Twinning Project on Equine Piroplasmosis – Feedback from Candidate Laboratory

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**Equine Population**

- **Total world equine population = 58 millions**

<table>
<thead>
<tr>
<th></th>
<th>Country</th>
<th>millions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>USA</td>
<td>9.5</td>
</tr>
<tr>
<td>2</td>
<td>China</td>
<td>7.4</td>
</tr>
<tr>
<td>3</td>
<td>Mexico</td>
<td>6.2</td>
</tr>
<tr>
<td>4</td>
<td>Brazil</td>
<td>5.7</td>
</tr>
<tr>
<td>5</td>
<td>Argentina</td>
<td>3.6</td>
</tr>
<tr>
<td>6</td>
<td>Mongol</td>
<td>2.0</td>
</tr>
<tr>
<td>7</td>
<td>Russian federation</td>
<td>1.3</td>
</tr>
</tbody>
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Impact on Economy

- Horse industry – a report from AHC
  - Direct impact of $39 billion on the US economy
  - Overall impact of $102 billion
  - Additionally, this industry supports 1.4 million equivalent full-time jobs.
Indian Equine Population

Total Indian equine population: 1.77 million

- Thoroughbred Horses (~25000 heads)
- Race-horse industry (~15000 heads)
- Other Agencies (~10000 heads)

Indigenous Equids (~1.735 millions)

- Defined Indian horse Breeds (~10000 heads)
- Non-discript horses, ponies, donkeys and Mules (~1.735 millions heads)

14 April 2011
Equine Demography (India)

Per Cent Population Distribution

- Horse & Pony: 47%
- Mule: 12%
- Donkey: 41%

14 April 2011
Breeding Track of Horses in India

Marwari

Spiti

Breeding track of Horses in India
National Research Centre on Equine, Hisar

Established: January 7, 1986
Mandate

- To undertake research on health & production management in equines
- To develop diagnostics/biologicals for major equine diseases
- To act as national referral facilities for diagnosis, surveillance & monitoring of equine diseases, and
- To provide diagnostic, advisory & consultancy services
Equine Piroplasmosis

- **Two species**
  - *Theileria equi*
    - Prevalent in 90% of the world inhabited by horses.
  - *Babesia caballi*
    - Only Canada, United States, Australia, Japan, England and Ireland are not considered to be endemic areas.
Life cycle of *Theileria equi*

Different forms of *T. equi* merozoite inside the erythrocytes
Clinical Form

- Acute clinical cases
- Sub-acute
- **Chronic** – non specific clinical signs, mild inappetence, poor performance, drop in body mass, splenomegaly.

Aborted foetus

Aborted foetus - severe hepatomegaly and splenomegaly
Impact of Latent Infection

- The latent infection is common in non-descript equids in India.
- These animals act as nucleus herd for maintaining and spread of infection thru vector ticks
  - Diagnosis of sub-clinical infection is of more relevance as these animals remain carrier to the *T. equi* parasite throughout their life span.
- These latently infected animals may exhibit
  - low performance
  - in the event of physical/immunological/mental stress the underline parasitaemia can flare-up leading to clinical form of the disease condition.
World Epidemiology

Disease Distribution Map
Jan 2010


Disease Outbreak
Jan 2010 to Dec. 2011

1976 the Equine piroplasmosis was reported in a outbreak form in imported horses.

50.1% and 49.76% incidence was recorded in North-West India by CAT & Dot-ELISA.

Clinical incidence is common in foreign breeds of horses kept in enzootic zones.
# National Sero-surveillance Analysis

<table>
<thead>
<tr>
<th>Year</th>
<th>Theileria equi</th>
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<tbody>
<tr>
<td>2002-03</td>
<td>19.6 %, (125/636)</td>
</tr>
<tr>
<td>2003-04</td>
<td>27.04% (387/1431)</td>
</tr>
<tr>
<td>2004-05</td>
<td>21.80% (230/1055)</td>
</tr>
<tr>
<td>2005-06</td>
<td>17.7% (168/955)</td>
</tr>
<tr>
<td>2006-07</td>
<td>52.0% (610/1172)</td>
</tr>
<tr>
<td>2007-08</td>
<td>24.3% (170/698)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>28.58% (1690/5947)</strong></td>
</tr>
</tbody>
</table>

**Theileria equi**

![Graph showing sero-prevalence percentages over years](chart.png)

- **% Sero-prevalence**
- **Years**
- **2002-03**
- **2003-04**
- **2004-05**
- **2005-06**
- **2006-07**
- **2007-08**
Diagnostics

- Developed at NRCE
  - Capillary Tube Agglutination Test
  - Dot-ELISA
  - Complement fixation test
  - CCIP
  - Single Dilution ELISA
  - ELISA based on r-Ag.
  - PCR based on EMA-1 & EMA-2
  - MASP in-vitro culture ??
  - IFAT ??

