel B S²  
¹Junior Research Fellow, DBT network project on Brucellosis, Department of Animal Biotechnology and Microbiology, COVSc&AH, SDAU, Sardarkrushinagar-Dantiwada, Gujarat, India; ²Department of Animal biotechnology and Microbiology, COVSc&AH, SDAU, Sardarkrushinagar-Dantiwada, Gujarat, India; ³Principal Scientist, NIVEDI, Bengaluru, India |
| PS3-11 | Identification of potential risk factors for bovine brucellosis in organized farms of Karnataka, India  
Shome, R.¹, Suresh, K. P.¹, Krithiga, N.¹, Mangadevi, N.¹, Padmashree, B. S. ¹, Reshma, K.¹, Nagalingam, M.¹, Shome, B. R.¹ and Rahman, H.²  
¹ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (ICAR-NIVEDI) Bengaluru, India; ²Indian Council for Agricultural Research (ICAR), New Delhi, India |
| PS3-12 | Bovine brucellosis: Evaluation of brucellosis management practices and vaccination campaign in two districts of Buenos Aires Province, Argentina.  
Aznar, M. N.¹,², Arregui, M.², Irastorza, I.³, Rocha, P.⁴, Saegerman, C.⁵, Samartino, L.²  
¹INTA/University of Liège, Argentina/Belgium, ²INTA, Hurlingham, Argentina, ³SENASA Bransden, Buenos Aires, Argentina, ⁴Sociedad Rural de Navarro, Buenos Aires, Argentina, ⁵University of Liège, Liège, Belgium |
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| PS4-1 | Serological, Bacteriological, Molecular Techniques for Diagnosis of Brucellosis in sheep  
Department of Animal biotechnology and Microbiology, COVSc&AH, SDAU, Sardarkrushinagar-Dantiwada, Gujarat, India. |
| PS4-2 | Differential diagnostic methods to identify Rose Bengal false positive samples  
K. Goginashvili, T. Tigilauri, N. Toklikishvili, M. Donduashvili  
Laboratory of the Ministry of Agriculture, Tbilisi, Georgia |
| PS4-3 | A pilot study evaluation of lateral flow assay – a point of care diagnostic for brucellosis  
1Karnataka Veterinary Animal and Fisheries Sciences University (KAVFSU), Hebbal, Bengaluru-560024, Karnataka, India;  
2ICAR- National Institute of Veterinary Epidemiology and Disease Informatics (ICAR-NIVEDI), Yelahanka, Bengaluru-560064, Karnataka, India. |
| PS4-4 | Evaluation of Usage Indirect ELISA Using Antigens from Two Different *Brucella* Strains in Serological Diagnosis of *Brucella canis* infection  
Oktay KESKİN, Sevil Erdenliğ Gürbilek, Osman Yaşar TEL  
1 Harran University, Faculty of Veterinary Medicine Microbiology Department, 63200, Şanlıurfa, Turkey |
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1 Instituto Agroalimentario - Centro de Investigación y Tecnología Agroalimentaria de Aragón (IA2-CITA), Zaragoza, Spain;  
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1National Dairy Development Board, R&D Laboratory, Gachibowli, Hyderabad 500032, Telangana, India;  
2National Dairy Development Board, Anand 388001, Gujarat, India;  
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<td><strong>Federal Institute for Risk Assessment, Berlin, Germany</strong></td>
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<td>(^1)Translational Research Platform for Veterinary Biologicals, Centre for Animal Health Studies, TANUVAS, MMC, Chennai, Tamil Nadu, India; (^2)ICAR–NIVEDI, Ramagondanahalli, Yelahanka, Bengaluru, Karnataka, India; (^3)Deputy Director General (Animal Science), ICAR, Krishi Bhavan, New Delhi, India</td>
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<td>(^1)Department of Veterinary Preventive Medicine; (^2)Department of Veterinary Public Health and Epidemiology; (^3)Department of Animal Biotechnology, Madras Veterinary College, Chennai, India; (^4)Department of Veterinary Public Health and Epidemiology, Veterinary College Research Institute, Orathanadu, India</td>
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<td>(^1)Genomix Molecular Diagnostics Pvt.Ltd, 5-36/207, Prashanthinagar, Kukatpally, Hyderabad- 500072, India; (^2)Department of Biotechnology, Ministry of Science &amp; Technology, Block-2, CGO Complex, Lodi Road, New Delhi; (^3)ICAR- National Institute of Veterinary Epidemiology and Disease Informatics (ICAR-NIVEDI) Ramagondanahalli, Post Box No. 6450, Yelahanka, Bengaluru-560064, Karnataka, India; (^4)Indian Council of Agricultural Research, Krishi Bhawan, New Delhi- 110 001</td>
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1Genomix Molecular Diagnostics Pvt.Ltd, 5-36/207, Prashanthinagar, Kukatpally, Hyderabad, Andhra Pradesh. 2 Department of Biotechnology, Acharya Nagarjuna university, Nagarjuna nagar, Guntur, Andhra Pradesh

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Matías Arregui1, Luis Samartino1,2
1Pathobiology, National Institute of Agriculture Technology (Veterinary Research Center), Hurlingham, Buenos Aires, Argentina; 2Veterinary School, University of Salvador, Pilar, Buenos Aires, Argentina

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J. McGiven1, L. Duncombe1, L. Howells1, N. V. Ganesh2, S. S. Mandal2, S. Sarkar2, J. M. Sadowska2, D. Bundle2
1 FAO/WHO Collaborating Centre for Brucellosis, OIE Brucellosis Reference Centre, Department of Bacteriology, Animal and Plant Health Agency (APHA), Addlestone, Surrey, KT15 3NB, UK, 2 Department of Chemistry, University of Alberta, 11227 Saskatchewan Dr NW, Edmonton, Alberta T6G 2G2, Canada
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¹Department of Veterinary Preventive Medicine, Madras Veterinary College, TANUVAS, India; ²Translational Research Platform for Veterinary Biologicals, TANUVAS, India; ³Division of Medicine, Indian Veterinary Research Institute, Uttar Pradesh, India; ⁴Vaccine Research Centre-Viral Vaccines, TANUVAS, India; ⁵Centre for Animal Health Studies, TANUVAS, India. |
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¹Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Ciudad de México, México; ²Departamento de Biología Celular, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Ciudad de México, México; ³Centro de Investigación en Ciencia Aplicada y Tecnología Avanzada, Instituto Politécnico Nacional, Querétaro, México |
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Nur Mohammad Shafy¹, B. S. Ahmed¹, Roma Rani Sarker¹, Md. K. S. A. Millat¹, Md. Tuhin Hasan², P.K. Bhattacharjee¹, A. Chakrabarty¹ and Md. Siddiquur Rahman¹  
¹Department of Medicine, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh; ²Military Farm, Chittagong |
| PS8-7     | Seropositivity rates of Brucella spp. infection in dogs in Southeast Provinces of Turkey  
Osman Yaşar TEL, Sevil Erdenliğ Gürbilekand Oktay Keskin  
Harran University, Faculty of Veterinary Medicine Microbiology Department, 63200, Şanlıurfa, Turkey |
| PS8-8 | The role of serological testing in a brucellosis control program in Geghashen in Kotayk Marz, Republic of Armenia  |
|       | Pertsh Tumanyan  |
|       | The Republican Veterinary-Sanitary and Phytosanitary Center of Laboratory Services, SNCO, Yerevan, Republic of Armenia |

| PS8-9 | First Discovery of Brucella Infection in Georgian Bats  |
|       | L. Urushadze¹,², Y. Bai³, L. Osikowicz³, C. McKee³, I. Kuzmin⁴, A. Kandaurov², P. Imnadze¹ and M. Kosoy³  |
|       | ¹Lugar Center, National Center for Disease Control and Public Health of Georgia (NCDC), Tbilisi, Georgia; ²Ilia State University, Tbilisi, Georgia; ³Centers for Disease Control and Prevention, Fort Collins, CO, USA; ⁴Centers for Disease Control and Prevention, Atlanta, GA, USA |

| PS8-10 | Monitoring of brucellosis in wild boars in 2013-2014 in ukraine  |
|        | Alekseeva H., Petrenko O. and Nevolko O.  |
|        | State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise, (SSRILDVSE), 30 Donetska str.Kyiv, Ukraine |
Key Note Lecture

L1 - L3
L-1. The Janus-faced nature of Brucella-host interactions
Dr. Renee Tsolis, Professor
Department of Medical Microbiology and Immunology, University of California at Davis, Davis, California, USA

Brucella abortus can persist in the mononuclear phagocyte system, evading the immune system and eliciting only a mild granulomatous response. However in pregnant animals, it can grow rapidly in the placenta, causing necrotizing inflammation and triggering abortion of the fetus. This presentation will review the current state of knowledge on how a single pathogen can cause two such disparate disease presentations and present results on how one virulence factor, the type IV secretion system, plays a role in this process.

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L-2. Epidemiological status of bovine brucellosis and tuberculosis in South America

José Soares Ferreira Neto

Collaborating Center for Animal Health, Faculty of Veterinary Medicine and Animal Science, University of São Paulo, Brazil

South America (SA) comprises 13 independent nations and six territories linked to European countries; six of them are islands. It hosts 35% of the world cattle population and is an important dairy producer and a major beef exporter. About 76% of these animals are concentrated in Brazil and Argentina.

Bovine brucellosis and tuberculosis are endemic throughout the continent, except in Suriname, French Guiana and islands, where the bovine population is very small or simply does not exist. The South American countries have a long experience in controlling these diseases, but more organized strategies and attuned to the OIE recommendations emerged since the early 2000s. Usually the programs are initiated by vaccination against brucellosis followed by the accreditation of free herds, culminating with the implementation of surveillance systems. Often programs against brucellosis precede the programs against tuberculosis.

Regarding bovine brucellosis, the situation is heterogeneous among countries and some of them also feature internal heterogeneities. Chile and Uruguay are running eradication strategies; they have very low prevalence and the programs’ information is well documented and easily accessible. Argentina has a moderate prevalence and information about the program is available. Colombia is developing an aggressive program of accreditation of free herds and free areas, maintains updated information on the official websites, but the prevalence of the disease in the country is not well characterized. The epidemiological situation of the disease in Brazil is well described, it is very uneven amongst regions and vaccination programs implemented by states have produced encouraging results. The information available allows to say that in other countries the domestic situation is also heterogeneous and national studies are necessary to properly plan the disease control and eradication.

Bovine tuberculosis in SA is associated with larger intensive dairy farms and the epidemiological situation among countries is quite different. Uruguay presents a very low prevalence and is running the eradication phase, with well-documented and available information. Chile and Argentina have good surveillance systems, but the prevalence is still high, especially in Chile and in certain regions of Argentina. Colombia is developing an aggressive accreditation program of free herds and free areas, but the disease situation is not properly characterized in the country. The epidemiological situation of the disease in Brazil is well described, with very low prevalence in beef production regions and higher in dairy basins; the implementation of surveillance systems is still incipient in the states. In other countries the internal situation seems to be quite heterogeneous and national studies are essential to properly plan the disease control and eradication.

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Control of ruminant brucellosis has proved elusive in many areas, particularly where Brucella melitensis predominates, such as the Middle East. Vaccination of the ruminant reservoir is the backbone of brucellosis control. Reasons why brucellosis incidence remains high in countries where control programs have been in place for years include poor compliance due to concerns over safety of live vaccines, perceived lack of cost effectiveness of vaccination and limited vaccine availability. Limiting factors for the success of control programs in these settings are not only technical and include the perceptions and motivations of livestock keepers and veterinarians and their willingness to comply.

Emerging livestock systems such as the periurban dairy sector that is expanding in many African countries to provide milk and dairy products to an increasingly urban and affluent population present another challenge for brucellosis control. Although limited, some evidence suggests that brucellosis is becoming established as endemic at high levels in these systems in countries where official, sustained control programs are lacking. Given the role of these farms as providers of milk and dairy products and the inadequate enforcement of food safety regulations the endemicity of brucellosis in this system poses a major public health challenge. Lack of financial resources to sustain a vaccination effort, limited capacity of local veterinary services and logistical and regulatory barriers that affect the supply of vaccines remain key barriers towards effective control.

This presentation gives an overview of current challenges for brucellosis control in 3 different settings: West African periurban dairy farms, small ruminant flocks on the Middle East and dairy farms in India Punjab. Despite their differences, the three systems share some common characteristics, namely: i) that they are low resource-settings where livestock production is critical for local livelihoods, ii) available information suggests brucellosis is endemic in the ruminant populations at high levels and iii) conditions that have made brucellosis control programs successful in other settings are largely absent.

Building on results of previous and ongoing field and simulation studies in these three contexts, conclusions that are relevant for brucellosis control in these and other resource-scarce settings are drawn, in particular, the role of intangibles such as farmers’ willingness to engage with the programs and unknowns such as the role of different species in systems where interactions between different hosts are common are discussed.

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Oral Abstracts
Session 1: Brucella Pathogenesis & Host-pathogen interaction

Oral Presentations

OS1-1. Characterization of an atypical Brucella spp. isolate from a Pac-Man Frog (Ceratophrys ornata) reveals characteristics departing from Classical Brucellae

Pedro F. Soler-Lloréns1,2, Chris R. Quance3, Sara D. Lawhon4, Tod P. Stuber3, John F. Edwards4, Thomas A. Ficht5, Suelee Robbe-Austerman3, David O’Callaghan1,2 and Anne Keriel1,2

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Brucella are highly infectious bacterial pathogens responsible for brucellosis, a frequent worldwide zoonosis. The Brucella genus has recently expanded from 6 to 11 species, all of which were associated with mammals; The natural host range recently expanded to amphibians after some reports of atypical strains from frogs. Here we describe the first in depth phenotypic and genetic characterization of a Brucella strains isolated from a frog. Strain B13-0095 was isolated from a Pac-Man frog (Ceratophyurus ornata) at a veterinary hospital in Texas and was initially misidentified as Ochrobactrum anthropi. We found that B13-0095 belongs to a group of early-diverging brucellae that includes Brucella inopinata strain BO1 and the B. inopinata-like strain BO2, with traits that depart significantly from those of the ‘classical’ Brucella spp. Analysis of B13-0095 genome sequence revealed several specific features that suggest that this isolate represents an intermediate between a soil associated ancestor and the host adapted ‘classical’ species. Like strain BO2, B13-0095 does not possess the genes required to produce the perosamine based LPS found in classical Brucella, but has a set of genes that could encode a rhamnose based O-antigen. Despite this, B13-0095 has a very fast intracellular replication rate in both epithelial cells and macrophages. Finally, another major finding in this study is the bacterial motility observed for strains B13-0095, BO1 and BO2, which is remarkable for this bacterial genus.

This study thus highlights several novel characteristics in strains belonging to an emerging group within the Brucella genus. Accurate identification tools for such atypical Brucella isolates and careful evaluation of their zoonotic potential are urgently required.

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OS1-2. Cloning, expression and characterisation of the immunoreactive *Brucella suis* 18 kDa cytoplasmic protein encoded by *Brucella* Lumazine Synthase gene

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*Brucella* species are the causative agents of brucellosis in many animal and humans. Serological tests for the diagnosis are based on the detection of anti smooth lipopolysaccharide (LPS) antibodies but not against rough LPS. Therefore, antigen/s shared by all the *Brucella* species could be significant for diagnostic purposes. *Brucella* Lumazine Synthase (BLS) gene that encodes a 18 kDa cytoplasmic protein was cloned into pET-32a expression vector and expressed in *E. coli* (DH5a cells). The 6x histidine tagged recombinant protein (rBLS) was extracted and sequence analysis revealed a total of 158 amino acids constituting 71 hydrophobic, 27 hydrophilic, 15 basic and 18 acidic amino acid residues. Lysates of the induced and non-induced bacteria analyzed by SDS-PAGE revealed expression of the rBLS with a relative molecular weight of 36 kDa. Purification of rBLS protein with Ni-NTA affinity chromatography and western blot analysis revealed that the recombinant protein had good immunoreactivity. Western blot with infected bovine, sheep & goat, swine, canine and human sera samples suggested that rBLS might be of value as an antigen for the serological diagnosis of brucellosis in different mammals.

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OS1-3. How the transcriptional response of the two component regulatory system BvrR/BvrS from *Brucella abortus* is affected by changes in pH.

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Several two components systems (TCS) have been described in the *Brucella*. One of the most studied is the TCS BvrR/BvrS. This system is linked with *Brucella* virulence favoring adaptation from the extracellular to the intracellular milieu and vice versa. TCS BvrR/BvrS is associated with regulation of expression of genes related to virulence, carbon metabolism, nitrogen metabolism, synthesis of lipopolysaccharide, synthesis of outer membrane proteins and transcriptional regulator proteins. Finding environmental factors triggering TCS BvrR/BvrS response is therefore relevant. Changes in pH had been described as an activator of TCS orthologues in other alpha-\textit{Proteobacteria}. Kinetics of \textit{bvrR} promoter activity \textit{in vitro} and in cell culture macrophages Raw 264.7 was assessed by measuring luciferase activity from a transcriptional fusion of the \textit{bvrR} promoter and the luciferase genes \textit{luxA-luxB} at different times during bacterial growth.

Increase in acid medium condition induced low levels of luciferase activity, resulting in down regulation of \textit{bvrR} promoter \textit{in vitro} as compared to a neutral pH. The highest signal of promoter activation during Raw 264.7 macrophages infection was detected at 24 hours post-infection, compared with 0, 4, and 48 hours post-infection. This indicates that, acidification of *Brucella* environment causes down regulation of \textit{bvrR} promoter \textit{in vitro}, and this is consistent with the data obtained in Raw 264.7 macrophages infection and pH changes of the vacuole containing *Brucella* during intracellular trafficking.

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OS1-4. Comparison of potential protection conferred by three immunization strategies (Protein/Protein, DNA/DNA, and DNA/Protein) against *Brucella* infection using truncated Omp2b protein in BALB/c Mice

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In the present study, immunogenicity and protective efficacy of the *Brucella* truncated form of the *Brucella* Omp2b was evaluated in BALB/c mice using Protein/Protein, DNA/DNA and DNA/Protein vaccine strategies. The truncated form of the Omp2b has been designed based on the prediction of the conserved areas and the strong T cell epitopes using bioinformatics tools. The 3D model of the tOmp2b was predicted and validated using computational approaches. The predicted 3D model had a high confidence value and it was with correct topology. Moreover, structural analysis of the predicted model indicated that the model is stable and within the range of native proteins of the similar size. Immunization of mice with three vaccine regimens elicited a strong specific IgG response (IgG2a titers over IgG1) and provided Th1-oriented immune responses (IFN-γ levels over IL-10 and IL-4). Vaccination of mice with the DNA/Pro regimen induced higher levels of IFN-γ/IL-2 and conferred more protection levels against *B. melitensis* and *B. abortus* challenge than did the protein or DNA alone. In conclusion, tOmp2b is able to stimulate specific immune responses and to confer cross protection against *B. melitensis* and *B. abortus* infection. Therefore, it could be introduced as a new potential candidate for the development of a subunit vaccine against *Brucella* infection.

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OS1-5. Molecular mechanism of TIR domain-containing protein from Brucella (TcpB)-mediated suppression of Toll-like Receptor-4 signaling.

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Toll-like receptors (TLRs) are essential components of innate immune response, which is the first level of host defense against invading microorganisms. TLRs constitute an extracellular leucine rich repeat domain that binds the conserved pathogen associated molecular patterns and an intracellular TIR domain that interacts with TIR domain containing adaptor proteins to initiate the signaling process. Activation of TLR signaling by PAMPs leads to the activation of transcription factors including NF-κB that in turn triggers expression of many pro-inflammatory cytokine genes. Subsequent secretion of pro-inflammatory cytokines activates innate immune cells that lead to various anti-microbial responses and promotes adaptive immunity.

Microorganisms have developed various defense mechanisms to suppress TLR signaling to facilitate their survival in the host. One of those strategies involves the interference of TLR signaling pathways using microbe encoded TIR domain-containing proteins. Brucella spp are infectious intracellular bacteria that affect humans as well as domestic and wild animals, leading to significant impact on public health and animal industry. Brucella spp. encodes a cell permeable TIR domain-containing protein (TcpB) that impairs host innate immunity mediated by TLR2 and TLR4 signaling. TcpB inhibits cytotoxic T cell-mediated killing of Brucella-infected cells and activates unfolded protein response in macrophages. TcpB associates with microtubules and modulates the dynamics of microtubule assembly, which correlates with the TLR suppression properties. TcpB targets the TLR2 and 4 adaptor protein TIRAP, where TcpB induces the ubiquitination and subsequent degradation of TIRAP. However, TcpB does not possess ubiquitin ligase property for ubiquitination of TIRAP. The mechanism by which TcpB promotes the enhanced ubiquitination and degradation of TIRAP that attenuates the TLR4 signaling will be presented.

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OS1-6. Genome-scale reconstruction of transcriptional regulatory networks of Brucella

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Transcription regulation is the one of the main mechanisms in prokaryotes for quickly switching their metabolism in changing environments. Transcriptional regulatory networks (TRNs) are the complex systems that help microorganisms to adapt to the internal changes and the changes they encounter in the environment. In this study, we constructed the TRNs of the zoonotic pathogen, Brucella, based on the complete genome sequences of nine Brucella sp. We applied a workflow for generating large-scale TRN models that integrates comparative genomics data, global gene expression analyses, and intrinsic properties of transcription factors (TFs). The inferred TRN for Brucella comprises of 57 gene clusters and 1325 predicted protein-DNA interactions. Cluster 1 and 6 were found to have more number of genes. BMEI1294, a crp family transcriptional factor and BMEI1190, a nrdR family transcriptional factor are regulating these two clusters. Both of these TFs are transcriptional repressors. We found that ~70% of the predicted gene clusters in this TRN are involved in the central metabolism of carbohydrates, amino acids, and fatty acids; two-component systems; quorum sensing; beta-lactam resistance and virulence.

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OS1-7. Comparative transcriptome profile of *Brucella abortus* S19\(\Delta\)per infected mice

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Brucellosis is caused by *Brucella abortus*, a Gram negative cocoobacilli that mainly affects large ruminants and also infectious to human beings. Control of animal disease through vaccination is a way to protect human beings as stamping out is not a choice in Indian scenario. WHO recommends the use of *B. abortus* S19 for managing bovine brucellosis, but it is abortifacient and infectious to humans. Recently, *Brucella abortus* S19\(\Delta\)per, a perosamine synthetase gene mutant of S19 has been reported with improved properties of safety, potency and DIVA capability to serve as an alternate vaccine candidate for the control of brucellosis in large animals. However, the molecular changes leading to attenuated virulence and pathogenicity remains poorly understood. In this study, the global gene expression profile in spleen of BALB/c mice infected with *B. abortus* S19 and *Brucella abortus* S19\(\Delta\)per was evaluated 15 days post infection. Analysis of RNA-Seq data revealed a total 2924 differentially expressed genes (DEGs) in S19\(\Delta\)per in comparison to S19 parent strain (1574 up regulated & 1350 down regulated). On Gene ontology (GO) analysis, processes of mitosis were found to be most enriched among the upregulated DEGs suggesting induction of cell proliferation and maturation by S19\(\Delta\)per in animals. Downregulated DEGs were enriched among the innate and adaptive immune responses (leukocyte activation, cell migration, cytokine production, chemotaxis, phagocytosis, IFN-\(\gamma\) production, apoptosis) and cell metabolism (related to protein and phosphate metabolic process) suggested that S19\(\Delta\)per may use common mechanism to other smooth brucellae to facilitate intracellular survival and multiplication. This is the first report of evaluation of gene expression profile of S19\(\Delta\)per in comparison to S19 in murine model, which is important to understand the molecular attenuation mechanism of mutant brucellae and may help in development of new attenuated vaccine strains with improved efficacy.

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Session 2: Human Brucellosis

OS2-1. Human brucellosis in India: Systematized review and Meta analysis

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Brucellosis is the most widespread zoonosis in the world caused by bacteria belonging to genus Brucella. The objectives of this systematic review were to assess the occurrence, severity of clinical manifestations, clinical findings, and detection methods used for human brucellosis in India.

A systematic literature review was performed on NCBI Pubmed, Google scholar and IndMed database using terms ‘Brucella, brucellosis, human, occupational exposure, serology, diagnosis, treatment, India’ for article search. Abstracts were selected on the basis of type of research article, sample nature, sample size, risk groups involved, diagnostic techniques used and significance of the study.

A total of 39 studies reporting seroprevalence of human brucellosis from India were retrieved from PubMed and IndMed databases. Similarly, a total of 32 clinical case reports describing brucellosis complications were retrieved from PubMed. Of the 39 seroprevalence studies, 4 were found to be longitudinal, consisting of long term hospital based prevalence reports which were highly significant epidemiologically. The studies were further divided on the basis of risk groups and study populations involved. The studies comprised of risk groups only (21), apparently healthy (2), clinical cases (5), risk group + apparently healthy (5), clinical + apparently healthy (3) and undefined (3). Systematic review of the literature indicated that brucellosis was reported frequently from the states of Karnataka, Maharashtra, Delhi, Kerala and Kashmir. Significantly higher prevalence among field veterinarians had been reported in Delhi, Rajasthan, Uttarakhand, Kerala and Himachal Pradesh. High prevalence among butchers and abattoir workers was reported in Delhi. Majority of the studies (n=9) used SAT as the only diagnostic method. Other tests including RBPT, 2ME, ELISA, CFT and Coombs test were also employed for diagnosis. It was observed that studies describing well defined study design and methods were performed only in few states including Karnataka, Maharashtra, Kerala, Tamil Nadu, Jammu and Kashmir, Uttarakhand, Himachal Pradesh and Delhi. Prevalence had been reported in many states but robust measurements using precise tests were lacking.

Maximum numbers of cases were reported with Brucella spondylitis followed by acute polyarthritis. Data relating to duration of illness and risk factors were also extracted. Severe complications of brucellosis infection were also reported, with cases of endocarditis and neurological. Debilitating conditions such as arthralgia, myalgia and back pain were also reported. Significant delays in appropriate diagnosis and treatment were reported.

This systematic review adds to the understanding of the burden of brucellosis in India. The severe, debilitating, and chronic impact of brucellosis is highlighted. Current epidemiological scenario highlights the need of Brucella strain repository. Polymorphism from different strains can be studied my molecular subtyping methods such as PFGE, MLST, MLVA. Whole genome sequences of vaccine and field strains of Brucella have been generated in India, these studies will help to improve molecular and serological diagnosis of the disease. Well designed epidemiological studies from regions lacking in data would allow a more complete understanding of the clinical manifestations of disease and exposure risks.

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OS2-2. Human brucellosis: sero-prevalence and associated risk factors in Punjab

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Punjab is an agrarian state of India, wherein animal husbandry is the widely accepted diversification field adopted by the farmers to improve their socio-economic status. However, due to improper management practices, lack of awareness, improper biosecurity measures, close association with animals and inadequate vaccination, the transmission of zoonotic diseases from animals to human has become immensely important. Brucellosis is such an infectious disease which has been reported throughout India in animal and human population. The disease has a significant occupational risk in human beings, mainly common in individuals who are in constant contact with diseased animals.

During the three years of study period from October 2012-2015, 3020 human blood samples from occupationally exposed risk groups (veterinarians, para-veterinarians, dairy farmers, animal handlers) and general human population were collected from different districts of Punjab state. Detailed history and relevant epidemiological data was also recorded in the designed proforma. The serum was separated from the samples and subjected to the detailed serological investigations through tests like Rose Bengal plate test (RBPT), serum agglutination test (STAT) and enzyme linked immunosorbent assay (ELISA). Further, the blood samples were attempted for isolation of the Brucella organisms and their molecular characterization.

A total of 3020 human samples were screened from different districts of Punjab state like Ludhiana, Bathinda, Mansa, Barnala, Moga, Faridkot, Fazilka, Ferozepur, Patiala, Sangrur, Muktsar, Abohar, Fatehgarh Sahib, Ropur, Nawanshahar, Jalandhar, Hoshiarpur, Mohali, Amritsar, Tarntaran, and Gurdaspur. The initial screening by RBPT revealed 541 samples positive for Brucella agglutinins indicating the 17.9% prevalence. The doubtful or weak positive cases along with positive cases were further subjected to STAT for confirmation and determination of titre. Out of total samples tested by STAT, 492 samples were found to be positive with titer of ≥ 80 IU. After subjecting the samples to ELISA, 527 samples were found reactive to IgG antibodies and 77 to IgM. The ELISA confirmed some negative samples as weak positives and some positive samples as negative reflecting true positivity and high specificity. The main symptoms in human subjects recorded were PUO, biphasic fever, joint pains, lethargy, malaise etc. About 24 % cases were found serological positive even some with high titre; though no symptoms were recorded in such cases.

Veterinarians, para-veterinary staff, dairy farmers and animal attendants comprised the major high risk group. Overall prevalence in human beings was recorded as 17.9% in risk population. Majority of the patients responded to the treatment, with few relapses. The study highlights that Brucellosis as a significant veterinary and public health problem of economic importance in Punjab state, which needs to be addressed urgently so as to restrain its zoonotic transmission.

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OS2-3. Surveillance of human brucellosis in France from 2005 to 2015 highlights evolution of contamination origins

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In France, human brucellosis is now rare, as the country is officially brucellosis free in cattle since 2005, and the last positive small ruminant herd was identified in 2003. Notification of human brucellosis is mandatory in France. The national reference center (CNR) is in charge of validating positive serological tests and typing Brucella strains isolated from clinical specimens. Between 2005-2015, 269 human cases were reported: 233 cases were confirmed by culture and/or by PCR and 36 cases by serology. Most patients were infected abroad in countries where the disease is endemic. The majority of cases (n=175, 75 %) were imported from 4 regions: the Maghreb (n=73, 31.3%), Turkey/Balkans (n=48, 20.6%), the Iberian Peninsula (n=31, 13.3 %) and the Middle East (n=23, 9.9%). The geographical origin has evolved during recent years, with decreased numbers of cases from European countries and Turkey/Balkans. B. melitensis bv3 was most frequent, mainly isolated in cases imported from Europe, Balkans, Turkey and Maghreb countries. B. melitensis bv. 1 was also frequently isolated, imported from various regions of the world (Africa, Turkey/Balkans, Central Asia, Middle East, India, and South America). Amongst the cases confirmed by culture and/or by PCR, only 8 were domestic: Interestingly, 4 patients were infected with B. suis biovar 2, probably contracted through direct contact with wild boars. The other 4 patients (2008, 2011, 2012) had with B. abortus or B. melitensis. The two cases detected in 2012 were B. melitensis bv 3 contracted from cheese made with unpasteurized cow milk. Here, a wild ibex reservoir was found in the French Alps. Brucellosis is often contracted through professional contact with infected animals; no cases were reported in veterinarians or abattoir workers, however 16 cases (7 %) were reported to be laboratory-acquired contaminations, the last cases being observed in 2014.

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OS2-4. Neurobrucellosis: Clinical, laboratory and epidemiological aspects

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Brucellosis remains the world’s most common bacterial zoonosis. In humans, it manifests as an acute, sub-acute, chronic or relapse form. It can affect any organ/organ systems and complications can occur at any stage of the disease. Neurobrucellosis is a rare complication and is directly attributed to the presence of Brucella or its products in the central nervous system. Symptoms of neurobrucellosis are nonspecific.

Of the 736 human brucellosis cases, 21 were diagnosed to have neurobrucellosis. Of these 21 cases twelve had meningitis; seven were of meningoencephalitis and two of chorea. No difference was noted in the general clinical features of neuro and non-neurobrucellosis cases whereas neurological symptoms were mainly seen in neurobrucellosis patients. Fever was seen in all the cases of brucellosis but was not typically undulant. Neurological signs like behavioral changes, neck rigidity, apathy, ataxia, disorientation, involuntary movements etc were noted.

All the patients with neurobrucellosis showed significant (≥320IU) serum SAT and 2-ME titers. The agglutination titers in CSF ranged between 8-128 IU. Protein rich lymphocytic pleocytosis was noted in all patients. Glucose was decreased in 85.7% of cases. Brucella melitensis was isolated from blood in seven patients. None of the CSF culture grew Brucella. All the patients responded well to anti-Brucella treatment with reduction in serum and CSF agglutinins and CSF protein at the end of two months. All these patients were suspected and investigated to have cerebral malaria/enteric encephalopathy/tubercular meningitis/Neurocysticercosis etc. and brucellosis was never suspected and were treated by several local doctors with diverse antibiotics.

All of them had direct contact with animals, 90% had consumed raw milk and 81% stayed in villages. Five cases of delayed convalescence were noted in the study who recovered fully on psychiatric counseling. Brucellosis is endemic in India especially rural areas. Physicians practicing in these regions must be aware that it is an infection with protean clinical manifestations and can affect any organ or organ systems. Typical undulant fever pattern described in the literature may not be seen. Detailed history with emphasis on occupational, environmental and socio-cultural conditions is essential to aid the diagnosis. Patients with neurological symptoms including severe and persistent headache should be considered for potential neurobrucellosis. Specific laboratory tests if performed both on serum and CSF may help in early diagnosis and treatment and also decrease the morbidity.

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OS2-5. Is brucellosis a potential cause of fever of unknown origin in Madagascar?

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Human brucellosis is a common cause of fever of unknown origin (FUO). In Africa and other geographic regions endemic for malaria and typhoid fever, brucellosis cases may be overlooked due to the unspecific nature of clinical signs and symptoms. Recent epidemiological data on the prevalence of brucellosis in Madagascar are lacking. Therefore, we assessed the percentage of Brucella infections in Malagasy patients exhibiting FUO. In addition, we screened zebu cattle as a potential reservoir of the pathogen.

Between September 2011 and June 2013, a total of 1,020 EDTA-blood samples were collected from FUO patients (pyrexia on admission ≥38.5 °C) presenting to three clinics in central Madagascar (Antananarivo and the district of Arivonimamo). In October 2011, a total of 215 zebu cattle were sampled in three different slaughterhouses in central Madagascar (district of Antananarivo-Antsimondrano). Genus-specific real-time PCR assays targeting IS711 and bcsp31 were performed for human and zebu samples. In addition, zebu serum samples were screened for anti-Brucella antibodies using enzyme-linked immunosorbent assay (ELISA), serum agglutination test (SAT) and complement fixation test (CFT). Whole blood samples were cultured to isolate Brucella spp.

Overall, 15 samples (1.5%) of FUO patients, eight females and seven males, aged 7 to 65 years (median 24) were IS711-positive. Out of these, seven samples were confirmed to be Brucella-positive by the detection of bcsp31. All cases lived in rural villages in the district of Arivonimamo, maybe arising from a local outbreak while no cases were detected among patients resident in the country’s capital.

Brucella DNA (IS711) was detected in a single (0.5%) eight years old healthy zebu originating from the Bongolava region. Serological and microbiological testing revealed negative results in all zebu samples. These findings represent the first recent evidence of human brucellosis in FUO patients in Madagascar and suggest an under-diagnosis of cases, which may be explained by a lack of disease awareness due to unspecific clinical signs and symptoms and limited diagnostic capabilities. Since bovine brucellosis does not seem to contribute to human infections in Madagascar, other transmission routes have to be assumed.

