Antibody titres in FMD type A strains: comparison of methodologies to predict cross-protection

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Outline

- VP1 gene sequence of all 10 FMDV type A strains
- Difference between serological tests
  - Virus neutralisation test (VNT)
  - Neutralisation index test (NIT)
  - Liquid Phase Blocking ELISA (LPBE)
- Statistical analysis
  - Mean titres
  - r1 values
  - Scaling responses
  - Cluster analysis
  - Principal Component analysis (PCA)

Research goals:

- Comparison of test methodologies

✓ VNT, NIT, and LPBE

✓ Which serological methodology gives the highest confidence?

✓ Does difference between test methods predict significant variation?

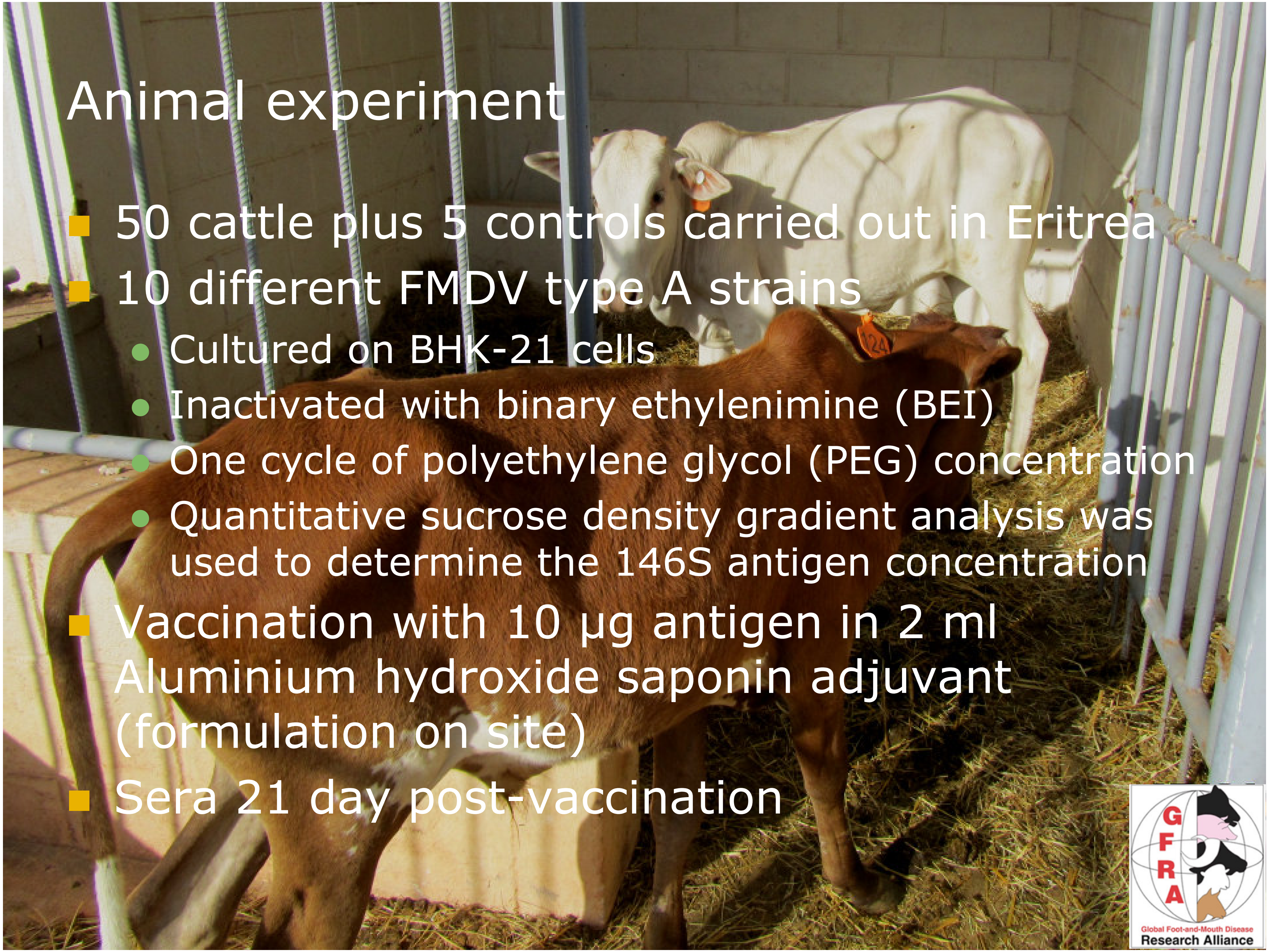
Source of the 10 FMDV Serotype A strains used in the study

WRLFMD, Pirbright, U.K.

- A/ERI/2/98
- A/SUD/2/84
- A/KEN/12/2005
- A/ETH/13/2005
- A/MAU/1/2006

CVI, Lelystad, Netherlands

- A22/IRQ/24/64
- A/TUR/20/2006
- A/TUR/14/98
- A/IRN/02/97
- A10/Holland/42



Animal experiment

- 50 cattle plus 5 controls carried out in Eritrea
- 10 different FMDV type A strains
  - Cultured on BHK-21 cells
  - Inactivated with binary ethylenimine (BEI)
  - One cycle of polyethylene glycol (PEG) concentration
  - Quantitative sucrose density gradient analysis was used to determine the 146S antigen concentration
- Vaccination with 10 µg antigen in 2 ml Aluminium hydroxide saponin adjuvant (formulation on site)
- Sera 21 day post-vaccination

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Serological test methods

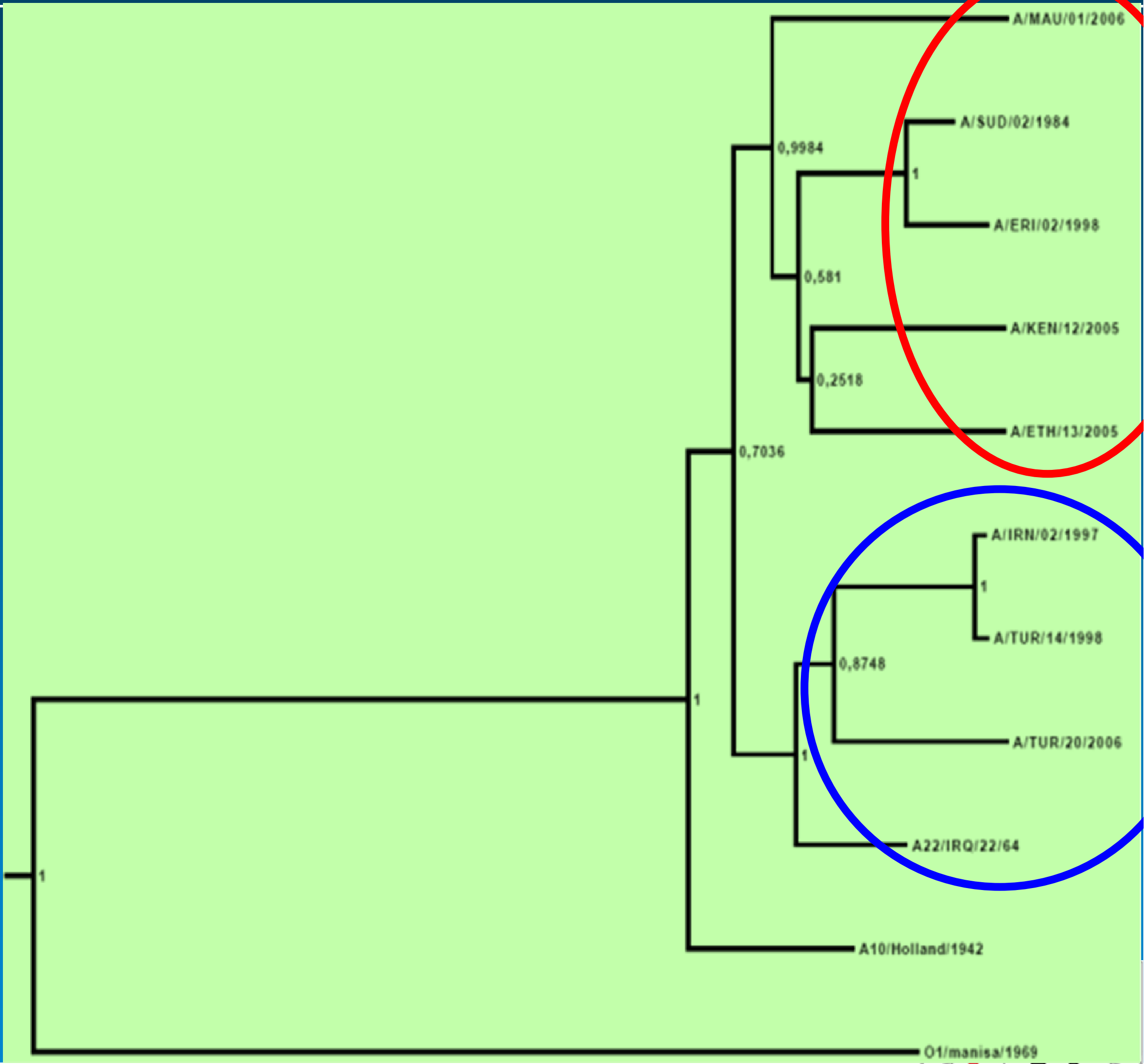
- Virus neutralisation
  - Varying serum dilution- versus fixed virus concentration
- Neutralisation index
  - Fixed serum dilution - versus variable virus concentration
- Liquid Phase Blocking ELISA
  - Using subtype specific Guinea-pig and Rabbit semi-purified Ig fractions (saturated Ammonium Sulphate)

