Laboratory proficiency testing for Rabies: an example of diagnostic support to national veterinary laboratories.

Claude Sabetta, PhD
Presentation ....

- Background & objectives
- Participating laboratories
- Composition of the panel
- Preparation of samples, validation of transportation and courier
- Results and discussion
- Recommendations and way forward
Follow up to the SEARG 2008 conference

• To conduct a theoretical and practical training on rabies diagnostics:
  – enhance skills and knowledge on current diagnostic tests and techniques available for use in the region,
• Identify and invite diagnosticians involved in rabies diagnosis in the SADC countries,
• Organise and coordinate logistical arrangements as well as identify a training coordinator,
• Prepare a draft report to be submitted to the ARCan and SEARG contact point,
Participants to the workshop

- 14 participants from all SADC countries (except Mauritius)
Course objectives ....

• Theory:
  – Describe the basic properties of the rabies virus, its transmission and disease course in Africa.
  – Recommend safe practices for those working in rabies diagnosis or shipping laboratory specimens.
  – Summarise quality control and quality assurance procedures for the rabies diagnostic laboratory.
  – Understand the concept of one health and positioning of rabies as a neglected zoonosis.

• Discussions:
  – Assess the role of the rabies laboratory in terms of diagnostic capability and the interpretation of laboratory results.
  – Review the various surveillance tools (antigenic typing, phylogenetic analysis and general case surveillance data) in rabies control (T & D).

• Practical:
  – Identify and prepare appropriate specimens for rabies diagnosis.
  – Demonstrating proficiency in observing fluorescent antibody test slides, detecting virus antigen when present and correctly interpreting difficult test results.
  – Understand the role of dog ecology in the context of rabies control.
Proficiency evaluation .....

- Participants split into 3 groups
  - Reading prepared slides (A)
  - Staining own samples (B)
  - Performing a rapid assay on samples brought from own lab (Moz, Na, Sz) (C)
- Comprehension of the FAT and reading of slides good
- Some participants did not obtain expected results [4A, 3B].
Proficiency evaluation …..

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What did we learn from the training?

- The theory and hands-on training workshop held in July 2009 was successful.
- Specific areas of further training were highlighted (above) and should be pursued in 2011.
- The OVI:
  - should co-ordinate an annual proficiency test for all the SADC member countries.
  - should supply key biologicals such as the conjugate for all the SADC member countries. The OIE sub-regional office should consider such a proposal.
- Individual laboratories within the SADC should be evaluated for their capability to perform these tests (human, infrastructure etc).
- Training should be expanded to include other non-SADC countries.
The first step was to harmonise the FAT protocol.

- Standardisation of diagnostic protocols in veterinary and animal laboratories
  - Ease of assessing competency of personnel involved in rabies diagnosis
  - Reliable surveillance data
The process of harmonisation of the FAT protocol.

- **Venue**: Onderstepoort, South Africa
  - Dr Sabeta (OIE Rabies Reference Lab, Onderstepoort, South Africa)
  - Dr Siegfried Khaiseb (CVL, Parasitology & Rabies, Namibia)
  - Dr Chanasa Ngeleja-Mpelumbe (CVL, Virology, Tanzania)
  - Dr Wonderful Shumba (OIE Rabies Reference Lab, Onderstepoort, South Africa)
The FAT test

• Gold standard for diagnosing rabies in brain tissues
• Recommended by both the World Health Organisation (WHO) and the World Animal Organisation for Health (OIE)
  – Fast (results obtained in <3 hrs)
  – Comparatively inexpensive
  – Accurate (can detect 97-99% positive specimens)
SOPs from 10 countries utilised …

- Botswana
- DRC
- Malawi
- Mozambique
- Namibia
- Onderstepoort (RSA)
- Swaziland
- Tanzania
- Zambia
- Zimbabwe
Safety considerations .......

- Personnel must be trained, competent and comply with biocontainment and biosafety regulations.
- Pre-exposure immunisation (inactivated vaccines)
  - Serological monitoring every 6 months (0.5IU/ml)
  - Vaccination with regular boosters
Staining of brain smears ..... 

- Polyclonal conjugate
  (Sanofi, Centocor, Chemicon, Onderstepoort)
- Incubate at 37°C for 1 hr (2)
- Apply conjugate (Evans Blue, 20% of the labs) and incubate at 37°C for 30 min (25-35) humidified chamber (8)
• Mounting fluid (50-90% glycerol) (4), Not defined (4)
• 70% glycerol, pH 8.76
  – [false negative results observed in pH range 7-7.5, Nanses].
• 1% glycerol [1].
• Two readers [1] and provide quantitative grades (intensity of fluorescence and distribution of antigen)
Quality control issues

• Quality of all reagents (acetone, conjugate, washing buffers) must be optimal [storage, pH].
• Routine use of pos and neg controls (use field/lab strains)
• Fluorescence microscope working properly, pH meter, Biological safety cabinet
• Conjugate must be broad spectrum
  – Concentrate dilutions must be mixed with glycerol, stored in aliquots.
  – Determine optimal working dilution of new batch of conjugate
• Fixed pos and neg controls stored at -80°C for 6 months.
• Staining – start with pos control and end with neg
Then what after the harmonisation of the protocol?.....

