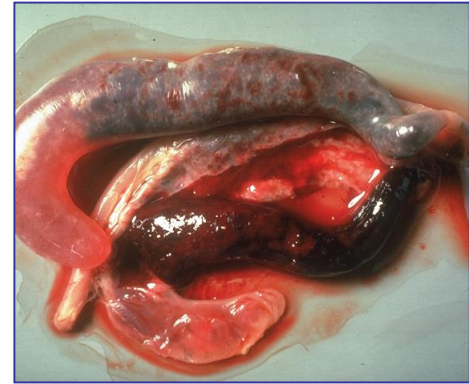
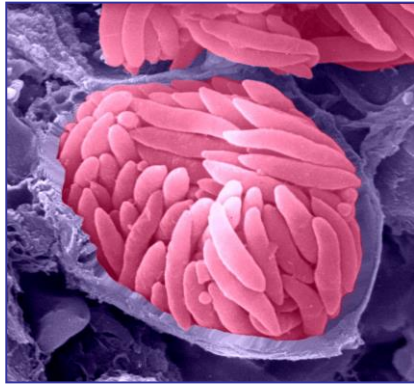


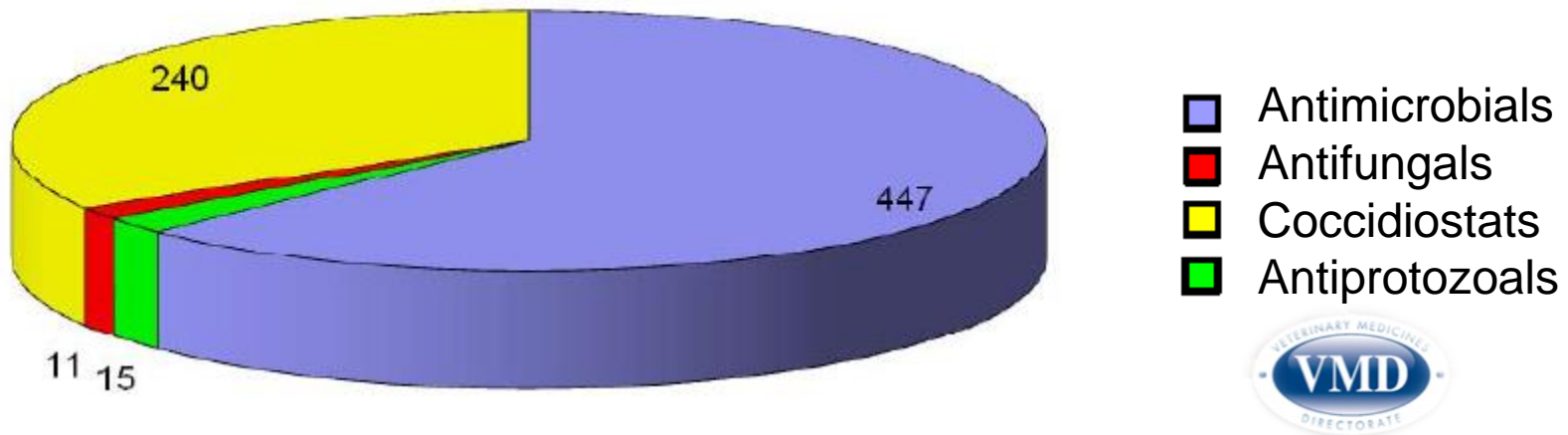
Population, genetic and antigenic diversity of *Eimeria*: prospects for novel vaccines



Damer Blake

The impact of coccidiosis

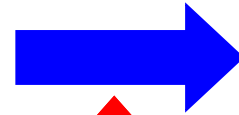
- Global cost **>£2.5 billion** per annum
- A **third** of drugs sold for use in UK agriculture are coccidiostats



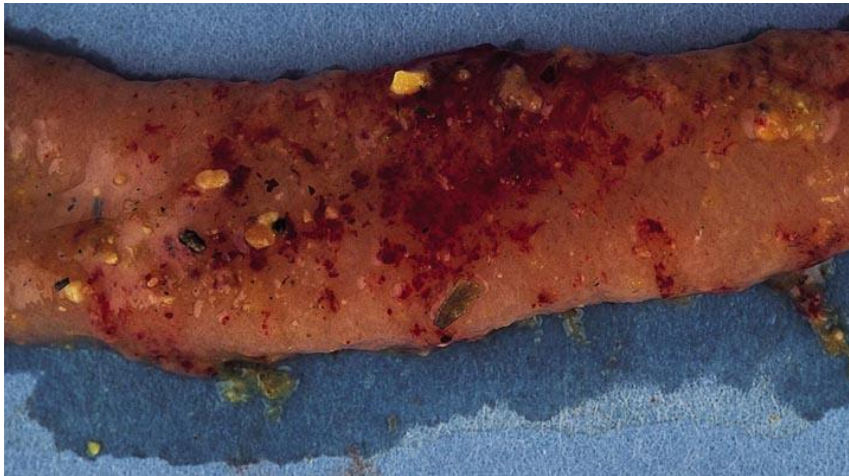
- Ranked in **Top 10 economically important** endemic veterinary diseases (UK)