Statistical analysis

- Mean titres
- r1-values
- Mean scaled titre (titre – mean)/sd
- Cluster analysis using Euclidian distance
- Principal component analysis (PCA)

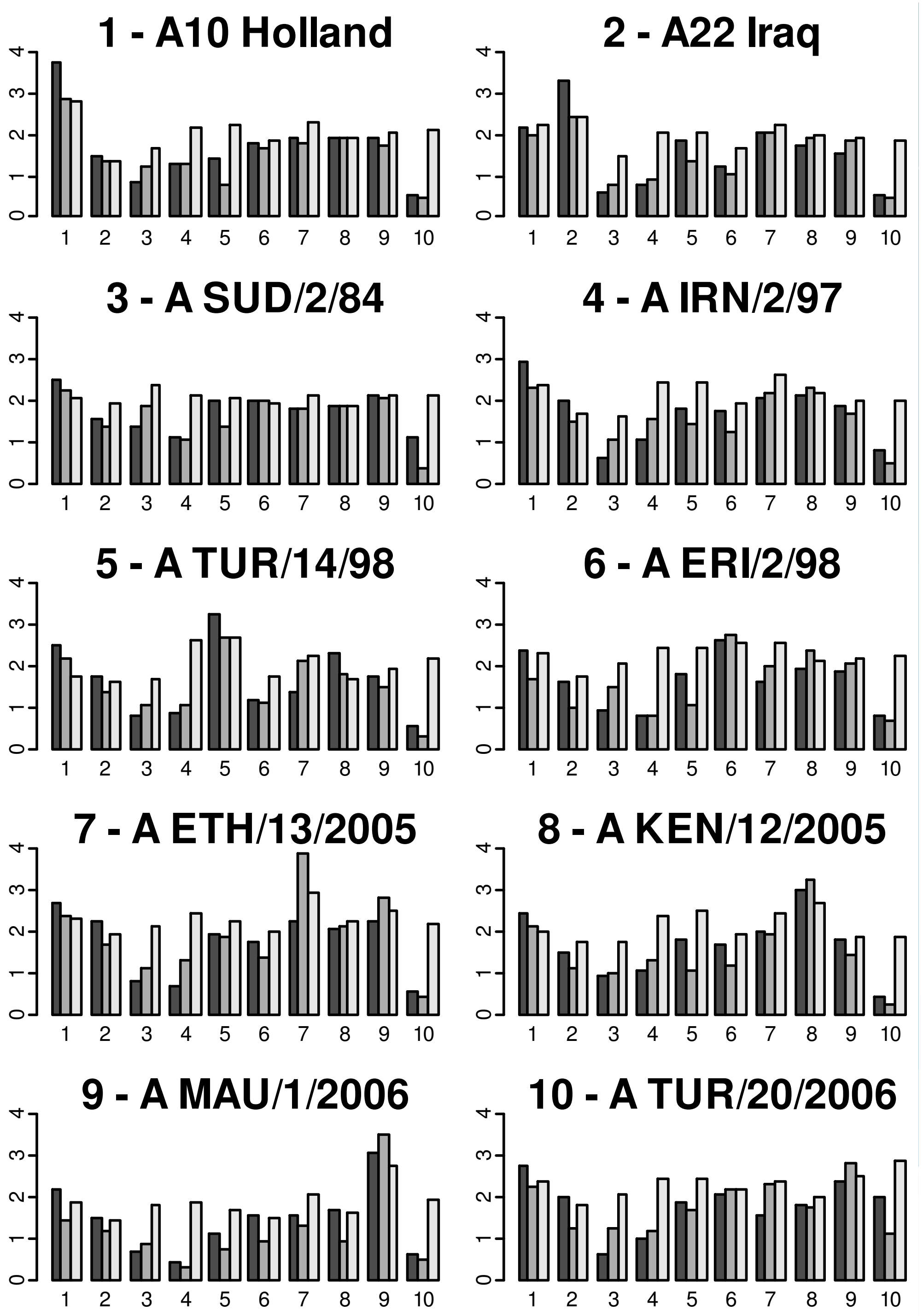
VP1 gene sequence of the 10 FMDV type A strains

- A dendrogram constructed from the VP1 gene of the 10 FMDV serotype A strains using BEAST displaying the posterior value (0 – 1) indicating the confidence of the split. FMDV serotype O1 manisa/1969 was used as an outgroup to construct the rooted tree.



Results of the 3 serological methods

- Huge dataset, difficult to interpret
- On the X-axis the different strains used in the test
- On the Y-axis the ^{10}log mean antibody titre
- For each vaccine barplots of VNT (black), NIT (grey) & LPBE (Light grey) results
- Very similar titres in VNT and NIT
- Mostly homologous titres are the highest

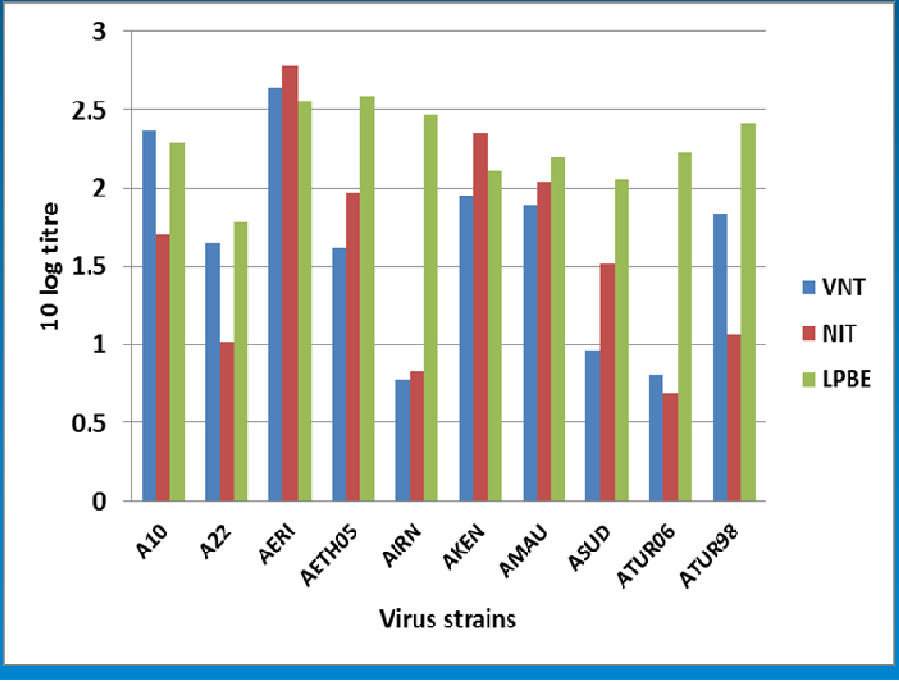


Comparison of vaccine response (African strains)

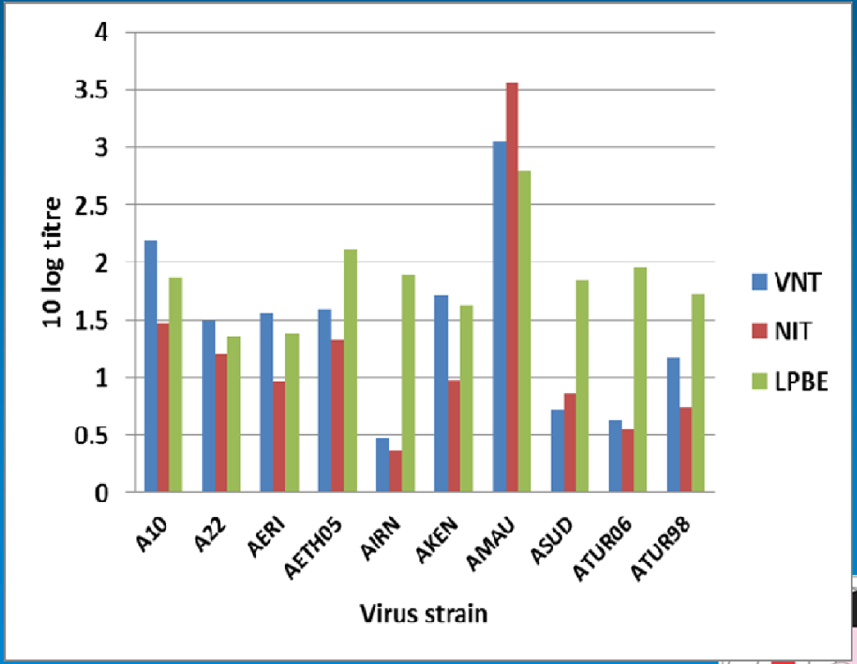
- Mostly homologous titres are the highest

- LPBE has a lower discriminatory capacity for differences between strains, but gives a more consistent result when compared with VN & NI

