Breeding sheep for parasite resistance: What traits to measure? Phenotypes and Genotypes

Animal Production and Health Laboratory, IAEA Laboratories, Seibersdorf
Why breeding sheep for parasite resistance?

- Helminthiasis (disease caused by gastro-intestinal nematodes) is the most important livestock diseases worldwide

- Use of anthelmintic drugs is increasingly regarded as unsustainable due to emergence of multiple drug resistant parasites

- Integrated parasite management strategies – Breeding sheep for parasite resistance is an important long term component objective to reduce the dependence on drugs for control
Why breeding sheep for parasite resistance?

• Acquisition and expression of immunity against GI nematodes is genetically controlled and varies between breeds and between individuals within breeds

Between breed variation

Red Maasai vs Dorper
Barbados Black Belly vs INRA composites
Garole vs Deccani
Santa Ines vs Suffolk
Ile de France vs Poll Dorset

Within breed variation

• Genetic resistance to parasites is a heritable trait
• Scope for selection of sheep for nematode resistance
Genetic components related to inheritance of parasite resistance characteristics include

Breed

Heritability

Genotype X Environment interaction

Correlations with other traits of economic importance

Host-parasite interaction
Selection focus

Resistance - Tolerance/Resilience

**Resistance** – Ability of the host to resist infection

**Tolerance** - Host is infected by pathogen but suffers little with adverse effect

Selection should be focused on reducing the transmission of infection (i.e. resistance) rather than reducing the clinical signs (resilience/tolerance)
What traits to measure?

Identification of the most appropriate indicator trait (phenotype) for parasite resistance is difficult

**Measures of resistance**
- Fecal egg count, Worm burden, Worm size and Fecundity

**Measures of immune response**
- Eosinophilia, Antibodies such as IgA (CarLA), IgG and IgM

**Measures of impact of infection**
- Anemia (PCV, FAMACHA), gastrin, pepsinogen or fructosamine concentrations

**Measures of resilience**
- Growth rate, Treatment frequency
What traits to measure? Fecal Egg Count (FEC)

Fecal egg count (FEC)

- Most within breed studies of genetic resistance use FEC as indicator trait
- Extensive within breed variation and significant heritability reported in small ruminants

**Sheep**
- $h^2 = (0.149)$ Avikalin
- $h^2 = (0.24)$ Muzafarnagri
- $h^2 = (0.4-0.5)$ Katahdin

**Goats**
- $h^2 = 0.13$ (Galla and East African)
- $h^2 = 0.11-0.16$ (Jamunapari)
- $h^2 = 0.37$ (Creole, French West Indies)
What traits to measure? Packed Cell Volume (PCV)

• Packed cell volume is a measure of RBCs and indicates the level of anemia in animals

• PCV has been used to evaluate within breed variations and one of the useful indicators for parasite resistance/resilience in sheep/goat

• Heritability estimates vary between 0.12 to 0.31 (e.g. Santa Ines sheep, composite of Dorset, Romney and Finn sheep)
What traits to measure?

FAMACHA

• FAMACHA system was initially introduced to manage haemonchosis in sheep and goats using targeted selective treatment (Vanwyk and Bath, 2002)
• FAMACHA scoring is based on the correlation between level of anemia and the colour of eye mucous membrane
• FAMACHA scoring is a practical and relatively easily obtained phenotype
• Riley and Vanwyk (2011) proposed genetic evaluations based on FAMACHA scoring combined with simple penalties
• Predicted breeding values based on FAMACHA scores can help to improve resistance and/or resilience with the ultimate objective of producing animals that survives and produce without deworming
What traits to measure? IgA (CarLA)

- Immunoglobulin IgA – Isotype closely associated with intestinal mucosal responses
- Prevents larvae from establishing in the gut and resulting in rapid expulsion
- Commercial saliva test available (CarLA) www.carlasalivatest.com

CarLA – T. colubriformis L3 carbohydrate surface antigen

- IgA antibody response to CarLA challenge
- Positively associated with resistance to parasites: High CarLA animals have low FEC and improved growth
## Phenotype ontology – An issue for consideration?