Coccidiosis - the problem

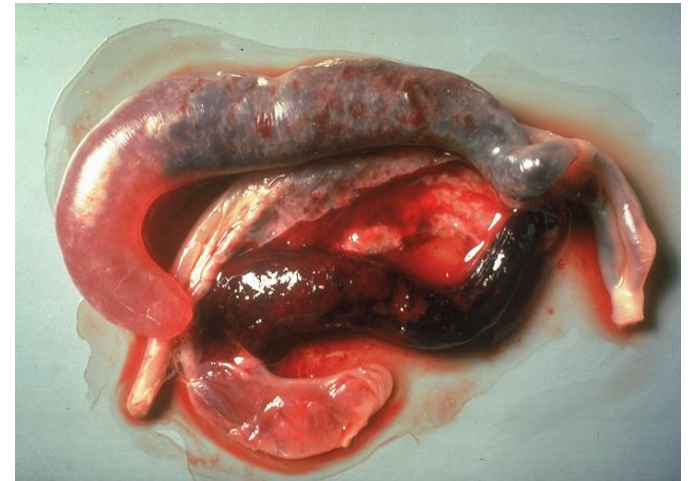
Eimeria spp.



New vaccines?

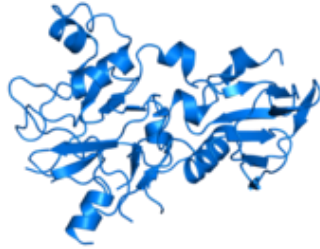


Eimeria maxima

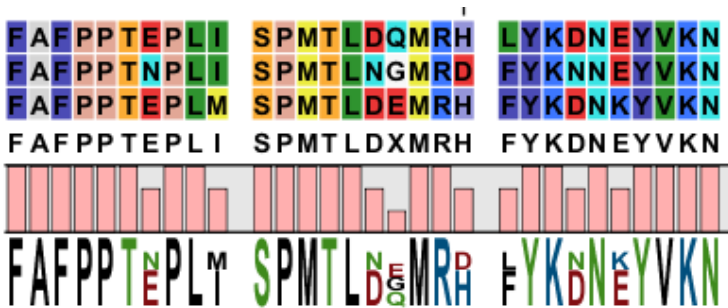


Eimeria tenella

Coccidiosis - new vaccines



- Multiple vaccine candidates
- AMA1, IMP1, MIC3...
- Efficient delivery is crucial
- Vectored, dietary....



- Genetic (antigenic) diversity?

Questions?

Prevalence of relevant genetic polymorphism?

- How effective will a defined subunit vaccine be?
 - What parasites are out there?
 - How diverse are they?
 - What population structure(s) define *Eimeria*?
 - How polymorphic is each vaccine candidate?

Global sample collection



- 512 samples collected (+ GenBank)
- 20 countries represented
- All six continents home to chickens

Genome-wide *E. tenella* genotyping

- *Eimeria tenella* (reference genome)
- NGS additional genomes
- Multiplexed MassARRAY SNP typing
- 54 *Eimeria tenella* SNPs (average 0.96 Mb SNP⁻¹)
- Field sample panel (known to contain *E. tenella*)
 - India: 139 (South: 53, North: 86)
 - Libya: 51
 - Egypt: 40
 - Nigeria: 14

