INTERNATIONAL SOCIETY FOR ANIMAL GENETICS

ISAG Conference 2006, Porto Seguro, Brazil

Applied Genetics in Companion Animals Standing Committee Workshop

The workshop on Applied Genetics in Companion Animals met on Sunday, August 20, 2006 from 13:30 – 15:30 in Room 2 at the Convention Center in Porto Seguro, Brazil, as part of the ISAG 2006 biennial meeting.

Standing Committee:

Present

- H. Van Heringen (Co-chair)
- Alan Guthrie (Comparison Test Duty Lab)
- Leslie Lyons (co-chair)
- Alison Ruhe
- W. van Haeringen (Comparison Test Analysis Lab)

Absent

- Andreas Helman

Approximately 80 ISAG members were in attendance and signed a participant's roster.

Agenda

Welcome - chair

I. Comparison Test Reports
   - Duty Laboratory Report – Cindy Harper (South Africa)
   - Analysis Laboratory (Report) Wim van Haeringen (The Netherlands)

II. Report by Allison Ruhe on additional canine markers developed by UC Davis.

III. Report by Leslie Lyons on allele sequencing of panel markers for the cat.

IV. Discussion (chaired by L. Lyons)
   - ISAG Panel
     - Do they work well?
     - How many labs using these panels routinely?
     - Do we need changes?
     - Standards / Reference samples
   - Nomenclature
     - Keep the same of switch to alphabet or repeat-based models
   - Next Comparison Test
     - Should one be performed?
     - Selection of duty and computer laboratories

V. Cat Phenotypic and Heath Information Registry

VI. Any other business

VII. Election for new committee

VIII. Closing Remarks

Participants were welcomed by the chair and the agenda items immediately proceeded with the reports of the cat and dog comparison tests.

I. Comparison Tests

Duty Laboratory Report: (C. Harper)

- Onderstepoort VGL South Africa - duty lab
- Sent out 21 samples of various breeds and DNA from an ATCC cell line for canine and for feline as a standardized reference samples.

- 41 laboratories participated
- 19 requested both feline and canine samples
- 1 requested only feline samples
- 26 labs reported canine results
- 11 labs reported feline results

A few minor problems for shipment were encountered including:

- Tubes that did not seal caused some samples to evaporate
    - Samples were re-sent to labs with this problem
- Shipment problems
- Questions regarding data format for submission
    - In the future, should the duty lab not be data lab as well?

Reference data was provided for different samples at different loci to include the range of alleles.

- In the future, is it preferred to provide data on the single reference sample?

Computer Laboratory Report: (L. van de Goor, W. van Haeringen)

Once a list of participants was received, instructions for reporting results were sent to participants, including an Excel file and a Word file for comments. The results were compiled and sent out to *reporting* groups. Once comments were requested, a finalized version was returned.

For the dog, 26 reports were received from 14 countries. For cat, 11 reports were received from 9 countries.

For the dog comparison test, several labs have changed to recommended markers from ISAG2004. No comments received from labs after reporting final results. There were nomenclature issues for several markers:

AHT130 or AHTh130
AHT260 or AHTh260
C22.279 or CXX279

Many other comments were submitted. Other issues included,

Quote: 'AHTk211 produced inconsistent results'
Marker 'characteristics'
Reference genotypes sometimes different
Split peaks – 1bp

Genotype database (Italy)

For the cat, the set of recommended markers is overall consistent and worked well. Other comments included:

- Comments about base pair values of reference sample (non corresponding size differences between alleles),
- Other multiplexes have been designed,
- Less than 25 ul DNA received,
- Wrong sample received (Sample 20 Canine instead of Feline),
- Quote: 'some isolated alleles were found'.

Overall comparison test comments:

- Nomenclature issues about alleles:
  - Several labs perform well, but…
  - Homozygous allele calls: 148/ or 148/148.
  - Much confusion about reporting 'complications'
    - How to report a marker that could not be scored?
    - We have received notifications in blue, yellow, bold, italics…
    - Should we discuss the differences in detail? Guidelines?
- What are the correct genotypes?
- Requests from several labs to extend the deadline of reporting,
- Sex reported by some labs – difference(s) between labs,
- Several new labs without ISAG codes.
- Several aspects can be improved.