A/ERI/2/98 Vaccine

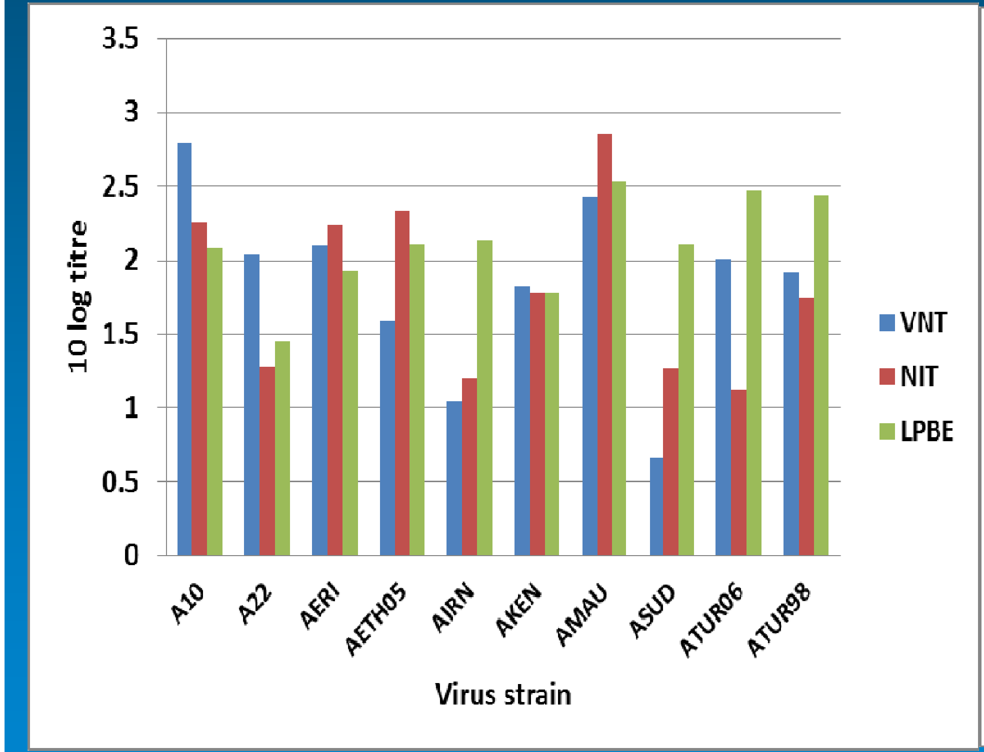


A/MAU/1/2006 Vaccine

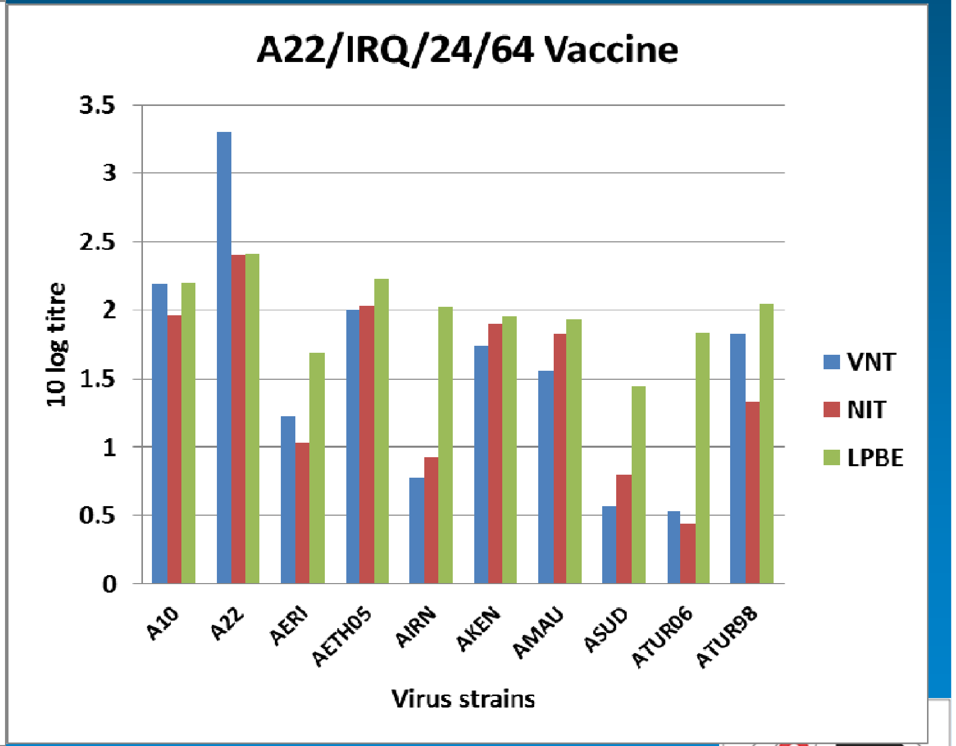


Comparison of vaccine response (Middle East strains)

A/TUR/20/2006 Vaccine



A22/IRQ/24/64 Vaccine



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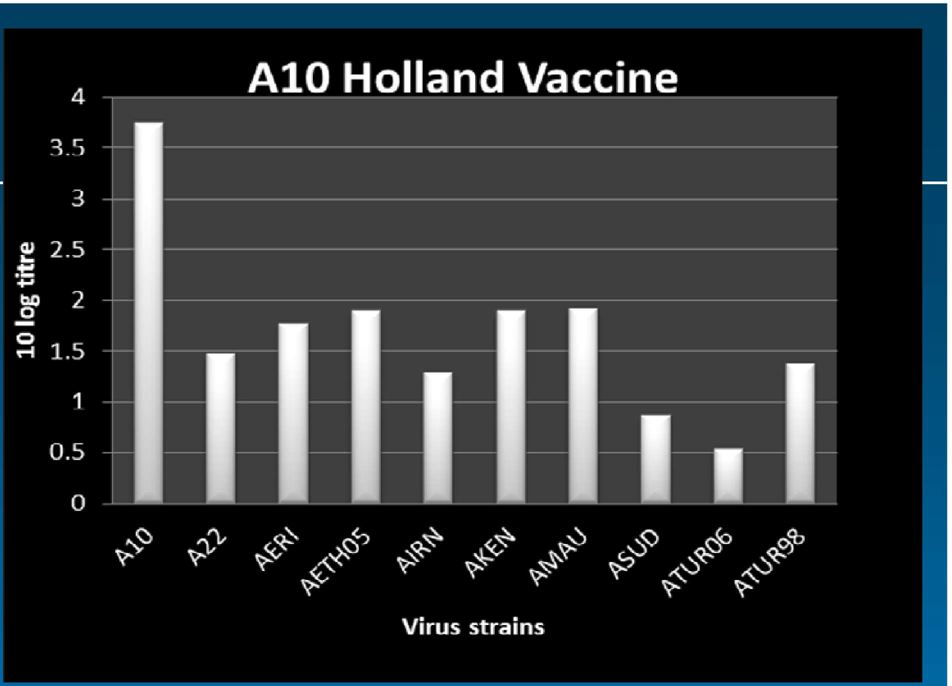
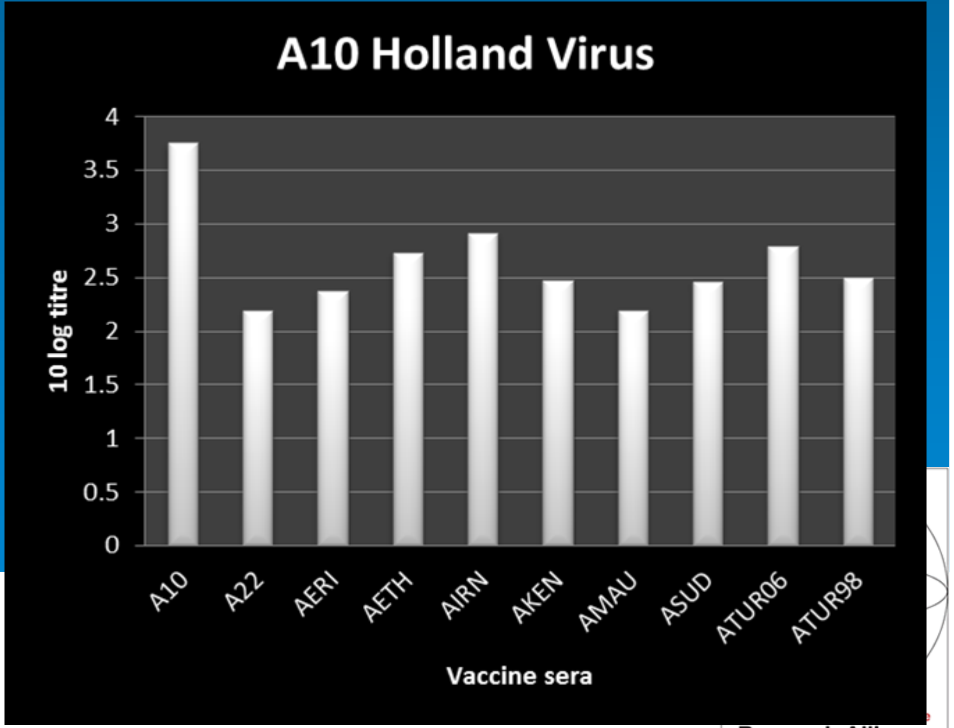
VNT Results