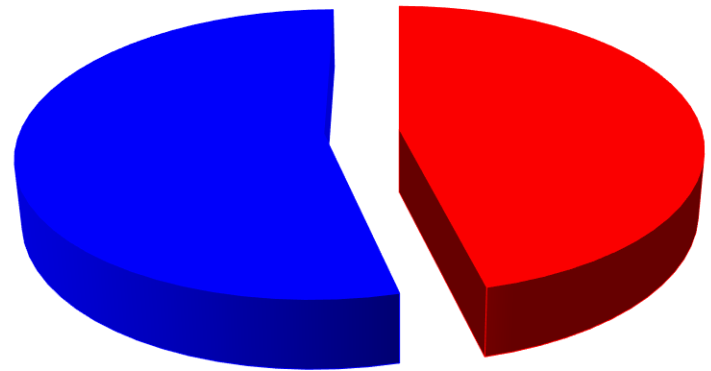
Genome-wide *E. tenella* genotyping

- 52/54 SNPs proved informative

- SNP profile

Monoclonal ●

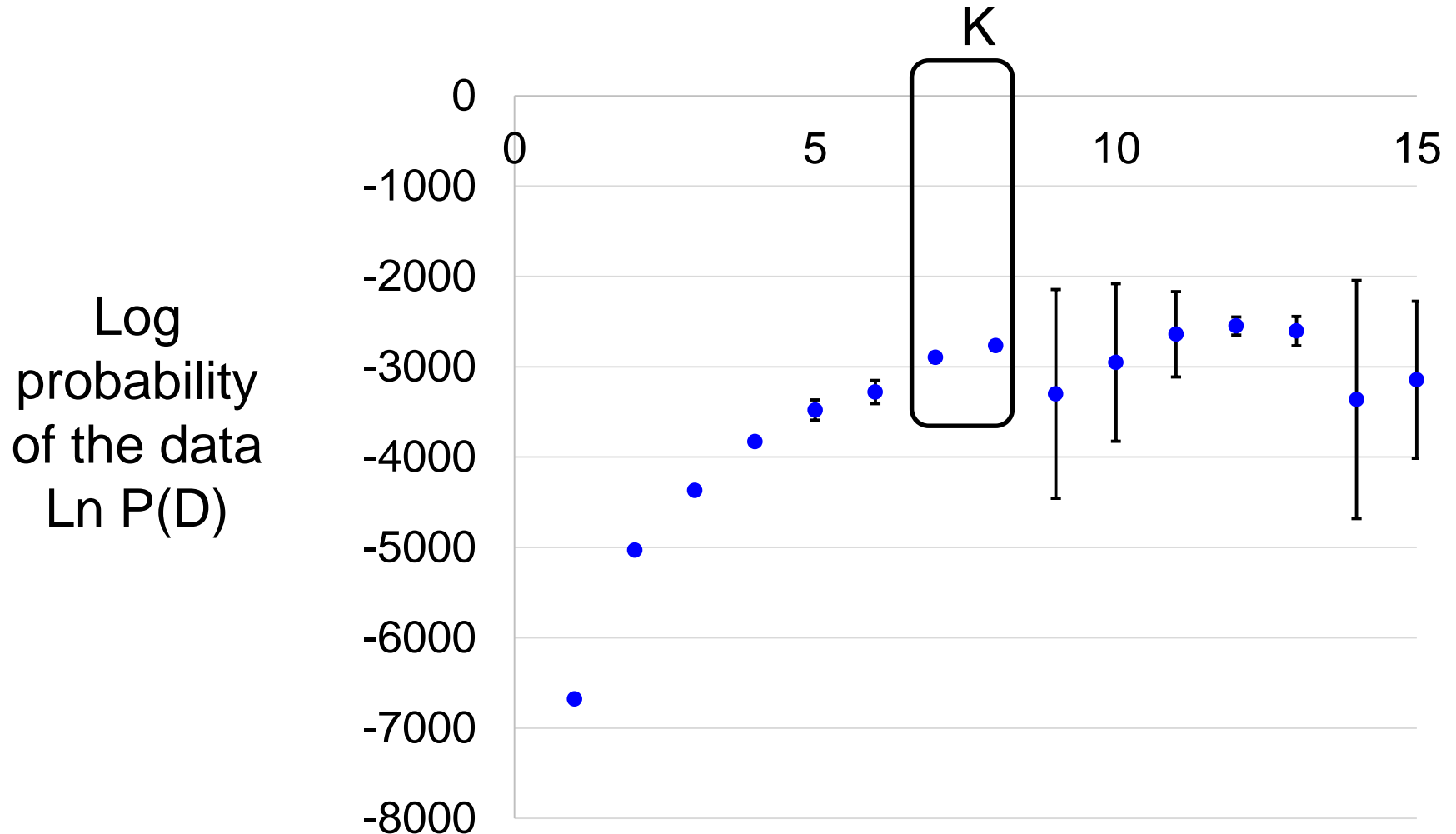
Polyclonal ●



- Population structure is important

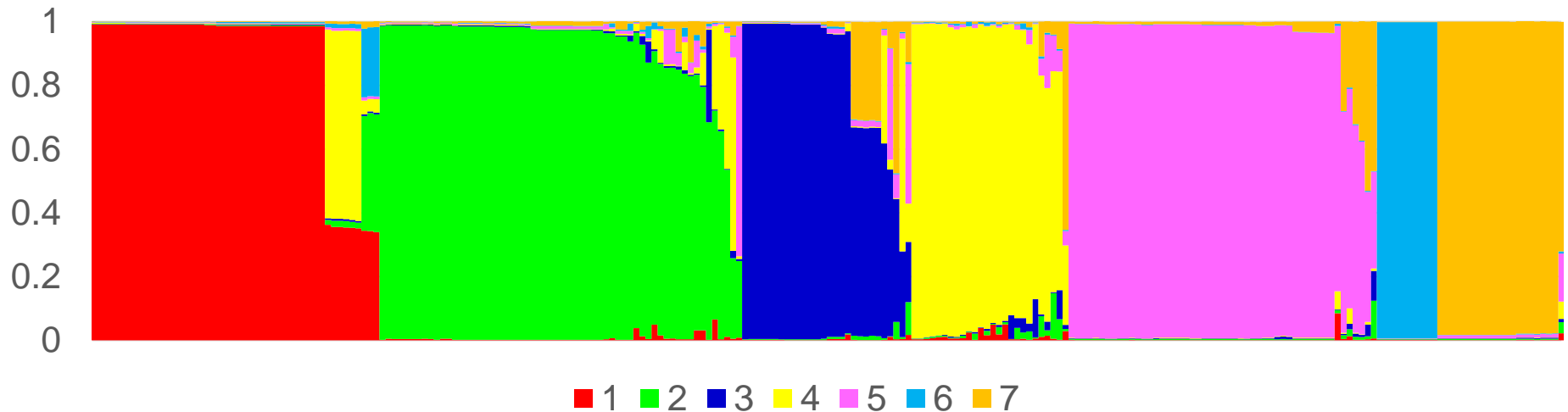
Population STRUCTURE

- *Eimeria tenella*: whole panel assessment



Population STRUCTURE