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OS2-6. Zoonotic impact and molecular characterization of Brucella species among human-animal interface in Cameroon

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Brucellosis is a zoonotic and economically important infectious disease in Sub-sahara Africa. With over 500,000 new human cases annually due to the neglected status, brucellosis still remains highly underreported in animals. Epidemiological studies of brucellosis in Sub-Sahara Africa have mainly been based on serology in few countries and no enough data are available on the molecular characterization of strains present in intertropical part of Africa including Cameroon. However, no vaccination is conducted on animal in Cameroon. The present study is designed with the objectives of evaluating the zoonotic impact of Brucella species in North (Garoua) and Centre (Yaounde) regions of Cameroon through molecular characterisation and antimicrobial resistance of isolates from both human and animal.

Two hundred cattle, sheep, goat and pigs each depending on the sex will be randomly and purposively sampled for blood, milk, vaginal secretions, abort swabs, testis biopsy at the central abattoirs in Yaounde and Garoua during ante-mortem and post mortem examinations. Abattoir visits will be organized twice a week for one year (2016-2018).Aborted cattle, sheep goat and pigs will also be sampled in these 2 regions during the study period. Blood samples will also be collected from selected farmers which record of abortion cases in their farms. Samples will be subjected to for culture, isolation and identification using Brucella agar and biochemical tests (H₂S production, Urease, Oxidase, CO₂ requirement, etc.). Sera will be screened using Rose-Bengal test. Antimicrobial sensitivity testing on Brucella isolates will be conducted for doxycycline, ciprofloxacin, streptomycin, trimethoprim-sulfamethoxazole, gentamicin, rifampicin, penicillin, erythromycin and oxytetracycline. Molecular testing for Brucella spp by qPCR on collected samples and genetic characterization using sequencing of genes of interest (both for genotyping and antibiotic resistant genes) will be conducted respectively for rpoB, recA, 16SrRNA,omp25, omp2a, omp2b, and also assess the antibacterial susceptibility (Shirley M Halling and Allen E Jensen, 2006); furthermore MLSA and MLVA will be performed based on housekeeping genes in addition to whole genome sequence of each representative Brucella sp. Alignment of sequences (VNTI, BioEdit) and phylogenetic analysis (BioEdit, Mega) using NCBI database will be used to compare isolates. Generated sequence data will be loaded into the GenBank.

Preliminary results indicate 10 Brucella strains (abortus and melitensis) isolated out of 208 samples from bovine, ovine and caprine with a seroprevalence of 11.5% based on Rose-Bengal screening test. Sequence analysis of 6 isolates based on omp2b and 16sRNA genes indicate variations among isolates with some 99% identity with the GenBank blast. Exact prevalence determination based on species and Brucella sp, antimicrobial susceptibility, genetic characterisation and phylogeny are yet to be conducted. Preliminary findings indicate that Brucellosis is endemic in Cameroon and that bovine, caprine and ovine species are infected. Furthermore isolates do present various genetic differences among strains. However no vaccination programme is organised in the country as in other diseases. Swine and human samples are yet to be collected. Findings from this study will help introduce animal vaccination programme to control this disease in the country. Vaccine matching will be an important step before selection of the candidate vaccine to be used in this endemic country with multi strain isolates.

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OS3-1. Meta-analysis reveals increased sero-prevalence rate of bovine brucellosis in India

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Brucellosis is an infection caused by organisms of the genus *Brucella* affecting livestock systems. Despite having the second largest population of cattle in India, brucellosis continues to debilitate the bovine population ever since its first detection in 1942. Presently, with the increase in demand for dairy and meat products accompanied by renewed and intensified farming practices in the country the disease is a major public health and economic concern. Therefore, it is very important to understand the epidemiology and its prevalence in India. Meta-analytical tools were employed to conglomerate the results published on brucellosis sero-prevalence. The distribution of sero-prevalence of brucellosis in bovines occurring across 8 different states of India from 2006 to 2015 having a sample size of 8407 from 19 studies were included in the study. The data was divided into two quinquennial periods (i.e. 2006-2010 and 2011-2015) to observe the trend in the disease sero-prevalence. Meta-analysis on the results of sero-prevalence rates was conducted using fixed effect model and random effect model. The heterogeneity being the main issue of meta-analysis was evaluated using Tau² value and level of significance. Results on meta-analysis for random effect model were used if the heterogeneity between studies were found to be significant with high Tau² value. The sero-prevalence of brucellosis in bovines was estimated to be 16% (95% CI: 11.0-23.0%) during the 2006-2010 period. Whereas, for the 2011-2015 period the prevalence was 24% (95% CI: 17.0-32.0%) indicating a considerable increase. Thus, these results would prove useful to get a more precise estimate of disease frequency such as disease prevalence proportions, based on the available data and thus, plan ahead for better interventions in the future.

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OS3-2. Cost-benefit analysis of vaccination strategies for brucellosis in small ruminants in Jordan.

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The economic losses due to brucellosis in livestock include both; direct and indirect losses. To estimate direct losses as a function of prevalence, a guess has to be made as to what proportion of seropositive animals would experience abortion. This is a major limitation that previous studies have overcome using speculative values from expert opinion. Therefore, production losses as a result of brucellosis would be better conceptualized and estimated as a function of incidence rather than seroprevalence. Vaccination with Rev-1 vaccine, a live attenuated vaccine against *B. melitensis* in sheep and goats, has proved successful at reducing prevalence in highly endemic settings. The overall aim of this study is to conduct a cost-benefit analysis (CBA) of vaccination strategies for brucellosis in Jordan.

Firstly, a brucellosis transmission model, developed to simulate the impact of control strategies in Egypt was adopted and reparametrized using Jordanian data. The model was used to simulate the effect of different combinations of Rev-1 vaccination against *Brucella* spp. on the incidence of new *Brucella* infections. Secondly, the economic losses imposed by brucellosis in small ruminants in Jordan were estimated as a function of the incidence of new infections. Finally; a cost-benefit analysis for selected vaccination strategies was conducted using the outputs obtained from the one and two. benefit-cost ratios (BCRs) and the net present value (NPV) were calculated for each vaccination strategy. Sensitivity analysis was carried out.

The average total losses due to brucellosis in sheep and goats in Jordan were estimated at 15,685,761 JD (95% CI: 7,072,889 - 26,233,016) and 5,867,005 JD (95% CI: 2,176,104 – 11,160,732), respectively. The loss per individual breeding sheep and goat per year was estimated at 6.8 (95% CI: 3.1 - 11.4) and 6.2 (95% CI: 1.7 – 12.3) JD, respectively. The average NPV for each of the studied vaccination scenarios (vaccination of 50% of adult, vaccination of 100% replacement and vaccination of 25% adult and 100% young replacement) were: 34,194,734 JD (90% CI: 13,695,597- 54,647,677), 36,862,975 JD (90% CI: 16,537,566 - 58,621,955) and 37,420,971 JD (90% CI: 17,135,902 - 58,581,289) respectively, and the averages of the BCR were 6.7 (90% CI: 3.4 – 10.0), 11.3 (90% CI: 5.7 – 17.6) and 14.2 (90% CI: 7.2 – 23.0) respectively. One JD= 1.4 USD.

Brucellosis results in heavy economic losses in small ruminants in Jordan. Using absolute risk reduction to estimate the number of animals that abort due to infection is more realistic and produces real estimates than using speculative values for the proportion of seropositive animals that will abort. Annual vaccination of replacement and young animals is predicted to be economically profitable for livestock owners. The net benefits of the vaccination strategy are a function of the absolute risk reduction and prices of sheep and goats milk.

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Brucellosis prevention and control interventions require involvement of public systems and communities. Therefore, this study was carried out to (a) identify stakeholders among the providers and users at different levels for human and animal health, (b) discover multi-sectoral linkages among stakeholders, (c) explore accountability, relationships among stakeholders.

Grounded theory was used to understand the transactions amongst different stakeholders. Documents were reviewed in light of information collected through in-depth interviews. The interface between human and animal health departments was studied using structural framework. Organizational structures of sectors were reviewed to understand reporting channels.

In-depth interviews were held with various stakeholders using semi-structures questionnaire. Focus group discussions (FGD) were conducted among cattle rearers at commercial and at household level, and among milk users using FGD Guides. Interviews were recorded using both audio aids and notes. After preparing transcripts, field notes were repeatedly discussed, and themes were identified after re-reading the memos. Findings from FGD, in-depth interviews, meetings and observations were triangulated in order to enhance quality of findings.

Stakeholders identified were: (a) Union government, Agriculture Ministry and State Department of Animal Husbandry and Dairy Development; (b) MILKFED (Punjab State Cooperative Milk Producers Federation and Private Milk Processing Industries; (c) Milk Vendors’ Union; (d) Food Safety and Standards Authority of India; (e) Human Health Services; and (f) Community. Policy makers from animal husbandry, dairy development and milk federation had good knowledge about brucellosis as compared to policy makers of human health sector. Veterinary doctors had better knowledge than veterinary inspectors. Among humans, tertiary hospitals were managing 6-10 cases of brucellosis every year. Most of these cases were among children and perhaps because of the use of processed cheese. Some veterinary health inspectors have suffered from brucellosis. Supplies of gloves, boots and glasses were quite erratic, and these were of poor quality. Community was not taking any precautions while handling the birthing process.

The vaccine to prevent brucellosis is produced in India, but it was provided to only half of the cattle population, due to poor resource availability. The records on which cattle were vaccinated were hardly available. Only one door is open for convergence between animal husbandry and human health through integrated disease surveillance program. Non-availability of equipment, lack of human resource, and infrastructures are barriers in following the guidelines. The milk users had no knowledge about the disease. They related the abortion in animals with ‘heat’ in animals. They did not know that it can infect humans. They were interested in knowing more about the disease as it is not only the economic loss but a disease that may hit them too.

Community is interested in vaccination of cattle and can raise this demand but they need more information. Mechanisms need to be developed for better coordination between human health sector and animal health sector.

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OS3-4. Seroprevalence and risk factors for human brucellosis in Ludhiana district in Punjab, India

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Brucellosis is endemic in Punjab, a state with over 27 million people and over 7 million cattle and buffalo, many of which are kept in household farms. Human cases of brucellosis are starting to be recorded as part of a hospital-based surveillance system, but as many infected people would not attend hospital, surveillance data is likely to be partial. Unbiased population-based age-specific seroprevalence in the general rural population in Punjab can however be used to fit mathematical models to estimate incidence and together with information on the main modes of transmission to humans, can be used to inform control measures at the animal-human interface.

A cross-sectional study was conducted using multi-stage stratified sampling. Within Ludhiana district, four out of seven administrative blocks were selected for logistical accessibility. Sixty villages were then randomly selected with probability proportional to the size of the villages (PPS). Households were sampled by simple random sampling. Finally, two subjects per household aged five years and older were randomly sampled stratified by age and gender until the required sample size of 30 individuals (15 males and 15 females) was met.

Questionnaire information was obtained from the head of household and the selected individuals together with a blood sample. Serological tests using commercial ELISAs against Brucella sp antibodies and the Rose Bengal test were carried out. Seropositives were defined as either Rose Bengal or IgG ELISA positive. Robust 95% confidence intervals were calculated to take into account clustering. The strength of statistical evidence for any apparent association between seroprevalence and age-group, gender, contact with livestock (via slaughter, parturition, abortion or milking), types of dairy produce consumed (milk, bauli, cheese or ice-cream) and how the dairy products had been processed was assessed. Multivariate logistic regression analysis was conducted.

Based on interim results of more than 600 interviews, only a tenth of those aged 16 and over noted agriculture as their primary occupation. A larger proportion (16%) however reported contact with livestock e.g. milking cattle. More than 99% drank milk and more than 99% of those reported boiling raw milk before consumption. Overall seroprevalence was low at about 4% but increased after young adulthood. Assisting cows with abortions (p value <0.001) and not boiling raw milk (p value <0.01) were both independently associated with an increased risk of being seropositive after controlling for gender and age.

Serological evidence for human brucellosis at the rural population level suggests poorly controlled zoonotic risk from household cattle ownership. However risk to humans can be mitigated by avoiding consumption of unpasteurised dairy products and good awareness and use of protective measures when assisting cows with parturition. Cattle vaccination programmes should include household herds.

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OS3-5. Estimation of economic losses due to brucellosis in small ruminant of Uttar Pradesh

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Brucellosis is one of the most economically important infectious zoonotic diseases mainly affecting cattle, sheep, goats and pig. A cross sectional seroprevalence study of brucellosis was conducted in small ruminants of Uttar Pradesh. A total of 1848 blood samples of small ruminants comprising of 378 sheep and 1470 goats of Uttar Pradesh were collected using multistage sampling method. The serum was separated and tested with RBPT, STAT, MAT and i-ELISA. Serum sample positive in 2 or more tests was considered as positive. The seroprevalence of brucellosis in sheep and goats was 10.48 % and 3.72 %, respectively. The economic losses due to brucellosis were calculated by including economic losses due to abortion, sterility, loss of milk yield, cost of treatment, loss of carcass weight and opportunity cost. In India small ruminants are mainly reared for meat purpose. Protein scarcity in human diet being quite common prohibits the total condemnation of meat and is practiced only in acute abortive form. However, in mild forms meat is passed after removal of the affected parts. These meat judgments were taken into consideration for economic loss calculation. The annual economic loss was estimated to the tune of Rs. 44.02 crore due to brucellosis in small ruminants (Rs. 4.97 crore in sheep and 39.05 crore in goats) in Uttar Pradesh.

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OS3-6. Serological Investigation Indicates Incidence of Brucellosis in Livestock along the Livestock Movement Routes in Meghalaya


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Brucellosis still remains a major endemic disease in India with considerable public health and economic significance. To understand the epidemiological status of brucellosis in livestock population of Meghalaya, sero-prevalence study based on ELISA and RBPT was undertaken from October 2012-June 2016, along with molecular characterization of the isolates.

A total of 4672 sera samples were collected from cattle (n=1903), buffalo (n=21), pig (n=2430) and goat (n=318) scattered over Meghalaya. Besides, clinical samples viz. vaginal swabs/discharge of cattle and goat (n=73), joints aspirate from cattle (n=2), tissue samples such as placenta and uteri of cattle (n=5) and swine (n=5) and blood samples (cattle-165, swine-17) were processed for isolation and identification of \textit{Brucella} spp. by standard protocols. Presumptive isolates were further characterized by PCR, real time PCR and sequence analysis of \textit{BCSP31} gene.

Overall, sero-positivity of brucellosis was 5.12% by ELISA and 2.59% by RBPT taking all species into consideration. Bubaline and caprine samples were found negative by both the tests. Analysis of spatial trends of disease occurrence in cattle indicated high incidence of brucellosis in RiBhoi district during 2013-14, 2014-15, 2015-16 and East Khasi Hills districts during 2015-16. These two districts incidentally form a contiguous livestock movement route from adjacent state of Assam to neighbouring country of Bangladesh. However, for pigs only West Khasi Hills recorded higher incidence (P<0.005) during 2013-14. Analysis of temporal trends of incidences revealed significantly higher incidence in RiBhoi and East Khasi Hills districts during 2014-15 (P<0.0001). Comparison of variance in incidence of brucellosis among age groups and sex revealed no significant difference in mean sample positivity. Moreover, ten \textit{Brucella} isolates was recovered (cattle - 8, goat – 1, pig - 1) by culture and isolation. Molecular characterization by AMOS and Bruce ladder PCR and bidirectional sequencing of \textit{BCSP31} gene confirmed the cattle and goat isolates as \textit{Brucella abortus} and swine isolate as \textit{Brucella suis}.

Results of the study underscore the incidence of brucellosis in cattle and pigs in Meghalaya. Interestingly, sero-positivity was noted primarily in adjoining areas of Assam and along the livestock movement routes touching up to international borders. Thus, it is imperative to monitor livestock movement for effective control and timely prevention of this important zoonosis.

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OS3-7. Seroprevalence studies on brucellosis in cattle and buffaloes in Ludhiana distt. of Punjab, India.

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India is now the world’s leading milk producer. This study was conducted in Punjab, which compared to other Indian states, produces the most cattle and buffalo’ milk per capita. Endemic and emerging livestock diseases pose a threat to the security of dairy industry due to reduced productivity of livestock, trade barriers and, if foodborne zoonoses, posing a threat to consumer health. Bovine brucellosis, caused primarily by Brucella abortus, is a reproductive disease which can result in abortions and/or diminished fertility in affected livestock. The disease is also zoonotic, spread via close contact with livestock, particularly those assisting calving or abortion, or via unpasteurized dairy products. There is some surveillance and research into Brucella spp. in the Punjab with previous studies estimating that up to 34.2% of dairy animals (DA: cows and buffalo) are affected. However, the majority testing is targeted towards DA’s with clinical signs of brucellosis such as abortion and many previous studies provide inadequate description of the study design and target populations. Therefore, the aim of this study was to provide updated estimates of Brucella spp. seroprevalence and identify high risk livestock populations. This study was conducted in parallel with a study of occupational exposure to brucellosis and employed a multi-stage sampling design. In the first stage, ~40 villages were randomly selected from four out of seven blocks of Ludhiana district using sampling probability proportional to human size. Within villages, up to eight dairy farms were selected using simple random sampling, in consultation with veterinary officers. Within selected farms, up to nine lactating dairy animals (DA: cattle and buffalo) were sampled using systematic sampling. Where possible, milk samples were collected from every animal and blood samples from every other animal studied. These samples are currently being tested by Rose Bengal test (RBT) and commercial and novel indirect ELISA’s. Based on a case-definition of serum samples testing RBT positive, univariate risk factor analysis was performed using chi-squared or Fisher’s exact tests. Serum samples (n=455) collected from cattle (315) and buffaloes (140) have been subjected to Rose Bengal test for the detection of Brucella spp. antibodies. Preliminary seroprevalence estimates based on RBT are 56 (12.3%), with 13.1% in cows and 10.7% in buffaloes. Based on preliminary risk factor analysis, vaccinated animals (P=0.02), animals that had aborted (P=0.04) and animals that were bred on the farm had 3.6, 3.7 and 10.4 times the odds of testing seropositive using RBT, respectively. Holstein-Friesian cattle which were reportedly purebred were also more likely to test seropositive for RBT compared to HF-cross (P<0.001).

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Session 4: Brucella research in India
Oral Presentations

OS4-1. Brucella research in India

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Ever since the discovery of the causative agent, brucellosis remains one of the most important and widespread zoonosis all over the world. Brucellosis causes considerable economic losses through reduced productivity, abortions and weak offsprings of livestock, which is a major stumbling block for trade and export. Brucellosis is prevalent in developing countries where humans and animals live in close proximity. Prevalence in humans is directly proportional to animal disease. Animal handlers are specifically more susceptible due to their occupation.

Though brucellosis was reported to have existed in India since 1879, the methodical study was taken up as late as 1943, in animals and man in Madras presidency. However, contagious abortion in livestock associated with brucellosis was first investigated in India by the Imperial Veterinary Research Institute, Mukteswar in 1918. Since then the disease has been reported from all the states of the country.

In India, the disease is showing an upward trend and there has been a staggering increase in the prevalence and incidence of the disease. The changing husbandry practices from traditional to modern livestock rearing, coupled with stocking of more number of animals per unit area have resulted in spatial clustering of both the infection and the disease. The situation is further complicated by unrestricted movement of animals, desperate sale of positive animals, etc.

National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI) (Formerly PD_ADMAS) has carried out systematic and exhaustive epidemiological study taking consideration of different risk factors associated with brucellosis in organized farms.

The economic losses due to brucellosis by sourcing prevalence data from epidemiological surveys conducted in India. The disease causes significant economic losses in the country and should be controlled on a priority basis.

Department of Biotechnology took a mega initiative to address all aspects of Brucella research in India. The initiative resulted in economic mapping of the disease, a national repository of the isolates, whole geneme sequencing of field isolates and vaccine strains, developing epidemiological tools, user friendly diagnostic kits, a safe and DIVA enabled vaccine candidate.

Brucellosis Control Programme (Brucellosis-CP) initiated by DADF, Govt of India in 12th Plan envisages vaccination of female calves between 6-8 months in all states.

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OS4-2. Brucellosis Epidemiology in India

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Brucellosis was first recognized in India during 1942 and since then the disease is showing a slow and steady raise in its prevalence in all livestock systems making the country a hot spot for brucellosis. Till date, many reports proved the prevalence of the disease in almost all the states of the country either through routine serosurveillance studies or screening organized farms or investigation of abortion storms. A serological survey of brucellosis in cattle and buffalo performed in 23 States of India way back at 1998 showed an overall prevalence rate of 1.9% in cattle and 1.8% in buffalo. From there onwards, long-term serological studies indicated 5% of cattle and 3% of buffaloes were infected with brucellosis during 2002. Similar nationwide seroprevalence studies indicated 6.5% bovine brucellosis in 17 states of the country during 2011-2015. With special reference to small ruminant brucellosis, the overall seroprevalence of 6.2% has been observed similar to that of bovine brucellosis in 18 states of India from 2006-2015. Even in organized dairy farms, 6.1% and 8.2% brucellosis prevalence was observed in cattle and buffaloes, respectively. Brucellosis outbreaks have also been recorded in the country and seroprevalence of 36% to 54% have been recorded among bovine, small ruminants and swine herds. In continuation to serosurveillance studies in livestock, national human brucellosis prevalence of 10% was recorded among the high-risk individuals specifically targeting people associated animal health care. This comprehensive information on countrywide prevalence of livestock and humans will be a definite input for the ongoing Brucellosis Control Program, One Health Approach for brucellosis control and research efforts, which in turn helps in strategizing evidence based disease control measures.

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OS4-3. Genomic insights into the host specificity of Brucella

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Brucella spp. are facultative intracellular bacteria that cause major zoonotic disease brucellosis worldwide. Brucella infects a wide range of mammals including humans. Brucella species are highly conserved in nucleotide level. Recombination analysis using complete genome sequences of Brucella revealed that recombination events are less frequent, and the impact of recombination occurred is negligible on the evolution of Brucella. This leads to the view that Brucella is clonally evolved. Host specificity of Brucella remains obscure. We predicted species-specific sRNAs in B. melitensis, B. abortus, and B. suis by performing a genome-wide computational analysis. The putative target genes that could be regulated by the identified sRNAs were predicted computationally. Also, we constructed a Gene Ontology based regulatory network of the predicted mRNA targets. GO network analysis revealed the species-specificsRNA-based regulatory networks in B. melitensis, B. abortus, and B. suis. To understand more about host specificity of Brucella, we analysed the secretion system and its interaction with the host proteins. Overall, 10.26 to 14.94% of total proteome were found to be either secreted (secretome) or membrane associated (membrane proteome). Approximately, 1.7 to 3.0% of total proteome were identified as T4SE proteins. This study has identified nine proteins encoded by Brucella spp. to have eukaryotic-like-domain which are likely to interact with host proteins leading to modulation of function. Prediction of protein-protein interaction showed 29 and 36 host-pathogen specific interactions between Bos Taurus (cattle)-B. abortus and Ovis aries (sheep)-B. melitensis, respectively. Phylogeny of 258 Brucella strains based on genome-wide SNPs was studied. This SNP-based phylogeny could differentiate the Brucella at the species level, and we have identified species-specific SNPs. For example, we identified 208 species-specific SNPs in Brucella abortus that are conserved 138 B. abortus genomes. These SNPs might have originated very early during the evolution of B. abortus and might be responsible for the evolution of B. abortus with cattle as the preferred host. This information may help to solve the mystery behind the Brucella pathogenicity and host specificity.

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OS4-4. Initiatives in vaccine development and control of bovine brucellosis in Indian scenario

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Brucellosis is a bacterial zoonosis widely prevalent across the globe. India is world’s largest milk producer and hosts more than 1 billion people. Brucellosis being endemic in India causes huge economic losses to livestock industry and a recent report states the loss to the tune of 3.4 billion USD. The threat of brucellosis is beyond imagination in rural areas where more than 65% of human population is residing in close proximity to animals. In addition, limitations of the currently available diagnostic and prophylactics and complex epidemiology have exacerbated the situation. *Brucella abortus* S19 strain is the recommended vaccine for cattle and buffaloes in India. S19 strain is a potent immunogen conferring solid protection and lifelong immunity to vaccinated animals. However, S19 strain possesses a number of drawbacks including residual virulence and pathogenic to human. Thereby, field veterinarians need to handle vaccine with additional precaution. A number of initiatives have been taken to circumvent drawbacks of existing vaccine and to develop an alternate vaccine for control of bovine brucellosis. Immunodominant antigens like L7/L12 ribosomal protein, P39 antigen, Cu-Zn SOD etc have been expressed and recombinant antigen cocktail used for immunization with immunoadjuvant as well with novel delivery system. DNA prime-protein boost strategy has been evaluated. Immunogenic potential of outer membrane vesicles of *Brucella abortus* S19 have been studied in experimental mice model. Although subunit vaccine candidates were superior in terms of safety, they conferred lower level of protection against virulent challenge in comparison to live S19 vaccine. Further, to reduce the virulence of S19 strain, mutants were generated by targeting LPS biosynthetic genes *per* and *rfbD* encoding perosamine synthetase and transport permease protein, respectively. The developed mutants, S19Δper and S19ΔrfbD exhibited altered LPS structure and also resulted in varying degree of attenuation of S19 strain. S19Δper mounted strong immune response in Swiss albino mice and conferred protection similar to S19 strain whereas S19ΔrfbD was found less effective. Both S19Δper and S19ΔrfbD showed DIVA capability. Efficacy of developed mutant is being evaluated in target host, buffaloes. Government of India has initiated national brucellosis control program. Mass vaccination of cattle and buffalo calves aged between 4-8 months with S19 strain is recommended. In brucellosis free countries, test and slaughter of infected animals played crucial role in stamping out the disease. However, from socio-economic point of view, test and segregation of infected animals in conjunction with vaccination perhaps the best choice in control of brucellosis in India. Efficient veterinary services, periodical surveillance, supply of clean semen and castration of infected bulls, disease awareness and education to farmers, compensation scheme, sanitary and biosecurity measures at farm level are essential components to be in place for effective control of brucellosis in India.

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OS4-5. Point of care Diagnostics for resource limited Areas: Shielding the Livestock from Brucellosis

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Brucella is a highly neglected and the commonest zoonotic disease transmitted animals to humans by consumption of raw milk, meat, ingestion of infected food products, and contact with body fluids. Since there is no therapeutic intervention of this disease process, segregation is the only option in some countries other than culling of the infected animal in controlling the disease. The burden of Brucellosis in endemic countries is enormous and per year toll has been estimated 4 billion dollars in India alone due to brucellosis in livestock especially in cattle, goat, sheep and pigs apart from animal handlers. This is due to uncontrolled animal movement, poor awareness of the disease and unavailable of diagnostics assays/kits at point of care areas.

Though several classical and gold standard methods of detecting Brucella are in place including blood and bone marrow cultures, they were failed with limited performance and poor success rate regarding their specificity and sensitivity and complexity of their use at point of care areas. The other serological methods like Rose Bengal plate agglutination test (RBPT), Complement Fixation test (CFT) using purified sLPS, which is the best known immunogenic component, have proven some success, still have limitations to perform at point of care areas due to lack of cold chain preservation of reagents. These assays also suffer lack of simple sample procuring methodologies or techniques that can be easily able to preserve and transport to the primary or tertiary health care centers. Molecular methods like PCR, Real time PCR have been proven very well and have been primary tools in detecting Brucella species in endemic or in screening aborted cases. These methods can be carried out in established laboratory set ups but their use in epidemiological surveillance or for point of care use at the resource limited areas have never been successful due to complexity of the assays and underlying expensive instrumentation. There is tremendous need for point of care diagnostic tools that are user friendly, cost effective, simple and affordable and reliable with good sensitivity and specificity. Here we report, sample collection devices or disks that are impregnated with chemicals that will stabilize biological fluids, proteins, DNA and RNA for later use. This is including brucellosis infected/suspected biological fluids from aborted fetuses, blood or serum and milk samples can be stored at room temperature and can be transported at ease. Lateral Flow assays using sLPS purified from s99 strain were developed and were validated for their use at point of care areas with approximately 85% sensitivity and 100% specificity. Dipstick LFA kits were also developed and validated in-house to test the suspected milk samples from organized and unorganized farms. sLPS based indirect ELISA kits were developed and are validated. Several attempts using recombinant proteins including OMP19, OMP28, BP26, BCSF31, P39, P19, 25E, BSL, V protein did not yeild any encouraging results when tested with sera from Brucella infected animals. Isothermal PCR (LAMP) assay coupled with LFA has been developed and the results suggest that this nucleic acid based assay can be performed at the point of care resource limited areas. This simple, point of care, inexpensive Brucellosis diagnostic detection assays/kits will be valuable tools for epidemiological screening, disease surveillance for Brucella endemic countries. The rapid diagnostic LFA kits will be a valuable tool for the farmers who want to purchase Brucella free animals and customers who want Brucella free organic milk as well as to screen milk collection centers at large. Future assays aiming at microfluidic devices at an affordable price to detect Brucellosis at point of care areas will improve the sensitivity and specificity of the diagnostics arena.

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Session 5: Canine and Wildlife Brucellosis

Oral presentations

OS5-1. Cross-species transmission and seroprevalence study of brucellosis in livestock and canine populations in India

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Brucellosis is a re-emerging anthropo zo onotic disease with multifaceted epidemiology and socioeconomic implications worldwide. *B. abortus*, *B. melitensis*, *B. ovis*, *B. suis* and *B. canis* are the classical species, preferentially affecting cattle, small ruminants, swine and dogs respectively, that are having a great impact on domestic livestock productivity and human health. Despite the species preference or specificity, there may be breaches in the animal host boundary causing cross-species transmission in mixed or integrated farming systems or at the livestock-wildlife interface. Based on the epidemiological data of active surveillance programme, it is estimated that there is a loss of 58.8 million US$ per year in India due to brucellosis. Indian Council of Agricultural Research (ICAR) recorded the national seroprevalence of brucellosis in cattle from 2012–2013 as roughly 13.5% which is at a stable, endemic equilibrium. As compared to cattle, there is limited information on brucellosis in small ruminants, swine and canine populations of India. The aim of the study is to determine the prevalence and possibility of cross- species transmission of brucellosis among small ruminants, swine and canine populations in the Tamil Nadu state of India. Anti-*Brucella* antibodies were detected by RBPT, i-ELISA and dot-ELISA in 340 small ruminants (145 sheep and 195 goats), 321 pigs and 300 dogs recording an overall seroprevalence of 11.45%, 10.1% and 7.33% respectively. Spillover infections of *B. abortus* were detected in small ruminants and swine by culture isolation and identification; which was further confirmed by PCR assays, clearly indicating the evidence of the breaches in host specificity. In addition, using the gold standard method for diagnosis, *B. melitensis* biovar 2, *B. ovis*, *B. suis* biovar 1and *B. canis* were isolated from the goats, sheep, swine and canine study populations respectively. Thus, the combined seroprevalence study and bacteriological methods gave a clear picture to confirm the endemicity, cross-species transmission of brucellosis causing sustained and spillover infection in the study populations. It can be suggested that the serosurveillance in all the susceptible species should be done for effective prevention of brucellosis in an endemic country like India, rather than focusing fully on dairy animals. It is concluded that the determination of cross-species transmission of *Brucella* organism is an important step for epidemiologic characterization of the disease to design the control and eradication programs.

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OS5-2. Characterization of novel Brucella species isolated from bats from a cave in Georgia (Caucasus)

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Bats have long been recognized as reservoirs for zoonotic pathogens and have demonstrated co-evolutionary adaptation with viruses, protozoa, and bacteria, due to the resilience of bats to infection by intracellular pathogens. In 2012, a field study in the country of Georgia collected >200 bats for a multi-pathogen discovery project. Bat tissues were processed and screened by molecular methods for multiple bacterial agents including Leptospria, Bartonella spp., and Brucella species. Four spleen tissues from two different bat species (Myotis blythii and Miniopterus schreibersii) collected in the same area tested positive for Brucella spp. by real-time PCR targeting the IS711 gene element. Fresh frozen tissues (spleen, liver and kidney) from the IS711 PCR positive bats were submitted to the Bacterial Special Pathogens Branch for Brucella isolation. Spleens were processed for culture and Brucella colonies were identified from two of the four spleen samples and purified for further characterization. The isolates were initially characterized by the standard microbiological testing algorithm for Brucella spp. consisting of phenotypic and molecular tests including the hydrogen sulfide test, urease test, gel formation test and Tbilisi phage typing in addition to the detection of the IS711 element by real-time PCR, Bruce-ladder and AMOS PCRs. By these standard tests, the two bat isolates were confirmed as Brucella spp. but did not have phenotypic or molecular signatures that matched with any of the classical or recently characterized Brucella spp.. The Bruce-ladder profiles of these bat isolates aligned with B. suis; by AMOS PCR they had an amplicon consistent with B. ovis and by Suis-ladder PCR; the two bat isolates had a unique banding pattern, inconsistent with any of the known B. suis biovars. By whole genome SNP analysis, the bat isolates clustered most closely with B. suis biovar 5 strain 513, isolated from mouse-like rodents in the 1960’s-1980’s USSR. Despite this close clustering, the bat isolates contained ~12,000 SNP differences from the representative strain 513 exceeding the intra-biovar diversity typically seen within Brucella species. By multi-locus sequence typing (MLST), the bat Brucella isolates demonstrated three unique SNP positions and one deletion, clustering closest to B. neotomae ST22. Clustering analysis of protein profiles obtained by MALDI-TOF analysis showed the two bat isolates in a singular clade demonstrating unique ribosomal protein expression compared to the other Brucella species. These preliminary data indicate that these bat isolates likely represent a novel species within the genus Brucella.

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OS5-3. Canine brucellosis in Brazil: assessment of genetic diversity

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Canine brucellosis caused by Brucella canis (BC) remains endemic in Asia, Europe, North America, and South America, including Brazil. Although BC is considered as zoonotic, its importance in Public Health is not clear and its occurrence in the world seems underestimated in humans, as well as in animals. Treatment of infected dogs involves long-term antibiotic combination, generally tetracycline with aminoglycoside or rifampicin but relapses are frequent showing that BC can persist. Several serological investigations report the widespread distribution of BC in Brazil, but the genetic diversity of BC strains circulating in the country is unknown. MLVA-16 (Multiple-Locus VNTR Analysis) is the current optimal strategy of Brucella molecular typing to track the infection source and the spread over time of local/global outbreaks. The aims of this study were i) to assess the genetic diversity among BC strains isolated from São Paulo (SP) Brazil, and to investigate their possible epidemiological relationship with worldwide strains and ii) to monitor the evolution of the BC infection after a 10 year-period on a local scale.

A total of 70 BC canine strains isolated in SP from different kennels (k): k0 (2003), k1 (2005), k2 (2005), k3 (2005), and k4 (2015) were characterized phenotypically and by MLVA-16. The obtained results were compared with previously published BC genotypes (n=95), isolated from Europe, North America, Asia. Hunter-Gaston diversity index (HGDI) was determined to assess MLVA-16 as typing tool for BC epidemiology. Antimicrobial susceptibility testing was performed by disk-diffusion method on Mueller-Hinton agar plates, supplemented with 5% sheep blood, with the antibiotics of veterinary and human interest, following the Clinical and Laboratory Standards Institute (CLSI) recommendations.