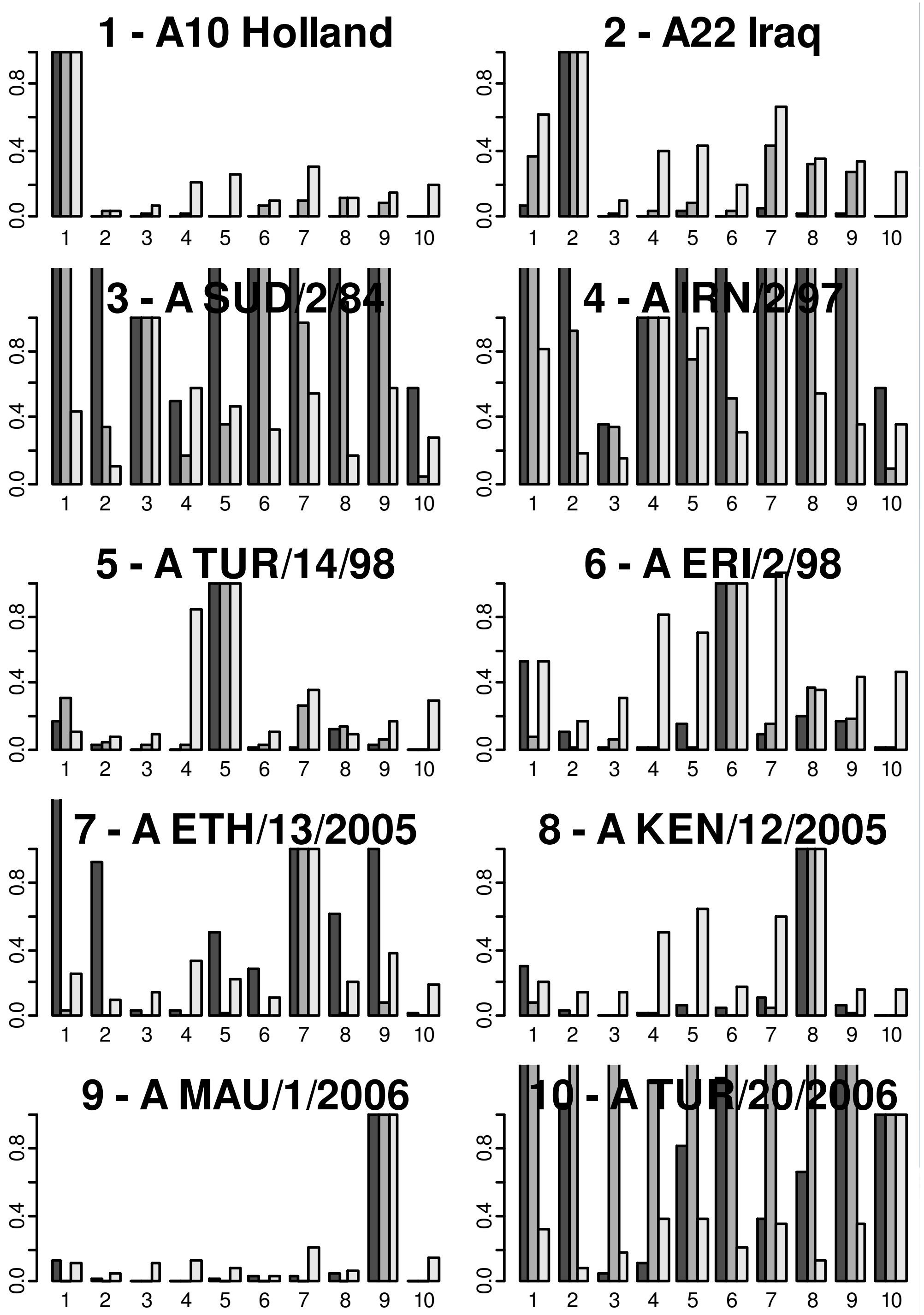
- Difference between antibody response induced by the vaccine compared to the response of the virus to the sera made by vaccination against the other strains
- Low response of vaccine against heterologous strains
- Relatively good response by sera from heterologous strains





r1 Values

- Comparison r1-values VNT (black), NIT (dark grey) and LPBE (light grey)
- Huge difference between neutralising and ELISA antibodies
- A/SUD/2/84, A/IRN/2/97 and A/TUR/20/2006 showed high VNT & NIT r1 values (>1.0)
- Because responses in different tests are not on the same scale



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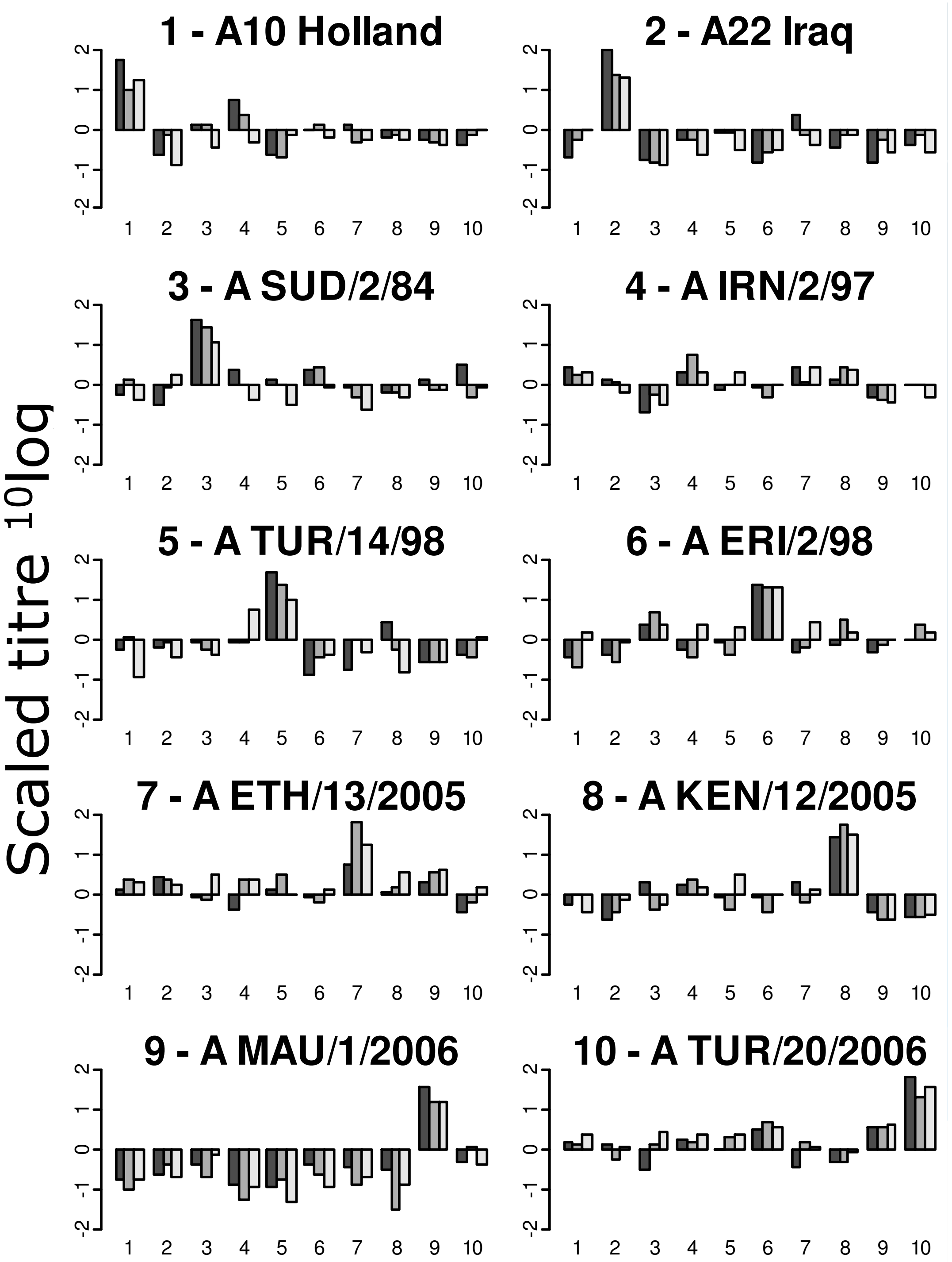
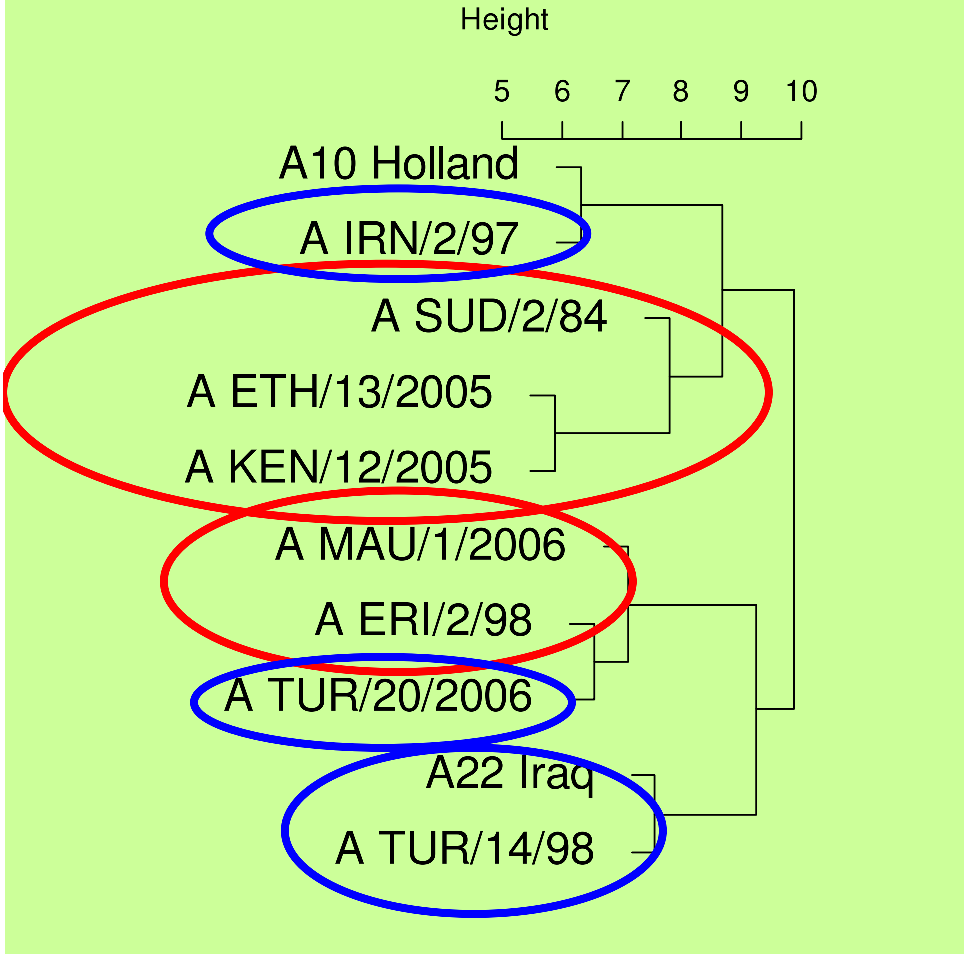
Scaled titres