- *Eimeria tenella*: whole panel assessment



- Bigger picture
 - *Toxoplasma* - largely clonal
 - *Plasmodium* - panmictic (? clonal)

Haplotypes (Specific)

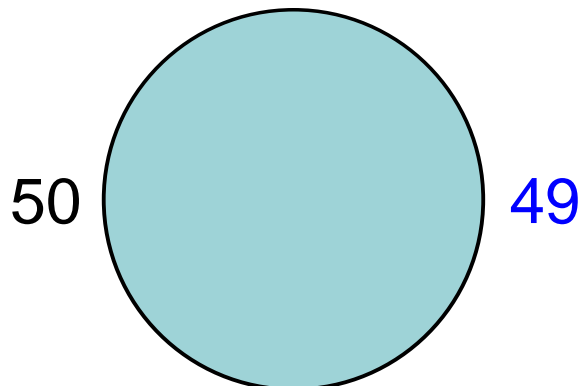
I_A^S

N. India (86)

8  7

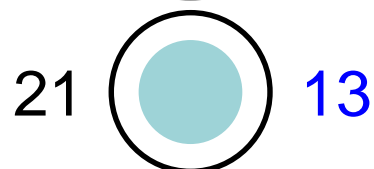
0.115*

S. India (53)



0.009

Egypt (51)



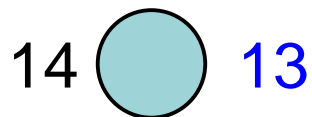
0.060*

Libya (40)



0.053*

Nigeria (14)