The global analysis shows that the BC strains are divided into 3 distinct clusters: a Brazilian, an Asian and a mixed group. These results suggest the existence of a Brazilian autochthonous lineage. MLVA clustering highlights a distribution of the SP strains into 2 groups, regardless of the isolation year, showing the circulation of different BC strains in a same area and time. Interestingly, within those SP isolates, the k3 strains are present into both clusters, one together with strains isolated from other SP kennels, and one close to Asian and Europe strains, suggesting a different contamination source, as a possible introduction of an animal from a foreign country. Moreover, our molecular results show an early branch divergence with strains isolated from the same place, but after 10 years (2015), reflecting the adaptation of BC strains and the evolution of the infection over time. Nevertheless, no difference in their antibiotic susceptibility pattern, vs 2005 strains, is noted. This work is the first molecular investigation regarding BC strains circulating in Brazil, providing new insights regarding the epidemiological situation of canine brucellosis.

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Session 6: Diagnostic methods

Oral Presentations

OS6-1. Conjugated O-polysaccharide antigen is more effective in iELISA at differentiating between sera from *B. abortus* S19 vaccinated and field infected cattle than sLPS or synthetic disaccharide antigens

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Control of brucellosis requires vaccination and eradication requires a serological test and slaughter programme. The universally recognised vaccines for brucellosis all have significant and well documented flaws. One of the most significant is that the smooth vaccines *B. abortus* S19 and *B. melitensis* Rev1 induce antibodies that react in conventional serological tests. This is due to the presence of abundant O-polysaccharide (OPS) on the surface of these vaccines and field strains plus the reliance upon OPS in serodiagnostic assays to provide sufficient sensitivity. This makes the differentiation of infected and vaccinated animals impossible in many circumstances.

The option to use non-OPS based antigens to resolve confounding serology is unviable due to the insufficient sensitivity of protein or glycolipid antigens. We therefore undertook a pilot study in cattle to evaluate the potential of novel OPS based antigens to differentiate between antibodies induced by field strains of *B. abortus* or S19 vaccine. The serological study was complemented by classical tests including CFT and RBT.

Three OPS based antigens were evaluated using standard iELISA methods. These were a standard preparation of smooth lipopolysaccharide (sLPS) from *B. abortus* S99, purified OPS derived from *B. abortus* S99 which had been modified and conjugated to a carrier to assist attachment to the ELISA plate surface (cOPS) and a synthetic ‘M’ disaccharide BSA conjugated antigen. The disaccharide is formed from two of the sugars that constitute the *Brucella* OPS homopolymer and which are linked in a α (1→3) manner.

These antigens were evaluated against the following serum panel (there was no multiple sampling of animals: 41 samples from non-infected cattle, 18 samples taken 25 days after vaccination, 20 samples taken 45 days after vaccination, 60 samples taken from herds confirmed by culture as infected with a field strains of *B. abortus* and 28 samples from serologically positive herds of unknown vaccination status. In addition 7 negative and 7 positive controls were applied. Vaccination was performed via the conjunctival route using a dose of 5-10 x 10⁹ CFUs of *B. abortus* S99.

The sLPS antigen was the most effective at differentiating between the samples from the infected and non-infected animals. As might be expected, it was also the most susceptible to reaction with sera from vaccinated animals, although this only occurred in the 45 day post vaccination group. Both the cOPS and ‘M’ disaccharide antigens detected sera from the infected herds. The principle finding was that the cOPS antigen was less sensitive in detecting vaccine induced antibodies whilst retaining sensitivity against field induced antibodies due to true infection. This proof-of-concept study suggests that the cOPS antigen may be a superior serological tool in areas where vaccination with *B. abortus* S19 is taking place.

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OS6-2. Evaluation of various blood components for the molecular detection of *Brucella* spp from human.

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The diagnosis of human brucellosis is demanding due to its indefinite sign and symptoms. Although, the conventional diagnosis of this disease is based on the serological tests, molecular methods are gradually getting importance due to rapid diagnosis and identification of species of the invading pathogen. Although the causative organism, *Brucella* spp are facultative intracellular pathogen, most of the studies are based on the detection of *Brucella* DNA from the sera samples only. Based on the evidences that *Brucella* spp can invade the macrophages and erythrocytes, we evaluated various blood components to identify the target blood component maximizing detection of *Brucella* DNA by PCR. A total of 50 seropositive samples were selected randomly for the study. Three different components, serum, buffy layer (WBC) and whole blood (plasma and formed elements) were separated from each patient. DNA were extracted from each of the individual cell components and subjected to bscp genus specific PCR. The detection of *Brucella* spp DNA was 10% from sera, 12% from the WBCs and 28% from the whole blood cells. Interestingly the positive samples from the blood components showed negative results in the sera samples of the same patient. The present study indicates the importance of whole blood as the target sample for the increased molecular detection of brucellosis in human.

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Session 7: Vaccines & Immunology

OS7-1. Meta-analysis and advancement of brucellosis vaccinology

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In spite of all the research effort for developing new vaccines against brucellosis, it remains unclear whether these new vaccine technologies will in fact become widely used. The goal of this study was to perform a meta-analysis to identify parameters that influence vaccine efficacy as well as a descriptive analysis on how the field of Brucella vaccinology is advancing in regard to type of vaccine, improvement of protection on animal models over time, and factors that may affect protection in the mouse model. A total of 117 publications that met the criteria were selected for inclusion in this study, with a total of 782 individual experiments analyzed. Attenuated (n = 221), inactivated (n = 66) and mutant (n = 102) vaccines provided median protection index above 2, whereas subunit (n = 287), DNA (n = 68), and vectored (n = 38) vaccines provided protection indexes lower than 2. When all categories of experimental vaccines are analyzed together, the trend line clearly demonstrates that there was no improvement of the protection indexes over the past 30 years, with a low negative and non significant linear coefficient. A meta-regression model was developed including all vaccine categories (attenuated, DNA, inactivated, mutant, subunit, and vectored) considering the protection index as a dependent variable and the other parameters (mouse strain, route of vaccination, number of vaccinations, use of adjuvant, challenge Brucella species) as independent variables. Some of these variables, including: type of vaccine, mouse strain, route of vaccination, number of vaccinations, challenge Brucella species, influenced the expected protection index of experimental vaccines against Brucella spp. in the mouse model.

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OS7-2. Attenuated *Brucella neotomae* as a vaccine against *B. suis* challenge

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Brucellosis is the most common zoonotic disease worldwide. Almost 500,000 new human cases occur each year; yet, there is no vaccine for human use. Most of the live modified vaccines used in animals can cause infection in humans. *B. neotomae* has not been reported to cause infections in human and thus can be manipulated to generate a human vaccine. We generated an attenuated *B. neotomae* strain by deleting the *wboA* gene encoding a glycosyltransferase and creating a rough strain. This strain lacks lipopolysaccharide (LPS) in its O side chain and thus the vaccinated animals can be differentiated from the infected animals. Protection against a *Brucella* challenge is mediated by strong CD4+ Th1 and CD8+ Tc1 adaptive immune responses. To induce a robust adaptive immune response, an enhanced innate response is required. We tested the efficacy of a rough *B. neotomae* to stimulate dendritic cells compared to the smooth wild type. Based on cytokine (TNF-α and IL-12) production, our data suggests that a significantly higher stimulation was obtained when dendritic cells were stimulated with the rough vaccine strain compared to the smooth wild type *B. neotomae*. Furthermore, the rough mutant was cleared from mice within 4 weeks even at a dose as high as 2 x 10^8 CFU/mouse. Vaccinated mice showed significantly higher protection against a *B. suis* 1330 challenge compared to the control mice. Antibody titers in the mice and cytokine production by the splenocytes from the vaccinated mice were evaluated to delineate the type of immune response generated by the vaccine.

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OS7-3. Pharmacologic effects of the macrolide antibiotic tulathromycin on Brucella melitensis infection in open and pregnant goats.

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The objective of the current study was to evaluate the pharmacologic effects of the macrolide antibiotic tulathromycin (Draxxin™) on Brucella melitensis infection in non-pregnant and pregnant goats. Tulathromycin, a macrolide antibiotic in the triamilide subclass, is long-acting (up to 14 days with a single dose) with a broad spectrum of activity and is known to preferentially accumulate in high concentrations in neutrophils and macrophages. Goats were randomly assigned by breed to pregnant and open groups, and further subdivided into antibiotic treated and untreated groups (n=8 or 9 per group). Goats were conjunctively challenged with 1 x 10⁷ CFU of B. melitensis 16M under BL3 containment either 10 weeks before initiation of natural breeding (open treatment), or at approximately mid-gestation (pregnant treatment). Experimental challenge was confirmed by recovery of the challenge strain from conjunctival swabs at 5 days post challenge. Treated goats in the non-pregnant group received tulathromycin (2 mg/lb IM) at 58 days whereas in the pregnant group antibiotic treatment was administered at 21 days after experimental challenge. Goats were euthanized after abortion, parturition or by 6 months after initiation of natural breeding. Antibiotic treatment of pregnant goats did not (P>0.05) influence abortion, time to abortion, or colonization (colony-forming units/gm tissue) of fetal or maternal tissues. Abortion was only observed in goats challenged while pregnant, and antibiotic treatment of goats infected in the absence of pregnancy did not influence (P>0.05) colonization in maternal or fetal tissues. The results of this experiment suggest that tulathromycin is not an effective pharmacologic treatment for control of brucellosis in goats.

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OS7-4. Immune response of calves vaccinated with Brucella abortus S19 or RB51 and revaccinated with RB51

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Brucella abortus S19 and RB51 strains have been successfully used to control bovine brucellosis worldwide, however, currently, most of our understanding of the protective immune response induced by vaccination comes from studies in mice. The aim of this study was to characterize and compare the immune responses induced in cattle prime-immunized with B. abortus S19 or RB51 and revaccinated with RB51. Female calves, aged 4 to 8 months, were vaccinated with either vaccine S19 (0.6-1.2 x 10¹¹ CFU) or RB51 (1.3 x 10¹⁰ CFU) on day 0, and revaccinated with RB51 (1.3 x 10¹⁰ CFU) on day 365 of the experiment. Characterization of the immune response was performed using serum and peripheral blood mononuclear cells. Blood samples were collected on days 0, 28, 210, 365, 393 and 575 post-immunization. Results showed that S19 and RB51 vaccination induced an immune response characterized by proliferation of CD4⁺ and CD8⁺ T-cells; IFN-γ and IL-17A production by CD4⁺ T-cells; cytotoxic CD8⁺ T-cells; IL-6 secretion; CD4⁺ and CD8⁺ memory cells; antibodies of IgG1 class; and expression of the phenotypes of activation in T-cells. However, the immune response stimulated by S19 compared to RB51 showed higher persistency of IFN-γ and CD4⁺ memory cells, induction of CD21⁺ memory cells and higher secretion of IL-6. After RB51 revaccination, the immune response was chiefly characterized by increase in IFN-γ expression, proliferation of antigen-specific CD4⁺ and CD8⁺ T-cells, cytotoxic CD8⁺ T-cells, cytotoxic CD8⁺ T-cells and decrease of IL-6 production in both groups. Nevertheless, a different polarization of the immune response, CD4⁺- or CD8⁺-dominant, was observed after the booster with RB51 for S19 and RB51 prime-vaccinated animals, respectively. Our results indicate that after prime vaccination both vaccine strains induce a strong and complex Th1 immune response, although after RB51 revaccination the differences between immune profiles induced by prime-vaccination become accentuated.

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OS7-5. Immunogenicity and efficacy studies of a Glyco-conjugate vaccine developed against Bovine Brucellosis

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Bovine brucellosis is an economically important disease for the dairy industry. The disease is also of high importance due to its zoonotic nature. As it also causes disease in humans associated or consuming unpasteurized animal products. Presently the disease is controlled by vaccination but the conventional vaccines has the limitations pertaining to their restricted use according to age and gender of the animal, insufficient protection and risk of excretion of live vaccine strains from immunized animals. In the process of preparing a better vaccine we have developed a vaccine against bovine brucellosis by chemical conjugation of lipopolysaccharide (LPS) and outer membrane protein (OMP) part extracted from Brucella abortus S19 strain (S19-GC). The vaccine has found to exhibit a good immune response, protective and therapeutic efficacy in challenge studies in mice. Immunogenicity studies in cattle and buffalo calves revealed cell mediated immune response as evidenced by stimulation of interferon gamma in ELISPOT and ELISA and humoral response (IgG1 and IgG2 subtypes) in in-house standardized ELISA tests. None of the animals showed any adverse clinical reactions upon vaccination. The vaccine was further tested for its prophylactic and therapeutic efficacy in Brucella infected farms which mimics naturally challenged conditions. Two cattle farms were screened for brucellosis by cultural, serological and molecular methods and Brucella negative adult female cattle were used for prophylactic study whereas Brucella positive adult female cattle were included for therapeutic study. A modified Brucella selective media for the isolation of Brucella wild strains from the field was adopted to study the shedding of Brucella organism. A quantitative real-time PCR (qPCR), developed and validated in house, was used to study the shedding pattern of Brucella genome in vaccinated and control animals. Three different experiments were performed. In ‘Experiment I’, vaccination with sub-cutaneous dose of 50µg/ml/dose could stop the Brucella shedding in all the Brucella positive animals atleast till 60 days post-vaccination (DPV). In Experiment II, a group of Brucella positive cattle vaccinated with 50µg/ml/dose followed by a booster done on 90DPV showed protection in 83.3% of animals in 250 days long study. Subsequently in ‘Experiment III’, upon immunization of a cattle group with 100µg S19-GC by sub-cutaneous route without booster resulted in complete stoppage of shedding; as evidenced by culture and qPCR assays till the end of study i.e. till 120 DPV. Hence it could be concluded that the S19-GC vaccine was safe and could trigger both humoral and cell mediated immune responses. The involvement of Th1 and Th2 cells was marked by pronounced antibody response which in turn could stop shedding in case of infected animals and prevented brucellosis free animals from acquiring fresh infection from in-contact animals carrying Brucella infection. The above observations needs to be further studied by inclusion of larger number of animals and challenge studies with wild strain of Brucella abortus 544.

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OS7-6. Evaluation of the immune response against graded doses of *Brucella abortus* S19 (calfhood) vaccine in buffaloes, India

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*Brucella abortus* S19, is a live vaccine recommended by OIE for the prevention of brucellosis in cattle and buffalo and minimum dose specified by OIE followed in Indian Pharmacopeia is 40 x 10⁹ CFU/dose. Many reports have stated lower doses of 3 ×10⁹ CFU in calves produce an immunity which is comparable to the one obtained with higher dosages (40 ×10⁹). Hence, a study aimed at the evaluating the existing dose with the reduced graded doses (1/10th, 1/20th and 1/100th) to determine the effective immunizing dose of *B. abortus* S19 specifically in Indian context for buffaloes. A total of 25 buffalo calves in the age of 4-5 months were allotted 5 each randomly in to each graded dose vaccine groups and were injected subcutaneously with specified dose of vaccine. Blood and serum samples were collected from 0, 14, 28, 45, 60, 90 and 120 days post vaccination (DPV) and processed for innate immune response (TNF-α, IL-6, IL-8, IL-12), humoral immune response (RBPT, SAT and iELISA) and CMI response (IFN-γ, CD4⁺ & CD8⁺ counts). The peak response of innate marker TNF-α was observed on DPV 14 and 28 and similar responses were noticed in full dose, 1/10th and 1/20th in comparison to control group. The antibody detection by RBPT was similar in full, 1/10th and 1/20th doses and antibody titer by SAT was same till DPV 60 between full and 1/10th doses. In iELISA, peak antibody response at 28 days post vaccination was taken as yard stick to compare the graded doses and the significant difference could not be observed between the full dose to 1/10th and 1/20th reduced doses in buffaloes. IFN-γ, a critical marker of CMI response increased at DPV 14 and 28 and decreased thereafter and significant IFN-γ response was noticed only in full dose compared to control group. The CD4⁺ cell count drastically increased from DPV 14, peak response was observed on DPV 28 and 45 days and the decreased concentrations were observed between full dose to 1/20th and 1/100th doses. Similarly, CD8⁺ cell count gradually increased from DPV 14, peak response was observed from DPV 60-150 days and decreased concentrations were observed between full to 1/10th , 1/20th and 1/100th accordance with the dose. Overall results obtained in this study suggest that the vaccine induced response in buffalo is similar in full dose and 1/10th graded dose till 120 days. The standard dose of calfhood vaccine can be further reduced to 1/10th without affecting the host immune response in Buffaloes.

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OS7-7. Characterization of the NOD-\textit{scid} IL2r\textsuperscript{γnull} Mouse Model to Study the Safety of \textit{B. abortus} S19 ΔvjbR Vaccine Candidate in \textit{Brucella}-induced Osteoarticular Disease

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Osteoarticular brucellosis is the most common chronic complication in \textit{Brucella}-infected humans. It has been reported that 46.5\% of \textit{Brucella} infected patients can develop osteoarticular disease, regardless of the age, sex, or immune status. The mechanism of bone destruction caused by \textit{Brucella} species remains unknown, which is partially attributable to the failure to identify an animal model to study \textit{Brucella}-induced osteoarticular disease. Development and use of such a model would be invaluable not only to determine the pathogenesis of osteoarticular damage, but also as a tool to screen potential side-effects associated with vaccination using live attenuated candidates. Among laboratory animals, mice have proven to be a valuable tool to understand basic host-agent interactions during \textit{Brucella} infection. However, osteoarticular disease is not typically manifested in this host species, and in the few cases it is observed development of osteoarticular damage can take longer than 6 months to develop, making it very challenging to study. In the present study, we explored the suitability of the NOD-\textit{scid} IL2r\textsuperscript{γnull} mouse model (characterized by the absence of mature T and B lymphocytes, natural killer cells, and the failure to respond to interleukin-2) as a tool to further examine the safety of vaccines candidates. To test the suitability of this strain for \textit{Brucella}- induced arthritis, mice were inoculated intraperitoneally with single dose of either 1x10\textsuperscript{4}, 1x10\textsuperscript{5}, or 1x10\textsuperscript{6} CFU of \textit{B. abortus} S19 or \textit{B. abortus} S19 ΔvjbR vaccine candidate, and monitored for the development of adverse side effects for up to 13 weeks including development of osteoarticular disease. Grossly, all animals inoculated with \textit{B. abortus} S19 developed a deviation and thickening of the tail region. Histopathology, revealed 100 \% of mice inoculated with \textit{B. abortus} S19 had tissue sections exhibiting severe dose-dependent inflammation in the tail vertebrae characterized by recruitment of neutrophils and macrophages, as well as proliferation and activation of osteoclasts and fibroblasts leading to bone resorption, destruction of the intervertebral discs, as typically observed in human \textit{Brucella} infection. In contrast, histopathological bone sections from mice inoculated with \textit{B. abortus} S19 ΔvjbR were unremarkable. Other clinical signs observed with \textit{B. abortus} S19 vaccination were hypothermia, weight loss, and malaise at 8 weeks post-inoculation. Splenomegaly and reduced liver weight was also recorded in this group correlating with high bacterial burden in liver and spleen a dose depended manner, whereas, mice inoculated with \textit{B. abortus} S19 ΔvjbR did not show any clinical signs along with a greatly reduced bacterial burden in the spleen, liver, and lung. In an effort to characterize histopathological lesions, along with bacterial tropism, immunohistochemistry, immunofluorescence, fluorescent in situ hybridization, and confocal microscopic analysis were performed on formalin fixed paraffin embedded tail tissue sections. The results revealed that \textit{Brucella} antigen was widely spread in the area of inflammation with a tropism to osteoclasts. Overall, these results not only demonstrate the safety of \textit{B. abortus} S19 ΔvjbR vaccine candidate, but also provide support for the potential use of this mouse model when evaluating vaccine safety.

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OS7-8. The application of synthetic oligosaccharides to develop a DIVA vaccine and diagnostic partnership based on the induction and detection of epitope specific anti-
Brucella OPS antibodies

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Control of brucellosis requires vaccination and subsequent eradication requires a serological test and slaughter programme. The current universally recognised vaccines for brucellosis all have significant and well documented flaws. For example, they are all live, possess residual virulence in livestock and are pathogenic to humans. The smooth vaccines B. abortus S19 and B. melitensis Rev1 also induce antibodies that react in conventional serological tests. This makes the differentiation of infected and vaccinated animals impossible in many circumstances.

We consider that anti-Brucella antibodies are an important part of immune protection against smooth species of Brucella (B. abortus, B. melitensis and B. suis). The surface of these cells is dominated by the O-polysaccharide antigen (OPS) which also dominates the antibody response. Consequently the presence of OPS within serodiagnostic antigens provides them with excellent sensitivity whereas antigens without OPS are comparatively ineffective diagnostics. Therefore the OPS are a necessary component of optimal diagnostics and vaccines.

Our approach was to resolve this conflict by manipulation of the A and M epitopes within the Brucella OPS. The aim was to develop an immunogen that induces a strong antibody response against smooth Brucella cells without inducing reaction against effective serodiagnostic antigens. The original concept was to develop a putative DIVA partnership using a chemically synthesised A epitope antigen, conjugated to tetanus toxoid, to induce anti-A (and C/Y) antibodies in combination with the previously described M epitope serodiagnostic antigen that has shown excellent diagnostic sensitivity and discriminates against anti-A epitopes antibodies.

Mice were immunised with the synthetic A immunogen and the resultant sera demonstrated significant titres against B. abortus S99, B. melitensis 16M and Y. enterocolitica O:9 sLPS antigens and also against synthetic oligosaccharide conjugates. Although the reaction against the M type synthetic antigens was up to 1.5 log10 lower (on average) than against the A type there was significant remaining titre. To refine our concept additional oligosaccharide structures were synthesized and were applied to the mouse serum and sera from Brucella infected cattle. The results provided fresh insights and prompted the preparation of novel OPS glycoconjugates. The sera derived from mice immunized with the novel OPS conjugates possessed strong antibody responses to both serotypes (A and M) of Brucella whole cell and sLPS antigens. Critically there was a complete absence of reaction against some synthetic antigens that were highly sensitive diagnostics in cattle. We will describe how the specific interactions between the antibodies and antigen structures have provided a means by which to break the OPS paradox and assist in the development of new Brucella vaccines.

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Poster Abstracts
Session 1: Human brucellosis

PS1-1. Seroprevalence of human brucellosis and their work practice analysis among veterinarians in Gujarat

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Brucellosis is an occupational and neglected zoonotic disease which has been reported endemic in many parts of the world. In India the agriculture and cattle rearing are main source of income. Gujarat state is the industrial hub and the dairy industry is one of the largest industry and dairy co-operative societies playing a major role in white revolution. This disease is generally overlooked and misdiagnosed because of lack of awareness among the community and health officials. Veterinarians are always at great risk as they handled diseased animals directly. Further the situation worsens due to lack of proper diagnostic facilities at the community health center (CHC) or public health center (PHC) level. The study was carried out to investigate the seroprevalence of brucellosis among high risk group of veterinarians and their work practices to understand the source of infection in the field condition.

Veterinarians are at high risk cause of handle animals directly. The serum of 365 veterinarians working in government and private sectors situated Gandhinagar, Anand and Vadodara districts of Gujarat of animal husbandry were evaluated using the Rose Bengal Plate Test (RBPT) Institute of Animal Health and Veterinary Biologicals (IAHVB, Bangalore) and commercially available Enzyme-linked immunosorbent assay (ELISA IgM and IgG) (NovaLisa, NovaTech Immundiagnostica, GmbH, Germany). The data was also collected on work practices and use of personal protective equipments while working in the field condition. The seroprevalence of brucellosis by RBPT has been 13.6 %, recent exposure to Brucella infection has been 16.1 % (IgM) and the past exposure has been found to be 20.2 % (IgG) have been established. This indicates that the prevalence of brucellosis among the veterinarians is very high. The data on the behavior of veterinarians for the prevention of Brucella infection indicates that these people are not adhering to the set procedures and norms to be followed while working in the field. Similarly the data on the use of personal protective equipment while handling animals with different ailments and clinical signs indicates that about 50 % of the veterinarians are ignorant of the use of PPE, this may be due to non-availability of PPE in field condition or lack of awareness. In India more work needs to be carried out on this aspect for the better management of human brucellosis among this highly susceptible group.

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PS1-2. Occupational risk factors associated with Human brucellosis among dairy farm workers in Gujarat

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Yearly half a million humans working in different occupation are affected by brucellosis, which is most wide-spread zoonoses throughout the world including India. Gujarat is one of the largest milk producing states in India with the contribution of 7.75 % share in the total milk production of India. As per state census data, out of about 102 lakhs total household of Gujarat, 42.6 lakhs households are engaged in Dairy and Animal Husbandry sectors as a primary or secondary source of their income. Ruminants are reservoirs of the microorganism. Hence, close association between human and animals, stray cattle, consumption of unpasteurized milk and dairy products and inappropriate waste disposal are well established and some of the principal factors perpetuating infection in humans.

The study was carried out among dairy farm workers to find out the Seroprevalence of Brucellosis in three district of Gujarat state. 2586 milk samples were screened to establish the exposure of dairy farm workers to Brucella infection were enrolled from 12 villages spread across 3 districts of Gujarat. Out of 2586 milk samples of household 385 customers (Registered with Cooperative and Private Milk societies) were found positive by Milk ring test antigen (MRT), Institute of Animal Health and Veterinary Biologicals (IAHVB, Bangalore) which represent each house hold at village level. Total 400 blood samples of dairy farm workers (male 183 and female 217) were screened by RBPT (IAHVB, Bangalore) results showed (43) 10.75 % positive cases. Confirmatory ELISA test (NovaLisa, NovaTech Immundiagnostica, GmbH, Germany) methods for human brucellosis showed (68) 17 % in IgM and (70) 17.5 % in IgG. The results showed high seroprevalence of brucellosis among dairy farm workers at village level in gujarat.

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PS1-3. Development of novel immunodiagnostic test for screening of human brucellosis cases using the whole cell antigens of *Brucella abortus* S19

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Brucellosis is worldwide disease and emerging cause of zoonotic infection in humans in developing countries. Existing test for diagnosis of brucellosis suffer from sensitivity and specificity limitation and therefore are unable to discriminate between the true positive and false positive serological results. In the present study, the diagnostic utility of in house designed ELISA assay using the whole cell antigens of *Brucella abortus* S19 was evaluated and compared with commercially available tests for the development of better tests for brucellosis.

A total of 568 serum samples were collected from a high risk Human population, which included i) malnourished population with high exposure to animals, ii) meat eaters from high the TB endemic area, iii) zoo-keepers, animal handlers and farmers. Samples were evaluated by indirect ELISA using the whole cell antigens of *Brucella abortus* S19. To determine the specificity and sensitivity of developed test, the results were compared with the commercially available IgG detection ELISA kit. Sensitivity and specificity, as given by the manufacturer was > 95 % for IgG as while that was also > 95 % for IgG for the in house designed protocol. Of all the samples tested, 7.4 % positivity was obtained in malnourished population with high exposure to animals, 14.4 % in meat eaters, 6.6 % in zookeepers and animal handlers, 31.2 % in farmers. On comparing the results of in house ELISA with that of commercial ELISA, the sensitivity and specificity of 94.4 % and 100 % was obtained respectively. Among the baseline characteristics, it was found that the major symptoms associated with the disease were fever, body ache, joint pain, lower back pain, loss of appetite and weight loss. The in house developed indirect ELISA had a better sensitivity and specificity than the commercially available kit. The two tests had a positive correlation.

In conclusion, a novel, simple, fast and cost effective ELISA method which employed the use of whole cell antigens of *Brucella abortus* strain S19 as antigens was developed. This assay could be used for further diagnosis of brucellosis and can be used in place of costly commercially available ELISA assay.

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PS1-4. Seroprevalence of human brucellosis in Telangana and Andhra Pradesh

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The present study was undertaken to know the sero prevalence of brucellosis in humans. A total of 1124 sera samples from human beings belonging to three regions (Telangana-401, coastal Andhra-502 and Rayalaseema-221) were tested for brucellosis by ELISA. An overall prevalence of 11.21% (126) was observed in three regions, prevalence of 11.47 % (46) was observed in Telangana State, 11.16 % (56) in Coastal andhra region and 10.86 % (24) in Rayalaseema region respectively. 1124 human serum samples screened in this study are from different occupations i.e., veterinarians (371), para veterinary staff (387), dairy farm workers (197), shepherds (104) and others (65) respectively. Out of 371 samples from veterinarians 39 (10.51 %), out of 387 samples from para veterinary staff 47 (12.14 %), out of 197 samples from dairy farm workers 23 (11.67 %), out of 104 samples from shepherds 12 (11.53 %) and out of 65 samples from others five (7.69 %) were positive.

Out of 1124 human serum samples screened from three regions, 900 were men and 224 were women. Out of 900 from men 106 (11.77 %), whereas from 224 women 20 (8.92 %) were positive ELISA respectively. Out of 1124 human serum samples tested from three regions under this study categorized as less than 10 years (51), 10-20 years (174), 20-40 years (510) and above 40 years (389). Out of 51 samples from less than 10 years age 3 (5.8 %), out of 174 samples from 10-20 years of age 13 (7.47 %), out of 510 samples from 20-40 years of age 69 (13.53 %) and out of 389 samples from more than 40 years of age 41(10.54 %) were positive respectively.

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PS1-5. Occupational Health Risk and Zoonotic hazards among Veterinarians

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To identify major occupational hazards prone to zoonoses in veterinary occupation, a questionnaire based study was conducted among veterinarians (n=124) at Gujarat and Maharashtra state. Study subjects comprised 62 % graduate and 31 % had post-graduate veterinarians in different disciplines of veterinary science and most of them (58 %) and (30 %) were working at Government and dairy sector respectively. Out of total interviewed maximum number (75 %) of veterinarians were involved in large animal practice and one fourth (25 %) of total veterinarians interviewed were not covered by any accidental insurance policy. Study findings revealed a high proportion (75 %) of veterinarians faced needle stick injury during their practice and it resulted in accidentally injecting rabies vaccine (5 %), nonspecific drug (71 %) etc. Biting and scratching injuries due to animals which may cause zoonotic diseases were found among 26 % and 57 % of veterinarians respectively. Though, prophylactic vaccination is an important intervention for zoonotic protection for veterinarian, it came out surprisingly to know that a large number (50 %) of veterinarians did not even receive common vaccination such as rabies and tetanus. Though being a fatal rabies disease very few no of vets (41%) have received rabies as prophylactic vaccine only. During the regular screening test of zoonotic diseases, most of veterinarians unable to screen themselves (51 %) for any zoonotic diseases whereas out of total screened for zoonotic diseases, only (46 %) reported to have tested for brucellosis. Most of the veterinarians (56 %) were mentioned that they are suffering with different ailments such as allergies, diabetes, enteric disorders etc. Only 1% of total interviewed veterinarians undergone routine (within 1 yr) medical health check-up. A majority of veterinarians (61 %) agreed that they were not following routine de-worming schedule. It was found that zoonotic hazards were the main reason of worry for most (79 %) of veterinarians and they need a proper training on occupational health risk in order to protect themselves from zoonotic hazards. Very few veterinarians (12 %) were found to be following the scientific method of hand washing in-between examinations of the patients. Though it may not be possible to completely prevent occupational zoonoses in veterinary medicine, but it can be reduced by taking preventive measures, creating awareness and including occupational health risk in Continuing Veterinary Education (CVE) program.

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PS1-6. Human brucellosis: Review of 61 cases from a tertiary care hospital of southern India

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Brucellosis is one of the major multi-system zoonosis of global importance. Brucellosis presents as acute, sub-acute or chronic febrile illness with protean clinical manifestations. Due to non-specific clinical symptoms it can be easily misdiagnosed with similar pathologies such as all of fevers of unknown origin that may be caused by infectious diseases, malignancies, collagen vascular diseases including tuberculosis, malaria, rheumatic fever and leishmaniasis. This study was carried out to describe the clinical, epidemiological, laboratory features, treatment options and outcome in patients with brucellosis.

This prospective observational study was conducted in a tertiary care hospital in southern Karnataka, India during April 2015 to July 2016. Diagnosis of brucellosis was made by isolating Brucella spp. from blood using automated blood culture system (BacT/ALERT, BioMérieux) and/ or by paired testing the sera for anti-Brucella agglutinins using the rose Bengal test and standard agglutination test (SAT). A titre of 1:160 or more was considered as significant. SPSS version 22 was used for all statistical analysis. During the study period, a total of 61 cases of brucellosis were diagnosed. Mean age of the patients were 41.8 (±16.4) years. Of these 61 patients, 52 (85.2 %) were male. Forty (65.5 %) and 11 (18 %) patients had history of contact with domestic animals and consumption of unpasteurized dairy products. Symptoms included fever (61, 100 %), myalgia (33, 54 %), musculoskeletal symptoms (23, 34 %), headache (16, 26.2 %), gastrointestinal symptoms (7, 11.4 %) and altered sensorium (2, 3.3 %). Twenty three (37.7 %) had hepatomegaly, 21 (34.4 %) had splenic enlargement, 5 (8.2 %) patients had cervical lymphadenopathy, 5 had hepatosplenomegaly and 2 (3.3 %) got meningeal signs. Anaemia was observed in 13 (21.3 %) cases, high erythrocyte sedimentation rate (ESR) was present in 51 (83.6 %) cases, leucocytosis in 12 (19.7 %), leucopenia in 7 (11.4 %), thrombocytopenia in 12 (19.7 %) and thrombocytosis in 1 (1.6 %) cases. Thirty one (50.8 %) of the patients were treated with combination composed of two (Streptomycin + Doxycycline or Rifampicin + Doxycycline), and 4 (6.5 %) with three antimicrobial agents. Relapse was registered in 1 (1.6 %), and 2 (3.3 %) patients were expired.

Diagnosis of brucellosis is difficult due to wide spectrum of clinical manifestations and non-specific clinical signs. Diagnosis needs to be supported by more sensitive, specific and rapid tests. Neurobrucellosis should always be kept in mind in patients with any neurological symptoms residing at endemic areas of brucellosis. Effective treatment is available for the human disease but prevention is the ideal, through control of the infection in animals. Timely recognition as well as prompt and proper treatment of the disease gives hope to its favourable outcome.

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PS1-7. Human brucellosis sero-surveillance using monoclonal antibody based blocking ELISA in high risk groups

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Brucellosis is one of the most widespread emerging zoonotic diseases. Human brucellosis is caused mainly by Brucella abortus, B. melitensis and B. suis through contact from cattle, goats/sheep and pigs respectively. Therefore, it remains an occupational hazard to high risk groups viz, veterinarians, butchers, slaughter house workers, farmers and laboratory personnel. Surveys from different parts of the world revealed that high risk of brucellosis (0.8 % to 58.6 % seroprevalence) in butchers and slaughterhouse workers than others. Early brucellosis diagnosis helps in appropriate treatment, to prevent carrier status and in differential diagnosis with other diseases like malaria, typhoid and rheumatic fever. Present study was carried out to detect brucellosis seroprevalence among slaughter house personnel using monoclonal antibody (MAb) based blocking ELISA co-developed by TRPVB and M/s. Ingenasa, Spain. A total of 115 personnel took part in the study from four sheep and goat slaughter houses in Chennai. An informed consent was taken from each individual with details of their age, sex, working experience and previous illness. Twenty nine samples (25 %) showed positive for detectable levels of human brucellosis IgG antibodies. The positivity indicates past or recent episodes of brucellosis. The observed seroprevalence was high (26.4 %) in maleworkers (n=106) than females (n=9; 11 %). The age group 30 to 65 was found more prone (n=26; 90 %) to get Brucella infection than youngsters between 18 to 27 years. Majority of the seropositive participants had more than ten years of experience in slaughter house. None of them revealed their previous illness as they were unaware of the etiology apart from indicating diabetes, blood pressure and mild fever. In addition, human sera samples (n=142) collected from Hyderabad slaughter house personnel and tested in this ELISA showed 16.2 % sero-positivity. Thus the MAb- based blocking ELISA is an affordable assay for human brucellosis sero-surveillance. The high prevalence in this risk group emphasizes the need for creation of better awareness, periodical screening and education on personal hygiene to avoid the occupational hazards.