- A clear difference in the scale of the response for each test strain
- For each test strain (titre-mean titre)/sd
- Comparison scaled titres VNT (black), NIT (dark grey) and LPBE (light grey)
- In all tests all homologous scaled titres are the highest

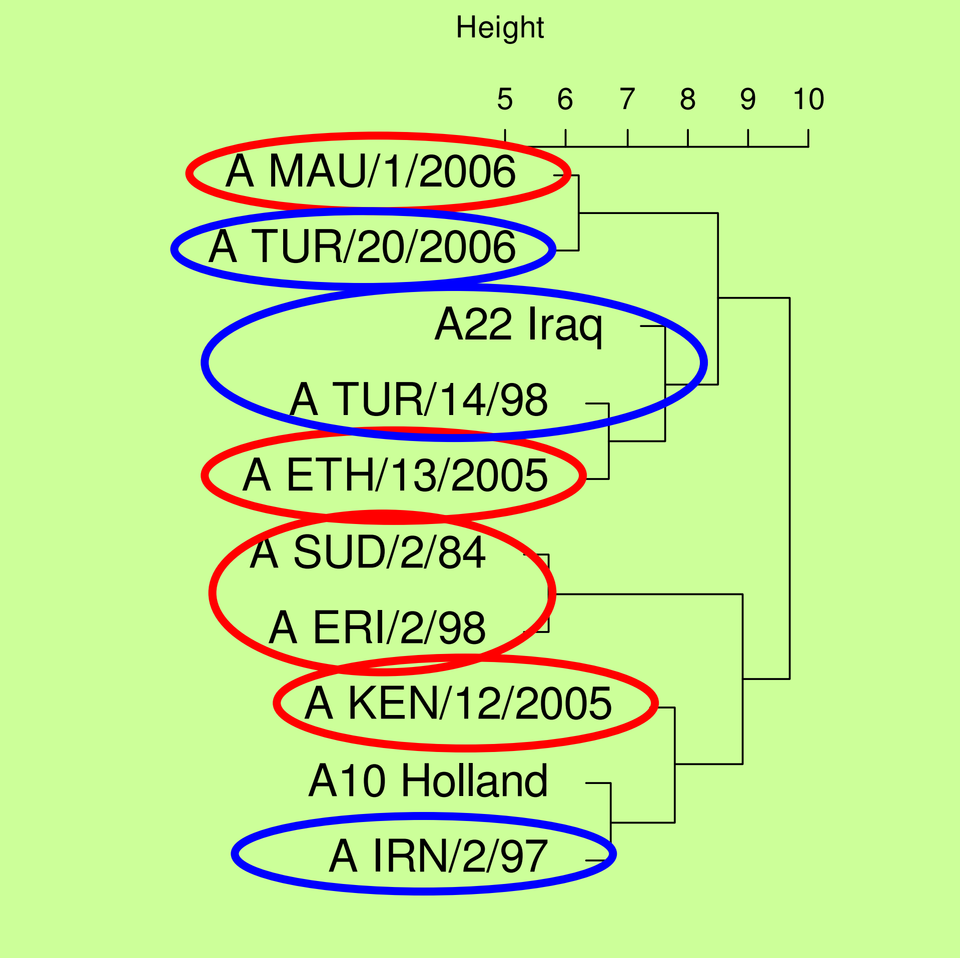


Clusters based on Euclidean distance

Based on scaled VNT titres

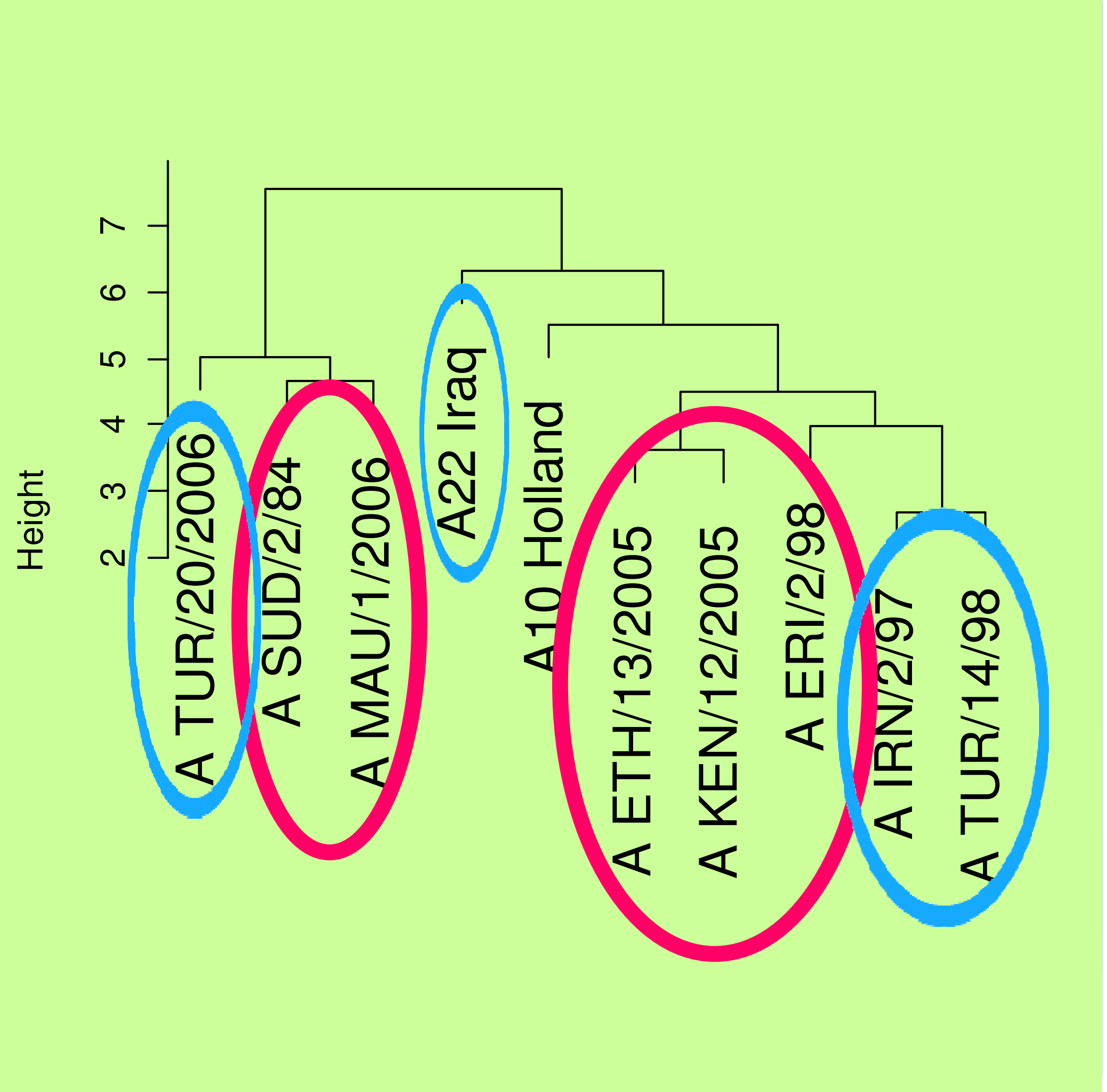


Based on scaled NIT titres



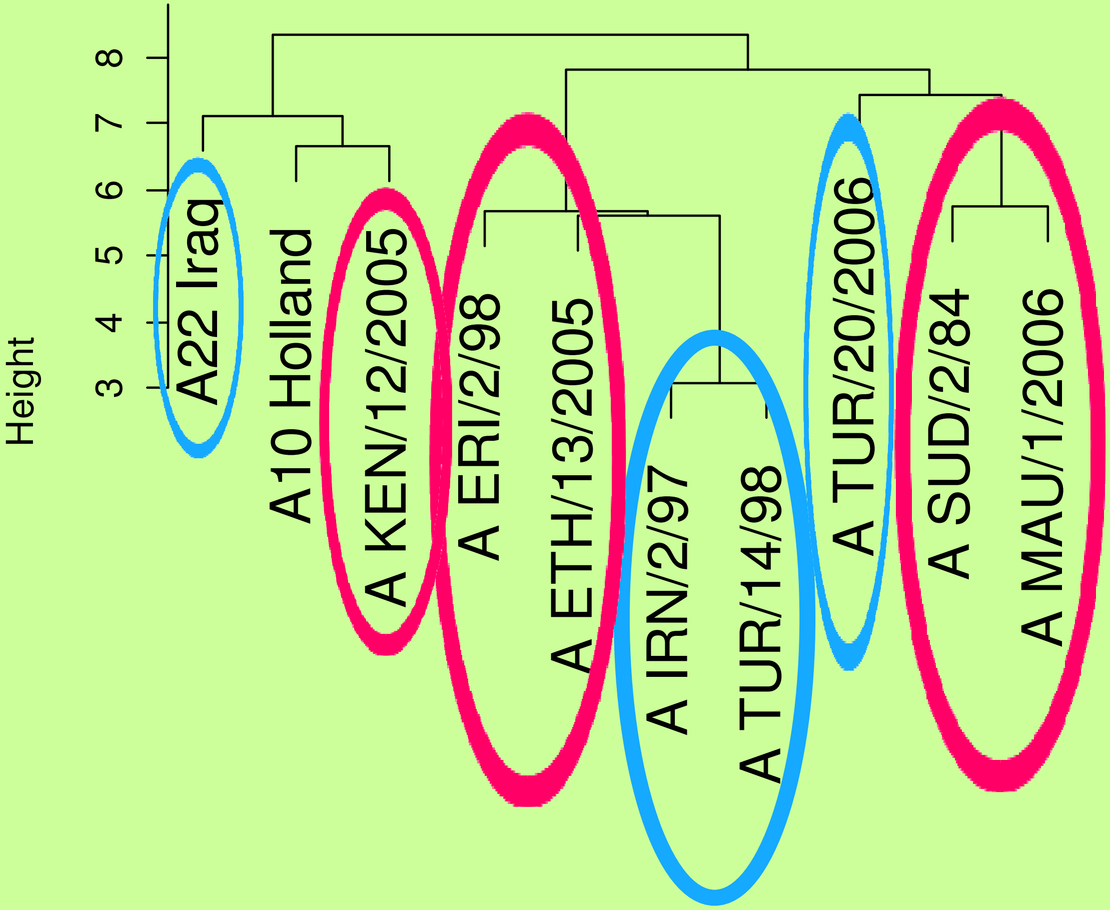
Clusters based on Euclidean distance

- Scaled LPBE titre