0.002

Equilibrium

$(I_A^S = 0)$



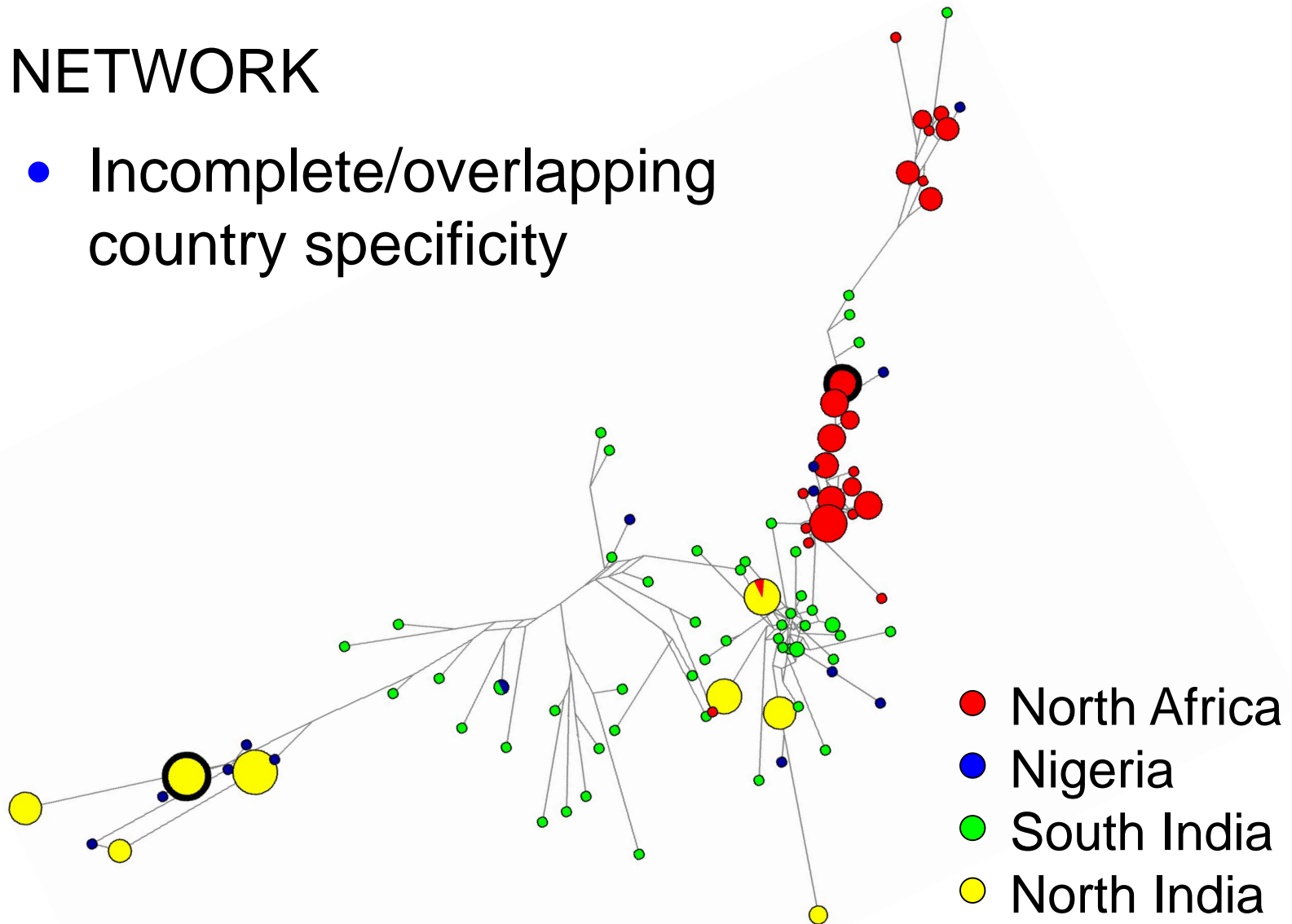
Disequilibrium

$(I_A^S > 0)$

*p<0.001

Population structure

- NETWORK
 - Incomplete/overlapping country specificity



Summary

- Populations of *E. tenella* are genetically diverse
- Polyclonal populations are common
- Genome-wide diversity is influenced by geographic location
 - Largely region-specific haplotypes
 - Population structure – varies
 - *Suggests:*
 - Background level of recombination
 - Potential for panmixia
 - Restraint = ? Environmental? Behavioural?