<table>
<thead>
<tr>
<th>QTL_symbol</th>
<th>Trait_name</th>
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<tbody>
<tr>
<td>FECGEN</td>
<td>Fecal egg count</td>
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<tr>
<td>FOC</td>
<td>Fecal oocyst count</td>
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<tr>
<td>HFEC_1</td>
<td>Haemonchus contortus FEC1</td>
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<tr>
<td>HFEC_2</td>
<td>Haemonchus contortus FEC2</td>
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<tr>
<td>IGE_2</td>
<td>Immunoglobulin E nematode challenge 2</td>
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<tr>
<td>LATRICH_2</td>
<td>Abomasal Trichostrongylus sp adults and larvae challenge 2</td>
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<tr>
<td>LSITRICH_2</td>
<td>Small Intestine Trichostrongylus sp adults and larvae challenge</td>
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<tr>
<td>NFEC</td>
<td>Nematodirus FEC</td>
</tr>
<tr>
<td>NFEC_1</td>
<td>Nematodirus FEC1 (August)</td>
</tr>
<tr>
<td>NFEC_2</td>
<td>Nematodirus FEC2 (September)</td>
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<tr>
<td>NFEC_3</td>
<td>Nematodirus FEC3 (October)</td>
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<tr>
<td>NFEC_AVE</td>
<td>Nematodirus FEC Average</td>
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<tr>
<td>SFEC</td>
<td>Strongyle FEC</td>
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<tr>
<td>SFEC_3</td>
<td>Strongyle FEC3</td>
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<tr>
<td>SFEC_AVE</td>
<td>Strongyle FEC average</td>
</tr>
<tr>
<td>TC_IGG_2</td>
<td>Trichostrongylus colubriformis serum IgG challenge 2</td>
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<tr>
<td>TFEC_1</td>
<td>Trichostrongylus colubriformis FEC1</td>
</tr>
<tr>
<td>TFEC_2</td>
<td>Trichostrongylus colubriformis FEC2</td>
</tr>
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</table>

(Animal QTL db, http://www.animalgenome.org/cgi-bin/QTLdb/index)
Genetic markers

QTLs associated with parasite resistance

MAS (Marker assisted selection (QTLs), candidate gene markers

Genomic selection
Sheep QTLs

- 753 QTLs from 86 studies in sheep for various economic traits
- 81 QTLs related to parasite resistance
- Chromosome 3 with 16 QTLs followed by Chromosome 14 with 7 QTLs
- QTLs related to parasite resistance are w.r.t. Haemonchus, Trichostrongyles, Strongyles and Nematodirus
QTLs related to various GINs in sheep

QTLs for HFEC = 44  
(Haemonchus Fecal Egg count)

• 4 QTLs – Chr 3, 7

• 3 QTLs = Chr 1, 16, 22
Candidate genes involved in innate and adaptive immune pathways
~80 candidate genes were re-sequenced in a panel of eight unrelated sheep

- Candidate genes
  - Pattern recognition receptors
    - Toll like receptors
    - NOD like receptors
    - RIG I like receptors
    - C type Lectin binding receptors
  - Cytokine genes (e.g. Interleukins, Interferons)
  - Ovine Histocompatibility genes
SNPs identified in Sheep

- 208 SNPs were identified
- 174 KASP SNP assays were developed
- No. SNPs per candidate gene varied from 1 to 9
- Among CDS/Exon SNPs, 51 were non-synonymous and 61 were synonymous

<table>
<thead>
<tr>
<th>Genic Region</th>
<th>No. SNPs</th>
<th>% SNPs</th>
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<tbody>
<tr>
<td>3'UTR</td>
<td>17</td>
<td>10</td>
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<tr>
<td>Intron</td>
<td>44</td>
<td>25</td>
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<tr>
<td>CDS/Exon</td>
<td>112</td>
<td>64</td>
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<tr>
<td>5' flanking region</td>
<td>1</td>
<td>1</td>
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</table>
Distribution of SNPs in Sheep genome

- Significant number of the identified SNPs (48) are located in chromosome 3.
- Many are involved in different immune pathways.

Candidate Gene Study

Goat

• Candidate genes involved in innate and adaptive immune pathways
  • ~72 candidate genes were re-sequenced in a panel of eight unrelated goats

• Candidate genes
  • Pattern recognition receptors
    • Toll like receptors
    • NOD like receptors
    • RIG I like receptors
    • C type Lectin binding receptors
  • Cytokine genes (e.g. Interleukins, Interferons)
  • Caprine histocompatibility genes
- 187 SNPs were identified
- 141 KASP SNP assays were developed
- No. SNPs per candidate gene varied from 1 to 7
### Status of genotyping sheep samples from RCHs