- African strains (red circle) cluster more closely than the Asian strains (blue circle)
- Different serological techniques produce different clusters
- Different statistical techniques produce different clusters (not shown)

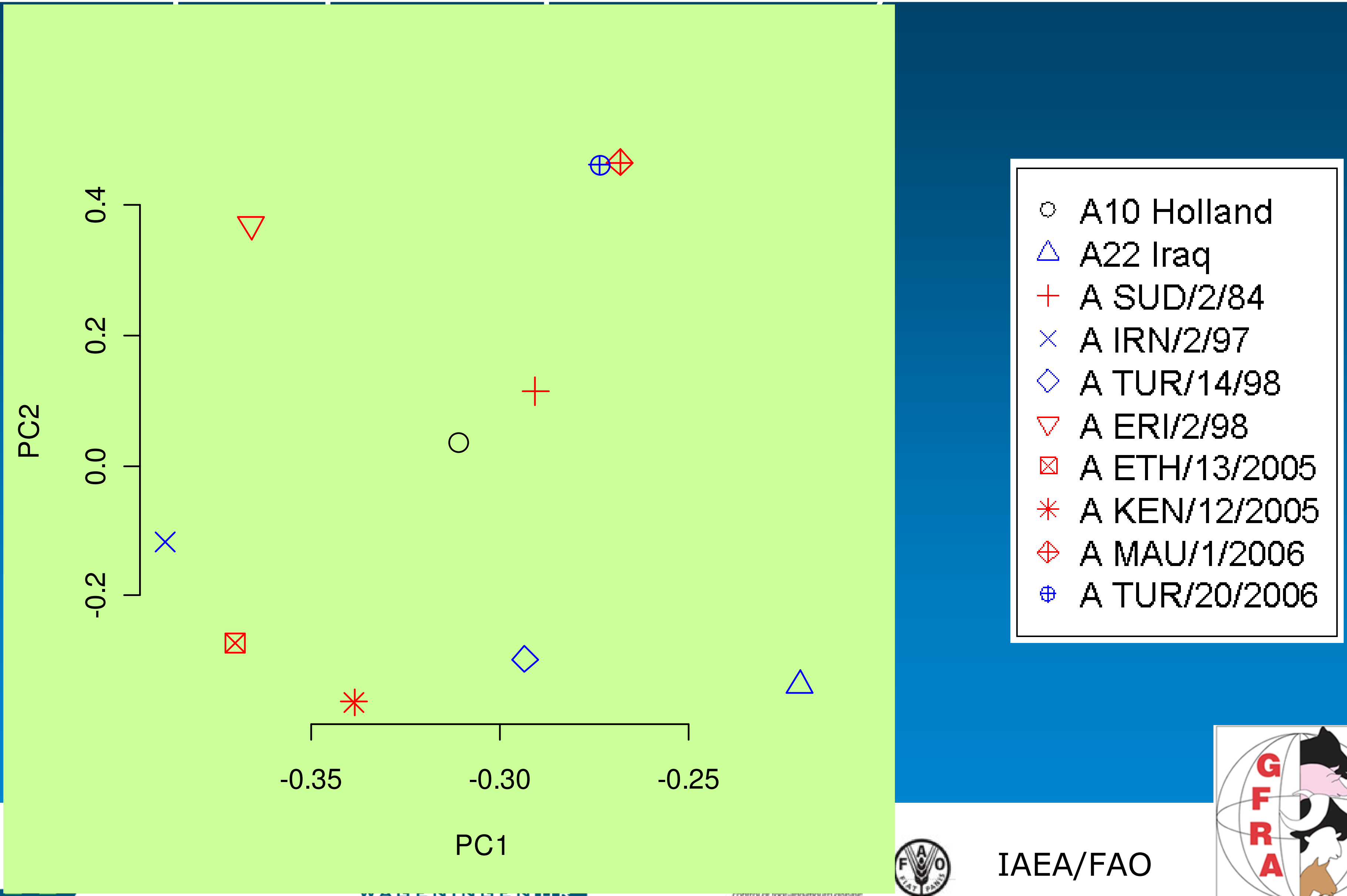


Clusters based on Euclidean distance

- LPBE scaled r1 cluster
- Clustering of the African and Middle East strains in multi groups indicating no antigenic similarity between the strains
- African strains clustered slightly better than the Asian strains