- OIE approved
  - IFAT
  - C-ELISA
  - CFT
  - PCR
NRCPD, Japan Previous Collaboration

- Under JSPS I joined as Post-doctorate (Nov. 2001 to March 2004).

- We worked on host-parasite relationship of *T. equi* and explored the drug targets for chemotherapy.


Expression of EMA-1 and EMA-2

- during the different developmental stages of merozoite
- detected by IFAT (confocal microscopy)
Twinning Project Objectives

- **Capacity building** of National Research Centre Equines, Hisar, India so that it can act as a referral centre in the Indian sub-continent with particular reference to SAARC countries.

- Development of sensitive & specific molecular diagnostics (based on recombinant antigens, qPCR techniques) as per OIE guidelines and training of scientific staff from selected laboratories in Indian sub-continent.

- Organization of training programmes/workshops for researchers working in this area of SAARC countries.

- Application submission to the OIE for designating NRCE, India laboratory as OIE reference laboratory for equine piroplasmosis.
Activities under Twinning Project

- **In-vitro cultivation by MASP technique.**
- **qPCR for quantification of parasitic load** in the latently infected equines specially donkeys and horses.
- **Genomic variability** of EMA-1 and EMA-2 gene in Indian equine population, if any.
- **Development of ELISA using expressed protein** from EMA-1 and EMA-2 and its validation on equine samples collected/obtained from different geographical origins.
- Development and validation of **ICT as a pen side field diagnostic test.**
- **IFAT facility** at NRCE, Hisar, India.
In-vitro Cultivation

Normal double gas CO$_2$ Incubator

- Initiated culture, parasitaemia of 7-8% and 5-6% was achieved in *T. equi* and *B. caballi* parasites.

Using Anaero Pouch®

- The parasite were observed on 14$^{th}$ day of the culture [both *T equi* (1-1.5% and *B caballi* (0.5-1%)].
- Upon subculture parasitaemia of 3-4% was observed in both the culture.
Protein Expression

21-22aa
249aa

Theileria equi EMA-1

22-23aa
255-274aa

86aa

Target region

Theileria equi EMA-2
Expression and Immunoblotting

Expressed in pGEX 4T-1 vector  Immuno blotting with +ve serum
Primers for Real Time PCR – 18s RNA (Kim et al 2008)

- Be18SF: 5’-GCG GTG TTT CGG TGA TTC ATA-3’
- Be18SR: 5’-TGA TAG GTC AGA AAC TTG AAT GAT ACA TC-3’
- TaqMan probe: 5’ AAA TTA GCG AAT CGC ATG GCT T-3’

- More data to be generated using some other primers and probe so as to be used as a diagnostic assay on field samples
qPCR

Real Time PCR of Field Genomic sample

Plasmid Copy no.
30
300
3000
30000
300000
3000000

Detector: B. equi 18S Sanjay
Plot: ΔRn vs. Cycle
Threshold: 0.20
Immuno Chromatographic Test (ICT)

- We prepared about 400 strips using EMA-2 (t) antigen of *T. equi*.
- These ICT strips will be validated on serum samples collected from Indian equine samples.
Theileria equi

Babesia caballi
NRCPD Experience

- State-of-art research laboratories equipped with all modern facilities.
- Imparted excellent technical/scientific support for capacity building of NRCE scientists under this OIE Twinning Project.
Future Activities

- The ELISA will be optimized using the EMA-1 and EMA-2 expressed antigens.
- The results as obtained in ELISA will be compared with OIE approved CI-ELISA kit, IFAT and in-vitro culture.
- This ELISA will be further transformed in the form of a diagnostic kit and validated.
- qPCR as a diagnostic test for accessing parasitic load in latently infected equids.
THANKS