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PS1-8. Evaluation of Fluorescence Polarization Assay Technology as a Human Brucellosis Diagnostic in Georgia

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Brucellosis occurs throughout the world and is a serious threat to public and animal health. Brucellosis is responsible for significant economic damage and human morbidity world-wide and it continues to be a major public and animal health problem in Georgia. Human brucellosis is diagnosed based on clinical symptoms and laboratory results, including bacteriological and serological tests. Laboratory testing is essential for diagnosis brucellosis. Serological tests are used for rapid detection of infected animals and humans. The fluorescence polarization assay (FPA) is widely used in animals; however it is not validated for human samples. In this study, we report evaluation of a new FPA Kit (Brucella FPA III) for testing human samples.

For the evaluation of the new FPA technology, delipidated serum samples from 19 confirmed (culture, ELISA, and tube agglutination) Brucella-infected patients were used. Brucella-infected samples were tested in conjunction with serum samples from 22 non-infected (culture confirmed negative, IgM and IgG ELISA negative, and tube agglutination negative) humans. Samples included 16 positive and 22 negative sera from GG-17 and three positive sera from GG-21. The samples were examined for the detection of Brucella specific antibodies using the FPA test by two people on separate days. Three positive sera were tested, in triplicate, again on separate days by two people to determine precision, reproducibility, accuracy, sensitivity, and specificity. The assay was conducted using the single tube format (Sentry 201).

Results were interpreted according to the manufacturer’s instructions (Ellie LLC, USA). Sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of the FPA were: 0.895, 1, 1, 0.917, and 0.951 respectively. The coefficients of variation of three positive sera tested by one staff were: 11 %, 9 %, and 12 % respectively. The coefficient of variation obtained by the other staff was: 26 %, 38 %, and 50 % respectively. According to our results sensitivity was somewhat low, but specificity was acceptable.

As a result of testing, the FPA technology hasn’t been adopted by the National Center for Disease Control (NCDC) at this time and further investigation is necessary for the diagnostic to be validated. While this kit, as tested in our laboratory, is very specific, the sensitivity was low. Precision needs to be improved. The single tube format is more cost-effective, rapid, easy, and accurate when compared with ELISA. Because of the ease of the procedure, it could be easily adopted for use in clinical laboratories.

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Ophthalmic brucellosis, a case report from India.

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Brucellosis— one of the major zoonotic diseases, still remains an uncontrolled problem, in regions of high endemicity. Ophthalmic brucellosis is not studied and overlooked in most developing countries including the South East Asian countries. In this paper we put forward a case report of a 40 years old female patient with Brucella uveitis. The patient presented with one and a half year history that started with sudden onset of redness and pain in the right eye, which subsided on routine medication that was subsequently followed by a recurrence of similar symptoms with bilateral involvement of both eyes. Associated with eye involvement, the patient also gave history of fever which subsided on medication and was of remittent type. Blood sample collected from the patient was subjected to five serological and one genus specific molecular investigation for the detection of Brucella infection. Conjunctival swab was also collected to know presence of any pathogenic microbial flora on the eye surface, including Brucella. After collection, the swab was inoculated immediately on to nutrient agar, blood agar, SDC slants (Sabouraud dextrose agar with chloramphenicol) and in biphasic Castaneda medium. Cultures on nutrient agar and blood agar were observed after overnight incubation at 37°C to find presence of any common pathogenic bacteria; culture on SDC was incubated at 25°C in BOD incubator and observed up to 21 days; while culture in Castaneda media was observed for any growth of Brucella up to 21 days post inoculation, and in subcultures on Brucella selective agar. Blood sample was subjected to Rose Bengal Plate Agglutination Test (RBPT; IAHVB, Bangalore), Standard Tube Agglutination Test (SAT; Tulip Diagnostics Pvt. Ltd.), ELISA (Immunolab GmbH, Germany) for the detection of IgM and IgG antibodies and 2ME test. For genus specific PCR (Prime), the serum sample was first subjected to DNA extraction using QIAmp DNA Mini Kit (Qiagen). The extracted DNA was then subjected to PCR. Patient was also subjected to Mantoux test, X’ray chest PA view, Toxoplasma IgM and IgG antibodies test. Patient showed positive results in all the serological tests (the SAT titre being 1:320 & with 2ME being 1:640) followed by PCR confirmation. Results of all other tests were negative. After treatment of brucellosis, patient recovered uneventfully. From this finding we can conclude that every case of systemic brucellosis should undergo routine ophthalmological evaluation and similarly all patients suffering from uveitis should be screened for brucellosis by available laboratory tests. This could reduce the possibility of blindness associated with brucellosis.

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PS1-10. Anti-lipopolysaccharide antibodies against brucellosis among risk groups in Central India

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Brucellosis is an important zoonotic disease caused by Brucella organism and exists worldwide. The bacteria are transmitted from animals to humans by ingestion through infected food products specifically unpasteurized dairy products and raw milk, direct contact with an infected animal, or inhalation of aerosols. In present study, a total of 546 human sera samples were collected. To detect the brucellosis in human the combination of serological tests including Rose Bengal Plate Test (RBPT) and IgG based Indirect Enzyme Linked Immunosorbent Assay (i-ELISA) using lipopolysaccharide (LPS) antigen were employed. The human included in the study were veterinarians, Para veterinarians, and labors of organized dairy farm and zoo, farmers and livestock owners. All humans included in the study were showed close association with the animals. On RBPT no sample turned positive while thei-ELISA showed 20 (3.66 %) serum samples positive.

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PS1-11. Human brucellosis: A study on seroprevalence and potential risk factors among the occupational high risk groups

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Brucellosis is one of the important occupational zoonotic threats in humans. The occupational source of exposure predisposes the framers, animal handlers, animal health care workers and slaughter house workers to a greater risk of contracting the disease. Determination of the seroprevalence and the major risk factors among high risk groups are very important to mitigate the disease acquisition. The study design included 464 samples consisting of veterinarians (n=43), livestock inspectors (n=100), artificial inseminators (n=104), farm workers (n=96), milk testers (n=12), semen collectors (n=9) and blood donors (n=100). The samples were screened for anti-Brucella antibodies using RBPT, SAT and IgM and IgG iELISA. Data on demographic and risk factors were collected in a structured questionnaire and analyzed by Logistic regression model RV 3.1.1. Regression analysis revealed the demographic parameters, like males (OR=1.82, 95 %; CI, 0.69-4.76) and 41-50 years (OR=1.73, 95 %; CI, 1.04-2.87) and 51-60 years age groups (OR=1.96, 95 %; CI, 1.14-3.35) were significantly correlating with the seropositivity. Among the high risk group, veterinarians (OR = 2.24, 95 %; CI, 1.11-4.53), artificial inseminators (OR = 2.13, 95 %; CI, 1.26-3.59) and livestock inspectors (OR = 1.34, 95 %; CI, 0.77-2.34) showed more risk for the disease with Odd Ratio (OR) ranging from 2.24 to 1.34. The association of animal health care activities when compared with brucellosis seroprevalence, contact with animals (OR=7.20, 95 %; CI, 3.05-16.97), raw milk consumption (OR=5.94, 95 %; CI, 2.67-13.21), handling aborted material (without gloves) (OR = 2.23, 95 %; CI, 1.37-3.64), veterinary care during parturition (OR = 1.80, 95 %; CI, 1.10-2.92) and animal shed cleaning and feeding (OR = 1.02, 95 %; CI, 0.50-2.06) were figured important in relation to seropositivity. Brucellosis symptoms such as fever, myalgia and joint pain (OR = 17.61, 95 %; CI, 7.14-43.41) and fever and myalgia (OR = 6.20, 95 %; CI, 3.26-71.78) were significantly correlated with seropositivity. The overall apparent prevalence (AP) was recorded very high upto 17.5% (95 %; CI, 14.3-21.2) in the tested samples. The study on risk variable indicates high seroprevalence of brucellosis among the veterinarians and para veterinarians and hence special attention to control disease in livestock and hygienic handling of the animals is necessary to reduce the risk of brucellosis in these occupational groups.

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PS1-12. Prevalence of Neurobrucellosis from Central India: A Hospital based study

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Neurobrucellosis is referred to involvement of the nervous system in the disease which is rare but a very serious condition, occurring in 5–10% of cases having brucellosis with high mortality rate if not checked. Permanent neurological disorders are common leading to burden of the disease on socioeconomic status of the patients. We aimed at diagnosing and showing the prevalence of Neurobrucellosis in a hospital based study in Central India population by detecting IgG and IgM antibodies specific for *Brucella* in CSF samples using ELISA based detection assay. For this, a total of 60 patients were retrospectively studied and screened for the disease using commercially available IgG and IgM antibody detection kit with little modifications for CSF samples. A total of 8 (13 %) patients showed IgG antibody positivity against *Brucella* while 2 (3 %) cases were positive for IgM antibody out of 60 screened cases. There were 4 (6 %) cases which showed both IgG and IgM antibodies positive.

Our study showed prevalence of neurobrucellosis in Central India population. Physicians should consider the likelihood of neurobrucellosis in the patients showing high risk for brucellosis with unexplained neurological behavior. Diagnosis of neurobrucellosis using ELISA based detection assay showed high sensitivity and specificity.

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**PS1-13. Rapid diagnosis and treatment follow up of human brucellosis by SYBR green based quantitative real-time polymerase chain reaction (qRT-PCR)**

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Brucellosis is a neglected zoonotic disease in humans. The endemicity of the disease in the region of high livestock population with non-specific symptoms and non-availability of any follow up serological test has led to many misdiagnosed and mistreated cases of brucellosis in humans. The aim of this study is to develop a rapid and efficient method to diagnose the disease by quantitative Real Time PCR and follow the treatment efficacy of the therapy. In this study, \(bcs31\) gene, a shared gene of \(Brucella\) species was cloned to pGEMT™ easy vector and the cloned plasmid was used to construct a standard curve, with linear range regression coefficient \((R^2)\) of 0.98 and efficiency of 1.89 over 7 orders of magnitude from \(10^7\) to \(10^1\) copies/µl. 188 high risk group suspected individuals were screened by serological tests of RBPT, STAT and ELISA. 55 serologically positive individuals were analyzed by PCR. 23 PCR and serologically positive individuals were recommended for 45 day therapy of Doxycycline (100mg BID) and Rifampin (600-900 mg/day) and were screened by qRT-PCR to quantify the load at different weekly intervals of treatment.

Out of 188 suspected individuals for brucellosis 85 (45.21%) were found to be serologically positive (RBPT and STAT). The intra- and inter-assay variation coefficients were 2 % and 1.97 %, respectively. The mean threshold cycle \((Cq)\) before treatment for the individuals recommended for therapy was 26.05 ± 0.35 (1,974.8 ± 549.6 copies/µl), which increased significantly \((p<0.05)\) to 32.7 ± 0.66 (142.5 ± 98.72 copies/µl) on 4th week during treatment, 35 ± 0.72 (14 ± 12.41 copies/µl) at 6th week on day of treatment completion, 35.66 ±0.66 (19.6 ± 9.11 copies/µl) on 21 day after treatment and 32.92 ± 1.08 (13.8 ± 7.47) on 18th week of treatment. No significant increase in cycle threshold was observed after the completion of therapy, but a significant reduction in DNA load (copies/µl) was observed during the above week intervals. Out of 19 PCR negative individuals, 13 individuals were qRT-PCR positive, although the Cq value mean for these 13 individuals was 33.38 ± 2.6 and mean copy number was 70 copies/µl. Symptomatic improvement was observed in 82% individuals after treatment, although weakness was a persistent symptom observed in individuals even after successful treatment. qRT-PCR is a rapid assay method which can be applied for rapid quantitative diagnosis of Brucellosis in humans with very high sensitivity. Therapy is more effective in acute cases of brucellosis than chronic cases.

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Brucellosis is one of the most common zoonotic infections transmitted to human through consumption of unpasteurized dairy product or through direct contact with infected animals or its body fluids after abortion, but is neglected disease in India, known to cause debilitating condition if not promptly treated. The purpose of this research is to establish health seeking behaviors of human brucellosis cases in rural as well as urban areas. Because, the signs and symptoms of brucellosis can be non-specific and can mimic those of many other diseases, therefore, meticulous attention is needed in making the confirmatory diagnosis and start specific treatment under the supervision of qualified physician.

A total of 474 sera were screened from human with the age ranged from 29 to 67 years, which included 291 male and 183 female respondents. These samples were received through local physician at Palanpur. Risk group comprising of Animal keeper (n=164), veterinarians (n=128), Para vets (n=49), Butcher (n=19) and general public (n=114). Among all these individuals, 350 individuals have one or more complaints of muscle pain, joint pain, back pain, headache, fever, sweating and orchitis. Rose Bengal plate test (RBPT), Standard tube agglutination test (STAT) and i-ELISA were performed. The cases found positive in RBPT were further screened by STAT and i-ELISA. In STAT tire ≥1:320 without symptoms or tire ≥1:160 with symptoms were considered as positive.

Out of 474 sera tested, 80 (16.88 %), 49 (10.34 %) and 23 (4.85 %) were detected positive by RBPT, i-ELISA and STAT. Among 291 samples of males, 47(16.15 %), 27 (9.28 %) and 15 (5.15%) and of 183 samples of females 33(18.03 %), 22 (12.02%) and 08 (4.37 %) were detected positive by RBPT, i-ELISA and STAT. A variable rate of seroprevalence was recorded in a person/s showing symptoms viz., muscle pain, joint pain, back pain, headache, fever, sweating and orchitis. Among the risk group persons Veterinarian (n=128) 17 (13.28 %), 09 (7.03 %) and 06 (4.69 %), Paraveterinarian (n=49) 13 (26.53 %), 07 (14.29 %) and 04 (8.16 %), Butchers (n=9) 03 (15.79 %), 01 (5.26 %) and 00 (0.0 %), Animal Keepers (n=164) 27(16.46 %), 20 (12.20 %) and 09 (5.49 %) and general public (n=114) 20 (17.54 %), 12 (10.53 %) and 04 (3.51 %) were detected positive by RBPT, i-ELISA and STAT respectively. Those samples which were detected positive in any of the two tests were considered as positive and were further subjected to the detection of Brucella in serum as well as in blood sample by conventional as well as real time PCR. Serum and blood samples of 08 persons who had one or more symptoms viz., back pain, join pain, intermittent fever suggestive of brucellosis were detected positive in real time PCR, which were undergone a treatment at local physician Palanpaur(Shah’s hospital, Palanpur) and they were monitored every months and after the 03 months regular medication with Doxycycline and Rifampicin they were detected negative and there was disappearance of the symptoms.

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PS1-15. Discussion of the rationale for urgent need of a national study for accurate nationwide estimates of human brucellosis in India

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According to various estimates India is considered to be endemic for brucellosis but WHO refer that that no country wide data on human brucellosis prevalence is available and all agencies project India to be having very high prevalence of human brucellosis. However; the data based on small sporadic studies and even multi-centric studies carried out in India have several limitations. Generally the greatest limitations in these studies have been the use of diagnostic test for the confirmation of disease has always been in question. Recent epidemiological studies by the NIOH research group on prevalence of this disease both in the high risk group of veterinarians and para-veterinarians along with the dairy farm workers at village level in Gujarat has revealed that the disease is prevalent in the general community and among occupational groups. Given that there is growing concern with the increase in the number of brucellosis cases in India, reliable and informative epidemiological evidence is vital to quantify impacts and predictors of disease and facilitate the formulation of prevention and control strategies. Currently there are large data deficits regarding the distribution, trends, determinants, and disease outcomes and where information is available, vast State wise heterogeneity and variable quality limits its value.

Keeping in view this present scenario the paper attempts to stress the need of a national study on brucellosis which should address the following questions i) what is the prevalence of brucellosis in India? ii) Whether Brucellosis can be classified as Occupational disease in India? iii) What is the rate of difference in the prevalence in rural and urban population? iv) Are there really regional disparities in the prevalence of occupational brucellosis in veterinarians and para-veterinarians India? v) If so, are these differences due to different work practices and PPE use by, veterinarians and para veterinarians, or differences in level of exposure at different work stations i.e. mobile unit, clinic or government job, dairy cooperatives etc. The present paper would highlight the some of the questions that needs to be answered and field experiences with respect to problems faced in the diagnosis and treatments of brucellosis. The public health draft for control and prevention of brucellosis among the community needs to be program need to be taken up and models for the containment of disease in the country where dairy and its subsidiary activities are the second largest employer so occupational health needs to be ensured.

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PS1-16. Neurobrucellosis –Microbiological and clinical evaluation

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Brucellosis is the most common zoonosis in the world, accounting for the annual occurrence of more than 500,000 cases. Although brucellosis and its means of transmission were discovered over 100 years ago, the disease remains a worldwide problem, predominantly so in developing countries. Human brucellosis is characterized by protean clinical manifestation. It is a multisystem disease that may present with a broad spectrum of clinical manifestations.

CNS involvement is a serious complication of brucellosis and clinical presentation is heterogeneous and the incidence ranges from 1 to 25 %. Neurobrucellosis may develop at any stage of the disease and diagnosis is usually made 2–12 months after the onset of symptoms in most cases. With this background the objective of the study was to analyze cases of Neurobrucellosis for its microbiological features and clinical correlation in a tertiary care neurocenter using different diagnostic modalities. Neurological cases with abnormal CSF and cases with neurological dysfunction not explained by other neurologic diseases were included in the study. The study was funded by ICMR and approved by the institution ethical committee. A total of 473 cases were analyzed. Clinico-demographic details were collected in a semi-structured proforma. Blood and CSF samples were collected and subjected to culture, Rose Bengal Plate Test, Standard Agglutination Test and Enzyme linked immunosorbent assay and Polymerase chain reaction.

Out of 473 cases, 195 (41 %) were negative and 278 (58.8 %) were positive in serum and or CSF by any method. Out of 278 positive cases, 123 (26 %) cases were positive only in serum by any test and 83 (17.54 %) cases were positive in serum and CSF by any test. Out of 83 cases, 50 (10.5 %) cases which were positive for Brucella antibodies and or PCR in serum and CSF 23 (4.86 %) showed antibodies in serum, 20 (4.22 %) showed antibodies in CSF. In addition 7 (1.4 %) cases were positive by PCR in serum and CSF. Neurobrucellosis should be considered in patients presenting with unexplained neurological symptoms. High index of suspicion and multimodal approach is essential for the diagnosis of Neurobrucellosis.

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Session 2: Host-Pathogen Interactions

PS2-1. Patho-physiological response of experimentally infected mice with Brucella abortus S19 vaccine and S19Δper mutant strain

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Brucellosis is a global anthropozoonotic infectious disease having considerable social, economic and international trade importance. Despite past and current efforts to eradicate brucellosis, a large number of new human cases are reported annually worldwide. Brucellosis in animal is characterized by reproductive failure such as abortions in the third trimester of pregnancy, premature or still births, reduced milk yield, high frequency of retained placenta, infertility, and prolonged calving interval in females, and orchitis with sterility in males. In this study, Swiss albino and BALB/c mice were infected with B. abortus 544, B. abortus S19 and its perosamine synthetase gene mutant, Brucella abortus S19Δper. The complete blood and differential leukocyte counts and detailed serum biochemistry profile for albumin, globulin, blood–urea-nitrogen (BUN), cholesterol, creatinine, total protein, triglyceride, glucose, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was determined 15 days post infection. In addition, histological examination of all major vital organs and fluorescence and immunohistochemical based detection of pathogen in all infected mice were also conducted. Higher serum AST and ALT level and observation of microgranuloma indicated that acute infection damages the liver. Other hematological complications, such as leucopenia and thrombocytopenia and moderate anemia were also observed. Therefore, measurement of hematological parameters with emphasis on hemoglobin concentration, platelets count, serum AST and ALT level may offer reliable indication of brucellosis irrespective of its causative strain.

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PS2-2. Infection of human placental trophoblasts by *Brucella papionis*

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The placenta, an organ bearing the responsibilities of nourishing and protecting the foetus, can be the target of infections altering pregnancy outcomes. *Brucella* are bacterial pathogens well known for causing huge reproductive losses in livestock, but several recent studies suggest that they could also cause complications in pregnant women. Notably, several zoonotic species of these intracellular bacteria can multiply efficiently within human trophoblasts, some specialized cells in the placenta. Such infections may alter the immune and hormonal functions of trophoblasts, which are essential during placental development. To investigate this possibility, we studied the infection of human trophoblasts by two strains of *Brucella papionis* that caused stillbirth in non-human primates. We found that *B. papionis* can invade both human cytotrophoblasts (CTB) and extravillous trophoblasts (EVT), but are only able to replicate within CTB, suggesting that EVT can restrict the intracellular growth of *B. papionis*. Both types of intracellular behaviour could affect the physiological functions of trophoblasts. In the placenta, CTB are stem cells that differentiate into 1) syncytiotrophoblasts, forming an epithelium in direct contact with the maternal blood, or 2) EVT, that have phagocytic properties and constitute the first immune barrier in the placenta. EVT also participate in the anchorage of the embryo and in the remodelling of uterine blood vessels to allow proper blood supply in the placenta. We are thus evaluating the consequences of *B. papionis* infection on the functions of trophoblasts *in vitro*, as well as their production of pro-inflammatory cytokines. We focus on CD98hc, a eukaryotic protein involved in the transport of amino-acids, the regulation of integrin-dependent signalling and cell fusion events and that was recently found to be important for *Brucella* replication in some cells. We will evaluate whether *B. papionis* infection alters the migration capacity of EVT or the ability of CTB to fuse together, both properties requiring CD98hc.

This work will help understanding the basic mechanisms of placental dysfunctions that could be induced by *Brucella* infections in pregnant women. Studying the role of CD98hc in this process, as well as the intracellular signalling pathways regulated by this protein, may also identify new therapeutic targets to fight infections.

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PS2-3. Omp31 plays an important role on outer membrane properties and intracellular survival of *Brucella melitensis* in murine macrophages and HeLa cells

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Brucellosis is an infectious disease that affects practically all species of mammals, including man, and is a major zoonosis worldwide. *Brucella* spp are facultative intracellular pathogens that have the ability to survive and multiply in phagocytic and non phagocytic cells such as trophoblast and epithelial cells. Among the six recognized species of the genus *Brucella*, *Brucella melitensis* is the main etiological agent involved in goat brucellosis and is also the most pathogenic for human. It causes significant losses in livestock production as a result of abortions, metritis, infertility and birth of weak animals. Outer membrane proteins (OMPs) are exposed on the bacterial surface and are in contact with cells and effectors of the host immune response, whereby they could be important virulence factors of *Brucella* species. To evaluate this hypothesis, the gene encoding for the major outer membrane protein Omp31 was amplified, cloned into pUC18 plasmid and inactivated by inserting a kanamycin cassette, rendering pLVM31 plasmid which was transformed into *B. melitensis* wild type strain to obtain LVM31 mutant strain. The Outer membrane (OM) properties of the mutant strain were compared with *B. melitensis* Bm133 wild type and *B. melitensis* Rev1 vaccine strains, in assessing its susceptibility to polymyxin B, sodium deoxycholate and nonimmune serum. The mutant strain was assessed in vitro survival assays in murine macrophages J774.A1 and HeLa cells. Our results demonstrate that LVM31 mutant is more susceptible to polymyxin B, sodium deoxycholate and nonimmune serum than control strains; moreover Omp31 mutation caused a decrease in the internalization and a significant decrease in the intracellular survival compared with the reference strains in both cell lines. These results allow us to conclude that Omp31 is important for maintaining OM integrity, but also it is necessary for bacterial internalization, establishment and development of an optimal replication niche, essential for survival and intracellular multiplication.

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PS2-4. Chronic low grade Th1 inflammation generated by Brucella infection induces selective alterations of marginal zone macrophages in spleen

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The spleen is known as the main filter for blood-borne pathogens. These later are trapped by specialized macrophage populations of the marginal zone (MZ); the CD209⁺ MZ macrophages (MZMs) and the CD169⁺ marginal metallophilic macrophages (MMMs). Acute systemic infection by various virus and protozoa strongly impact the location of T and B cells and the MZ populations. This phenomenon has been linked to a reduction of chemokine secretion by stroma cells in response to inflammatory reaction. Brucella spp. are the causative agent of brucellosis, a wide spread zoonotic disease leading to debilitating and febrile illness in humans. Here, we used B. melitensis infection as a model to investigate the impact of chronic stealthy infection on splenic MZ macrophage populations. During the late phase of Brucella infection, we observed that both MZMs and MMMs durably disappeared, leading to a reduction of the ability of the spleen to uptake fluorescent antigens and particles. This effect appears selective as all other analyzed lymphoid and myeloid populations increase during infection. Comparison of wild type and deficient mice suggest that MZ macrophage populations’ loss is dependent of IFN but independent of T cells or TNF signaling pathways and not correlated to an alteration of CCL19, CCL21 and CXCL13 mRNA expression. Our results suggest that both MZMs and MMMs are particularly sensible to persistent low grade Th1-mediated inflammation and that Brucella infection could reduce the ability of spleen to perform some MZMs and MMMs-dependent tasks, such as control of systemic infection, antigen specific response and tolerance.

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PS2-5. SytV gene silencing and its effect on early stages in phagocytosis in human macrophages infected by *Brucella melitensis*

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Brucellosis is one of the most important zoonotic and infectious diseases distributed worldwide that is characterized by chronic infection in humans and animals. This disease is caused by multiple species of *Brucella*, although *B. melitensis* is the species most frequently associated with human brucellosis, and it is the etiologic agent of the disease in goats. More than 500 000 new cases per year are reported globally predominantly in developing countries. All species of the *Brucella* genus are facultative intracellular pathogens and possess the ability to survive and multiply in professional and nonprofessional phagocytic cells. It has been found that *Brucella* is able to modify proteins that control intracellular traffic in the course of invasion and colonization. Otherwise during phagocytosis occurs the exocytosis, in these events the soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) associated to the endoplasmic reticulum are required, as well as Synaptotagmins. In our research group we have studied SNAREs proteins as Sintaxin 4 (STX4), Vamp 3 or SNAP-25. When the expression of STX4 mRNA was inhibited by silencing RNA, *B. melitensis* survival in THP-1 cells was significantly reduced at 12 h post-infection. In contrast, in infected and silenced murine macrophages J774.A1 with *B. melitensis*, the results indicated that VAMP3 is not involved in *Brucella* survival. Synaptotagmins are a large family of transmembrane proteins characterized by the presence of tandem C2 domains which act as a Ca⁺²-sensors, and regulate membrane fusion during exocytosis via interaction with SNAREs proteins. Syt V is predominantly associated to exocytosis of dense-core vesicles in neural cells and pancreatic cells. This protein can also be found in macrophages and plays a role in the regulation of phagocytosis. The objective of this work is to evaluate the role of Synaptotagmin V during *Brucella melitensis* infection by genetic silencing in human macrophages THP-1 trough qPCR and Western blot, and determinate the intracellular survival of *B. melitensis* before and after the genetic silencing. The partial results obtained are the purification of recombinant Syt V protein fragment and the production of a polyclonal antibody anti-SytV in rabbits to detect SytV protein in human macrophages by Western blot. Beside it, we determinated the quantitative expression of Syt V in non-infected cells by qPCR.

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The mbfA gene is carried by all Brucella species and is conserved in α-proteobacteria. mbfA encodes a chimeric protein (MbfA) comprising an N-terminal di-Fe peroxide reductase (erythrin) domain fused to a C-terminal, membrane-embedded, iron-export protein (VIT1, vacuolar iron transport 1). Both domains are anticipated to contribute to oxidative-stress resistance and thus MbfA could play an important role in the intracellular survival of Brucella. Our experiments show that mbfA complements an E. coli mutant devoid of catalases/alkyl-hydroperoxidases, enhances growth of a fur mutant with deregulated iron uptake, and impairs growth of a mutant lacking iron-uptake capacity; this supports a role in peroxide resistance and iron export. MbfA was shown to export 55Fe when expressed in E. coli, and this activity was found to be H2O2 dependent. In addition, MbfA mediated the decomposition of exogenous H2O2. A B. suis mbfA mutant displayed enhanced sensitivity to both Fe2+- and H2O2-mediated oxidative stress. In macrophages, mbfA enhances intracellular survival during the early colonization phase in the endosomal BCV, whilst in vivo mbfA contributes significantly to the maintenance of chronic infection within the liver and spleen. The isolated erythrin domain was shown to bind two iron atom (as expected) which stabilized the protein, but no substantive peroxidase activity was exhibited. The results are therefore consistent with a role for mbfA in intracellular survival and redox stress resistance.

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Session 3: Epidemiology

PS3-1. Assessment of cattle brucellosis surveillance from 2011-2013 in the Republic of Armenia

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Brucellosis is endemic in Armenia. More than 4,280 cases were reported in cattle from 2011-2013. *Brucella* can be preserved in the mammary glands for up to nine years. In order to assess the surveillance system for brucellosis in the Republic of Armenia I used data from the EpiInfo electronic database and paper-based records of examinations conducted by the “Republican Veterinary Sanitary and Phytosanitary Laboratory Services Center” State non-commercial organization.

The Rose Bengal test results from 2011 produced 839 positive responses from the tested cattle population of 553,885 (0.15 %). In 2012 there were 3,127 positive responses from 592,883 cattle (0.53 %). In 2013 there were 321 positives among 658,181 cattle tested (0.048 %). The suspected positive samples from the Marz laboratories are re-examined at the Republican laboratory for the final determination. Of the 43,475 positive samples from the Marz laboratories re-examined at the Republican laboratory in 2011, 839 samples (1.93 %) showed a positive response. In 2012, 3127 of 35,149 samples (8.89 %) registered positive. In 2013, 321 of 11,904 samples (2.7 %) were verified positive. Out of 9303 samples of cattle examined by means of the Rose Bengal test at the Marz laboratories from January-June 2014, 592 samples reported positive (6.3 %). Upon re-testing by the Republican laboratory using an ELISA, just 521 samples were positive. The RBT error rate can be calculated as (592-521)/592*100 %=11.99 %.

The analysis of the disease reveals that the increase of percentage of cases in 2012 is due to the improvement in both the detection of infected animals and in the organization of testing. Positive animals were subject to obligatory slaughtering. Reducing the rate of brucellosis in cattle requires continued testing and culling. Alternative diagnostics used in parallel with the Rose Bengal test (e.g. agglutination reactions or complement fixation reactions) should be adopted so as to avoid slaughtering animals that are not actually positive. Livestock owners should also be compensated for the slaughter of infected animals to reduce the likelihood that these animals will be hidden to avoid testing.

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PS3-2. Herd and Individual Prevalence of Brucellosis in Georgia

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Brucellosis is an endemic disease of animals and humans in Georgia, which leads to thousands of cases in human per year. In 2015, in accordance with the long term brucellosis prevention and control strategy of Georgia, cattle in high prevalence territories including the regions of Kakheti, Tbilisi (capital with surrounded villages) and Adjara were sampled (e.g. blood) and tested for the presence of brucellosis. The aim of the sero-survey was to identify new brucellosis foci and to determine the prevalence of disease among herds (village). In total 65,626 cattle were sampled throughout Georgia. Blood samples were taken from cattle up to 12 months of age. Blood samples were taken only from ear-tagged animals so traceability could be ensured. Data regarding sample collection (e.g., demographic information, animal ID, gender, and age) was collected using paper forms, which were entered in the Electronic Integrated Disease Surveillance System (EIDSS). All samples were sent to the Laboratory of the Ministry of Agriculture (LMA) following cold chain principles. The Rose-Bengal test was used for screening for brucellosis followed by enzyme linked immunosorbent assay (ELISA) or fluorescence polarization assay (FPA) as a confirmatory test. All positive animals have been culled and farms were disinfected. No compensation was issued to animal owners. Results showed that out of 754 investigated villages, 205 (27 %) were positive for brucellosis with at least one positive animal in a herd. The prevalence within the villages varied from 1-43 % with an average of 6 % of investigated animals; the mode within villages was 3 %. Brucellosis is widespread in Georgia (with 27 % of infected herds), but individual prevalence seems to be less than 2 %.

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PS3-3. Determination of the Geographical Distribution of Brucellosis in Georgia Using Geographic Information Systems

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Brucellosis is a widely spread disease among animals and humans in Georgia. Recently, the Repository of Bacteria and Viruses at the National Center for Disease Control and Public Health of Georgia (NCDC) collected more than one hundred Brucella strains, isolated during active surveillance activities in the country funded by the Defense Threat Reduction Agency (DTRA). The purpose of this study was to examine and describe the spatial aspects of the distribution of human and animal brucellosis in Georgia since 2008. A Geographical Information System (GIS) was used to identify the spatial distribution of brucellosis cases in Georgia. All passport dates of Brucella strains that are kept in the National Repository of Bacteria and Viruses of NCDC were mapped. Among the total number (n=116) of collected strains, 62.3 % (n=73) were classified as Brucella melitensis and 29.3 % (n=34) as Brucella abortus. The remaining 7.7% (n=9) were classified Brucella spp. Most strains of B. melitensis (n=64) were isolated from humans and animals in the east part of Georgia, particularly in the Kakheti region (n=20) and the Shida Kartli region (n=35). Also, there were some cases of B. melitensis in the Mtskheta-Mtianeti region (n=3). In the western part of Georgia B. abortus circulated mostly.

According to this data, a positive association was observed between the frequency of B. melitensis and the density of sheep in the eastern part of the country. Also, a positive association was observed between the frequency of disease cases caused by B. abortus and the density of cattle in some regions. Extrapolations of the results are crucial for monitoring the spread of brucellosis and for predicting future disease demographics and occurrences. The results indicate that brucellosis cases cluster geographically by species and the concentration of their animal hosts. In addition, for future study, it is necessary to evaluate geographic phenomena, such as microhabitats, that allow for the interaction of disease agents and hosts.