Dog comparison test summary:

The dog test pre-Tokyo 2004 (as given on the ISAG website) included 23 markers, six of which were licensed and several others "FH" markers were difficult to genotype. Following the Tokyo 2004 meeting, 25 additional markers were tested by the VGL at UC Davis as a "mini-test" for consideration in the 2006 comparison test. The markers listed in the table below were the finalized list that were included in the 2006 comparison test.

A few issues that make data analyses difficult and need to be rectified included:

- Data not standardized (ND, N/D, n.r., n.d. and blank cells – 118/120, 118-120, 118 / 120)
- Data tables not standardized e.g. loci names different (REN105103 and REN105L03)

No data analyses were provided by the computer laboratory for these reasons. However, there appeared to be consensus for the markers that were tested but an "official" panel should be clearly defined.

Table 1. Markers for the ISAG 2006 Dog Parentage Panel Comparison Test

SA-1	SA-2	SA-3	SA-4
(FH2001)	INU030	INU005	AHT121
REN54P11	INU055	AHTh130	INRA21
AHTk253	REN105L03	REN64E19	REN169018
(FH2328)	AHTh137	REN162C04	
CXX279	FH2848	AHTk211	
FH2054	REN247M23		
AHTh171	LEI004		
	AMEL		
	REN169D01		
	AHTh260		

Cat comparison test summary (Detailed data analysis summary attached):

The same markers as the unofficial cat comparison test from Tokyo 2004 were distributed. Initial reference results sent with the samples, however, following feedback and suggestions, updated reference results were sent in February 2006. FCA649 did not amplify for the duty lab, thus, no reference results for provided this marker.

No analyses were provided by the computer laboratory for the reasons listed above. Marker FCA678 on sample FeWCT21 genotyped as 194/194 by the duty laboratory, other reports indicated a 230 allele (null allele) as well (UC Davis showed this amplified using high concentration of primer).

The same markers as in Tokyo 2004 were suggested as the core panel.

General comparison test comments:

- Appears to be no real standardization to date, hence, should the ATCC reference should be implemented?
- Allele calling rules also changed between previous CT's on same markers, thus the reference sample and an allelic ladder would resolve this problem.
- Are there better markers following completion of canine genome and advances on feline genome? (See presentation by Alison Ruhe, UC Davis)
- Should the test(s) be changed again?
    - Can the number of markers in the test be reduced? The dog test is 24 markers plus + AMEL, but these are likely needed for the diverse breeds. The cat test is already low.
- Can the data as it is formatted now be used to make informed decisions about the test?

II. Report by Alison Ruhe on additional canine markers developed by UC Davis.

The canine research team at UC Davis has identified, genotyped and is in the process of mapping several thousand perfect repeat microsatellites. These markers have been evaluated for

efficient genotyping and will be listed on the VGL web site. Many indels have been identified and genotyped as well. Markers could be selected from this pool if required for the canine parentage panel if replacement markers are required.

III. Report by Leslie Lyons on allele sequencing of panel markers for the cat.

Data was presented for the feline ATCC reference sample and sequencing of at least one common allele in three diverse cats for each of the "core" markers. This information is provided in Table 2 of the detailed cat report.

IV. Discussion (chaired by L. Lyons)

A very lively discussion ensued regarding the use of di- versus tetranucleotide repeats, the standardization with forensics and the use of alphabetical or repeat length based nomenclature. In addition, the comments presented above by the duty and computer laboratories were addressed.

Adopted actions:

Dog Comparison Test

The decision on the "core panel" for the dog parentage test was deferred to a decision by the standing committee. Repeat lengths and types are not yet available for all markers, hence this information should be considered. It is likely that the dog will need a core of approximately 20 markers, for now, the 21 markers and AMEL will be adopted, but be reviewed for their repeat type within 30 days by reviewing Genbank and published data. Complex repeats will be dropped and replacement markers will be suggested from other markers that have been analyzed by the UC Davis VGL. Others suggestions for markers will be solicited and considered. UC Davis will publish the list of dog marker possibilities along with the comparison test report on their website.