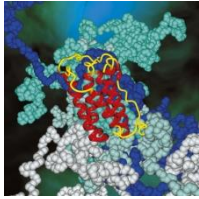
Global sample diversity



Eimeria tenella, gene specific

Apical membrane antigen-1

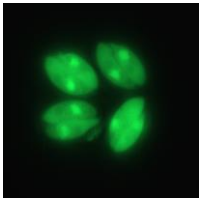
- *Eimeria maxima*



- Recombinant EmAMA-1 protein: 23% protection

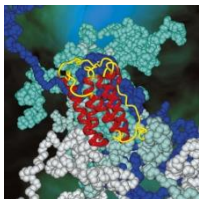


- EmAMA-1 DNA vaccine: 42% protection



- Transgenic *Eimeria tenella*: 40-50% protection

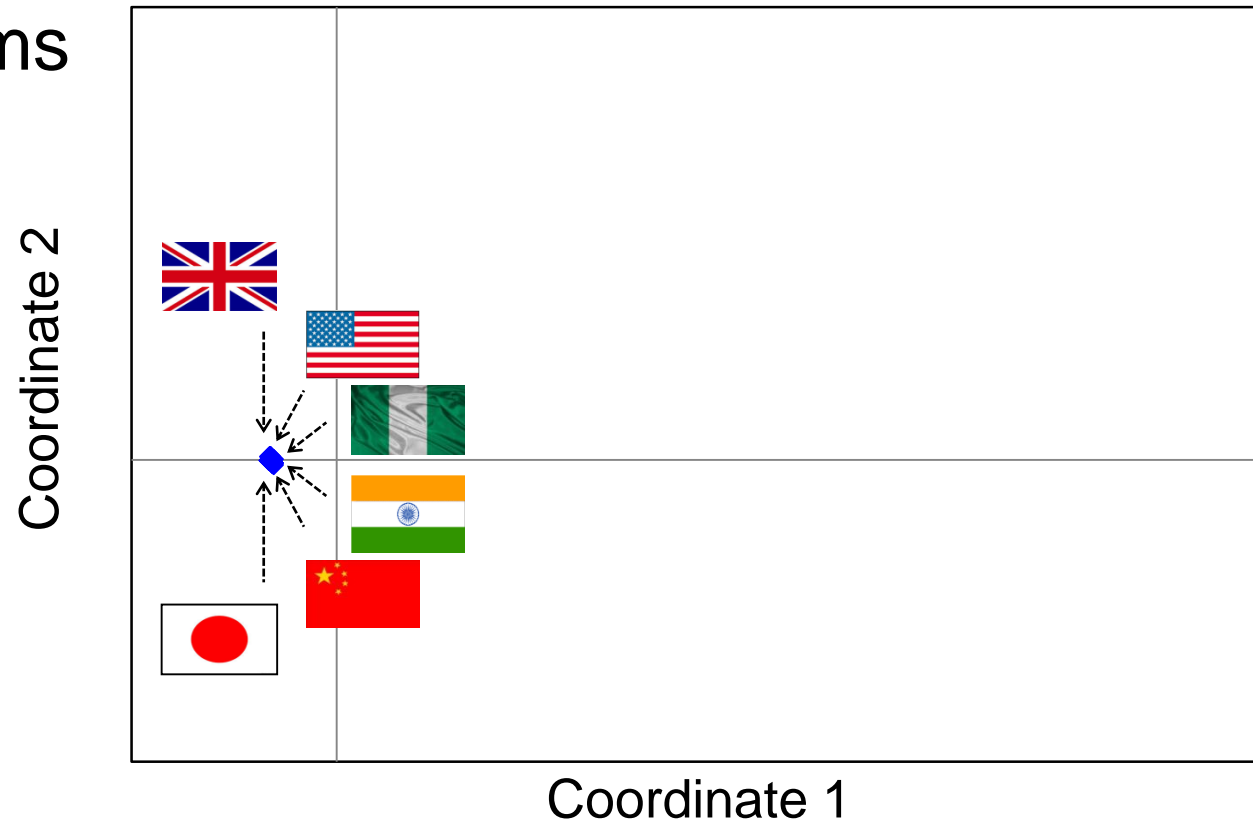
- *Eimeria tenella*



- Recombinant EtAMA-1 protein: 66% protection

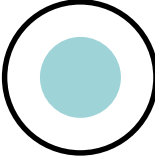

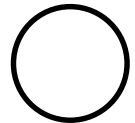

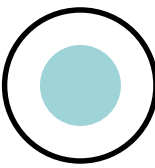
Candidate: apical membrane antigen-1

- 56 full length coding sequences (31 Nigerian)
- 10 countries represented, including five continents
- Overall mean diversity (amino acid): 0.006 ± 0.002
- Seven isoforms
- Within farm
- PCoA