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The mithun (Bos frontalis), a unique ruminant of NEH region of India, is a semi-domesticated animal, managed in fenced tracts of forest rather than being kept in or near villages. Mithun, basically a meat animal, has a higher dressing percentage (58.82%) than cattle (55.96%). Many infectious diseases have been reported in mithuns including brucellosis. Brucellosis is one of the most common zoonotic diseases in the world also affects mithun but extent of disease is ill understood. The present study was targeted to find out the status of brucellosis in different strains of mithun with respect to age, sex and place using detection of antibodies and antigen in serum. Antibodies for Brucella were detected with Rose Bengal plate agglutination test (RBPT) and antigens were detected using polymerase chain reaction (PCR) with universal primers for 16s rRNA gene of bacteria and Brucella genus specific primers using genomic DNA template extracted from serum. In the study, a total of 702 serum samples of mithun collected with relevant epidemiological information (history of sickness if any, place, strain, sex, and age) were tested. Out of 702 serum samples of mithun, 234 (33.33 %) samples were positive for 16s rRNA bacterial gene. Among 234 positive samples for bacterial genome, Brucella genome could not be detected in any of the serum samples. Brucella antibodies were detected in 63 (8.9 %) mithun serum samples. About 2.6 % calf, 4.7 % young, 11.4 % adult and 19.1 % aged mithun were positive with RBPT. Difference in seropositivity for Brucella agglutinins with reference to age was statistically significant between calves and aged (p, 0.015), young and adult (p, 0.003), and young and aged (p<0.001) mithun. About 7.6% serum samples of mithuns from Nagaland, 16 % from Arunachal Pradesh, 14.8 % from Mizoram and 15.6 % from Manipur were positive for Brucella antibodies with RBPT. There was no statistically important difference for Brucella-sero-positivity of mithun of different regions except the mithuns in Nagaland region were more often seropositive than mithuns in Arunachal Pradesh (p, 0.038). Among the four strain of mithun, serum samples of Nagaland strain were more positive (14.2 %) for Brucella antibodies than mithuns of other three strains. Brucella sero-positivity among male mithuns (7.4 %) was less than in females (9.5 %). It might be due to higher susceptibility of females to brucellosis. Brucella genome could not be detected in serum indicating that serum PCR is not much sensitive for diagnosis of Brucella infection in mithun.

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PS3-5. Unusual productive and reproductive behaviour in cows affected with brucellosis – a field study in Odisha, India

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Altered productive and reproductive performances have been well documented in cows suffering from brucellosis. However, the present field investigation shows deviation to the earlier recorded behaviour that creates hindrance in effective control strategy of this occupational disease. Perusal of communication revealed that subsequent to the episode of abortion in a central Govt. Dairy farm during late 2009, complaints of abortion started pouring from different private dairy units from surrounding areas which prompted to initiate exploration. The study area covers 800 square km in Koraput district of Odisha state, India, a hilly area situated at 18° 49' 0" North and 82° 49' 48" East with a cattle population of 39,352 (2012 census).

Blood samples collected from 402 cattle including heifers and cows with/without the history of abortion(s) were tested during June 2015 to May 2016 for Brucella specific antibodies using indirect ELISA test kits procured from Genomix Molecular Diagnostic Pvt Ltd. where OD values of > 0.4 were considered positive. A total of the 197 (49.0 %) heads were confirmed to be seropositive reactors to Brucella antibodies. Further, Polymerase Chain Reaction (PCR) assay of clinical samples like vaginal discharge, aborted foetal contents and placental fluids from aborting cows tested positive for Brucella genus by generating an amplicon size of 223 base pair using bscp 31 primer and Brucella abortus species using IS711 primer producing an amplicon size of 498 bp which ultimately confirmed association of Brucellasp with the abortion storm. Information with respect to productive and reproductive performances were made available either from the cattle owner or health record maintained in the veterinary hospital. Carpal joint hygroma, repeat breeding and retention of placenta were recorded in 41 (20.81 %), 7 (3.5 %) and 134 (68.02 %) cases, respectively. Of the seropositive cows, 144 (73.09 %) cows had history of abortions. Further distribution as regards to number of abortion(s) revealed that 34.02, 15.97, 28.47, 13.88 and 7.63 % cows had abortion for 1, 2, 3, 4 and 5 times, respectively. There were continuous abortions with no live fetus in between. Rest 36.8 per cent seropositive heads had no history of abortion. Brucella abortus infection failed to exert any negative impact on conception rate i.e., cows continued to conceive after repeated abortions. In other words, conception to calving period (CCP) was extended as a result of multiple abortions. Maximum abortion was recorded in 7th month of pregnancy (28.47 %) followed by 6th and 8th (20.13 %), 9th (14.58 %), 5th (9.7 %), 4th (4.86 %) and 3rd (2.08 %) months. Average daily milk yield was 8.6 litres and lactational length was 248 days in seropositive cows with history of abortion(s). There was no significant variation in milk yield before and after abortion as the cows, following abortion, showed usual conception with continuation of lactation to its full term. In spite of such unusual behaviours i.e., absence of disruption in the continuity of conception with desired milk yield, dairy farmers of the area have been rearing brucellosis infected cows with least interest towards Brucellosis control programme. Supplementary work is in progress to surface more epidemiological picture, a prerequisite for appropriate control measures thereof.

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PS3-6. Epidemiological investigation and molecular detection of *Brucella* spp. in cattle at Mymensingh district of Bangladesh

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This study was undertaken for epidemiological investigation of brucellosis and molecular detection of *Brucella* spp. in cattle of Mymensingh. A total of 274 samples comprised of blood (n=250), placenta (n=4), vaginal swabs (n=10) and milk samples (n=10) were collected from cattle at farms, slaughter houses, veterinary hospital and villages of Mymensingh district. A structured questionnaire was used to collect information on animals age, sex, management practices and reproductive disorders. Serum was separated from blood samples for rose Bengal plate Test (RBPT) to know *Brucella abortus* specific antibody response. Placenta, vaginal swabs and milk samples were processed and inoculated onto both *Brucella* agar and blood agar media and incubated at 37°C for 7 days under supply of 5 % CO₂. Identification of *Brucella* spp. was performed by colonial morphology, Gram’s staining and biochemical test. Molecular detection of *Brucella* spp., at genus level was performed by polymerase chain reaction (PCR) assay targeting 16srRNA gene. Female cattle exhibited more prevalence of brucellosis (8.31 %) as compared to male (6.3 %). Sexually mature cattle over 4 years showed higher prevalence of brucellosis (10.15 %) as compared to young (below 4 years- 2.7 %), prevalence of brucellosis was higher in slaughter house (15.09 %). Cattle reared under semi-intensive showed higher brucellosis prevalence (9.20 %) as compared to cattle reared under intensive condition (4.59 %). Overall seroprevalence of brucellosis in cattle was (7.6 %). Three of 43 samples were found positive in bacteriological cultural, staining, and biochemical test. PCR assay confirmed only one cultural positive isolate as *Brucella* spp.

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PS3-7. Seroprevalence of brucellosis in goats at the selected areas of Mymensingh district


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This study was undertaken for seroprevalence of Brucella spp. in goats in selected area of Mymensingh. A total of 470 samples comprised of blood (n=450), spleen (n=15) and vaginal swabs (n=5) were collected from goats at slaughter house, veterinary teaching hospital and some villages of Mymensingh district. A structured questionnaire was used to collect information on animal’s age, sex, management practices, pregnancy status and reproductive disorders. Serum was separated from blood samples for Rose Bengal Plate Test (RBPT) to know Brucella spp. specific antibody response. Spleen and vaginal swabs samples were processed and inoculated onto both Brucella agar and blood agar media and incubated at 37ºC for 7 days under supply of 5 % CO2. Identification of Brucella spp. was performed by colonial morphology, Gram’s staining and biochemical tests. Overall seroprevalence of brucellosis in goats was 3.33 %. Higher prevalence was recorded in goats above 3 years (4.82 %). Female goats showed higher prevalence of brucellosis (5.64 %) as compared to male (0 %). Pregnant goats exhibited more prevalence of brucellosis (9.38 %) as compared to non-pregnant goats (5.94%). The prevalence of brucellosis was 22.22 %, 14.29 % and 4.76 % in goat with the previous history of abortion, retained placenta and infertility respectively. Prevalence of brucellosis was higher in veterinary teaching hospital, BAU (5.38 %). Goats reared under semi-intensive condition showed higher prevalence of brucellosis (4.06 %) as compared to goats reared under intensive condition (0.95 %). Findings of this study suggest that brucellosis was endemic in goats in the study area.

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PS3-8. Risk analysis of concurrent occurrence of Brucellosis and Infectious Bovine Rhinotracheitis in organized dairy farms

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Brucellosis and Infectious Bovine Rhinotracheitis (IBR) affects bovines and causes significant economic losses to dairy farmers in India. In the present study, risk analysis of brucellosis and IBR in organized dairy farms was done. Sera samples (n=1140) were collected from ten organized dairy farms in southern India. The major reproductive problems observed in the study area were repeat breeding (12.6 %), abortion (4.3 %), mastitis (1.4 %), metritis (0.4 %), anoestrus (0.2 %) and retention of placenta (0.1 %). The chi square analysis for determining associations and risk analysis were done. The apparent and true prevalence were 20.1 and 22.0% for brucellosis, 62.4 and 67.1 % for IBR, 14.6 and 15.8 % for both Brucella and IBR antibodies, respectively. Higher age groups (>6 years) showed increased both brucellosis and IBR positivity (4.9 %). Seropositivity was high in Females (14.8 %) for both brucellosis and IBR. The animals with history of abortion had higher seropositivity for both (32.7 %), which indicated that these diseases might have caused the abortion. The relative risk of occurrence of both brucellosis and IBR in cattle (0.45) was more than buffaloes and in females (0.64) was more than males. The attributable risk of occurrence of both brucellosis and IBR were 123.2 % in cattle than buffaloes and 5 per 100 females than males respectively. In cattle, the Odds ratio for both brucellosis and IBR was 0.38 compared to buffaloes. Females showed Odds ratio for disease (0.60) compared to males. The relative risk, risk difference, attributable risk and odds ratio of IBR positive animals were 1.62, 9 per one animal, 38.5 % and 1.81, respectively to become brucellosis positive animals. In conclusion, the risk results implies though there is variation in risk levels between species for both brucellosis and IBR occurrence, there is a need for vaccination for IBR and strengthening of Brucellosis control programme in organized dairy farms. Further, these diseases in organized dairy farms in southern India may be controlled effectively for better productivity and profitability of the dairy farmers.

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PS3-9. Molecular Epidemiology of *Brucella abortus* isolated from cattle in Brazil, 2009 – 2013

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Albeit eradicated in some countries, brucellosis remains one of the most economically important zoonosis, endemic in some regions of the world such as Latin America, Middle East, Africa and Asia. Since 2001, Brazil has started the Programa Nacional de Controle e Erradicação da Brucelose e Tuberculose (PNCEBT) (National Brucellosis and Tuberculosis Control and Eradication Program), which aims to reduce the prevalence and incidence of brucellosis in the country. Thus, the aims of the present study were to genotype *Brucella abortus* strains isolated from cattle in Brazil between 2009 and 2013, and to analyze their distribution to support the PNCEBT. One hundred forty *B. abortus* strains isolated from cattle in Brazil between 2009 and 2013 were genotyped using a set of 18 variable number of tandem repeats (VNTR) (MLVA16+HOOF-Print 3 and 4). The multiple locus VNTR analysis (MLVA) composed by eight markers (MLVA8) revealed eight different genotypes among *B. abortus* strains, including five previously described and three new ones. Analysis of the MLVA16 loci revealed fifty-eight distinct genotypes, from which three were identical, thirty-eight were considered very close, and seventeen were considered distant compared to those previously described and deposited in MLVAbank. Analysis of the HOOF-Prints 3 and 4 revealed the larger number of different alleles among all VNTR assessed, exhibiting maximum resolution when associated with MLVA16 markers. Our results validate the usefulness of the MLVA technique, especially MLVA16, in the molecular epidemiology of *B. abortus*. This study also provides insights on the genotypes of *B. abortus* circulating in Brazil, which certainly contribute for the better understanding of the epidemiology and control of bovine brucellosis in the country. Moreover, our data showed a high genetic diversity among the *B. abortus* isolates and a close relationship among these strains and Brazilian *B. abortus* deposited in MLVAbank, demonstrated by micro-evolutionary divergence arising from mutations in hyper-variable loci.

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PS3-10. Clinico and Molecular Epidemiological Characteristics study of Brucellosis in Animals.

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The poster describes the clinico epidemiological observations of Brucellosis over three years of period (2012-15) including molecular epidemiology. A total of 6410 sera from animals having different clinical conditions, cattle (2723), buffaloes (894), sheep (1072), goats (1281), camel (438) and equine (02) were collected and screened for the presence of anti *Brucella* antibodies by RBPT and i-ELISA. The overall seroprevalence in animals was 12.85 % (824/ 6410) and 11.87 % (761/6410) by RBPT and i-ELISA, respectively. The data were further analysed on the basis of clinical signs accordingly and compared with clinically healthy or incontact animals. The rate of seroprevalence in animal having heifer, clinically healthy, history of abortion, hygroma, pregnancy, non-pregnant, status unknown, still birth, Retension of placenta, Repeat breeding and orchitis was 4.65 and 6.97 percent, 7.23 and 6.56 percent, 25.58 and 29.09 percent, 13.33 and 11.66 percent, 5.45 and 4.21 percent, 7.81 and 6.17 percent, 7.74 and 6.16 percent, 16.00 and 20.00 percent, 14.22 and 11.71 percent, 14.22 and 11.71 percent, 19.70 and 14.35 percent, 34.34 and 18.18 percent respectively by RBPT and i-ELISA. Milk ring test was also performed for the milk collected from aborted animals. A total of 744 milk samples from cattle, buffaloes and camels were screened for presence of *Brucella* antibodies by MRT and of these, 152 milk samples were found to be positive. *Brucella abortus* was isolated from cows and buffaloes, while *Brucella melitensis* was isolated form sheep by processing the various clinical samples. MLVA and MLST typing of two isolates (sKN-1 and skn-2) revealed that they belong to ST-1 group.

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PS3-11. Identification of potential risk factors for bovine brucellosis in organized farms of Karnataka, India

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Brucellosis is a complex contagious, zoonotic and economically important bacterial disease of animals caused by Brucella spp. Disease is influenced by several factors including factors associated with disease transmission between herds, factors influencing the maintenance and spread of infection within herds, type of the animal, farm management and the biology of the disease. Understanding these factors is therefore vital for strategizing evidence based disease control measures and such information is inadequate in India. In the study, risk factors for bovine brucellosis in 24 organized farms of Karnataka, India for 3610 animals (cattle, n=3221 and buffaloes, n=389) were investigated by collecting farm and animal data in structured questionnaire. The seroprevalence was recorded by combination of RBPT and iELISA serological tests and seropositivity was compared with farm and animal data by logistic regression model using SPSS software version 15.0. The study revealed apparent and true prevalence at animal level as 6.1 (95 %; CI, 5.30-7.00) and 1.2 (95 %; CI, 0.00-2.40) for cattle; 8.2 (95 %; CI, 5.90-11.40) and 6.9 (95 %; CI, 3.60-10.30) for buffaloes and 6.3 (95 %; CI, 5.60-7.20) and 1.5 (95 %; CI, 0.30-2.70) in cattle and buffalo farms, respectively. In univariate analysis, seroprevalence was significantly associated with Holstein Friesian breed (P<0.001), animals in age group of 4-6 years (P<0.001) and cows after 3-5 calvings (P<0.001). At farm-level, the seropositivity was significantly associated with farms of rural (P<0.001), than peri-urban and urban regions, cleaning without disinfectant (P<0.001), stone flooring in the shed (P<0.001), monthly veterinary services (P=0.001), disposing manure in pits (P=0.047), accessibility of stray animals in the farm (P=0.047), non vaccination of brucellosis (P=0.062) and lack of brucellosis awareness among the farmers (P=0.069), animals procured from other farms (p=0.022), open discard of aborted materials (P=0.036) and cleaning the animal shed twice in a week (P=0.018).

Multivariate logistic regression model identified overall seven risk factors for brucellosis viz., absence of separate sheds for sick, calves, heifer and pregnant animals (OR=3.02; 95 %; CI, 0.63-14.43), non vaccination of brucellosis (OR=1.784; 95 %; CI, 0.95-3.34), disposing manure in pits (OR=1.464; 95 %; CI, 0.63-3.41), cleaning without disinfectant (OR=1.22; 95 %; CI, 0.67-2.21), sex (female, OR=1.15; 95 %; CI, 0.47-2.79), cleaning the animal shed twice in a week (OR=1.09; 95 %; CI, 0.66-1.82) and monthly veterinary services (OR=1.040; 95 %; CI, 0.42-2.61). The current study highlights 6.1 and 8.2 % seroprevalence of brucellosis in univariate and multivariate analysis. A concerted effort to control the disease is achieved by vaccination and instituting the complimentary measures for the potential risk factors for brucellosis in organized dairy farms.

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PS3-12. Bovine brucellosis: Evaluation of brucellosis management practices and vaccination campaign in two districts of Buenos Aires Province, Argentina

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In Argentina, bovine brucellosis is endemic. Vaccination of female calves between 3 and 8 months with Brucella strain S19 is mandatory. The SENASA entrusts the vaccination campaign execution to Local Sanitary Entities (LSEs).

Objective: to evaluate farmers’ and veterinarians’ management practices regarding the disease and to assess the vaccination campaign and vaccine coverage in two districts of Buenos Aires Province.

Methods: 4 different questionnaires were performed to 113 dairy and beef farmers, 11 veterinarians, 13 vaccinators and 2 people responsible for the LSEs. Also, 5 brucellosis experts answered the farmers’ questionnaires, giving their opinion on what should be the ideal and “unacceptable” answers. To check the vaccine coverage, serum samples of 20 animals vaccinated 21-50 days earlier from 25 of the 113 farms were randomly taken. The buffered plate antigen test (BPA) verified the vaccine exposure. Farms with at least 18 BPA positive calves were considered as “corrected vaccinated”.

Results: a) Farmers: 65% of farmers tried to diagnose the cause of reproductive disorders (Neospora caninum was the most common cause). Dairy farmers do more diagnosis (P<0.05) than beef ones. 42% of the farmers purchased cattle in the last year, however, only 38% did so from free certified farms and 27% tested the purchased animals, although all veterinarians suggested this useful practice. In beef farms, more “unacceptable” answers were found. b) Vaccinators: 77% calibrated the syringe, 40% homogenized the vaccine and 75% injected it again if some vaccine dropped. c) Vaccine coverage: 78% of heifers was BPA+, which is significantly lower (P<0.05) than the expected coverage of 100%. At all the farms at least one heifer was BPA+, but only 44% of them can be considered “corrected vaccinated”.

Conclusions: although farmers are well advised by their veterinarians, they should improve some management practices. The vaccination campaign is globally well implemented but some aspects should be improved. The low coverage is not due to the quality of the vaccine but more related to the lack of good vaccination practices.

Relevance: Knowledge of brucellosis practices and vaccination is useful to improve and adequate the National Control Program.

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Session 4: Diagnostics

PS4-1. Serological, Bacteriological, Molecular Techniques for Diagnosis of Brucellosis in sheep

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The present study reports the diagnosis of brucellosis in sheep using serological, bacteriological and molecular techniques. For serological study a total of 1373 samples were screened for the presence of Brucella antibodies by RBPT and i-ELISA. Of these, 94 and 66 were found positive for the Brucella antibodies respectively by RBPT and i-ELISA. For bacteriological study attempts were made to isolate the Brucella by processing a total of 89 samples viz., vaginal swabs (having previous history of abortion), cotyledons, aborted foetal organs (lung, liver, spleen, heart blood and stomach contents), hygroma and orchitis fluid, milk and blood (from aborted animals) on Brucella agar medium (BAM) with selective antibiotic supplements with or without 5%CO2 and incubated for 3-7 days. Of these, 03 Brucella isolates were obtained from a total of 03 isolates were obtained from vaginal swab, vaginal discharge and placenta. The colonies were identified as Brucella based on Gram’s staining, MZN staining, positive agglutination with serum, genus specific PCR. These were further identified as Brucella melitensis based on species specific PCR omp31 and Bruce ladder multiplex PCR. These samples were also processed for DNA extraction to detect Brucella by PCR directly. Detection of Brucella in various clinical samples collected from sheep and goats was attempted using B4/B5, JPF/JPR and F4/R2 genus specific PCR. Out of 89 clinical samples screened, 3 samples (2 vaginal swabs and 1 placental cotyledon) indicated the presence of Brucella. Species specific PCR based on omp31, B. abortus +IS711 and ORF A0503 was performed for the confirmation of Brucella species. All the three samples yielded amplicon of 720bp by primers omp31 but none of the isolates showed amplicon of 249 bp and 498 bp by ORF A0503 and B. abortus +IS711 primers, respectively. Amplicon of 720bp by omp31 primers indicates all the three samples of sheep to be Brucella melitensis.

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PS4-2. Differential Diagnostic Methods to Identify Rose Bengal False Positive Samples

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The Laboratory of the Ministry of Agriculture (LMA) is an international, ISO17025 accredited laboratory, working on animal diseases in Georgia. Animal brucellosis is an issue for Georgian farmers and animal owners. The risk of brucellosis cases in humans is very high. LMA uses a comprehensive series of assays to detect Brucella, including Rose Bengal as a screening test. Also, the laboratory performs testing of samples submitted by private farmers. LMA receives suspect Brucella samples through routine work throughout the year. To date, in this study, we identified 338 and 339 suspect Brucella spp. in 2015 and 2016, respectively. From these, 17 were Rose Bengal positive, however they were also Brucella ELISA and FPA negative. These samples are very interesting, indicating some type of cross-reactivity, so, we attempted a differential diagnosis for Coxiella burnetii. We tested these samples using the Coxiella burnetii detection algorithm (ELISA; IFA). We found that 48 % (eight samples) were positive by both tests (Brucella RB+ and Coxiella ELISA/IFA positive). These samples show that Coxiella positive samples can cross-react with the Rose Bengal test. LMA has a very important role in the brucellosis surveillance process in Georgia, and it is imperative that we can exclude Brucella false positive results and detect such contiguous zoonotic diseases such as Q fever. The laboratory continues to implement diagnostic methods for new diseases and for other diseases that may cross react with the Rose Bengal test, such as Yersinia enterocolitica, Escherichia coli, Salmonella spp., and Francisella tularensis. The increased capabilities of LMA will ensure increased detection of zoonotic diseases, which are important for disease control and public health and for expansion of the animal husbandry industry in Georgia.

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PS4-3. A pilot study evaluation of lateral flow assay – a point of care diagnostic for brucellosis

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Brucellosis is important bacterial zoonoses caused by different species of the genus Brucella in multiple livestock species and diagnosed by battery of laboratory based tests. Lateral flow assay (LFA), a rapid, user friendly diagnostics are gaining importance in the recent years. Brucellosis is endemic in India and field level diagnostics like LFA are highly suitable compared to existing sero-diagnostics. The present study highlights the evaluation of LFA using either blood or serum for diagnosis of brucellosis in comparison to gold standard RBPT and iELISA tests. A total of 792 both serum and blood samples from bovines (208), sheep (140), goats (219) and pigs (225) were evaluated. Comparative test evaluation of the LFA test with RBPT and iELISA were analyzed using chi square/fisher exact test, kappa, sensitivity and specificity using online software VASSARSTAT. True prevalence of brucellosis was found to be 6.70, 12.85, 2.73 and 39.11 by RBPT; 10.57, 16.42, 5.93 and 46.60 by iELISA; 5.28, 8.57, 2.73 and 31.10 by blood -LFA and 1.44, 10.71, 3.19 and 32.88 per cent by serum-LFA in bovines, sheep, goats and pigs, respectively. Overall seropositivity was found highest by iELISA followed by RBPT and LFA. The relative specificity (sp) for all the species was more than 99 per cent for LFA (blood) and 92 per cent for LFA ( serum) whereas relative sensitivity (se) ranged from 50 to 83.33 per cent by blood- LFA and 53.85 to 84.09 per cent by serum-LFA, respectively for all species compared to RBPT and iELISA. Overall Kappa agreement co-efficient value ranged from 0.61 to 0.80 (p<0.001) and 0.56 to 0.86 (p<0.001) for blood and serum LFA indicating good agreement characters of LFA test for both blood and serum samples. The present study concludes that either blood or serum can be used in the LFA test unlike other serological tests which requires only serum. The test can be used as a point of care diagnostics for surveillance of brucellosis for adaptation on-farm, market, and slaughter, clinical and pre-purchase test in the country.

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The aim of this study was to develop two indirect ELISAs (i-ELISA) using heat-extracted antigens prepared with the *Brucella canis* M- and *Brucella abortus* RB51 strains for the serologic diagnosis of *B. canis* infection in dogs. Reference sera consisted of 16 agar gel immunodiffusion (AGID)-positive sera from animals with clinical signs and positive bacterial isolation and 24 AGID-negative sera from healthy animals with no history or evidence of canine brucellosis were used to assess diagnostic sensitivity and specificity of the tests. Using validation panel, the cut-off values of OD \(_{414}\) 0.243 and 0.354 were selected to give 100 % diagnostic specificity and sensitivity for *B. abortus* RB51 i-ELISA and *B. canis* M- i-ELISA, respectively. Based on these cut-off values, 113 serum samples taken from dog shelters in Şanlıurfa and Bursa metropolitan municipalities tested by rapid slide agglutination test (RSAT) and both i-ELISAs. Of the 47 dog sera tested from Şanlıurfa, 6 (12.8\%) were positive by RSAT, 3 (6.4 \%) were positive by *B. abortus* RB51 i-ELISA and two of the samples (4.2 \%) were found positive by *B. canis* M- i-ELISA. Of the 66 serum samples from Bursa, 11 (16.6 \%) were found positive by RSAT, 5 (7.6 \%) and 4 (6.1 \%) of which were confirmed by *B. abortus* RB51 i-ELISA and *B. canis* M- i-ELISA, respectively.

In conclusion, both *B. abortus* RB51 i-ELISA and *B. canis* M- i-ELISA yielded the same diagnostic specificity and sensitivity with minor differences and it was thought that they could be used successfully as a confirmatory test together with RSAT as a screening test.

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PS4-5. A novel close-tube Loop mediated isothermal Amplification (Br-LAMP) assay for rapid detection of Brucella

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Loop mediated isothermal amplification (LAMP) assay is one of the robust nucleic acid detection platforms. Especially, the technique is suitable for laboratory-deficit field conditions for a rapid as well as accurate disease diagnosis. In the present report, two sets of LAMP primers targeting omp2b gene of Brucella are designed and standardized against all the major Brucella spp. The adoption of a close-tube format has made the technique possible for nucleic acid detection without any persistent contamination as an added advantage from previous detection strategies through LAMP. This is the first report of development of a close-tube Brucella genus specific LAMP (Br-LAMP) technique targeting omp2b gene.

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PS4-6. CITA enrichment broth is suitable for the direct isolation of *Brucella* spp. from field contaminated samples and for direct PCR

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The identification and typing of *Brucellae* requires their bacteriological isolation. However, because these bacteria are easily overgrown by contaminants on isolation plates the usefulness of bacteriological culture is limited when samples are contaminated (a very common situation) or numbers of *Brucellae* in tissues are low. To overcome these difficulties, the objective of this work was to develop an enrichment broth that would favor the growth of *Brucellae* and could be combined with PCR for direct diagnosis and typing.

Based on existing knowledge, we assessed the effect of basal broth components in combination with detoxifying agents and antimicrobials to support the growth of the major *Brucella* species in the presence of bacterial and fungal contaminants common in field samples. In a first step, we determined the growth of representative strains of *B. melitensis*, *B. abortus*, *B. suis* and *B. ovis* and field contaminants (*Escherichia coli*, *Enterococcus faecalis*, *Streptococcus thoraltensis*, *Kocuria* spp., *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*). For this, each basal broth was inoculated with 10\(^2\) CFU/mL of each bacterium and the colony forming units (CFU) and pH were determined after 4h, 24h, 48h, 72h and 6 days of incubation at 37ºC in a 10 % CO\(_2\) atmosphere.

A selective broth containing Mueller-Hinton as basal component supplemented with yeast extract, newborn calf serum, CAPS [N-cyclohexyl-3-aminopropanesulfonic acid] and the antibiotics used in the selective CITA agar medium (amphotericin B, colistin, nitrofurantoin, nystatin and vancomycin) plus phosphomycin, inhibited all contaminants while allowing the growth of all *Brucella* strains up to 10\(^4\) to 10\(^8\) CFU/mL (depending on the strain and incubation time) after 48-72h. Additional experiments on spiked samples (spleen and milk from sheep or pig) and tissues (lymph nodes and seminal vesicles) from naturally infected sheep or wild boar showed that CITA enrichment broth supported growth of *Brucella* spp. from less than 10 CFU/mL to more than 10\(^4\) CFU/ml after 48-72h of incubation, making possible their detection by direct PCR of the broth.

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Evaluation of filter papers as a novel method for transportation of specimen for diagnosis of brucellosis in animals

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Bovine brucellosis, caused by \textit{Brucella abortus}, is a highly contagious zoonotic disease affecting cattle and buffaloes causing infertility and abortion. The major impediment in the diagnosis and surveillance of the disease is the requirement of cold chain for transportation of the samples from remote location to the laboratory. Cellulose based filter papers can be considered as safe and easy mode of transhipment of clinical material. In the present study filter paper was evaluated as the means for transportation of serum and clinical specimens for diagnosis of brucellosis through antibody detection by ELISA and antigen detection by real-time PCR respectively.

Initially, \textit{B. abortus} 544 strain spiked in different matrix viz. bacteriological culture media, PBS, milk, nasal swabs (NS) and genital swabs (GS) of cattle and spotted on to the FTA\textsuperscript{®} elute card. Subsequently, DNA was extracted from FTA card and the organism was detected by real time PCR targeting BCSP-31 gene. The detection limit was found to be 10 bacteria and 480pg DNA. The laboratory validation of method suggested high repeatability with coefficient of variation less than 5\%. For field evaluation, nasal swab (NS), genital swab (GS), lacrimal swab (LS) and milk samples were collected from 87 \textit{Brucella} sero-positive animals and spotted onto FTA cards in the farm. Liquid samples were transported to the laboratory in the cold chain whereas FTA cards were transported at ambient temperature. Diagnostic sensitivity and specificity of FTA spotted samples was found to be 75 \% and 99.62 \% respectively as compared to their respective liquid samples. The sensitivity of FTA card for NS, GS, LS and milk was 88.89 \%, 82.35 \%, 66.67 \% and 40 \% respectively. The specificity of FTA card was found to be 100 \% except for GS (98.57 \%). The strength of agreement between both the two methods was very good (k= 0.823). NS was found to be the best clinical sample for \textit{Brucella} detection followed by GS, LS and milk. Screening of 182 FTA spotted clinical samples of blood, aborted material, milk, nasal secretion, genital secretion from animals of reproductive disorder revealed 6.5 \% positivity.

In another study, serum samples spotted onto Nobuto’s filter paper (Advantec, Japan) were evaluated for detection of \textit{Brucella} antibody by indirect ELISA. The protocol was optimised by panel of reference serum samples maintained in our laboratory and comparison of the data with direct serum sample revealed 5-14 \% coefficient of variation. Comparison of spotted filter papers with direct serum sample from 300 animals (collected from farm and field) revealed 97.27 \% and 100 \% sensitivity and specificity respectively. The strength of agreement was found to be good (k=0.972). The antibody could be detected after one month of spotting onto the filter paper without affecting sensitivity.

These results suggest FTA\textsuperscript{®} elute card and Nobuto’s filter paper can be used for transportation of clinical materials and serum samples for detection of bovine brucellosis by employing ELISA and molecular technique respectively. These filter paper offer safe, easy and affordable alternative solution for transport of \textit{Brucella} suspected clinical samples.

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PS4-8. Detection of *Brucella* spp. in milk by fluorescence *in situ* hybridization

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The detection of zoonotic bacteria like *Brucella* spp. is dominated by conventional cultivation techniques, which are sensitive but slow and laborious, and molecular assays like PCR, which are rapid but are unable to differentiate between dead and viable bacteria. Fluorescence *in situ* hybridization (FISH) is a promising alternative technique combining the ability to detect viability markers in whole cells like ribosomal RNAs with the advantages of rapid, culture-independent detection methods. In this work, we developed a reliable FISH assay for the detection of *Brucella* spp. and tested its performance in spiked milk samples.

For FISH probe development alignments of 16S as well as 23S rRNA sequences of various *Brucella* strains were used and subsequently evaluated in probeCheck. Hence, a target site in the 16S rRNA region was identified, which was exclusively found in 97.6 % of all *Brucella* strains. *In silico* and *in vitro* analysis revealed a high specificity and sensitivity of the chosen probe sequence Bruc-236 and no cross-reactivity was observed for closely related bacterial groups like *Ochrobactrum* spp. Lysozyme pretreatment significantly improved permeabilization and FISH probe penetration. Strong fluorophores in combination with a double fluorophore labelling of the probes resulted in bright and consistent FISH signals. To test the suitability of the *Brucella* FISH assay in food samples, unskimmed milk was artificially contaminated with *B. melitensis* strain 16M in concentrations ranging from 1 to 10^7 CFU/ml. The universal bacterial probe EUB338 was used simultaneously to monitor the occurrence of the accompanying flora. High spiking concentrations could be detected directly in the milk whereas for a low inoculum of 1 CFU/ml, a selective enrichment period of 24 to 48 hours was necessary. The milk ingredients, especially the milk fat, caused background fluorescence. Nevertheless, the signal-to-noise ratio still allowed unambiguous results. In contrast to FISH, the detection of *B. melitensis* by conventional cultivation in *Brucella* (selective) broth and subsequent plating took at least 4 to 5 days for all tested spiking inoculums.

In conclusion, FISH represents a suitable bridge technology between other rapid molecular methods, which are severely impaired to reliably identify viable bacteria, and the slow cultivation procedures and has proven to detect *Brucella* spp. with a high specificity in spiked food samples.

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PS4-9. Establishing a standard reference sera panel for validating sero-diagnostic tests for Brucella

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International standard sera are primary reference standards, which are necessary for the standardisation and harmonisation of diagnostic test methods for the detection of antibody to infectious pathogens. These international reference standards should be regarded as valuable commodity and should only be used for specified purposes. These standards should in turn be used as prototypes for the production and cross-standardisation of national standards. Such national or secondary standards should be prepared by the national authority to calibrate test methods in regional or state laboratories.

In this context, we have prepared two secondary standard anti-Brucella sera panels (in-house) comprising a total of 9 numbers of sera samples. This qualitative panel consists of 2 strong positive, 3 weak positive and 4 negative sera for validation of qualitative assays. These sera were calibrated alongside the three international reference standards from OIE Brucella reference laboratory and revealed 100 % relatedness by an indirect ELISA that has been calibrated according to OIE specifications and Annexure C of the European directive CEE 64/432.

The sequential sera panel consists of 60 sera samples sourced from 6 vaccinated cattle. These sera where collected from naïve vaccinated (Jersey cross bred) calves every three days since 0 day to 35 days post vaccination. This panel is intended for validating assays, which are developed for early detection anti-Brucella antibodies. The third panel consists of 2 numbers of anti-Brucella sera which are negative in culture tests for Brucella species and with assorted degrees of reactivity from field cases from different geographical areas. This panel can help in assessing the performance sensitivity across different diagnostic methodologies to detect Brucella antibodies. Development of a fourth panel of sera samples from animals that are positive for brucellosis by culture test (clinical or subclinical infection sera panel) is underway. Together these four panels have the potential to be recognized as a full-fledged national Brucella reference sera panel.

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PS4-10. Isolation and identification of *Brucella* spp. from farm animals.