VNT principal component analysis on strains



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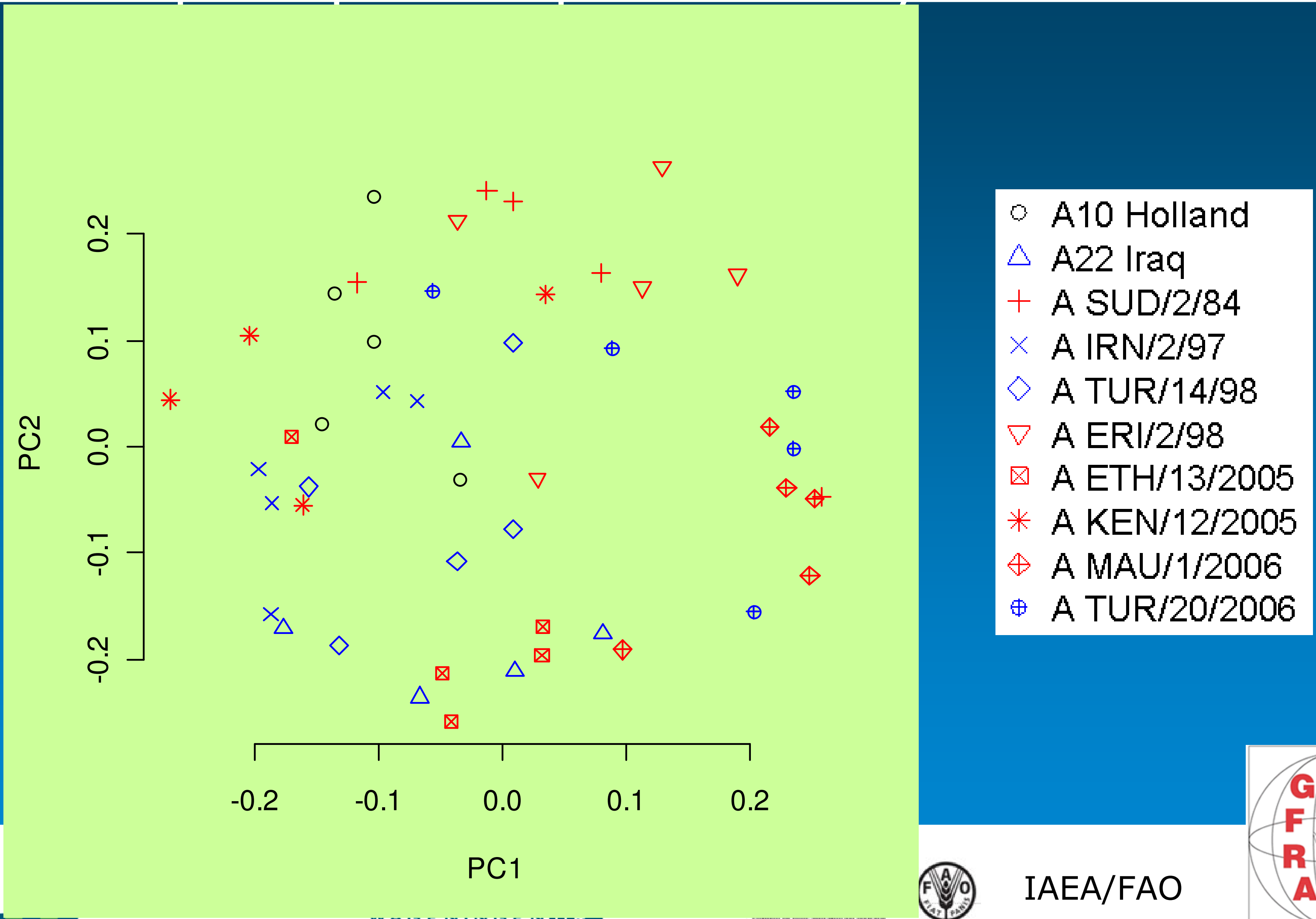
VNT principal component analysis on sera



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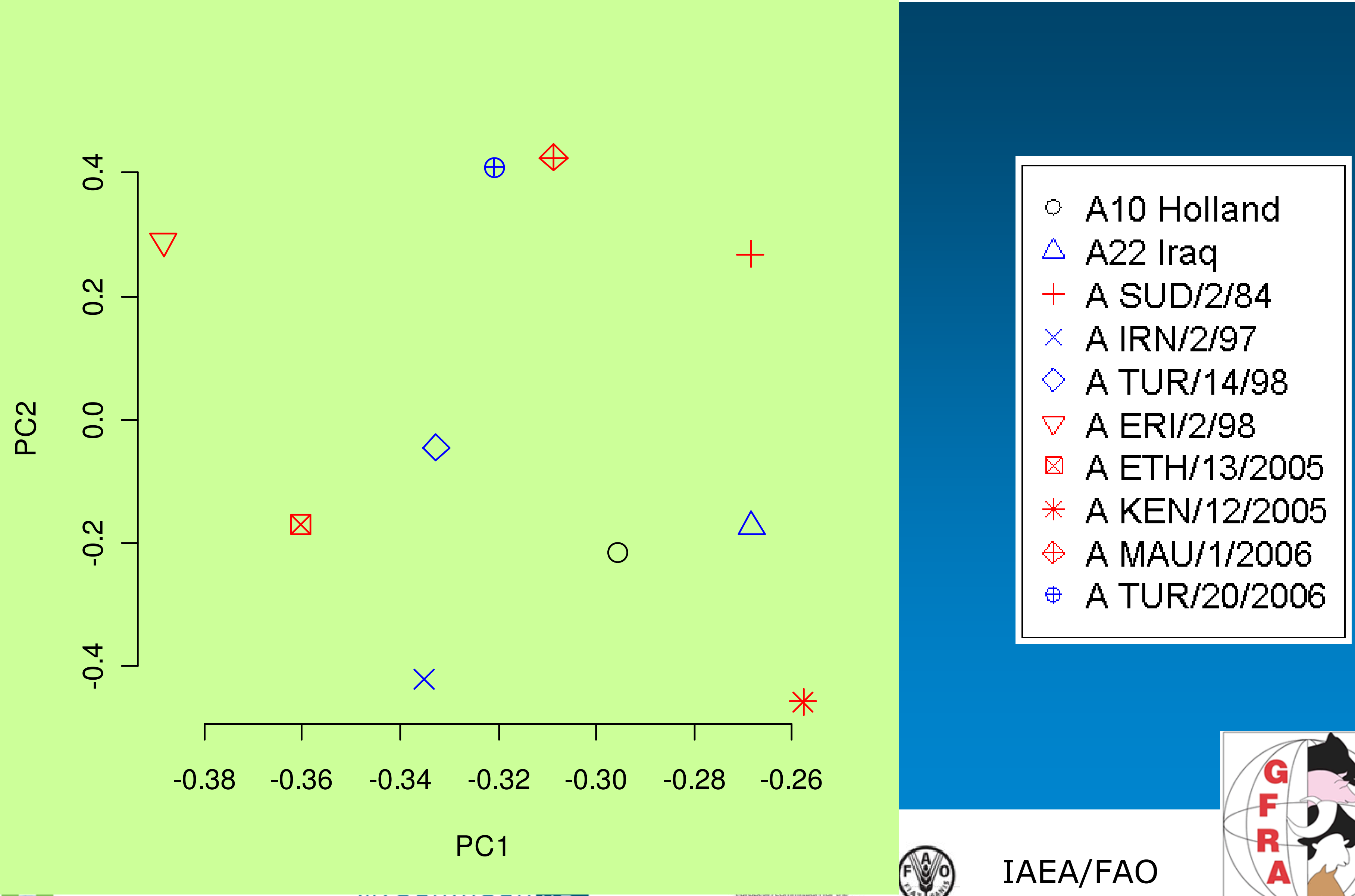
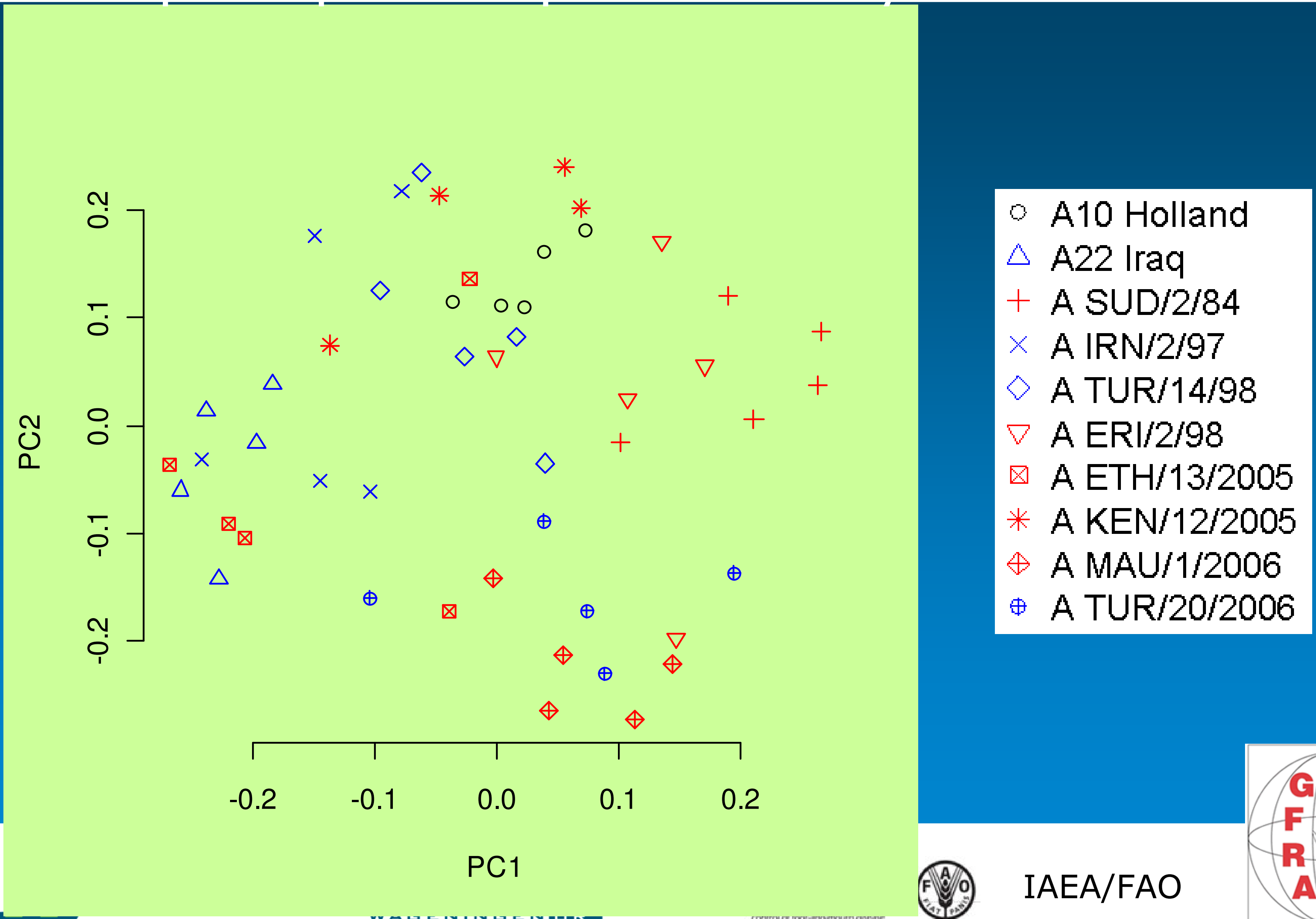
NIT principal component analysis on strains