The repeat-based nomenclature can not be adopted in the dog unless a variety of alleles are sequenced in different breeds for each marker. The VGL in South Africa will sequence the alleles of the ATCC reference sample and expand to common alleles for each marker. This work will be in conjunction and coordinated with efforts by the Van Haeringen laboratory.

Cat Comparison Test

1. The cat parentage core panel may consider removing FCA310 since it is a complex repeat and is not amenable to standardized nomenclature based on repeat length, however, this marker performs very well in the test and could still be used. Both of the X-linked markers can be replaced with more the efficient markers, AMEL and ZFXY, since these markers produce both an X and Y-based fragment. These markers and primers are published and the gender based markers are currently incorporated into the cat panel performed at the VGL at UC Davis.

2. Since the repeat length and type of each marker has been determined for at least one common allele for each marker in the cat and since the reference values from the ATCC cell line were available, a table with the cat instrument size of the allele, the sequenced size of the allele, and the appropriate repeat-based and alphabetical nomenclature will be published with the comparison test report.

3. The data for the next cat comparison test will be presented in both nomenclature formats to determine effectiveness and ease of transition to a repeat based nomenclature.

4. The allele frequency, allelic range and breed data for these markers needs to be published by the Lyons laboratory.

5. The next cat comparison test may want to consider the addition of any other suggested markers and /or diagnostic markers. Agouti, albino, dilution, and blood type are sized-based variants and could be incorporated into an ABI type scoring system.

General Comparison Test Recommendations:

Comparison test procedures are not currently sufficiently detailed to allow an efficient distribution or reporting of information. Thus, the standing committee has been charged with drafting SOPs that would pertain to companion animals but could be adoptable by all comparison test committees.

In addition, many of the data information issues could be resolved by the development of a website for comparison test details, contacts, guidelines and data submission. Thus, the committee strongly recommends that ISAG consider the addition of appropriate pages on the ISAG web site.

UC Davis will re-establish their website for the presentation of the cat and dog comparison tests reports and the associated information that is mentioned above. This will be a protected site, only accessible by ISAG members and the site will have a link to the ISAG main webpage.

Although, standardization with forensic DNA marker panels is appreciated, the participating laboratories have very limited forensic services and a switch to different panels would require a significant financial and labor investment for parentage testing laboratories, thus, the forensic's community is encouraged to consider the ISAG panels as the databases are significantly larger, worldwide and more comprehensive at this time. In addition, the cat and dog forensic panel datasets are not freely available and may never be, however, the difficulty with dinucleotide repeat stutter bands in court interpretations is appreciated.

V. Cat Phenotypic and Heath Information Registry

Due to a lack of time, this information will be posted on the UC Davis web site with the comparison test information.

VI. New / other business

None

VII. Elections:

Dr. Van Haeringen stepped down as chair due to other ISAG committee responsibilities. For the 2008 comparison tests, Dr. Perrota volunteered to be the dog duty and computer laboratory, Dr. Lyons volunteered for as the cat duty and computer laboratory. Dr. Helman was removed from the committee due to lack of interest. The committee chairs were selected by the committee.

New Standing Committee:

Cindy Harper (Co-chair) – University of Pretoria, Onderstepoort, South Africa
Nigel Holmes - Animal Health Trust, New Market, UK
Leslie Lyons (Co-chair, cat duty and analysis lab) – UC Davis, USA
Alison Ruhe – UC Davis, USA
Giovana Perrota (Dog duty and analysis lab) – Laboratori Genetica e Servizi, Cremona, Italy

Timeline for next comparison test:

September 30, 2006
- submit reports to ISAG Executive Committee
- establish cat and dog comparison test report website and link to ISAG
- evaluate microsatellite repeats within the dog for each of the 21 markers
- suggest deletions and additions to the dog core panel and present with the report and on the web page.

November 30, 2006	allele sequencing evaluations for reference sample in dog and cat
March 31, 2007	allele sequencing evaluations for core markers – dog
September 01, 2007	Call for participants in the comparison tests dog and cat
October 01, 2007	Distribution of DNA dog and cat
June 01, 2008	Close of website and form data submission dog and cat
July 01, 2008	Return of the report to the participants dog and cat
July 20 – 24, 2008	ISAG 2008