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Samples (n=338) consisting of vaginal swabs, placenta, uterine discharges, foetal stomach content and milk were processed for isolation of *Brucella abortus*. Of these 338 samples, 220 samples were from cows and 46 samples were from buffaloes having a history of reproductive disorders and abortions, 65 samples were from brucellosis suspected dogs, six samples were from pigs and one sample was from sheep. The samples were streaked on *Brucella* agar base that was made selective by incorporation of *Brucella* selective supplement (Polymyxin B sulphate, Bacitracin, Nystatin, Cycloheximide, Nalidixic acid and Vancomycin). The inoculated plates were incubated at 37°C for 3-4 days in the presence of 5-10% CO₂. Out of 338 samples, 10 samples consisting of uterine discharges (03), vaginal swabs (04) and foetal stomach content (03) from cows (08), buffaloes (01) and pig (01) were positive for *Brucella* spp. by isolation. On *Brucella* selective agar, small, convex, smooth translucent colonies that were showing Gram negative coccobacilli on microscopic examination were further identified by biochemical tests like catalase, oxidase, urease and nitrate reduction test. All the isolates were MZN positive, non motile and failed to grow on MacConkey’s Lactose agar. All the isolates were oxidase, catalase, nitrate and urease positive. All the isolates produced H₂S and the reaction of all isolates was alkaline on triple sugar iron medium. All the isolates were negative for indole. Slide agglutination test was performed with the standard antiserum obtained from IVRI. All the isolates were agglutinated by *B. abortus* antiserum.

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PS4-11. Serum PCR for the diagnosis of brucellosis in dairy animals

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A total of 761 serum samples were collected from cattle and buffaloes from various organized and unorganized farms in and around Ludhiana. The samples were tested for brucellosis by polymerase chain reaction (PCR). DNA was extracted from the serum samples. Briefly, 100 µl of the serum sample was mixed with 100 µl of the lysis buffer. Proteinase K was added to a final concentration of 60 µl / ml and the mixture was incubated for 60 minutes at 55°C. Proteinase K was inactivated by heating the mixture to 95°C for 10 minutes followed by centrifugation at 12000 g for 10 minutes at 4°C. The supernatant was collected in a fresh centrifuge tube to which 0.1 volume of sodium acetate (3M) and 0.6 volume of isopropanol were added. The contents were mixed gently and kept on ice for 1 hour and then centrifuged at 8000 g for 10 minutes. The pellet was washed with 70 % alcohol twice and dried at 37°C. Finally, the pellet was suspended in 20 µl of Tris EDTA buffer and stored at -20°C till further use. The extracted DNA was subjected to PCR using B4/B5 primer pair derived from BCSP31 gene of B. abortus. of the 761 serum samples, 295 (38.76 %) samples were positive by PCR and an amplicon size of 223bp was obtained in positive control as well as in the samples.

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Brucellosis, a zoonotic disease is caused by Gram negative intracellular bacteria and leads to heavy economic losses to human and dairy animals in India as well as other developing countries. For effective disease prevention and control, early and precise diagnosis of disease is important. At present, the diagnosis of brucellosis in India is done by conventional serological test (rose Bengal test or standard tube agglutination test) based on the antibody detection against lipopolysacchide (LPS) or whole bacterial lysate. These tests may give false positive results due to cross reactivity to other pathogenic organisms. The present study was designed to develop a recombinant outer membrane protein, which may be used to develop the sensitive and specific diagnostic tests for bovine brucellosis. During the present study, the omp28 gene of Brucella abortus was amplified by PCR amplification using specific primers. The amplified omp28 gene was digested with the restriction enzymes (BamHI and HindIII) and ligated into the pMAL vector. Thereafter, the ligated product was transformed into the expression host i.e. E. coli DH5 α competent cells. The recombinant cells were confirmed by blue white screening, colony PCR. The omp28 protein was purified by Ni-NTA chromatography and further confirmed by western blotting with positive control bovine serum. This recombinant omp28 protein may be used for the development of suitable diagnostic test such as ELISA or latex agglutination test.

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PS4-13. PCR based detection of *Brucella abortus* in aborted cattle

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Brucellosis is a zoonotic disease that primarily affects animals, which act as reservoir for human infections. The disease is transmitted by direct contact with the secretions of *Brucella* infected animals which have aborted or by contaminated dairy products from infected animals. Reliable and sensitive diagnostic tools play a crucial role in the control of brucellosis in livestock and humans. The diagnosis of brucellosis is currently based on serological and microbiological tests, which are time consuming, low specific, cross reacting and represent a risk for laboratory personnel. In this connection molecular diagnostic techniques represent an important breakthrough in the diagnostic practice by targeting specific gene sequences for detection of *Brucella* organisms from clinical specimens. In the present study, 87 aborted tissue samples were collected from aborted cattle and subjected to PCR employing self designed in-house primer targeting IS711 gene which is specific for *Brucella abortus*. Results of this study showed, 16.09 per cent (14/87) of the samples were positive for *B. abortus* which yielded a specific product size of 378bp. To further validate our results, representative samples were sent for sequencing. The analyzed sequence was submitted to Gen bank and was provided with an accession No: KX528918 which showed 99.7 per cent homology with *B. abortus* IS711 sequence (AF148682). Phylogenetic analysis showed that the isolates obtained in this study formed a clad with other Indian *B. abortus* isolates. To conclude IS711 PCR assay developed in the present study was highly specific and sensitive for detection of *B. abortus* from aborted cattle.

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Assessment of laboratory diagnosis and field screening test for identifying brucellosis in herds with reproductive disorders

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Brucellosis is a zoonotic disease which causes reproductive disorders in livestock, such as abortions, infertility and repeat breeding. It is caused by several species of Brucella, mainly Brucella abortus in cattle and Buffalo, Brucella suis in pig, Brucella melitensis in goat and Brucella ovis in sheep. Though Brucella melitensis is predominant in other countries Brucella abortus is the endemic and predominant species in Sri Lanka. Brucella abortus bio type 3 was the mostly reported bio type in cattle. Bovine Brucellosis has higher economic impact on cattle farming. It had been introduced to the country through cattle importation during the 2nd World war and first clinical outbreak of bovine brucellosis was reported in 1956. The incidence of bovine brucellosis is more prominent in dry zone of the country raised under the extensive management system. According to the studies the sero-prevalence of brucellosis in that area was 4.7% and 4.2% in cattle and buffalo respectively, and it has been considered that the Up country wet zone is free of Brucella abortus. According to the Epidemiological Bulletin of Department of Animal Production and Health, Sri Lanka, 164 Brucella abortus suspected cases were reported in 2015. Under the national disease prevention and control program, the heifers at 6 months in endemic areas have to be vaccinated with locally produced S-19 vaccine. Along with the vaccination program, Brucella suspected herds which showed high incidence of abortions in all over the country are screened with milk ring test and Rose Bengal Plate Test for serological diagnosis by the range Veterinary Investigation officer (VIO). All positive sera for RBPT are confirmed by Complement Fixation Test (CFT). The Veterinary Research Institute (VRI) is the national reference laboratory where Brucella isolation and serological confirmation by CFT are being done in Sri Lanka. This study was conducted to assess the seropositive animals in brucellosis suspected herd with history of abortions during the period from 2010 to 2015 and to differentiate them from the vaccinated animals from non-vaccinated animals by laboratory confirmation. During the study period, 2189 cattle and 175 buffalo serum samples were submitted to the VRI as RBPT positive and out of those 390 and 17 were respectively confirmed as Brucella abortus positive samples by CFT. The aborted fetal samples were cultured for isolation of Brucella during the study period. Out of 133 foetal samples from cattle 4 were culture positive and confirmed as Brucella abortus. However, none of the samples out of 19 aborted foetuses obtained from buffaloes were found as culture positives. The results revealed that although high numbers of seropositive animals were found by RBPT, only 17.2% animals were confirmed as Brucellosis positive. Rest of the RBPT positive results may be due to the vaccination in one time in their life time.

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PS4-15. Development of Simple, Rapid and Inexpensive Point of Care Dip stick Diagnostic kit for Brucellosis to screen milk and body fluids


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Brucellosis is a reemerging and commonest zoonotic disease in the world especially in central and near east asia. Brucellosis is a febrile disease in human and typical symptoms range from undulant fever, septicemia, arthralgia, myalgia, spondylodiscitis of lumbar spine accompanied by sacroiliitis are very characteristics of the disease. It causes abortion in cattle or premature calving of recently infected animals, most often still births between the fifth and eight month of pregnancy leading to poor milk yield and economical loss. Recently, incidence of bovine brucellosis has been increased, possibly due to escalated free trade and rapid movement of livestock as well as poor awareness of the disease in dairy field, animal handlers and in public at large. In the humans, the infection is known to occur through consumption of infected raw milk, milk products and undercooked or raw meat apart from handling the body fluids of infected animals, aborted featuses. The diagnosis of Brucellosis is a very complex procedure. Generally, Brucellosis is diagnosed by identification of specific antibodies present in serum, plasma or other body fluids of suspected animals. A definitive diagnosis of Brucellosis is by isolation of Brucella species from specimen is a time consuming, very laborious and hazardous process. In general the control and eradication of this Brucellosis is based on the serological detection of disease specific antibodies that are present in the collected specimens and segregating or culling the infected animals.

There is a great demand for a simple, rapid, inexpensive on the spot detection device that can screen the raw milk and suspected animal body fluids for Brucellosis. Here, we describe a simple Lateral Flow based dip stick assay that can detect antibodies against Brucella species (Brucellaabortus, B.melitensis and B.suis). The device comprises a plastic backing laminated with one end sample pad followed by a conjugation pad that is impregnated with protein-G conjugated with gold nanoparticles and the other end absorption pad and both of them connected with a nitrocellulose membrane that has been immobilized with Brucella specific sLPS at test line and specific analytes at test line to validate the performance of the assay. A total of 538 milk samples from lactating cattle from organized and unorganized sources were tested and compared with indirect ELISA and milk ring test. The data suggests the dip stick kit is 100 % specific and 88 % sensitive compared to indirect ELISA. These kits are inexpensive 15 cents (fraction of a dollar) and can be stored at room temperature and will be useful for resource limited and point of care areas.

These kits will be of great help and handy to screen the milk centers for survilance of Brucellosis infected milk samples as well as to rule out Brucella related still births in livestock. Especially, these kits will be very valuable for individual house hold who are keen on organic milk, the concept has been picking up increased demand now a days. Further more, these inexpensive kits will be great assets to screen the animals before purchasing for new dairy farms.

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PS4-16. Membrane Filters Used to Stabilize Biological Samples for Brucellosis Disease Diagnosis in Economically Challenged Areas.

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Proper disease diagnosis and surveillance of emerging and re-emerging diseases like Brucellosis are often facing many difficulties of sample collection, storage and the transportation throughout the world most commonly in endemic and economically challenged areas. Most of the biological samples undergo degradation either by proteases, nucleases or due to high temperatures. A large variety of products are available in the developed countries to meet the requirements for sample collection, storage and transportation like FTA membrane by Whatman Inc, USA. Though these membranes are widely used across many molecular disciplines for sample capture, storage and analysis but they are much costly (about 3-4 dollars) to utilize in economically challenged resource limited areas.

Here we present Sample Stabilization Membrane Filters that are developed using a polymer based technology coupled with protease and nuclease inhibitors to stabilize and protect biological sample for long term storage and transportation at room temperature. The membranes prepared with combination of polaxomers, carbohydrates and other polymers were tested for their suitability in stabilizing the biological activity or integrity of the molecules. Biologically active components are physically entrapped, immobilized and stabilized for storage at room temperature. Samples collected on these filters are studied for their temperature stability and biological integrity and assay suitability for PCR, ELISA, Lateral flow and immune-blot methodologies. While testing, rehydrate the dried membrane with the sample diluent and then sample can be tested for diagnosis applications of wide range of veterinary and human/zoonotic diseases like Brucellosis, ParaTB, Thilaria at Penside and malaria, Microfilaria in the jungle and tribal areas that are hampered with limited resources. Comparative analyses between commercially available filters and filters prepared by us are carried out on samples of Whole blood/ Serum/ Plasma/ Nucleic Acids and body fluids from aborted fetuses for disease diagnosis purpose especially for Brucellosis and thilaria at Pen side.

The data obtained from this study using these Sample Stabilization Filters are comparable with commercially available expensive filters and samples can be used for RDT, ELISA and nucleic acid analysis especially PCR/LAMP assays at a later stage validation. The samples can be stored up to six months with 95% sensitivity and specificity when compared with data obtained on fresh samples. Thus these filters will be very useful to collect and store samples in bulk from resource limited areas and perform the Diagnostic assays at a later stage for epidemiological purposes.

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PS4-17. Comparison of serological tests based on microbiological culture for the diagnosis of canine brucellosis

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Canine brucellosis is a zoonotic disease, caused by Brucella canis, with global importance. In dogs, this disease is a reproductive disease characterized by retained placenta and impaired fertility. As there is risk to human infection in public health, diagnosis for brucellosis should be conducted quickly and correctly. But the present screening diagnosis method still has problem of non-specific reaction. Thus, this study is to compare conventional serological tests with microbiological culture which is considered as a golden standard for the diagnosis of canine brucellosis, ultimately to find out improvement of the diagnostic tests. A total of 347 whole blood-samples were collected from kennel dogs in South Korea in January to June 2016. Serological tests conducted in this study included rapid slide agglutination test (RSAT), RSAT with 2-mercaptoethanol (2-ME RSAT), and immunochromatographic assay (ICT). In comparative study, bacterial isolation was attempted by direct microbiological culture from whole blood of dogs which were interpreted as positive in serologic tests (2-ME RSAT and/or ICT). All Brucella-suspected cultures were confirmed as B. canis by differential multiplex PCR. Of 347 dogs, positive dogs in RSAT, 2-ME RSAT, and ICT were 101 (29.1%), 43 (12.4%), and 36 (10.4%), respectively. Of 45 dogs which were positive in 2-ME RSAT and/or ICT, B. canis were isolated from 11 dogs (24.4%). Correlation between bacterial isolation and serological test, 2-ME RSAT and ICT, were 25.6% and 30.6% respectively. As a result, positive rate of RSAT was the highest among serological tests, but correlation with bacterial isolation was the highest in ICT. Although we couldn’t find out significance among serological tests in this study due to small amounts of samples and seropositive individuals, in the future study, we will reach the reliable results with more samples to establish the ways that can supplement discordance among serological tests.

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Evaluation of storage stability in LPS antigen for indirect milk-ELISA screening dairy brucellosis

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The Brucella abortus smooth LPS has broadly used to an indirect ELISA to detect B. abortus antibodies in both bovine serum and milk samples. But practically little has been known about stability of LPS antigen during storage after coating in plate of ELISA for diagnosis of dairy brucellosis with milk samples. Thus, we investigate the storage stability of Brucella LPS antigen coated in ELISA format to establish the storage period and develop the indirect milk-ELISA for detecting Brucella infection.

The assays used B. abortus smooth LPS antigen that was produced by hot-phenol extraction. Following by coating LPS in 0.01M phosphate buffered saline on a polystyrene matrix; all plates were stored at 4°C and then evaluated8 times for 24 weeks. A total of 43 bulk milk samples were selected from 3573 dairy herds whose results of MRT were positive or negative together with comparing to commercial milk-ELISA and serological tests. The cut-off value determined using receiver operating characteristic (ROC) analysis was fixed at 450nm OD of 0.22 [21.9 likelihood ratio (LR)] giving the sensitivity of 93.8 % with a 95% confidence interval (CI) of 73.97–99.02% and the specificity of 93.62% (CI, 94.64–99.42).

One week after coating LPS antigen on plate, percent positivity (PP) was 35.14 with an acceptable sensitivity (90.91%) and specificity (99.87%). Since then it has kept the level of more than 27 PP until 16 weeks (r²=0.6314). At 20 and 24 weeks, milk-ELISA showed 18.92 and 8.11 PP, respectively, indicating a significant decrease comparing to 1 week after coating. The result depending on storage time exhibited correlation with commercial milk-ELISA test and serological tests; from 1 to 16 weeks moderate or good correlation with commercial milk-ELISA test (kappa, 0.63-0.811; CI, 0.362–1.00), with a range of sensitivity (58.33-83.33%) and specificity (88-100%) and with serological tests (kappa, 0.46-0.708; CI, 0.252–0.973).

In conclusion, this study validated that LPS-coated indirect ELISA designed for diagnosis of dairy brucellosis with milk samples had reliable storage stability at least for 16 week. Moreover, the newly designed LPS-milk ELISA could support more accurate diagnosis for diary brucellosis.

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PS4-19. Specific identification of the genus *Brucella* using SNP of *bcsp 31*

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Brucellosis is known as a widespread zoonotic disease caused by *Brucella* spp. There are several diagnostic techniques for brucellosis, but it’s difficult to establish phenomenon by non-specific reaction. Among a diversity of tests, especially in case of PCR, perhaps cause of non-specific reaction is considered to attribute highly homologous bacteria with *Brucella* spp. Therefore, we investigated discriminable sites in *Brucella* spp. comparing with these genetically similar bacteria. To begin with, we selected 16S rRNA gene, because it has used for phylogenetic studies as it is highly conserved in different species of bacteria and archaea. We designed primers for 16S rRNA gene and performed conventional PCR for 10 *Brucella* reference strains and 3 *Brucella* vaccine strains. As expected, sequences of all of them were equal except their single nucleotide polymorphism (SNP). Then, amplified sequences of 16S rRNA were aligned with a number of bacteria in the NCBI Blast. Owing to identities over 90% with *Brucella* species, it was difficult to assort between these and selected sequence. Alternatively, among a variety of genes explored in this study, *Brucella* cell surface protein 31 (*bcsp*31) gene was selected because many researchers had confirmed that the *bcsp*31 gene was highly conserved in *Brucella* strains. Like the 16S rRNA, primers were made for *bcsp*31 gene and PCR products of *Brucella* species were compared each other in sequential order. Finally, through comparison with genomes of a number of bacteria in the NCBI Blast, there were identified specific SNP to differ from them. In summary, we aligned 16S rRNA and *bcsp*31 gene to find a discrimination point between *Brucella* species and the other bacteria. Then, we identified the genus *Brucella* specific SNP at *bcsp*31 gene, and it realized *bcsp*31 PCR could use a genetic based diagnostic tool or molecular analysis.

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**PS4-20. Fast methodology to diagnosis caprine brucellosis**

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Brucellosis in goats is an endemic disease in specific regions in Argentina. Precise diagnosis is a major tool to control the disease. However, some regions either do not have laboratories facilities or do not have the chance to do major test who can define the presence of brucellosis in a flock. We present here a simple methodology which is possible to be done in a very basic laboratory.

**Methodology**

598 blood samples from goats were collected. Serological tests performed: Buffered Plate Antigen Test (BPAT), was used as screening and Fluorescence Polarization Assay (FPA) as confirmatory test (cutoff 85 mP). Complement Fixation test (CF)(cut off:20 UIFC) was also done. Tissue samples were taken to do bacteriological studies. Briefly, material of abortion or lymph nodes, spleen, and testicle taken from positive animals were analyzed. Tissues were homogenized and plated in Tryptose Agar, incubating 7 days at 37° and plates were checked every day. Contaminated plates were disposed.

Complement Fixation and bacteriology has been done only to bring complementary information.

**Results**

BPAT was positive in 127 samples. From these, 96 were positive to FPA. These results were also confirmed with CF given the same results. 471 animals were negative to all the tests. *Brucella melitensis* biotype 1 was isolated from testicle and lymph nodes from 3 different animals.

**Conclusion**

Good serological methods are necessary to control any diseases. Brucellosis has very well-known serological techniques. However, it is very important the decision of the test to be used which allows bringing precise results as fast as possible. We show here a very precise methodology using BPAT as screening and FPA as confirmatory which can be implemented in any place. BPA has been demonstrated to be an excellent screening test for caprine brucellosis and FPA shows the same specificity than CF, thus is an excellent methodology to be applied to any control program.
PS4-21. The application of synthetic oligosaccharide antigens to false positive serological reactor samples in cattle suggests at least two populations derived from different cross-reactive organisms

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Serology based on the detection of antibodies to the Brucella OPS is the principle method for the detection of brucellosis in livestock. Sera from infected animals are typically rich in anti-OPS antibodies and therefore such assays have good diagnostic sensitivity. However, in regions that have low prevalence of disease or are free of brucellosis false positive serological reactions (FPSR) may become a significant problem. These arise due to infection with Gram-negative bacteria that are unrelated to \textit{Brucella} but which possess OPS which has structural similarities. The archetypal field FPSR inducer is considered to be \textit{Y. enterocolitica} O:9. However, other bacteria with similar OPS structures, such as \textit{E. hermannii}, also possess this FPSR potential.

The \textit{Brucella} OPS is an unbranched homopolymer of 4,6-dideoxy-4-formamido-D-mannopyranosyl residues (D-Rha4NFo) that are variably $\alpha(1\rightarrow2)$ and $\alpha(1\rightarrow3)$ linked. In addition to the structure of the D-Rha4NFo sugars themselves, the manner in which they are linked is an important part of the specificity of the antibody epitopes. The OPS of \textit{Y. enterocolitica} O:9 is an exclusively $\alpha(1\rightarrow2)$ linked D-Rha4NFo homopolymer. The OPS of \textit{E. hermannii} is a homopolymer of D-Rh4NAc (acetylated rather than formylated) sugars that are variably $\alpha(1\rightarrow2)$ and $\alpha(1\rightarrow3)$ linked. In \textit{Brucella} OPS the proportion of $\alpha(1\rightarrow3)$ linkages varies from 0 (in \textit{B. suis} biovar 2) to between 2% (in A dominant strains) and 20% (in M dominant strains). In \textit{E. hermannii} the proportion of $\alpha(1\rightarrow3)$ linkages is much higher, between 40% and 60%.

We obtained samples from a cattle herd that was presenting an atypical serological profile and no epidemiological evidence of brucellosis. We applied a synthetic ‘M’ antigen (comprising two D-Rha4NFo sugars linked in $\alpha(1\rightarrow3)$ manner) to five sLPS cELISA and CFT positive serum samples from this herd. These samples gave high titres against the ‘M’ antigen. They gave very low titres against a synthetic exclusively $\alpha(1\rightarrow2)$ linked type ‘A’ hexasaccharide (D-Rha4NFo) antigen. This did not fit the serological profile from true \textit{Brucella} or \textit{Y. enterocolitica} O:9 positive samples but indicated that the antibodies may have been induced by \textit{E. hermannii} type OPS.

To test this hypothesis further we purified OPS from \textit{E. hermannii}, deacetylated it and then either reacetylated or formylated it and applied these antigens to samples from cattle infected with \textit{B. abortus} or \textit{Y. enterocolitica} O:9 and field FPSRs. We found a positive correlation between the FPSR titre against the ‘M’ disaccharide antigen and a preference for binding to acetylated versus formylated \textit{E. hermannii} OPS antigen. We believe this is evidence that these atypical FPSRs are induced by an organism that is in possession of OPS units that are acetylated and frequently $\alpha(1\rightarrow3)$ linked, like those of \textit{E. hermannii}. On the basis of this information we are producing an synthetic $\alpha(1\rightarrow3)$ linked D-Rha4NAc disaccharide antigen that we hope will enable, when used in combination with the ‘M’ disaccharide and ‘A’ hexasaccharide, the differentiation of antibodies induced by \textit{Brucella}, \textit{Y. enterocolitica} O:9 and \textit{E. hermannii} like organisms.

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Session 5: Control and Eradication

PS5-1. Evaluation of immune response against reduced dose of *Brucella abortus* strain 19 vaccine administered through conjunctival route in cattle

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A study was undertaken to evaluate the immune response against *Brucella abortus* S19 reduced dose vaccine administered through conjunctival route in comparison with *B. abortus* S19 standard dose administered through subcutaneous (S/C) route. In all, 38 *Brucella* seronegative cattle were grouped as Group-1 with 24 animals (>8 months), Group-2 with 8 female calves (4-8 months), Group-3 with 6 animals (>8 months). On day 0, Group-1, 2 and 3 animals were administered with reduced dose (5-8×10⁹CFU/dose) vaccine through conjunctival route, standard dose (40-80×10⁹CFU/dose) vaccine through S/C route and normal saline through conjunctival route respectively. Blood and serum samples were collected on 0, 21, 60, 120, 150 and 180 Day of post vaccination (DPV). On 120th day, Group-1 animals received booster conjunctival vaccine dose (5-8×10⁹CFU/dose). The humoral and cell mediated immune (CMI) response induced by conjunctival and S/C route vaccine was determined by Competitive Enzyme Linked Immunosorbent assay (C-ELISA) and Interferon-γ (IFN-γ) assay respectively. The vaccinal antibodies on 21, 60, 120, 150 and 180 DPV were 33.3, 41.6, 22.7, 77.2 and 70.1 % respectively in conjunctival group and 100, 100, 50, 33.3 and 30.2 % respectively in S/C route vaccine group. Stimulation index (>8.89) for IFN-γ response on 21, 60, 120 150 and 180 DPV were 25, 70.8, 50, 69.5 and 20 % respectively in conjunctival group and 37.5, 37.5, 50, 33.3 and 16.7 % respectively in S/C vaccine group. The CMI response was comparatively high on 60 DPV in conjunctival group compared to S/C group animals. Humoral immune response of S/C group animals was 100% on 21 and 60 DPV. After administration of the booster dose vaccinal antibodies increased from 50 to 69.5% and stimulation index for IFN-γ response from 22.7 to 77.2 %. Conjunctival route vaccination is safer and easier to dispense than S/C route and it is effective in female cattle >8 months of age. Persistent antibody level is a disadvantage in S/C vaccine which can be overcome by using reduced dose conjunctival route vaccine. If a calf misses S/C vaccination in the age group of 4-8 months an alternate way to immunize animals through conjunctival vaccine can be adopted to achieve maximum vaccination coverage against brucellosis.

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PS5-2. A trivalent vaccine candidate against brucellosis

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Brucellosis is caused by Gram-negative bacteria of the genus *Brucella*, which infect different domestic and wild animals. So far, there is no available vaccine which is safe enough for humans. On this point, subunit vaccine has become the new breakthrough of conquering brucellosis. The main goals of the present study include determining the immunogenic potential of a trivalent vaccine candidate consisting of BP26, Omp25 and L7/L12 ribosomal protein from *Brucella abortus*. Bp26 is 753 bp, 26 kDa periplasmic protein, which is conserved throughout the *Brucella* genus. It induces a strong Th2 response with mild increase in IFN-gamma levels. Ribosomal protein of *Brucella* L7/L12 is highly conserved protein in *Brucella* species and found to be immunogenic. Moreover, Outer membrane protein 25 (Omp 25) is a highly conserved protein in *Brucella sp.* which is studied to be involved in virulence of *Brucella*. Omp 25 is linked to peptidoglycan and is responsible for inhibition of TNF-α production from macrophages. Further, Omp25 vaccine may help in generating an appropriate Th1 immune response for protection against *Brucella*.

In this study, we have evaluated the efficacy of combinatorial antigens against the *Brucella abortus* infection. The current work deals with the co immunization of mentioned 3 proteins (40µg rL7/L12+40µg rBp26+30µg rOmp25) and (20µg rL7/L12+20 µg rBp26+15 µg rOmp25) in mice. Further, to evaluate the humoral and cellular mediated immune response in mice. This study suggests future application of this trivalent protein as an improved vaccine against *Brucella* species infection.

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PS5-3. The possibility of applying vaccination within the framework of combating brucellosis in Armenia

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Brucellosis is endemic in the Republic of Armenia. The test and slaughter approach for control of brucellosis is presently being used. Sexually mature female cows and ewes are tested via Rose Bengal with positive animals subject to obligatory slaughtering. However, this approach has not yet resolved the problem, and every year contagious animals are detected in nearly all Marzes. With the assistance of the United Nations Food and Agriculture Organization, experimental vaccination activities are being implemented in Syunik Marz. Female lambs (3-8 months) are vaccinated with Rev-1 and calves (4-12 months) are vaccinated with RB-51. From 2010-2011, 21,306 cattle and 111,714 sheep were vaccinated, while from 2013-2015 40,534 cattle and 154,568 sheep were vaccinated. The objective of this research is to assess the influence of vaccination on brucellosis in Syunik Marz with the results used to enhance the effectiveness of brucellosis control efforts in other areas. From 2005-2014, 2218 brucellosis cases in cattle and 1450 brucellosis cases in sheep were registered in Syunik Marz. Dividing this period into (1) 2005-2009 when no vaccination activities were implemented and (2) 2010-2014, when vaccination activities were implemented at certain intervals – it can be seen that 72 % of the brucellosis cases among cattle and 94 % of brucellosis cases among sheep and goats occurred in the first period when no vaccination activities were carried out. While there are numerous factors influencing the epidemic situation, these have all been present during the entire period 2005-2014 and are presumed to have a similar influence.

This preliminarily analysis suggests that vaccination has improved the brucellosis epidemic situation in Syunik Marz. A more complete picture with holistic data is necessary to carry out comparable assessments in other Marzes of the Republic of Armenia. This can be further considered within the framework of combating brucellosis and improving the overall strategy to prevent the disease.

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PS5-4. Sustained release of the Brucella antigen rOMP28 from PLGA microspheres abrogates virulent Brucella abortus 544’s zoonotic infection

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Brucellosis is one of the most common bacterial zoonosis prevalent throughout the globe and caused by gram negative coccobacilli from genus Brucella. Classified as a category B bioterror agent by CDC, it causes late term abortions, still births and highly infected weak calves in cattle while it leads to chronic systemic infections of the order that includes arthralgia, myalgia etc. in humans. Till date, no licenced vaccine is available for human use but several attenuated vaccines are available in the market for vaccination of ruminants. In the current study we have encapsulated Brucella antigen rOMP28, a remarkable immunogen and a promising vaccine candidate into PLGA microspheres in order to evaluate its overall immune response against Brucella abortus strain 544 in murine model and the protection conferred thereof. Since these polymeric particles are bestowed with the feature of slow and sustained release of the entrapped content (OMP28), immunization with the vaccine formulation was performed in mice following a single or no booster regimen. Strong humoral response was elicited which continued to increase till 4 weeks after primary immunization therefore the booster was given at day 35th. Each mouse was immunized with a pre-weighed amount of particles containing 40 µg of rOMP28 (per dose). The particulate formulation was found to be safe to administer and extremely immunogenic. Moreover, the in vitro uptake and internalization of the particles by RAW 264.1 macrophages was found to occur within 4-6 hours. The immunization in mice with the formulation as a single dose as well as with a booster conferred significant protective immunity against a high dose of the virulent Brucella abortus 544. These results were further corroborated by histopathological examination of spleens in which the “Bp26-Mps group” (prime-boost regimen) was found to have absolutely healthy splenic parenchyma with an absence of any prominent signs of inflammation, necrosis/degeneration. This further confirms the prospective potential of the formulation to be used as an anti-Brucella prophylactic tool even in humans as well as being a stable formulation suitable for use in diagnostics.

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PS5-5. OMVs of *Brucella abortus* S19 and S19Δper mutant confer poor protection against virulent challenge

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Brucellosis is a worldwide anthropozoonotic infectious disease affecting animals, humans and marine mammals. Brucellosis causes huge economic losses (3.4 billion USD) in India, out of which cattle and buffalo industries accounted for 95% of total losses due to abortions, temporary infertility and sterility of adult animals. Control of brucellosis is a way to control the zoonotic diseases in human population. Vaccination is the preferred means of controlling brucellosis, as stamping out is not the choice in Indian scenario. *B. abortus* strain 19 is used as live attenuated vaccine in India and many other countries. However, S19 strain possesses residual virulence, not safe for pregnant animals, infectious to human beings. In this study, outer membrane vesicles (OMVs) from *B. abortus* S19 and S19Δper strain have been evaluated as vaccine candidate. As an acellular entity, OMVs are safe and non-infectious. Outer membrane vesicles (OMVs) were extracted using differential density ultracentrifugation. On immunoblot, OMVs have showed immuno-reactivity with mice antisera. Transmission electron microscopy revealed the presence of OMVs as spherical nano-sized structures emerging from bacterial surface. Swiss albino mice were immunized with S19 and S19Δ per derived OMVs. High antibody response was observed in immunized animals. Further, serum cytokine level and FACS based enumeration of CD4⁺ and CD8⁺ T cells revealed that OMVs of S19 and S19Δper elicit strong immune response. However, protective efficacy of OMVs from both S19 and S19Δper was much less than S19 live attenuated vaccine.

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PS5-6. Rough Brucella neotomae and Brucella suis overexpressing GnRH and FSH: A novel Brucella immunocontraception vaccine


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While brucellosis has been eradicated from domestic livestock in the United States, the causative agent is still present in wild life i.e., elk, bison, and feral swine. The interactions between the human population, domestic livestock, and infected wildlife poses a great health risk. In particular, the feral swine population has quadrupled in the past ten years and continues to expand nationwide, making their population control an important national objective. Furthermore, feral swine are known carriers of zoonotic diseases like leptospirosis, pseudorabies, and swine influenza, along with brucellosis. The current population control practices have neither minimized their spread throughout the United States nor lessened the conservative $1.5 billion dollars of damage a year to our agriculture. Thus, there is a need to effectively control the feral swine population and prevent the spread of zoonotic diseases like brucellosis. Two rough strains of Brucella, B. suis and B. neotomae, expressing gonadotropin releasing hormone (GnRH) and/or follicle stimulating hormone (FSH) were created. They have the potential to be an effective immunocontraceptive for feral swine management, while reducing the spread of brucellosis. These strains could pave the way for effective novel immunocontraceptive tools to be used in wildlife and domestic animal managements.

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Brucellosis is a highly pathogenic human and animal infectious disease. It has 7 subtypes. *Brucella* causes abortions in animals. Mostly *B. melitensis* is identified in human cases. Brucellosis is reported among animals each year in Azerbaijan, which results in human infection. Also, the disease has an economic burden for farms. Annual serological monitorings are conducted as part of disease-surveillance activities. Blood samples are obtained from livestock selectively and testing is performed. Rose-Bengal and CFT assays are used for this purpose. CompELISA has been used for the first time for the confirmation of submitted sero-monitoring samples. This assay allows differentiation between sick and vaccinated animals.

Samples include blood serum collected from large and small cattle. Samples were collected throughout the whole country. Rose-Bengal assay was used for initial screening. Screening was performed in 12 Zonal Veterinary Labs (ZVLs). Confirmation of positive tests occurred in Republican Veterinary Laboratory (RVL). CompELISA was utilized (manufacturer UK) for confirmation of screening results. Serologic monitoring results data was obtained from EIDSS. Each ZVL serves certain rayons. Blood samples were collected from respective rayons and stored in fridges at ZVL facilities. 92,000 blood samples were collected during this serologic monitoring. Positive screening results were confirmed using CompELISA in RVL. 1231 sick animals were identified as a result (1.3%).

A vaccination programme is implemented for brucellosis in Azerbaijan and the coverage is checked using routine serologic monitorings. Also, sero-monitoring allows RVL to keep control over epidemiologic situation. Considering the sensitivity of Rose-Bengal assay for all antibodies, compELISA was preferred as a more effective method.