<table>
<thead>
<tr>
<th>Country</th>
<th>Species</th>
<th>Samples arrived @ IAEA</th>
<th>Samples genotyped</th>
<th>No. SNP per sample</th>
<th>No. genotypes generated</th>
<th>Genotyping in progress</th>
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<tbody>
<tr>
<td>Brazil</td>
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<td>721</td>
<td>679</td>
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<td>106603</td>
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<td>Argentina</td>
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<td>1234</td>
<td>895</td>
<td>157</td>
<td>140515</td>
<td>339</td>
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<tr>
<td>Iran</td>
<td>Sheep</td>
<td>309</td>
<td>309</td>
<td>157</td>
<td>48513</td>
<td>0</td>
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<tr>
<td>Indonesia</td>
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<td>203</td>
<td>152</td>
<td>157</td>
<td>23864</td>
<td>51</td>
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<tr>
<td>Ethiopia</td>
<td>Sheep</td>
<td>423</td>
<td>113</td>
<td>157</td>
<td>17741</td>
<td>310</td>
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<tr>
<td>Burkina</td>
<td>Sheep</td>
<td>225</td>
<td>140</td>
<td>157</td>
<td>21980</td>
<td>85</td>
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<tr>
<td>Total</td>
<td>Sheep</td>
<td>3115</td>
<td>2288</td>
<td></td>
<td>359216</td>
<td>827</td>
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Additional samples from Austria, Bulgaria, India, Sri Lanka, Iraq, Peru and Pakistan have been genotyped.
Additional samples from Myanmar, Austria, India and Pakistan have been genotyped

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<thead>
<tr>
<th>Country</th>
<th>Species</th>
<th>Samples arrived @ IAEA</th>
<th>Samples genotyped</th>
<th>No. SNP per sample</th>
<th>No. genotypes generated</th>
<th>Genotyping failure</th>
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<tbody>
<tr>
<td>China</td>
<td>Goat</td>
<td>288</td>
<td>280</td>
<td>141</td>
<td>39480</td>
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<tr>
<td>Sri Lanka</td>
<td>Goat</td>
<td>567</td>
<td>518</td>
<td>141</td>
<td>73038</td>
<td>49</td>
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<tr>
<td>Bangladesh</td>
<td>Goat</td>
<td>233</td>
<td>227</td>
<td>141</td>
<td>32007</td>
<td>6</td>
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<tr>
<td>Nigeria</td>
<td>Goat</td>
<td>306</td>
<td>84</td>
<td>141</td>
<td>11844</td>
<td>222</td>
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<tr>
<td><strong>Total</strong></td>
<td>Goat</td>
<td><strong>1394</strong></td>
<td><strong>1109</strong></td>
<td><strong>141</strong></td>
<td><strong>156369</strong></td>
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</table>
Genotyping Method Employed for Candidate gene SNPs

**KASP Genotyping**

- **Conserved region**
  - **Allele 1**
  - **Allele 2**

**Design allele-specific primers (nucleotides 1–17)**

- **3’ Allele 1-specific (ASP1) primer**
  - 5’ A

- **3’ Allele 2-specific primer (ASP2)**
  - 5’ C

**PCR with ASP1 or ASP2 + conserved primer (CON)**
Competitive allele specific PCR (KASP Genotyping)

- PCR-based KASP genotyping assay is a homogeneous, fluorescence (FRET) based assay that enables bi-allelic discrimination of known SNPs and InDels.

- KASP genotyping chemistry requires no labeling of the target-specific primers/probes
Two allele-specific primers (one for each SNP allele). Each primer contains a unique unlabelled tail sequence at the 5' end.

One common (reverse) primer.
Work Flow of KASP Genotyping

2 x Reaction Buffer
- KASP assay
- water

Aliquot 8μl Mastermix to each PCR-tube

Transfer 2μl DNA or water to aliquoted Mastermix

[Image showing a diagram with steps for KASP genotyping process]

[Graph showing allelic discrimination with RFU values]

[Image of a PCR machine and a laptop]
The fluorescence data of each sample from both the dyes attached to SNP alleles can be plotted to visualize the cluster of genotypes.

Can be visualized in Kluster Caller or Allele Discrimination module incorporated in real time PCR systems.
Genome wide Association Study

- Custom designed 60K SNP array from Affymetrix will be used for genome wide SNP typing
- First set of 96 samples (48 samples from the tail of phenotypes; low and high FEC) from Argentina has been genotyped
- 384 additional samples will be processed with representation from indigenous breeds of different countries
Thank You