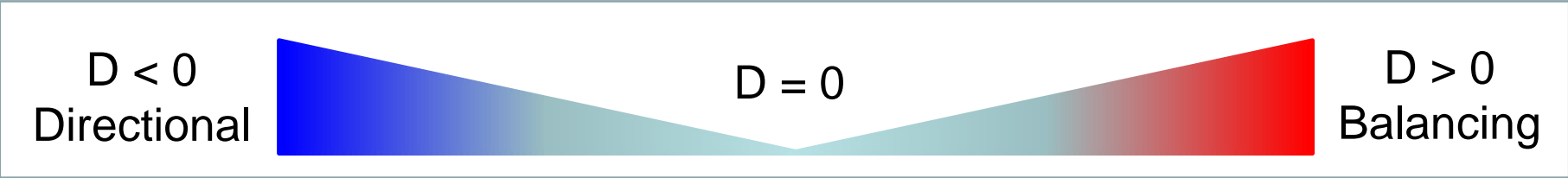
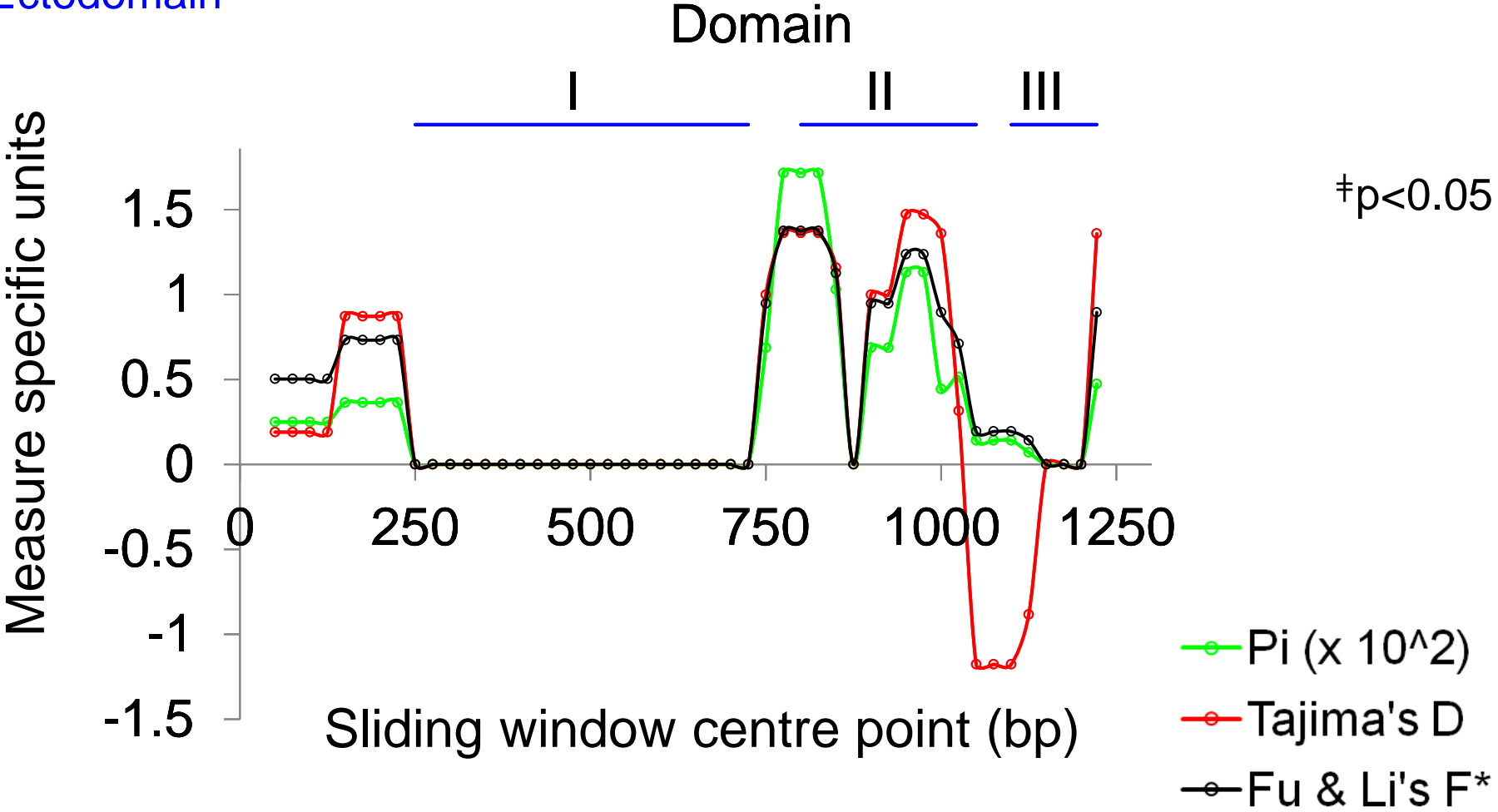
Candidate: apical membrane antigen-1

DNA haplotypes (Specific)

| | | | |
|---------------|---|---|---|
| Asia (7) | 4 |  | 2 |
| N. Africa (7) | 2 |  | 0 |
| Europe/US (6) | 3 |  | 0 |
| Venezuela (5) | 2 |  | 0 |
| Nigeria (31) | 4 |  | 2 |