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PS5-8. Brucellosis Control: Towards a holistic, one health model

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India is endemic to bovine brucellosis which causes serious economic losses to the dairy farmer. Human brucellosis is also a serious public health issue as it causes morbidity. It is important that a control programme for brucellosis deals with it holistically. It is also imperative that the veterinary and medical fraternity work hand in hand to control this scourge in our country. Towards this end, NDDB has been implementing a pilot project on brucellosis control since 2012-13, the main components being awareness creation, vaccination and identification of animals, disposal, tracing of the infected animals and humans, linkages with the medical doctors etc. Sample collection is also carried out using appropriate methodologies.

NDDB has been closely working with a Non-Government Organization (NGO), the Kutch Nav Nirman Abhiyan (KNNA) in Kutch district, one of the remotest districts in Gujarat, for the Pilot study. A total of 132 villages covering 6 talukas in the district are presently under the Project. Before initiating the Project in each village, extensive awareness campaigns were carried out with the support of the Animal Husbandry Department. Baseline surveys are conducted to assess the prevalence in each village. Calfhood vaccinations are then carried out and the animals identified by ear tags and registered under the NDDB’s Information Network for Animal Productivity and Health (INAPH) system. Milk Ring Test (MRT) and Rose Bengal Plate Test (RBPT) are carried out at village and farmer level respectively to identify positive animals, which was later confirmed by i-ELISA in a laboratory. The farmers in general are educated on how to dispose the afterbirth after normal calvings and, farmers with positive animals are specifically instructed on disposal of the aborted material and disinfection of the infected premises for which disinfectants are also provided. Video clippings on how to carry out proper disposal in suspected cases of brucellosis and the process of disinfection are screened in each village during awareness programmes. Posters on various steps in brucellosis control are also provided to all the milk collection centers. Booklets covering different aspects of animal health including control of brucellosis are provided to the farmers. Cases of abortion and ROP are regularly recorded. A novel and simple method of sample collection from abortion and ROP cases in suspected cases of brucellosis using Flinders Technology Associate (FTA) cards has been standardized and is being adopted extensively by which samples can be sent in an envelope through post to the laboratory without the need for cold chain.

In line with the ‘one health’ concept in diseases control, farmers with positive animals, especially those who show symptoms of brucellosis are tested using Lateral Flow Assay (LFA) kits at their premises to identify reactors. They are then directed to the local physician for specific treatment regimen. To create awareness among the medical doctors on brucellosis, symptoms in humans, treatment protocols and, to create linkages between all the stakeholders, a district level workshop was organized, inviting officials from both the medical and veterinary departments in which experts from the medical profession provided their insights on the disease and its treatment strategies in humans. These linkages were then utilized for identification and treatment of positive farmers in the project area.

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PS5-9. Comparative analysis of S19Δper and S19ΔrfbD as potential *Brucella* vaccine candidates

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Brucellosis in recent time gained more importance due to its zoonotic potential and bio-warfare implications. WHO declared brucellosis as one of the seven neglected endemic zoonoses under Animal zoonoses group 3 of the Neglected Tropical Diseases (NTD). In country like India where 70% of population lives in villages and suburban areas having close contacts with animal population are more susceptible to zoonotic diseases. As far as humans are concerned bovine brucellosis is more prominent owing to consumption of contaminated milk and meat products. Dairy products prepared from unpasteurized milk such cheese, yoghurts and ice cream may contain high concentration of bacteria and consumption of these may lead to brucellosis. *B. abortus* S19 is used as vaccine in India and many other countries. However, the drawbacks of S19 vaccine strain- it may cause abortion when applied during pregnancy, possesses residual virulence, interferes with clinical diagnosis of the disease. Smooth lipopolysaccharide (LPS) plays role for its residual virulence and serological interference. Rough strains are more attenuated and are non-reactive to conventional serological antigen. In this report we describe development of a gene knock-out mutants of *Brucella abortus* S19 vaccine strain targeting LPS biosynthetic genes *per* and *rfbD* encoding perosamine synthetase and transport permease protein, respectively. The developed mutants, S19Δper and S19ΔrfbD exhibited altered LPS structure. Phenotypic studies revealed that S19Δper is an intermediate rough type whereas S19ΔrfbD a rough phenotype. Deletion of *per/rfbD* gene also resulted in varying degree of attenuation of S19 strain. However, S19Δper showed balanced attenuation with optimum immunogenic properties. It mounted strong immune response in Swiss albino mice and conferred protection similar to S19 strain. S19Δper mutant immunized mice produced higher levels of IFN-γ, IgG2a and thus has immune response inclined towards Th1 cell mediated immunity. Both S19ΔrfbD and S19Δper mutant displayed more susceptibility to serum complement mediated killing. S19Δper conferred solid immunity against virulent challenge whereas S19ΔrfbD was found less effective. Sera from immunized animals did not show agglutination reaction with RBPT antigen and thus could serve as DIVA (differentiation of infected from vaccinated animals) vaccine. S19Δper mutant inherited desirable qualities of both smooth and rough strain displaying remarkable resemblance to S19 strain with improved properties of safety, immunogenicity and DIVA capability for control of bovine brucellosis.

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PS5-10. Study on immunogenicity of *Brucella abortus* S19 phage lysate in mice model

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This study aimed to evaluate the immunogenicity of *Brucella abortus* S19 phage lysate in swiss albino female mice. The experimental outlay consisted of five groups of 6 numbers each and inoculated with *B. abortus* S19 phage lysate in different doses subcutaneously (S/C). The group 1, 2and 3 were inoculated with 20µl (0.094 mg total protein and 0.06 µg total Carbohydrate), 40µl (0.18 mg total protein and 0.12 µg total Carbohydrate), 60 µl (0.282 mg total protein 0.24 µg total Carbohydrate) phage lysate respectively, 4th group vaccinated with *B. abortus* S19 vaccine at a dose of 1 X 10\(^5\)cfu in PBS and control group with only PBS. Thirty days post inoculation, mice were challenged with *B. abortus* S544 in a dose of 2 X 10\(^5\)cfu in 0.1 ml PBS intra-peritoneal (I/P) and mice were killed 15 days post-challenge. The spleens were collected, homogenized individually in 9 times to weight of the spleen in buffer saline solution and mean spleen counts of *Brucella* was enumerated by culturing it on *Brucella* Agar Media. The counts thus obtained were converted in term of Immunogenic activity = log (X/logX) where X = cfu/spleen. Mean of Immunogenic activity of phage lysate was found to be 3.3, 2.9 and 2.7 for the groups inoculated with 20µl, 40 µl and 60 µl, respectively whereas in S19 group 2.3 and 4.9 in PBS group were observed. Immunogenic activity of phage lysate was measured against *B. abortus* S544 in mice where phage lysate gave significant protection in comparison to *B. abortus* S19 vaccine in comparison to unvaccinated group. It showed that immunogenic activity conferred by phage lysate (60 µl) and S19 inoculated group was significant with respect to control group and does not differ significantly to each other (P<0.05). *B. abortus* S19 vaccine has showed higher potency than phage lysate but the levels of immunogenicity do not differ significantly. These results showed the potential safe and effective vaccine candidature of phage lysate in large animals.

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Session 6: Brucellosis in India

PS6-1. Epidemiology of brucellosis in an occupationally exposed group in Punjab, India

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Brucellosis, caused by Brucella spp., is a zoonotic disease affecting a wide range of domestic and wild animal species. Organisms are shed in milk and uterine discharges of affected animals for variable amounts of time. The disease is considered an occupational zoonosis and is transmitted to humans via direct contact, particularly during calving and/or abortion, or via consumption of unpasteurised dairy products. Although endemic in India, possibly at high levels the disease is often neglected. This study was conducted in Punjab State of India, where it is estimated that 70 million households in rural Punjab derive income or employment from the dairy sector. Despite the development of cooperatives in this State, the majority of milk is still sold via informal channels where there is little regulation of production and processing of milk and dairy products. In the absence of milk testing, raw milk contaminated Brucella spp., may enter the food chain, posing a significant health threat if it is not destroyed during processing. The aim of the study was to estimate the seroprevalence and risk factors for disease in occupationally exposed individuals.

This study was conducted in parallel with a study of brucellosis in the general population and employed a two-stage sampling design. In the first stage, 40 villages were randomly selected from four out of seven blocks of Ludhiana district using sampling probability proportional to human size. Within villages, up to eight dairy farms were selected using simple random sampling in consultation with veterinary officers. Within selected farms, all farm workers or family members in contact with the dairy animals were offered the opportunity to participate in the study. A blood sample was collected which was screened for Brucella spp. antibodies using Rose Bengal Test (RBT) and commercial ELISA kits for IgG antibodies.

A total of 26 (6.7 %) participants tested seropositive by IgG and RBT, all except one were male ($P=0.05$) and all reported assisting with calving. People testing seropositive had 3 times the odds of assisting with abortion or having occupational contact with sheep and goats, all these results were non-significant. The majority of persons reported never drinking pasteurised milk (98.2%), although most people always boiled their milk before consumption (88.3%).

Multivariable analysis with random effects will now be performed in order to identify factors associated with Brucella spp. status after controlling for the potential confounding effects of other variables. The correlation between seroprevalence in livestock will also be investigated. However, the results to date suggest that brucellosis remains an important occupational zoonosis in Punjab. Awareness campaigns are recommended and these results are being relayed to policymakers to inform public health policy regarding the targeting of resources towards high risk populations.

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PS6-2. Status of Brucellosis Infection in Jaffarabadi Buffalo of Gujarat

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To understand the genetics of brucellosis infection in domestic buffalo through Genome Wide Association Studies (GWAS), we are undertaking epidemiological survey on the status of brucellosis infection in this species maintained under village conditions in four districts of Gujarat. In total Three thousand three hundred eighteen Jaffarabadi buffalo owned by 1044 farmers in 85 villages of four districts, namely; Amreli (55 %), Bhavnagar (7 %), Junagarh (18 %) and Rajkot (20 %)) were tested for brucellosis using an ELISA method. Average herd size varied from 2.5 in Junagarh district to18.70 in Rajkot district. Out of the tested animals, 36 % were below 3 years of age, 28 % were between 3 to 6 years, 22 % between 6 to 9 years and the remaining 14 % were above 9 years of age. More than half (57 %) of the animals had no history of any vaccination against any epidemic disease while the remaining (43 %) were vaccinated against epidemic diseases, including Haemorrhagic Septicemia, Foot and Mouth Disease and Black Quarter. The majority (84 %) of buffalo owners had provision for drinking water for individual animals. More than half (56 %) of the animals were bred through natural service. A total of 121 (3.65 %) animals tested +ve for Brucella. Amongst these, more than half (52 %) were from Amreli district alone; 30 % were from Rajkot, 13 % from Junagarh and the remaining (5 %) belonged to Bhavnagar district. While infection equally affected all age groups of buffalo, region was a significant factor in defining the prevalence of the disease.

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PS6-3. Seroprevalence study of *Brucella* infection amongst cattle population in and around Rewa district

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The present work was conducted to study the seroprevalence of *Brucella* infection amongst the cattle population in and around Rewa district (Madhya Pradesh). Seroprevalence study was conducted using RBPT and SVANOVIR *Brucella* Ab c-ELISA kit. C-ELISA is OIE referred gold standard test for detecting *Brucella* antibodies. Around 50 serum samples were screened for the presence of *Brucella* antibody using both Rose Bengal plate test (RBPT), and Competitive Enzyme Linked Immunosorbent assay (c-ELISA) kit. Out of 50 samples tested with RBPT and c-ELISA, 42 % and 20 % were detected to be positive respectively. RBPT was not sensitive enough to detect weak positive sample, which were detected by c-ELISA, considering it as gold standard test for seroprevalence study of *Brucella* infection.

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PS6-4. A study to identify habits of cattle keepers that lead to spread of infection and the prevalence of Brucella-specific antibodies among the Buffalo population of Delhi.

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India has highest population of Buffalos in the world. Semen of Murrah breed from India is being exported to USA to achieve a new breed of healthier buffalos. This export has also helped Indian farmers to achieve better economic status. Furthermore, general practice of an Indian rural family is to rear buffalos in the house compound to meet daily needs of the family. In this scenario it is pertinent to screen the prevalence of Brucellosis in Indian Buffalos. Although there have been several reports of Brucellosis from different states of India like Panjab, Haryana, South Indian states etc., but Bovine Brucellosis in Delhi state has never been reported before. Our study is the first report about seroprevalence of Brucella-specific antibodies in Buffalo population of Delhi. Delhi has 11 districts and 369 registered villages. All the districts were surveyed by questionnaires in Hindi [native language of India]. The cattle keepers/ dairy farmers of all the districts in Delhi were surveyed for 1. Their knowledge of Brucella S19 vaccine; 2. Their habit like consumption of unboiled milk; 3. Their practices like isolation of sick animals; 4. Occurrence of miscarriages during 5th to 7th month of pregnancy in Buffalos. All these habits were correlated with the seroprevalence of Brucella-specific antibodies in Buffalo population of Delhi. Blood of 100 buffalos was randomly collected from different districts of Delhi and was screened with Rose Bengal test [RBT] and Brucella abortus Serum Agglutination Test [SAT]. We found that more than 50% of Buffalos tested in Delhi were positive for RBT and SAT. It was found that none of the cattle keepers were aware of S19 vaccine and none of the buffalos were vaccinated against Brucellosis. During our survey we distributed the directives of Indian Council of Agricultural Research for animal maintenance among the cattle keepers. As it was found that the cattle keepers were aware of vaccine for Foot and mouth Disease only, thus we told them about Brucella S19 vaccine and its easy availability from Indian Veterinary Research Institute. It was also found that other habits of Dairy farmers were very conducive for spread of infectious diseases. As the seroprevalence of Brucella-specific antibodies was very high amongst buffalos of Delhi therefore, there is a need to spread awareness about this debilitating disease. We have done our bit but efforts have to be made to curb the emergence of Brucellosis in Delhi.

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PS6-5. Isolation and Identification of *B*. *melitensis* from sheep and goat flock from Akola, Maharashtra

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Brucellosis is a notifiable disease distributed worldwide and responsible for causing the economic losses due to abortions and reduced milk production. In present study, a total of 251 samples comprising of blood (179) and vaginal swabs (72) were collected from migratory sheep and goat mixed flock from Akola district, Maharashtra. There was a history of abortions in the flock. Various diagnostic tools including serology, isolation of bacteria and molecular tests were used to detect the organism and diagnose the disease. On serology, combination of Rose Bengal Plate Test (RBPT) and Indirect Enzyme Linked Immunosorbent Assay (i-ELISA) by using smooth lipopolysaccharide antigen were used. On RBPT examination out of 179 serum samples 73 (40.78 %) were turned positive and on i-ELISA 122 (68.15 %) were found positive. On bacteriological analysis of 72 clinical samples (vaginal swabs) two *Brucella* isolates were recovered. On biochemical tests these isolates were confirmed as *Brucella*. The isolate were confirmed by using Polymerase Chain Reaction (PCR). The genus specific PCR for *Brucella* genus were performed to detect the *Brucella* Cell Surface Extractable protein (BCSP) gene amplified at 223 bp. Two isolates amplified at 223 bp and same isolates then subjected for species specific AMOS PCR. On AMOS PCR these two isolates were amplified at 731 bp confirms the isolates belong to *B*. *melitensis*.

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PS6-6. Seroprevalence of brucellosis in bovines of Chhattisgarh region

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Chhattisgarh is traditional area for livestock raising. However, there is no recognized breed of cattle in Chhattisgarh. The animals reared are mainly non-descript. Few cross bred (crossed with Holstein Friesian and Jersey) are available besides Indian milch breed, Sahiwal and Gir. Brucellosis, caused by Brucella abortus, is one of the most important infectious causes of reproductive disorders in domestic animals. It results in infertility, anoestrus, repeat breeding, retained placenta, abortions, abnormal termination of pregnancy, cervicitis, endometritis etc. and thus causes huge economic loss to the livestock industry. In the present study, a total of 345 serum samples from cattle were collected from six districts of Chhattisgarh. These samples were from different sectors (organized – n= 133; unorganized – n= 212), sex (male – n=13; female – n=332), breeds (exotic Cross bred – n=155; improved Indian breeds – n=65; non-descript breed – n=125) and clinical conditions in animals (with history of reproductive problems viz., infertility, anoestrous, repeat breeding, and abortion – n=115; without any clinical history – n=230). All the serum samples were subjected to indirect ELISA for detection of antibodies against bovine brucellosis using commercially available ELISA kits. Out of 345 serum samples, 53 were positive for brucellosis with an overall seroprevalence rate of 15.36 %. All the 53 positive samples were from female animals. About 25.16 % exotic cross bred, 13.85 % improved Indian breeds, 27.81 % animals from organized sector, 21.73 % of animals with clinical history were found seropositive. While, only 4 % non-descript breed, 7.55 % animals from unorganized sector, 12.17 % of clinically healthy animals were found positive for brucellosis. The findings of this study indicate that the problem of brucellosis is more prevalent in cross bred animals reared in organized conditions. Further in this study all the bulls were found negative to brucellosis. Thus, the findings support the fact that sexually mature females which are reared intensively are prone to infection. The results of this study showed the presence of brucellosis in the Chhattisgarh region, thus warranting a need for regular vaccination for brucellosis in the bovine population.

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PS6-7. Monoclonal antibody based blocking ELISA for detection of *Brucella* antibodies in Indian livestock population

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Diagnosis of Brucellosis is complicated due to its variable/prolonged incubation period, and absence of prominent clinical signs except during the late abortion stage. Sero-diagnosis of Brucellosis is one of the reliable methods for identifying the disease. Rose Bengal plate agglutination test (RBPT) is one of the widely used conventional serological methods for the diagnosis of Brucellosis. Though, the test is rapid, easy to perform and inexpensive, the sensitivity and specificity of RBPT is lower than that of ELISA. Most of the commercially available ELISA kits are imported, except for a few indirect ELISA kits available indigenously. The cross reactivity of *Brucella* LPS with other Gram negative bacteria is one of the major drawbacks of the indirect ELISA kits. Therefore, monoclonal antibody based blocking/competitive ELISA is preferred and it is recommended by OIE also. In the present study, a *Brucella* LPS specific mAb based blocking ELISA was developed and validated as per the OIE guidelines. The *Brucella* specific mAb was developed and characterized at Ingenasa at Spain. A cut-off of 40 % percentage inhibition (PI) was arrived based on the ELISA results of sera samples from Indian cattle population (n=200). The ELISA results showed 100 % correlation with culture negative (n=20) and culture positive (n=20) reference sera. Further the ELISA was compared with the commercially available cELISA kit from IDEXX. The results showed a perfect kappa agreement (k-value = 1) using a set of positive (n=50) and negative (n=50) sera samples. The ELISA detects *Brucella* specific antibodies as early as 5 days post S19 vaccination. The ELISA kit can be used for diagnosis of Brucellosis in India as the kit is much affordable compared to imported cELISA kits and a preferred test over indirect ELISA.

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PS6-8. Sero- surveillance of bovine brucellosis in selected districts of Tamil Nadu, India

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Brucellosis is one of the important reproductive diseases of dairy animals and it is highly prevalent among cattle population in India. The objective of this work was to determine the seroprevalence of brucellosis from selected districts of Tamil Nadu, India. A cross sectional serosurvey was conducted in cattle from eleven districts of Tamil Nadu (Erode – 125, Salem – 74, Kancheepuram – 83, Villupuram – 70, Tiruvannamalai – 114, Tiruvallur – 48, Tirunelveli – 51, Pudhukkottai – 89, Thiruvarur – 91, Virudhunagar – 61 and Chennai - 15). A total of 821 serum samples were collected and subjected to various serological tests viz., RBT, STAT and i-ELISA. The highest seroprevalence of brucellosis was detected by i-ELISA (6.70 %) followed by STAT (4.38 %) and RBT (4.02 %). Of all the sero diagnostic tests employed, i-ELISA was found to be the most suitable test for sero diagnosis of bovine brucellosis.

Presence of *Brucella* antibodies in this study area is of public health and economic importance which warrants the need for appropriate control strategies and measures to eliminate positive indicators to combat the spread of brucellosis among cattle in the study area.

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PS6-9. Sero-prevalence of *Brucella abortus* infection in nomadic flocks of small ruminants in South Gujarat

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An investigative study to find out sero-prevalence of *Brucella abortus* infection in nomadic flocks of small ruminants (sheep and goats) in South Gujarat was carried out during the year 2013-14. Four nomadic flocks of small ruminants facing problem of abortions were included. Serum samples were collected from all females having history of abortion along with few randomly selected animals from each flock. Total 89 serum samples were collected out of 514 small ruminants and subjected to RBPT and I-ELISA. The overall prevalence of *B. abortus* infection was 55.06 per cent on RBPT and 68.54 per cent on I-ELISA. The difference between results of RBPT and I-ELISA was statistically non-significant. The effects of flock (flock I-IV) and species (sheep/goats) on prevalence of *B. abortus* infection were also statistically non-significant (p>0.05). The prevalences in four different flocks were ranged between 47.37 to 73.91 per cent. The prevalence was comparatively higher in sheep (69.35 %) than goats (59.26 %). The prevalence of *B. abortus* infection was significantly (p<0.05) higher in females (71.83 %) than males (44.44 %). Further, the prevalence in females with history of abortions (87.23 %) was also significantly higher than females without history of abortions (41.67 %). It was concluded that the overall prevalence of *B. abortus* infection was very high in nomadic flocks of small ruminants having history of abortions. Mass movement of flocks and commingling at communal pastures and watering areas, frequent replacement of males without prior testing and absence of disease monitoring and control policy were the important predisposing factors to be considered for planning of control strategies.

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PS6-10. Seroprevalence of Brucellosis in cattle & buffaloes in Gujarat, India

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Purpose of this research is to determine the study of brucellosis in cattle and buffaloes belonging to Gujarat state by Rose Bengal Plate test (RBPT) and Indirect Enzyme Linked Immunosorbent Assay (i-ELISA). A total of 550 (323 cattle and 227 buffaloes) serum samples were screened for Brucella antibodies using Rose Bengal Plate Test (RBPT) and Indirect Enzyme Linked Immunosorbent Assay (i-ELISA). Out of 550 serum samples screened, 112 samples were found positive by RBPT and 75 by i-ELISA yielding overall seroprevalence rates of 20.36 % and 13.64 %, respectively. Species wise seroprevalence observed was 21.67 % and 14.55 % in cattle, where as 18.50 % and 12.33 % in buffaloes by RBPT and i-ELISA, respectively. Sexwise, age wise, farm wise and status wise seroprevalence was recorded and statistical analysis was carried out and results reveals presence of brucellosis in Gujarat state.

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PS6-11. Comparison of STAT, RBPT, ELISA and PCR tests for diagnosis of human brucellosis in and around Kolkata, India

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Brucellosis is one of the important emerging zoonosis in developing countries like India. The disease has manifold pathological consequences without specific sign and symptoms. The present study was aimed to compare results of different serological tests and PCR for diagnosis of brucellosis in patients suffering from PUO in Kolkata and in adjoining districts. A total of 2088 blood samples were collected from PUO cases during January 2013 to September 2015 from patients in Kolkata and from adjoining districts. The samples were tested by STAT, RBPT, ELISA (IgM, IgG) and *Brucella* genus specific PCR. The study revealed decreasing positive results by STAT (18.43 %, N=385), RBPT (12.59 %, N=263), IgM (7.66 %, N=160), PCR (4.21 %, N=88) and IgG (1.38 %, N=29). When serological tests were compared with PCR, it was found that STAT and PCR were positive in 84 samples (4.02 %), RBPT and PCR were positive in 65 samples (3.11 %), IgM and PCR were positive in 51 samples (2.44 %), IgG and PCR were positive in 9 samples (0.43 %).

Thus in this cross sectional study in a zonal population of India it was found that STAT was the most sensitive test for diagnosis of brucellosis alone or when it is further confirmed by PCR. However, four STAT negative samples showed positive results in PCR, which were positive by RBPT test. This indicates that if we combine STAT and RBPT for diagnosis of brucellosis then both sensitivity and specificity of the combined test will increase.

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PS6-12. Serological and Molecular Diagnosis of Brucellosis in Cattle in Assam


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Brucellosis is zoonotic disease of public health importance worldwide. The disease in bovine is caused almost exclusively by *Brucella abortus* and causes severe economic losses to the dairy industry worldwide. In this study, a total of 4198 serum samples were collected from local, pure and cross breed cattle of different age groups from 25 districts of Assam from October, 2012 to September, 2015 and screened for detection of *Brucella* antibodies employing the Rose Bengal Plate Test (RBPT) and Indirect Enzyme Linked Immunosorbent Assay (I-ELISA). Of these, 585 samples from 18 districts were found positive for *Brucella* antibodies by both the tests and thus seroprevalence of brucellosis in cattle in Assam was 13.93 %. Out of 585 serum samples of seropositive cattle, 285 samples were used for extraction of DNA using commercial DNA extraction kit and tested by *Brucella bscp* 31 genus-specific PCR and 137 samples (48.07 %) were found positive for *Brucella* DNA. Again, all the *Brucella* positive DNA samples were tested by *Brucella* species-specific Bruce Ladder PCR. All the DNA samples were identified as *Brucella abortus* DNA based on the results of by Bruce Ladder PCR. This study concludes that *Brucella abortus* is the *Brucella* spp. circulating among cattle in 18 out of 25 districts of Assam covered under the study. Cultural examination of a total of 176 clinical samples collected from cattle including vaginal swab, vaginal discharge, aborted fetus, placenta and hygroma fluid with history of abortion, retention of placenta and repeat breeding yielded 18 isolates of *Brucella abortus* which also indicated that *Brucella abortus* is the *Brucella* spp. associated with brucellosis in cattle in Assam.

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PS6-13. Epidemiology of *Brucella* infection in livestock in Assam

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Brucellosis is regarded as one of the most important zoonotic diseases of public health importance worldwide which is caused by different *Brucella* spp. in livestock and wild animals. It is endemic in India many countries of the world. It causes enormous economic losses to the dairy sector in India. In this study, a total of 5663 serum samples were collected from cattle (4198), goat (1025), sheep (53) and pig (386) from 25 districts of Assam from October, 2012 to September, 2015 and tested for detection of *Brucella* antibody by using the Rose Bengal Plate Test (RBPT) and Indirect Enzyme Linked Immunosorbent Assay (I-ELISA). Based on results of both the tests, the overall seroprevalence of brucellosis in livestock was found to be 11.09 %. The individual animal species seroprevalence was 13.93 % in cattle, 2.53 % in goat, 5.66 % in sheep and 3.63 % in pig. Farm-wise, prevalence of brucellosis was higher in large cattle farm (57.14 %) compared to small (31.58 %) and medium (24.62 %) farm. Prevalence of the disease was significantly higher in female (14.71 %) than in male (3.46 %) cattle ($\chi^2 = 28.39, P <0.0001$). It was non-significantly higher in female than in male sheep, goat and pig. In respect of age group, higher prevalence was recorded in cows from 6.1-8 years (17.61 %) of age and doe above 18 months (3.23 %) compared to other age groups. The disease was significantly associated with the age group ($\chi^2 = 59.59, P <0.0001$) of cattle. The prevalence was higher in Holstein Friesian (22.17 %) compared to cross breed (15.95 %), Jersey (11.58 %) and indigenous cattle (5.80 %). There was statistically significant ($\chi^2 = 111.91, P <0.0001$) association of breed with prevalence of brucellosis in cattle. In cattle, animals with history of abortion ($\chi^2 = 353.30, P <0.0001$), mastitis ($\chi^2 = 429.43, P <0.0001$), retention of placenta ($\chi^2 = 360.69, P <0.0001$) and repeat breeding ($\chi^2 = 368.79, P <0.0001$) showed significant relationship with prevalence of the disease. There was also significant association between the disease and abortion in goat ($\chi^2 = 44.22, P <0.0001$). A total of 204 clinical samples including vaginal swab (113) of cattle, goat and pig, vaginal discharge (53) of cattle and pig, aborted fetus (9) of cattle and goat, placenta (17) and hygroma fluid (12) of cattle with history of abortion, retention of placenta and repeat breeding were collected and processed for isolation of *Brucella* spp. Of these, 18 samples including 6 vaginal discharge, 5 vaginal swab, 3 placenta, 1 aborted fetus and 1 hygroma fluid of cattle, 1 aborted fetus of goat and 1 vaginal swab of pig yielded *Brucella* spp. The cultures initially identified by conventional methods were confirmed as *Brucella* by *bcsp* 31 genus specific PCR and as *Brucella abortus* by AMOS and Bruce Ladder PCR.

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PS6-14. Serological Detection of Brucellosis in Bovines of South Gujarat

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Brucellosis is a zoonotically important contagious disease of reproductively mature animals. The disease is economically most devastating in Bovines (cattle and buffalo). In most of laboratories, disease diagnosis is made upon the demonstration of antibodies as personnel safety and cost are major limiting factors in culture based and molecular diagnosis. In the present communication, brucellosis was detected using Rose Bengal Plate Test (RBPT) and antibody capture indirect ELISA upon sera collected from cattle and buffaloes and few milk samples were tested with milk ring test (MRT). A total of 2976 bovine sera and 51 milk samples were collected from different districts of south Gujarat region (having 7 districts) from 2012-2016. The 354 / 2976 (11.895 %) and 389/2359 (16.490 %) sera samples were found positive via RBPT and ELISA test respectively. Among the positive samples 9/72(9.72 %) belonged to male animals and rest were females. The year wise positivity was found as 03.09 % (2012-13) via RBPT only, then 14.15 % & 24.59 % (2014 ), 5.63 % & 1.70 % (2015 ) and 23.94 % & 17.04 % (2016) with RBPT and ELISA, respectively. Results were not found unequivocal as three types of differences observed in the result, viz. RBPT positive but ELISA negative, ELISA positive and RBPT negative and negative in RBPT but moderately positive in ELISA. In MRT, 9/51 (17.64 %) milk samples were found positive. The overall test results indicate higher incidence of Brucellosis in this area relative to reported national average.

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PS6-15. Canine brucellosis antibodies detection and differentiation as smooth vs rough strains in India

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Canine brucellosis is a worldwide bacterial disease caused by Brucella canis with emerging zoonotic and public health significance. The absence of apparent clinical signs, diagnostic dilemma, non-availability of vaccine and no fail-safe antibiotic therapy makes canine brucellosis a high health risk to pet owners and animal handlers. Data on the prevalence of canine brucellosis in India is scarce or rather unreported, thus the public significance is not known. Sera samples (N=150) were collected from dogs with clinical signs of brucellosis like abortion, conception failure, scrotal oedema and discospondylitis. Canine brucellosis antibodies were detected using Immunocomb Canine Brucella antibody test kit and Bru Alert monoclonal based blocking ELISA. From the clinically suspected dogs tested, 13.13 % turned seropositive. Of these, 12 % was positive for Brucella canis (rough) antibodies implying that these population got aborted due to the Brucella rough antigen and 1 % was positive for both rough and smooth antibodies indicating the cross reaction of other Brucella species. The prevalence of brucellosis was compared in the study population based on the age group, sex, breed and gestation length in which abortion had happened. Upon the data analysis, it was found that the prevalence was highest in 5-6 years age group and 33.33% dogs got aborted at a gestational length of 46-55 days. Statistical analysis revealed that there was a significant difference (p<0.05) in the prevalence of brucellosis among age groups and gestation length at which abortion happened. Statistical analysis revealed that there was no significant difference (p>0.05) in the prevalence of brucellosis among various breeds and sex of the dogs. It is concluded that routine serosurveillance of canines should also be done so that proper control measures can be taken especially in breeding and pregnant dogs, thereby preventing the spread of infection in kennels, veterinary institutions and associated zoonotic implications from aborted animals.

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PS6-16. Brucellosis amongs livestock population at organized farms from Central India


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Brucellosis is a zoonotic disease distributed worldwide and having economic as well as public health importance. The disease is mainly characterized by the abortion, retained placenta, orchitis, arthritis with excretion of organisms in uterine discharges and milk. The diagnosis of disease can be done by isolation of bacteria, serological responses to *Brucella* antigen and molecular tool like PCR. In present study a total of 995 of blood samples of animal origin including cattle, buffalo and goat were collected from the different organized farms having the report of outbreak of brucellosis. To diagnose the brucellosis various diagnostic tools used in the study includes Rose Bengal Plate Test (RBPT), Enzyme Linked Immunosorbent Assay (ELISA) and Polymerase Chain Reaction (PCR). The serological test performed by using RBPT and IgG based Indirect ELISA using smooth lipopolysaccharide antigen and for molecular detection blood DNA were extracted and subjected for PCR. Out of 995 serum samples 79 were found positive for brucellosis by RBPT (7.93 %) and about 88 (8.84 %) samples were positive by i-ELISA. PCR was performed to detect the *Brucella* Cell Surface Extractable protein gene (BCSP) amplified at 223 bp. Out of 995 blood DNA samples 77 (7.73 %) showed amplification at 223 bp confirming the detection of BCSP gene in particular DNA sample that means positive for brucellosis. The isolation of *Brucella* organism of near about 93 clinical samples including vaginal samples of suspected animals were attempted but no isolations were observed.

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PS6-17. Development of disease transmission models for bovine brucellosis in India

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India is now the world’s leading milk producer following significant development in the dairy industry, including the establishment of dairy cooperatives. Within India, the Punjab State produces a milk surplus and cattle and buffalo’ milk produced per capita is the highest of all the States. Rapid growth of the Punjabi dairy industry presents many opportunities for development, however there are also threats, including increased risk of disease due to higher livestock densities, increased trade and use of exotic breeds. In addition to causing financial losses to the industry, food-borne diseases such as brucellosis also pose a public health threat to consumers if present in the final product. As a result of its potential for direct transmission, brucellosis is also an occupational hazard for those in direct contact with livestock. Control of the disease in humans relies on control of the disease in livestock and milk hygiene practices. India is a unique setting as cattle slaughter is culturally unacceptable, therefore the productive-life of cattle is much longer than in other settings. This complicates the control of the disease as cattle are kept in the herd for a longer period of time and test and slaughter campaigns are prohibited. This study aims to develop a within-herd transmission model of brucellosis in commercialized dairy farms, and to simulate the potential effects of vaccination on disease transmission. The model is a within-herd Susceptible-Exposed-Infectious-Recovered-Susceptible (SEIRS) model with an additional compartment for persistently infected (PI) and vaccinated (V) individuals. The model is based on differential-equations and is age-structured in order to investigate the effect of increased productive life-span of Indian dairy cattle. The model is now being used to simulate the effectiveness of different vaccination strategies using a range of transmission parameters. The model outputs will also be used as inputs for a cost-benefit analysis to predict the private benefits of vaccination for dairy farmers. This work will also be expanded further and between-herd transmission models developed in order to inform policy decisions regarding publically funded brucellosis control campaigns. It is also hoped that the tools developed by this project can be adapted to other endemic settings.