NIT principal component analysis on sera



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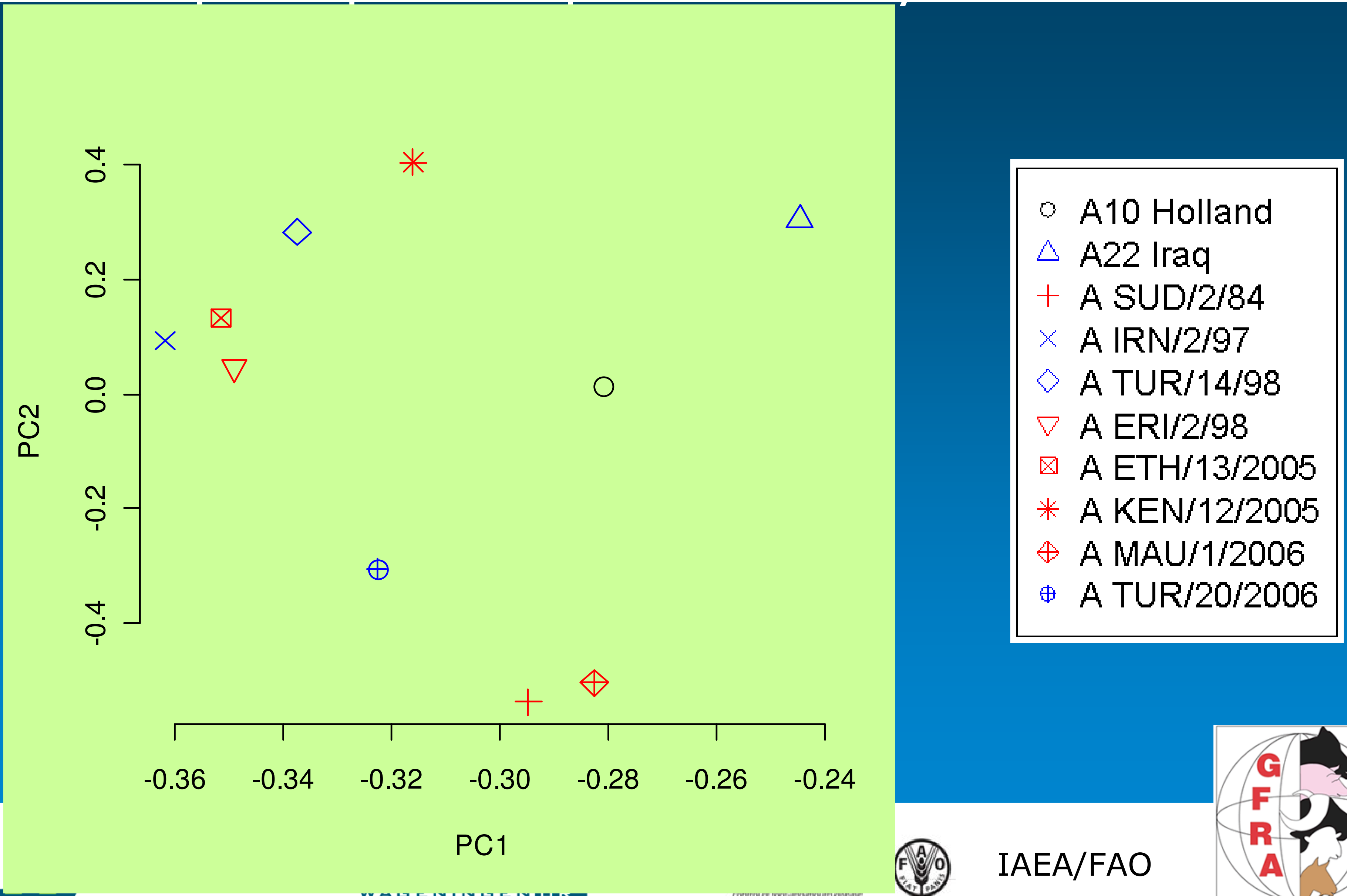
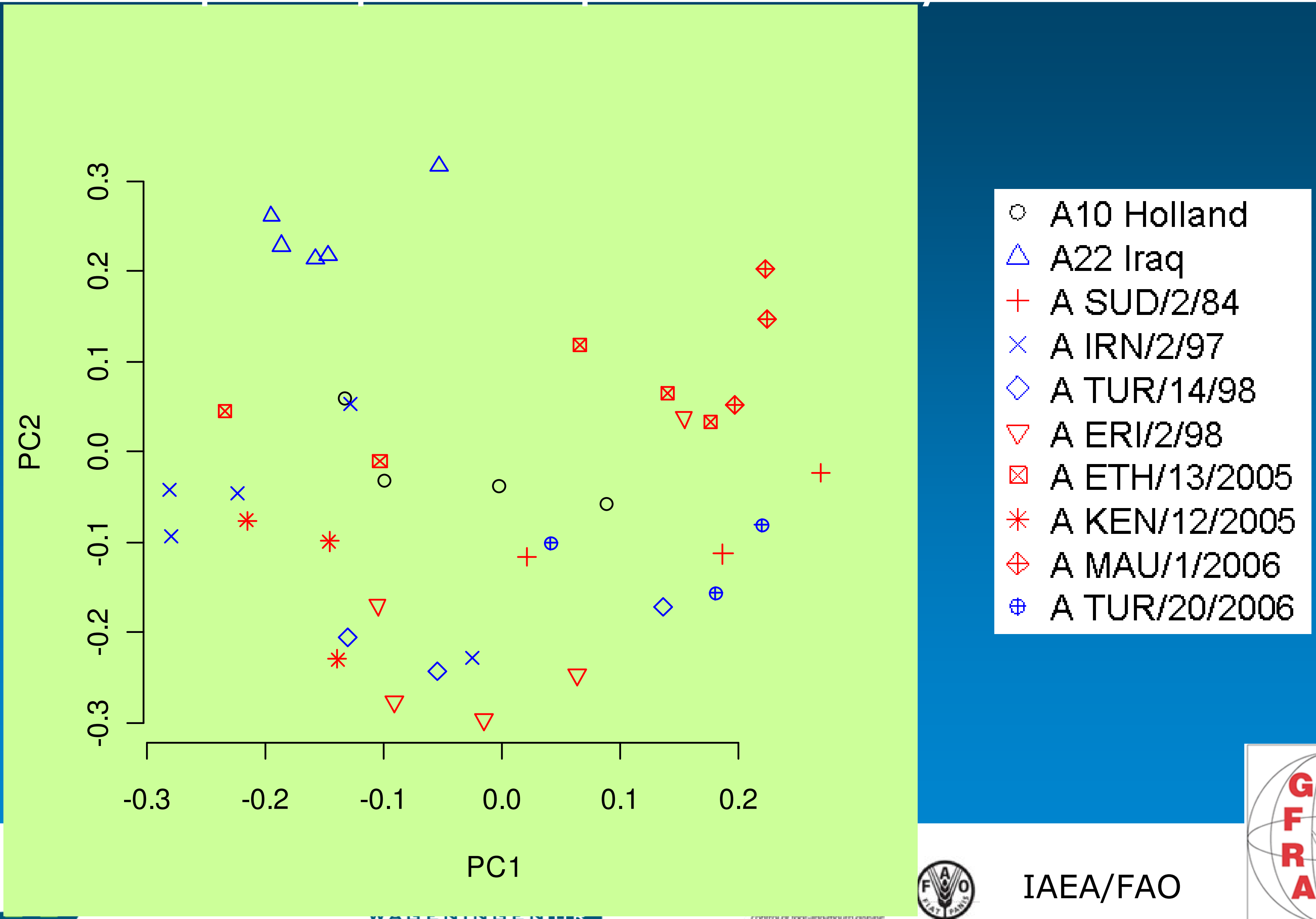
LPBE principal component analysis on strains



LPBE principal component analysis on sera



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Conclusion

- Neutralisation tests, VNT and NIT, have a higher specificity than LPBE
- Therefore neutralisation tests have a better discrimination capacity of strains
- r1 values are difficult to interpret as values greater than 1 are observed
- Scaled titres can reduce variation between tests
- Cluster analysis does not produce consistent results and not comparable with genetic information
- PCA showed high variations on sera and strains
- Antigenic evolution was found to be more punctuated than genetic evolution, as the change of virion proteins in response to antibody selection is more frequent.

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