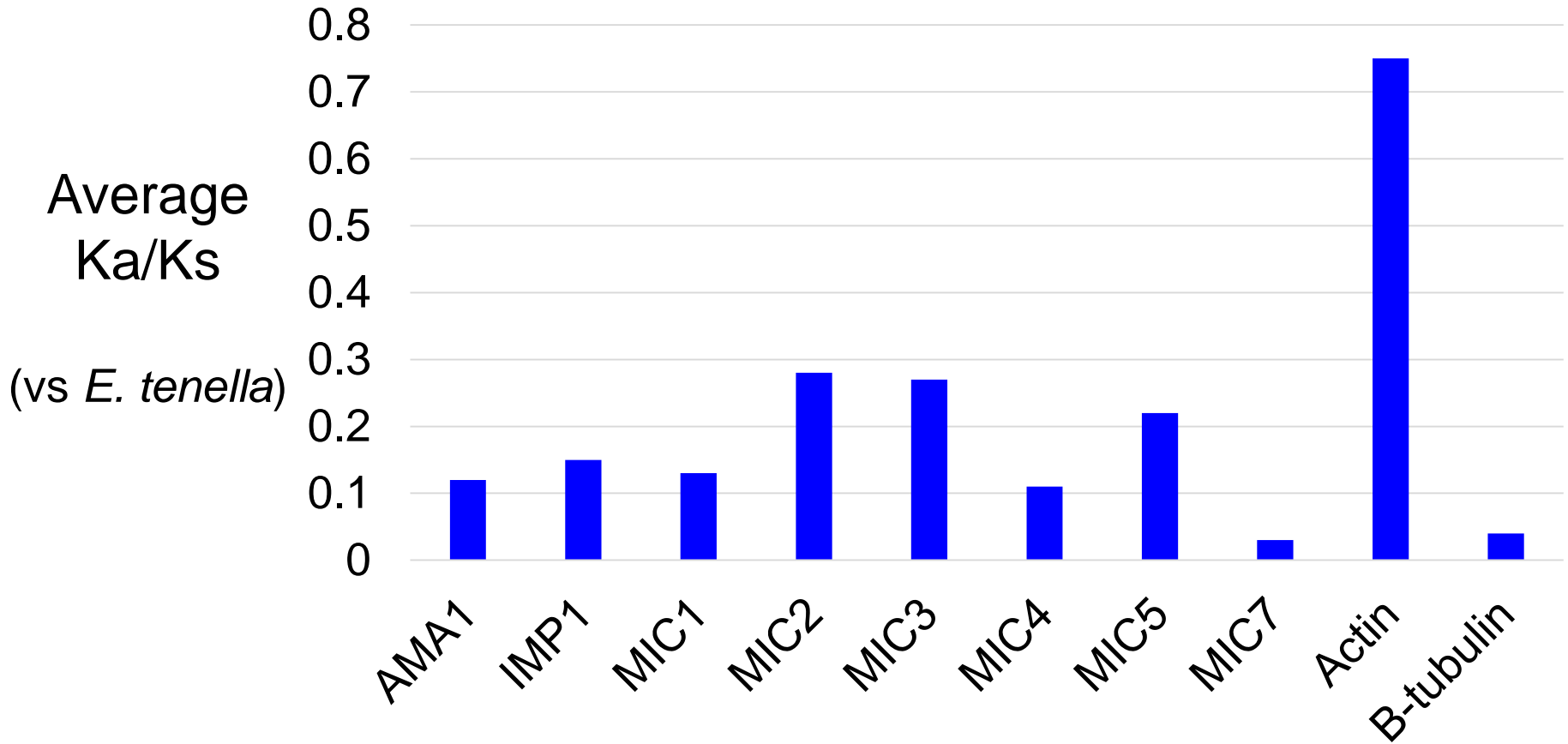
Candidate: apical membrane antigen-1

Ectodomain



Ka/Ks ratios

- Ratio nonsynonymous / synonymous substitutions



Summary

- Little/no evidence of a geographic effect
- Limited diversity at several anticoccidial vaccine candidate encoding loci
- Weak evidence of balancing selection (non-significant)

Genome



Gene

Conflict: genome vs gene?

- Genome - lifecycles

Parasite - 'hit and run', highly immunogenic, long-lived in the environment

Host - fast turnover, 6-7 weeks common, up to 20 weeks

- Gene - immunity

Parasite - multiple antigens, experimentally many shown to induce immune protection

Host - strong but often incomplete (initially), even against whole parasites

- Potential for genome-wide diversity is high, with antigen-specific balancing selection (immunity) diluted by availability of naïve hosts and the large number of antigens
- Antigen functionality dominates – vaccine prospects **GOOD**

Conclusions

- *Eimeria tenella* genomes are genetically diverse
- Population structure varies by region
- Limited antigenic diversity encourages vaccine development
- Weak signatures of balancing selection
- Strong prospects for anticoccidial vaccine development

Acknowledgements

Royal Veterinary College

- Emily Clark
- Sarah Macdonald
- Matt Nolan
- Fiona Tomley

India

TANUVAS

- V. Thenmozhi
- M. Raman
- G. Dhinakar Raj

IVRI

- K. Kundu
- A. K. Tewari
- R. Garg
- P. S. Banerjee



Sanger Institute

- Adam Reid

Egypt/Libya

Newcastle University

- Abdalqader Moftah

Nigeria

Ahmadu Bello University

- Isa Danladi Jatau



Acknowledgements

Sample donors/project partners...

China

Xun Suo

Ghana

Joseph A. Awuni

India

Arni S. R. Srinivasa Rao
Kumarasamy Thangaraj

Japan

Fumiya Kawahara

Nigeria

Simeon Ayoade
A. O. Adebambo

USA

Thilak Rathinam
H. David Chapman

Uganda

Claire M. Mugasa

Europe

Kimberly M. Fornace
Jonathan Rushton
Patrick Garland

Tanzania

Esron Karimuribo

Venezuela

Ramón Álvarez Zapata

Zambia

Boniface Namangala