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PS6-18. A meta-analysis of diagnostic test performance for bovine brucellosis


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Bovine brucellosis, a worldwide zoonotic disease caused by *Brucella abortus*, less frequently by *B. melitensis* and rarely by *B. suis*. For diagnosis of brucellosis, both serum and milk samples are extensively used for surveillance, monitoring and diagnosis by array of tests worldwide and OIE specifically recommends battery of 2 tests for diagnostic interpretation of brucellosis. The choice of the test/s depends on the availability, cost, expertise and infrastructure for performing the test. In such circumstances, the correlation of the various diagnostic tests tends to vary and difficult to interpret. Hence, the study was undertaken to evaluate the diagnostic performance of the tests used to detect brucellosis in serum and milk by meta-analysis of published reports. In the current study, data has been sourced from published papers, abstracts and reports using key words in the following electronic databases like PubMed, Google Scholar, Science Direct and Consortium for e-Resources in Agriculture. A total of 92 studies with total sample size of 49970 during 1995 to 2015 reported by 13 tests (RBPT, SAT, 2-ME, CFT, iELISA, AB-ELISA, cELISA, dot-ELISA, milk-ELISA, MRT, FPA, LFA and PCR) were taken into consideration for brucellosis detection. Meta-analysis of random effect model was used when significant heterogeneity and higher Tau2 value otherwise fixed effect model was used. The detection rate for serum samples was found to be highest for CFT 70 % (n=599; 95% CI: 28.0-93.0, T²=2.363) followed by cELISA 33% (n=3360; 95% CI: 2.0-91.0, T²=9.894), FPA 32% (n=3277; 95% CI: 0-98.0, T²=10.73) and other tests fall in the detection range of 9.0-20.0%. The tests like Dot-ELISA, LFA and 2-ME showed fixed effect model without heterogeneity between the results. In case of milk samples, milk-ELISA showed highest detection rate of 18% (n=2196; 95% CI: 8.0-34.0, T²=0.5604) followed by MRT 16% (95% CI: 7.0-35.0, T²=1.855). Based on meta-analysis, the test of choice identified is CFT and cELISA for serum and milk–ELISA for milk. Though CFT has highest detection rate but preference worldwide is ELISAs because of robustness. Similarly, RBPT is commonly preferred test in resource poor laboratories has low detection rate of 11% may be due to low specificity which again warrants its use as a sole test for diagnosis of brucellosis. This study is an report based systematic summary of the diagnostic test performance for selection of right choice of the test/s for bovines brucellosis.

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Knowledge of Smallholder Producers towards Bovine Brucellosis in Bihar, India

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Bovine brucellosis is one of the most common but neglected zoonotic diseases in the world. It is endemic in India and cause major health, economic and livelihood burdens. In absence of state-run vaccination programmes and a well effective vaccine to control the disease, it is important to understand the knowledge, attitude and practices of farmers to design an effective control programme acceptable to the community. However, knowledge of farmers towards the diseases and the practices that they followed to control the disease is not well understood. Therefore, an effort was made to understand the level of knowledge among the smallholder dairy producers in Bihar, India, about brucellosis through a cross-sectional study.

The study was conducted in urban, peri-urban and rural areas of three districts of Bihar, India during September to November 2015. The districts include Patna, Vaishali and Nalanda. In each district, one rural and one urban development block were selected. From each development block, 4 villages were selected and from each village 6-18 households were selected from the consenting producers available to take part in the study. In total 292 farming households were selected for the study. All selected households were interviewed using questionnaires.

In the study we found that level of knowledge among smallholder dairy producers in Bihar towards brucellosis is very poor. Only 6% producers (18/292) have heard about brucellosis and out of them only 7 reported that they know about brucellosis, the remaining have heard about the disease but do not know anything about it. Those who have claimed they know about brucellosis, majority of them reported that it affects cattle followed by buffalo, goat and human. Less than 3% of farmers claimed that they are aware about the symptoms of brucellosis which are mentioned as abortion, prolapse of uterus, pain, restlessness and bleeding in animals. Less than 2% farmers reported that brucellosis could be transmitted to human through consumption of unpasteurised milk. No one has reported that brucellosis had occurred in their family but 1 farmer reported that brucellosis could be transmitted from animal to human and 5 farmers reported that it can be transmitted from animal to animal.

In regards to practices of the dairy producers, majority of them throw away (52.78%) the aborted foetus followed by burial (44.44%). In regards to hygiene measures that the farmers follow to dispose aborted materials, many farmers take bath (47.92%) after disposing the materials, followed by washing of hands with soap (39.52%) and washing of clothes (10.42%). No farmers reported wearing of gloves during disposing of aborted materials.

From the study it is observed that knowledge about brucellosis is very scarce, almost negligible among smallholder dairy producers. In absence of effective vaccination programme against brucellosis, adequate knowledge and capacity of the producers are important to adopt useful control measures like better hygiene, quarantine, hygienic disposal of dead foetuses, segregation and slaughtering of affected animals etc. More participatory research will be required to understand knowledge, attitude and practices of the dairy producers and to design and implement an effective community lead control options.
Session 7: Genomics, Bioinformatics and Proteomics

PS7-1. Developing improved epidemiological tools for bovine brucellosis using whole genome sequencing

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Bovine brucellosis caused by Brucella abortus has been eradicated from the United Kingdom, with Great Britain (England, Wales and Scotland) declared officially brucellosis free (OBF) in 1985. However, there remains a risk that the pathogen could be imported via international trade. In order to be able to distinguish such an instance from re-emergence from an unidentified endogenous reservoir of infection, and to identify the likely source, we have sought to characterize the genetic diversity of Brucella abortus isolates in Great Britain (GB) prior to eradication. We have done this using whole genome sequencing of 89 isolates from the strain collection of the UK OIE/WHO/FAO Brucellosis Reference Laboratory. These span a wide geographic range and were isolated over a period of 42 years (1962 - 2004), including some from disease occurrences since eradication. Paired-end short sequence reads for all isolates were generated on the Illumina MiSeq platform. Short sequence read data were subsequently processed using an analysis pipeline involving trimming for read quality, filtering duplicate reads and mapping of the short reads to a reference genome (B. abortus biovar 1 field isolate 9-941). Following mapping, single nucleotide polymorphisms (SNPs) in the aligned sequences were identified. Sequencing of GB isolates generated an average 44-fold depth of coverage over the 3.3 megabases (Mb) of the B. abortus genome. Of those sequenced samples with more than 15-fold coverage an average of 99.7 % of processed reads per sample were mapped to the reference genome. Consistent with the high level of nucleotide similarity previously observed within the Brucella genus, the level of agreement with the reference genome was high in all sequenced isolates, with an average 99.2 % homology. Maximum likelihood phylogenetic analysis using SNP data demonstrated that two main B. abortus lineages existed within Britain prior to eradication, with one dominated by biovars 1 and 2, and the second composed primarily of biovars 5 and 9. Diversity in the number of SNPs observed amongst isolates of biovars 5 and 9 was low.

Further analysis of whole genome sequencing data will seek to reconstruct the B. abortus population structure in Britain prior to eradication with greater resolution, using Bayesian phylogenetic methods. In parallel, on-going work will seek to enlarge the database of available genomes from UK trading partners. This will provide a valuable resource for supporting traditional epidemiological approaches in any potential future occurrence of B. abortus in Britain.

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PS7-2. Isolation and molecular characterization of *Brucella abortus* from cattle and buffaloes in Gujarat, India


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The present study reports the isolation, identification and molecular characterization of *Brucella abortus* from various clinical samples collected from cattle and buffaloes in Gujarat. A total of 423 clinical samples viz., blood, vaginal swab, vaginal discharge, placenta, hygroma fluid, orchitis fluid, milk, foetal stomach content, foetal liver, foetal Spleen, foetal lung, foetal heart blood, uterine discharge and cotyledon collected from cattle and buffaloes, were processed for isolation of *Brucella* on BBL *Brucella* agar kept in 5.00 % CO₂. Colonies obtained on *Brucella* agar after 3-7 days were identified as *Brucella* based on Gram’s staining, MZN staining, Catalase, KOH test, genus specific PCR. Further the species level identification was carried out by species specific PCR, Bruce ladder PCR, which confirmed the isolates as *Brucella abortus*. Thus 15 isolates were obtained by processing 423 clinical samples. Of these, 8 isolates were from cows and 7 were of buffalo origin (vaginal swab-2, placenta-4, vaginal discharge -3, foetal stomach content-1, foetal liver-1, foetal Lung-1, foetal Heart blood-3). The genomics DNA of *Brucella abortus* SKN-13 was isolated and Sequenced with Ion Torrent Sequencing Technology with standard protocol then assembled with assembler MIRA version SOAPdenovo v. 4.0.2 by the de novo assembly method. This whole genome sequencing based on Ion torrent PGM revealed the *Brucella abortus* for confirmation.

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PS7-3. Outer membrane vesicles from *Brucella suis*.

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Gram negative bacteria shed outer membrane vesicles (OMVs) budding from the outer membrane and released into the environment. OMVs have been involved in many roles. Proteomic characterization from OMVs helps in understanding bacterial physiology and identifies protein sorting-cargo. *Brucella suis* is a Gram-negative pathogenic bacteria and ethologic agent of porcine brucellosis; at date they *B. suis* OMVs characterization has not been reported. The aim of this study is purification and characterization of protein-cargo from *B. suis* 1330 OMVs. The preparation and purification of OMVs was made from *B. suis* 1330 culture on agar plates by differential centrifugation. Purified OMVs were observed by electron microscopy. The protein content of the vesicles was analyzed by SDS-PAGE. Protein identification was made by proteomic analysis. The results showed that the vesicles purified from *B. suis* 1330 showed spherical shape, double membrane bilayer and a diameter between 49 and 130 nm. The electrophoretic profile showed around 11 bands of 8 and 80kDa. Proteomic analysis revealed 252 proteins present in vesicles. The identified proteins were classified regarding *in silico* analysis: outer membrane proteins, virulence factors, proteins response to external agents, enzymes, among others. *B. suis* OMVs purification was achieved, *B. suis* OMVs shown similar characteristics to other bacterial vesicles. Proteomic analysis revealed a great amount of identified proteins transported into the OMVs and released to the external milieu.

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PS7-4. Development of a PNA-FISH assay for the detection of *Brucella* spp.

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Fluorescence *in situ* hybridization using peptide nucleic acid probes (PNA-FISH) is a novel diagnostic technique for rapid and accurate diagnosis of infectious diseases. PNA-FISH technique is a combination of simple and traditional staining procedures with the unique performance of PNA probes. In the present study, PNA-FISH was standardized for detection of *Brucella* spp. *B. abortus* reference strain S99 and *B. abortus* isolates obtained from the clinical samples were used. The fluorescently labelled probe sequence selected was based on nucleotide sequence of 16S rRNA of *B. abortus* and were got synthesized from PNA Bios (PNA Inc. USA). Sequence of the probes was 5’-Flu-OO- labelled where ‘Flu’ is equivalent to 5,6-carboxyfluorescein and ‘O’ is equivalent to 8-amino-3,6-dioxaoctanoic acid. Yellow green fluorescent coccobacilli were seen when the smear was observed under the fluorescent microscope indicating that the PNA probe hybridized with *B. abortus*. Different concentrations of PNA probe prepared in hybridization buffer (50 nm, 200 nm and 500 nm per 20 µl of hybridization buffer) were tested for hybridization with the standard culture of *Brucella* S99 and maximum fluorescence was obtained at 500 nm/20 µl probe concentration. The PNA probe was evaluated for specificity by testing some commonly available bacteria (*E. coli*, *Enterococcus*, *Salmonella*, *Staphylococcus* and *Streptococcus*). No positive fluorescence signals were observed with the different bacteria tested indicating that the PNA probe was specific for *Brucella* spp.

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PS7-5. Typing of *Brucella melitensis* isolates from India by Multilocus Variable-Number Tandem-Repeat Analysis (MLVA)

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Brucellosis, a true zoonosis, is considered as one of the most widespread zoonosis in the world. This is a contagious disease with huge economic impact on livestock industry and severe hazard to human health. For eradication of brucellosis from livestock, continuous surveillance and epidemiological trace-back is necessary which requires strain-specific identification to know the source of infection. In the present study, a MLVA typing scheme was applied to differentiate the field strains of *B. melitensis* isolated from the different parts of India. A total of 65 *B. melitensis* strains along with 3 representative reference strains available in the repository of the *Brucella* Laboratory, Div. of VPH, IVRI, Izatnagar were subjected to MLVA-15 genotyping. After revival and characterization of the isolates by cultural, phenotypic and bio-chemical tests, the extraction of DNA from pure culture of *Brucella* was done. Molecular confirmation was done by genus specific PCR assay targeting 16S-23S r-RNA spacer gene. Then, all the *Brucella* isolates were subjected to PCR amplification with 15 primers corresponding to 15 VNTR loci (Panel 1 (Bruce 6, 8, 11, 12, 42, 43, 45, and 55) and Panel 2 (Bruce 4, Bruce 7, Bruce 9, Bruce 16, Bruce 18, Bruce 21 and Bruce 30) for MLVA typing assay. The MLVA-15 typing of 65 *B. melitensis* field strains and 3 reference strains (*B. melitensis* 16M, Rev 1 and Isfahan) resulted in 48 different profiles. Of these, 38 profiles were unique while remaining 10 profiles were shared by at least 2 strains. On clustering analysis, the 68 isolates were classified into 33 clusters corresponding to 48 genotypes. For *B. melitensis* strains, most discriminatory loci found were Bruce 4 and 16 having highest allelic diversity of 0.837 and 0.838, respectively. Bruce 45 showed no allelic diversity. The D value of MLVA-15 genotyping was 0.982 and type ability was 100 per cent. In the present study, MLVA-15 genotyping clearly discriminated Indian field isolates of *B. melitensis*. Even *B. melitensis* strains isolated from a limited geographical area (Karnataka) were sufficiently discriminated. On clustering analysis, grouping or specific pattern was observed according to epidemiological data/source of infection or exposure. Clustering analysis based on MLVA genotypes revealed no association between the different strains with the time of isolation. Overall, in the present study, MLVA-15 genotyping was found rapid, highly discriminatory and appropriate for epidemiological typing of strains of *B. melitensis*. This is the first report of VNTR analysis of Indian isolates of *Brucella*.

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PS7-6. Cloning and expression of immunogenic protein(s) of *Brucella abortus* in prokaryotic expression system and assessing their suitability for serodiagnosis of bovine brucellosis


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Bovine brucellosis caused by *Brucella abortus* is an economically important and zoonotic disease affecting cattle and buffaloes resulting in abortion, retained placenta, and reduced milk production in females and orchitis and epididymitis in males. For diagnosing bovine brucellosis presently used sero diagnostic tests/assays depend upon whole or smooth lipopolysaccharide (sLPS) antigen which can also detect antibodies against other cross reacting gram negative bacteria such as *Yersinia enterocolitica* O: 9 resulting in lowered specificity. Using immunogenic recombinant proteins of *B. abortus* in diagnostics may provide suitable solution for addressing the problems of cross reactivity and biohazard of handling *Brucella* organisms. Thus the proteins BP26, BLS and SOD C of *B. abortus* were identified, expressed in prokaryotic expression system, purified and characterized by SDS-PAGE and Western Blot. Using these recombinant proteins, checker board titration was performed for standardizing indirect ELISA with *Brucella* antibodies positive and negative cattle serum samples. Evaluation of assay was carried out with field serum samples and diagnostic sensitivity, specificity and other statistical parameters were calculated for the developed recombinant protein(s) based indirect ELISA. It is evident that this assay may be promising complement assay in addition to existing conventional sLPS antigen based diagnostic/surveillance tests/assays for brucellosis.

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PS7-7. Genome-scale metabolic network reconstruction of *Brucella abortus* 2308

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The reconstruction of genome-scale metabolic networks is an iterative process where information obtained from several data sources is combined to construct a preliminary set of reactions and boundaries. Genome-scale modelling of bacterial metabolic networks provides a powerful tool to identify and analyze pathways required for successful intracellular replication during host-pathogen interaction. We aimed to provide, through systems analysis, a basis for the characterization of the genome-scale properties of this pathogen’s versatile metabolic network. We used “metabolic models reconstruction using genome-scale information” (Merlin) tool for the metabolic network reconstruction of *Brucella abortus* 2308. Merlin uses metabolic information from KEGG (enzymes, metabolites, and reactions) to build the backbone of the network. It also assigns Gene-Protein-Reaction associations to enzymatic reactions in the network. The first draft of *Brucella abortus* 2308, the model, encompasses 1379 reactions (with pathway 1148 reactions), 2265 metabolic species and 778 genes. We employed flux balance analysis, which identified 232 genes as essential genes. This model will be used to assist in the study of the molecular mechanisms involved in virulence by studying the network topology and gene essentiality in different strains.

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PS7-8. Molecular identification and characterisation of *Brucella* spp. from farm animals.

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Ten isolates of *Brucella* spp. were isolated from cows, buffaloes and pigs suffering from reproductive disorders and abortions. The isolates obtained were confirmed biochemically and by polymerase chain reaction (PCR) using B4/B5 primer pair derived from *BCSP31* gene of *B. abortus*. An amplicon size of 223 bp was obtained in all the isolates and they were confirmed as *Brucella* spp. Bruce ladder PCR was employed for detection, differentiation and species typing of *Brucella* species i.e. *B. abortus*, *B. melitensis* and *B. suis*. Multiplex PCR (Bruce ladder) assay was carried out using 8 pair of primers. DNA was extracted from all the ten isolates and that from the vaccine strain S-19, standard *B. melitensis* and standard *B. suis* strains by using HiPurA Bacterial genomic DNA purification kit (Himedia). Bruce Ladder Multiplex PCR was carried out on DNA of the ten isolates. All the field isolates amplified showed fragments of 1682 bp, 794, 587, 450 and 152 bp size. Hence, it was confirmed that the isolates were that of *B. abortus*. With *B. melitensis* DNA, an additional 1,071-bp fragment was amplified and *B. suis* was confirmed by the presence of an additional 272-bp fragment. PCR with *B. abortus* S19 DNA did not produce the 587-bp fragment.

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Session 8: Wild-life brucellosis and world brucellosis

PS8-1. Epidemiological features of brucellosis among human in Azerbaijan

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Brucellosis is an endemic disease for Azerbaijan. Epidemiological situation on brucellosis in Azerbaijan remains stressed, caused by the presence of infection among agricultural animals which are the main source of human infections.

This study was carried out using the electronic database (EIDSS) of the Ministry of Health of Azerbaijan, Republican Anti-plague Station and State Veterinary Control Service for the period of 2013-2015. Analysis of seasonality of brucellosis morbidity for 2013-2015 showed an increase in registration of cases during the spring, summer and fall period. Analysis of case distribution by age groups and gender showed that generally people aged 15-59 become ill. The greatest proportion of the disease for 3 years is the proportion of age group from 30 to 59 years (347 cases) - active able-bodied age - men (232, 66, 7 %) and women (115, 33,3 %).

The evaluation of influence of the risk factors on patients with brucellosis showed that consumption of milk by patients occurred in 18,84-38,9 %; consumption of insufficiently thermally processed meat – in 5,86-9,2 %; contact with a sick animal occurred in 7,06-14,35 % of cases from all the informed ones for all study period. For animals, the statistics of disease cases showed similar trends over the years: in 2013: Cattle - 1163, small cattle - 431; in 2014: Cattle – 1289, small cattle – 493; and in 2015: Cattle – 1073, small cattle – 887. *Brucella* infection prevalence is higher in the summer pasture areas are lower than in villages. While performing analyses by age, trends showed highest infection amongst animals aged 2-4 years in the summer pasture, and older than 4 years in villages. The presence of animal markets and automobile road crossings increases the risk of spread of diseases among the animals.

Thus, the epidemiological situation on brucellosis in Azerbaijan remains stressed owing to remaining epizootic problems among agricultural animals. In this regard, it is important to improve tactics of epizootological and epidemiological inspection of brucellosis foci and work together to identify the factors affecting the proliferation of this infection.
PS8-2. Seroprevalence and identifying risk factors of Brucellosis and genetically characterization of *Brucella* spp. in Azerbaijan

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Brucellosis is considered an endemic disease in Azerbaijan since it is an important problem both for people and livestock. State Veterinary Control Service (SVCS) performs regular active observation of brucellosis in order to conduct surveillance as well as reduce the disease among livestock. Moreover, State Veterinary Control Service implements vaccination among small ruminants with REV1 eye drop vaccine in accordance with national epizootic plan and its performance. Every year along with 250-400 human cases, 1,100 cattle, and 1,200 small ruminants brucellosis cases are annually reported in AJ.

The main purpose of study is to evaluate the brucellosis national active surveillance and control measures using recommended sampling methodology by relevant international organizations in order to calculation of sero-prevalence. One of the purposes of study is isolation, molecular and phylogenetic characterization *Brucella* spp. which circulating among cattle and small ruminants in Azerbaijan for comparing brucellosis study with border country.

Cross sectional study including multistage random selection of animal and sample collection. As a sample will be collected milk and blood. Identification of the vaccination history, current symptoms and likely risk factor will be conducted. Samples will be taken from infected milk shall be isolated by culturing and DNA extraction according to *Brucella* spp. and send to Georgian Central Reference Laboratory for purpose of genetic subtyping.

Expected results: 1) will be able to evaluate control measure against brucellosis in Azerbaijan, 2) will identify the main risk factors of spreading brucellosis in Azerbaijan, 3) will study genetic characterization of *Brucella* spp. in Azerbaijan, 4) will provide isolated *Brucella* spp. for further studies.

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Brucellosis is a chronic infectious disease occurring in both humans and livestock/wild animals. Human Brucella infection is the leading cause of disability. Brucella negatively impacts production among agricultural animals and is one of the highest concerns due to resultant economic loss. Based on World Health Organization and World Animal Health Organization data, brucellosis is widely distributed in many countries of the world, including Azerbaijan. Despite implemented measures, complete eradication has not been accomplished.

Our purpose was to evaluate the epidemiologic situation for human and animal Brucella in Azerbaijan for the last three years. In order to evaluate current situation, statistics, laboratory data, as well as State Veterinary Control Service Data, and personal research data was used. Blood (serum) samples collected from large and small cattle in Azerbaijan’s regions were tested using serology assays (CFT, RBT). In total, 3,311.658 blood samples obtained from large cattle and 487.701 from small cattle during the period of 2013-2015 were tested. 3.939 samples were positive in serologic testing, of which 2503 belonged to large cattle and 1436 were collected from small cattle. Human brucellosis was identified in 543 individuals. Vaccination was conducted among large cattle and as a result 4.430.568 heads of animals received vaccine. Prevalence declined among small cattle during vaccination period.

Our analysis demonstrated Brucella prevalence among livestock in various regions of Azerbaijan. The most important steps in preventing the spread of the disease are establishment of animal identification system with the creation of relevant database and preventive vaccination among large cattle, considering the effectiveness of this measure among small cattle using eye drop technique is recommended.

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PS8-4. Distribution of *Brucella* Species in Georgia

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Brucellosis is the most common of all the bacterial zoonoses resulting in significant economic losses due to reduction in animal populations, as well as human death and suffering. Although the picture of global brucellosis morbidity is not completely clear, brucellosis is found on almost all continents and at least 500,000 people are infected annually. Human infection occurs from exposure to infected animals or contaminated animal products, and it is clear that control of the human disease can only occur through control and eradication of the disease in animals. In 2014, the Laboratory of the Ministry of Agriculture (LMA) completed the Phase I and Phase II *Brucella* State Surveillance program and found a prevalence of 6.4% across Georgia. In the last five years (2011-2016) as an additional study to the State program, 940 samples were received from farmers across Georgia. Several different types of samples from cattle and small ruminants were delivered to the Georgian Laboratory of the Ministry of Agriculture, summarized as: 635 blood samples; 635 serum samples; 286 milk samples; 17 aborted fetal tissues; and 12 vaginal swabs. Clinical samples were tested using serology (Rose-Bengal and FPA), microbiology, and PCR. Isolated cultures were retested by PCR and also ran against the Bruce ladder PCR assay to differentiate specific *Brucella* species (*B. abortus*, *B. melitensis*, *B. ovis*, and *B. suis*). The identification of *Brucella* spp. was conducted based on morphology, staining, and a metabolic profile (catalase, oxidase, and urease) and compared with the Bruce Ladder test results.

In this study we found a total prevalence of 5%, which is slightly lower than the results from the Phase I/Phase II study. In total, we isolated 48 cultures from both cattle and small ruminants, which were 35 *Brucella abortus* and 13 *Brucella melitensis*. Laboratory studies show that *Brucella* infection is endemic in Georgia. The main two species repeatedly found are *Brucella abortus* and *Brucella melitensis*. This work will help veterinarians decide which vaccine candidates are appropriate to conduct a vaccination campaign and improve the epidemiological surveillance program and reduce incidence of brucellosis in animals in Georgia.

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PS8-5. A retrospective analysis of the bovine brucellosis control program in Minas Gerais State, Brazil

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Brucellosis is one of the most important and widespread zoonoses in the world. Due to the direct and indirect losses triggered by the Brucella abortus infection in cattle, control and eradication of the disease have been a major goal of many countries where the disease is endemic, including Brazil, which since 2001 has the Programa Nacional de Controle e Erradicação da Brucelose e Tuberculose Animal - PNCEBT (National Program for the Control and Eradication of Animal Brucellosis and Tuberculosis). The present study aimed to review and evaluate retrospectively the data on combating bovine brucellosis in the state of Minas Gerais, considering the information generated by the four studies of seroprevalence of brucellosis in animals and properties carried out between the years 1974 and 2011 (1974, 1980, 2002 and 2011). A comparison of the results obtained in these studies identified a reduction in the prevalence of seropositive animals and properties from 17.70 % (95 % CI: 16.40 to 19.00) to 3.59 % (95 % CI: 2.76-4.42 %), and from 30.6 % (95 % CI: 5.88-6.72 %) to 0.81 % (95 % CI: 0.1 - 1.10), respectively. Taking into account that the average vaccinal coverage during the ten years between the last two cross-sectional studies (2002 and 2011) was 79.50 % and that there was a significant decrease in the apparent prevalence of properties and seropositive cows in this period, it is possible to attribute this reduction in the prevalence to the maintenance of the immunization coverage. Moreover, the other strategies proposed in PNCEBT and employed by Instituto Mineiro de Agropecuária (IMA), the Animal Health Protection Agency of Minas Gerais State, from 2001, such as transit control of animals for breeding and culling of animals diagnosed as positive for brucellosis, probably have also contributed to the control of the brucellosis in Minas Gerais State. The results in the control of bovine brucellosis achieved by the state of Minas Gerais demonstrated that the application of measures provided for in PNCEBT has been effective in reducing the prevalence of disease in the medium term. The experiences of the IMA in the control of bovine brucellosis, which preceded in several years the publication of the PNCEBT can be used by the animal health protection services from other Brazilian federal units in the control and eradication of this disease in their herds.

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PS8-6. Serological prevalence of ovine and caprine brucellosis in Bangladesh

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Brucellosis is considered to be the most widespread zoonosis throughout the world. It has a serious implication on human health as well as on the economic development in a developing country like Bangladesh. The objective of the present study was to determine the seroprevalence and to delineate the risk factors for Brucella seropositivity in small ruminants in Mymensingh district of Bangladesh. In the present study, serum samples were collected from a total of 2456 small ruminants (1710 goat and 746 sheep) from 13 upazilla of Mymensingh district. The data related to age, sex and location were also collected using a questionnaire. Serum samples were screened using Rose Bengal Test (RBT) and Enzyme Linked Immunosorbent Assay (ELISA). Seroprevalence of Brucellosis was 9.53 % (163 out of 1710) in goats and 9.92 % (74 out of 746) in sheep on RBT test. In goat, the highest Brucella antibody was observed in Mymensingh sadar upazilla (13 %) followed by Dhobaura upazilla (12.8 %). On the other hand, highest ovine Brucella antibody observed in Haluaghat upazilla (13.04 %) followed by Mymensingh sadar (12.5 %). The prevalence was more in adults (55.2 % in goats and 57 % in sheep) than young (8.6 % in goat and 8.1 % in sheep) and more in female goats (41.1 %) and sheep (39.2 %) than male goats (14.1 %) and sheep (18 %). Degree of agglutination Positive “+” 93.26 % (152), “++” 6.13 % (10) and “+++” 0.61 % (1) were observed in case of goats where as “+” 89.19 % (66), “++” 8.11 % (6) and “ +++” 2.70 % (2) in sheep in RBT. ELISA test showed 33.70 % (31 out of 92 RBT positive samples) positive reaction of total RBT positive reactors. Although Brucellosis is endemic zoonotic disease in Bangladesh, no vaccination programme yet started for controlling this disease. Result of this study can be useful for launching new initiatives in this area of works where the country will be benefited through the development of prevention and control strategy.

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PS8-7. Seropositivity rates of Brucella spp. infection in dogs in Southeast Provinces of Turkey
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The knowledge of the prevalence of brucellosis in dogs is very limited and mainly based on the results of couples of articles and almost all of them focused on B.canis infection. The main objective of this study was to determine seropositivity rates of infection caused by smooth and rough species of Brucella in dogs in two provinces located in Southeast part of Turkey. Smooth Rose Bengal test (S-RBPT) and rough RBT (R-RBPT) and two ELISAs using rough heat saline extract (HSE) of B.canis M- strain (R-ELISA) and lipopolysaccharide (LPS) of B. abortus S99 strain (S-ELISA) as antigens were used as serological tests.

A total of 151 serum collected from several dog shelters located in Sanliurfa and Diyarbakir Provinces. Of all the test sera, 53 (35.1 %), 22 (14.6 %), 32 (21.2 %) and 21 (13.9 %) were positive by S-RBPT, R-RBPT, S-ELISA, and R-ELISA, respectively. Ten of the serum samples were positive by both S-ELISA and R-ELISA. HSE that is used in R-ELISA as antigen contains both R-LPS and outer membran proteins (OMPs), the latter are common in all Brucellae. This probably explains the seropositivity shared by both ELISAs. While 10 dogs that were negative by S-RBPT presented positive reactions by S-ELISA, 29 dogs that were positive by S-RBPT were negative by S-ELISA. The first could be due to prozone phenomenon or chronic infection, the latter could be the result of false positive reactions or being at an early stage of infection. In conclusion, it was assumed that canine brucellosis is caused by mainly smooth Brucellae although rough Brucellae also play an important role in canine brucellosis. All preventive measures should be taken into account to control, prevent and eliminate the disease from dog populations. This will also reduce the risk for public health.

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PS8-8. The role of serological testing in a brucellosis control program in Geghashen in Kotayk Marz, Republic of Armenia

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Brucellosis testing in cattle has indicated that the Geghashen community in Kotayk Marz has a high prevalence of the disease in comparison of other communities which are similarly included in the national test and slaughter program. Blood samples were collected from cattle in Geghashen and subjected to the Rose Bengal Test. Positive samples were subsequently examined with a second assay, either ELISA or agglutination reaction methods, to confirm the initial diagnosis and reduce the chance of false positives.

More than one third of all reported brucellosis cases in cattle during the 2014-2015 period have been in Kotayk Marz: 346/929 (37.6 %) in 2014 and 358/1048 (34.1 %) in 2015. The Geghashen community is particularly afflicted. Of 2221 blood samples collected in 2014 from cattle of the Geghashen community, 186 registered positive for exposure to *Brucella* via the Rose Bengal test. These were subsequently examined by ELISA and agglutination reactions with 177 reporting positive (7.9 % of the total and 95.2 % of the original positives). Of 1488 cattle blood samples from Geghashen in 2015, 248 (16.6 %) were positive via Rose Bengal of which 230 (15.4 %) were positive upon re-testing (92.7 % of the original). In the 1st quarter of 2016, 62 positive samples out of 725 cattle blood samples (8.5 %) were registered in Gegashen of which 56 (7.7 %) were subsequently confirmed at the Republican Laboratories.

Brucellosis test results from 2014-2016 suggest that the incidence of the disease is decreasing in the Geghashen community. Control measures are still in progress and so, a further reduction of confirmed positive cases are expected. The measures include controlling the movement of animals, as well as enumerating and registrating animals.

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**PS8-9. First Discovery of Brucella Infection in Georgian Bats**

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Brucellosis is a bacterial disease transmitted to humans through direct contact with infected animals such as sheep, cattle, goats, pigs, and dogs. Multiple *Brucella* species are capable of causing health problems in both human and animal populations. No published data is available regarding *Brucella* detection in bats worldwide.

A study was designed to define a new possible reservoir for brucellosis by identification of *Brucella* spp. among Georgian bats. This effort was part of a study of multiple bacterial pathogens in Georgian bats. In total 200 bats from eight different species were collected from four regions of Georgia. A small piece of spleen from each bat was homogenized separately. DNA was tested for *Brucella* spp. using an IS711-based real-time PCR assay; further confirmation of positive samples was performed by *Brucella* specific PCR targeting bosp31 or conventional PCR targeting the 16S rRNA gene. Four spleen samples from two different groups of bats, *Myotis blythii* and *Miniopterus schreibersii*, were positive for *Brucella*. In addition, two samples were co-infected with two bacterial pathogens (*Bartonella* spp. and *Leptospira* spp.). All positive bats were females and were captured in the Tskaltubo or Imereti regions of west Georgia in July 2012. Positive samples were sent to CDC Atlanta for culturing and genetic analysis.

Results revealed that bats in Georgia may serve as an additional reservoir for *Brucella* spp. and can potentially transmit the infection to humans or other animals. Further data will assist in forming a better understanding of the pathogens host range, and with assessment of existing and potential threats for veterinary and human health.

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PS8-10. Monitoring of brucellosis in wild boars in 2013-2014 in Ukraine

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Brucellosis is an infectious zoonotic disease caused by bacteria of *Brucella* genus. Cattle, small cattle, pigs, camels, horses and dogs are susceptible to brucellosis. Some other ruminants and sea mammals could also be infected. Brucellosis is especially dangerous to humans. High brucellosis morbidity is observed in Mediterranean region countries, Iran, Pakistan, Afghanistan, African countries, China, India, Peru, and Mexico. Increase in animal brucellosis case number is being observed during the last years in the countries of the Central and South-Western Asia. The last case of the cattle brucellosis was registered in Ukraine in 1992. *B. suis* was isolated in domestic pigs in Crimea and Kherson oblast in 1999. According to the Ministry of Health, one case of human brucellosis is registered in Ukraine every year. Planned preventive serological testing of bulls, cows, heifers, calves, rams, ewes, breeding boars and sows for brucellosis is performed once a year. According to State Monitoring Plan, the following sera samples from domestic animals are tested for brucellosis each year: about 3,000,000 sera samples from cattle, 500,000 - from small ruminants, 200,000 - from swine, and 6,500 - from horses. Additionally, SSRILDVSE conducts serological study of samples from wild animals. Monitoring of brucellosis in wild boars in Ukraine to control the epizootic situation on brucellosis. Assessment of risk for possible occurrence of brucellosis in humans and animals upon contact. Blood sera from wild boars collected during hunting seasons of 2013-2014 in different regions of Ukraine were studied. The following serological methods were used during the study: complement fixation test (CFT) and rose-Bengal test (RBT).

In 2013, 694 sera of wild boars from 21 oblasts were tested for brucellosis. 97 samples (14%) were positive using RBT and 184 positive results (26,5%) were identified using CFT. In 2014, 666 sera of wild boars from 18 oblasts were analyzed using RBT, among which 93 (14%) were positive. Only 558 out of 666 samples were analyzed in CFT since 108 samples appeared inapplicable for this test. 134 positive results (24%) were identified.

The obtained results (14% of positives in RBT and 26.5% in CFT) demonstrate continuous circulating of brucellosis pathogen in wild animals in different regions of Ukraine and a threat of outbreaks in domestic animals. The study requires further analysis through the comparison of seroprevalence in domestic and wild animals and should be accompanied by the isolation of a *Brucella* culture.
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