• **The next steps:**
  - Ensure protocol is adhered to,
  - Provide equipment and infrastructure to all member country laboratories,
  - Good and high quality biologicals,
  - Improve (training workshops) and maintain competency of rabies diagnosticians through external quality control (proficiency tests).
Preparation of the PT exercise - Kopanong meeting ….

- LOA between the ARC and the OIE signed (R75 000 provided by the OIE)
- Preparation of documents
  - acknowledgement forms
  - report forms
  - send calls for participation
  - request for import permits
- Procurement of mice, materials for courier of samples and other biologicals
- Selection and preparation of samples for the panel, test stability of samples
  - Courier panel of samples and biological conjugate to national laboratories
  - Labs to inform Onderstepoort (receipt of samples)
- Results to be submitted to Onderstepoort by end of June
- Analysis and report – Mid July
- Communicate to Heads of Laboratories, Chair SADC lab sub-committee, funders (OIE and FAO)
## Participating countries...

<table>
<thead>
<tr>
<th>Country</th>
<th>Code</th>
<th>Participated</th>
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<tbody>
<tr>
<td>Angola</td>
<td>L01</td>
<td>N</td>
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<tr>
<td>Botswana</td>
<td>L02</td>
<td>Y</td>
</tr>
<tr>
<td>Democratic Republic of Congo</td>
<td>L03</td>
<td>Y</td>
</tr>
<tr>
<td>Lesotho</td>
<td>L04</td>
<td>Y</td>
</tr>
<tr>
<td>Madagascar</td>
<td>L05</td>
<td>N</td>
</tr>
<tr>
<td>Malawi</td>
<td>L06</td>
<td>Y</td>
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<tr>
<td>Mauritius</td>
<td>L07</td>
<td>N</td>
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<tr>
<td>Mozambique</td>
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<tr>
<td>Namibia</td>
<td>L09</td>
<td>Y</td>
</tr>
<tr>
<td>Seychelles</td>
<td>L10</td>
<td>N</td>
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<tr>
<td>South Africa</td>
<td>L11 &amp; L12</td>
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<td>Swaziland</td>
<td>L13</td>
<td>Y</td>
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<td>Tanzania</td>
<td>L14</td>
<td>Y</td>
</tr>
<tr>
<td>Zambia</td>
<td>L15</td>
<td>Y</td>
</tr>
<tr>
<td>Zimbabwe</td>
<td>L16</td>
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### Panel of samples

<table>
<thead>
<tr>
<th>Virus material</th>
<th>Laboratory reference no.</th>
<th>Genotype</th>
<th>Dilution</th>
</tr>
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<tbody>
<tr>
<td>Lagos bat virus</td>
<td>RA390</td>
<td>Genotype 2</td>
<td>Undiluted</td>
</tr>
<tr>
<td>Mokola virus</td>
<td>173/06</td>
<td>Genotype 3</td>
<td>Diluted</td>
</tr>
<tr>
<td>Duvenhage virus</td>
<td>SA06</td>
<td>Genotype 4</td>
<td>Undiluted</td>
</tr>
<tr>
<td>Mongoose rabies virus</td>
<td>1164/10</td>
<td>Genotype 1</td>
<td>Undiluted</td>
</tr>
<tr>
<td>Negative (bovine)</td>
<td>366/11</td>
<td>N/A</td>
<td>Undiluted</td>
</tr>
<tr>
<td>Positive A</td>
<td>341/11</td>
<td>Genotype 1 (mongoose)</td>
<td>Undiluted</td>
</tr>
<tr>
<td>Positive B</td>
<td>341/11</td>
<td>Genotype 1 (dog)</td>
<td>Undiluted</td>
</tr>
<tr>
<td>Positive C</td>
<td>343/11</td>
<td>Genotype 1 (dog)</td>
<td>1:5</td>
</tr>
<tr>
<td>Positive D</td>
<td>173/06</td>
<td>Genotype 3</td>
<td>1:400</td>
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<tr>
<td>Positive E</td>
<td>351/11</td>
<td>Genotype 1</td>
<td>1:100</td>
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<tr>
<td>Negative</td>
<td>367/11</td>
<td>N/A</td>
<td>Undiluted</td>
</tr>
</tbody>
</table>
Sending samples to participating labs

- Import permit
- Validation of transportation of samples
- Shipment of samples (ambient temperature according to international regulation) [UN2814]
  - Acknowledgement form (on receipt of samples, condition)
  - Store samples at 4 degrees until analysis
  - Stability will be tested before dispatch of samples (10 days at room temperature)
- Deadline (one month from receipt of samples)
- Result form
- Technical questionnaire (not circulated)
- Instructions to dilute conjugate
Results interpretation ....

- **Discrepancy**: result given by a laboratory different from the expected result (positive or negative), also include false positive/false negative.

- **Sensitivity**: \[
\frac{\text{No. of true pos. samples found by labs}}{\text{Total number of pos samples (True pos. + false neg)}} \times 100
\]

- **Specificity**: \[
\frac{\text{No. of true neg samples found by labs}}{\text{Total no. of negative samples (true neg + false pos)}} \times 100
\]

- Note: The sensitivity and specificity of the inter-laboratory proficiency test cannot be compared to that of classical sensitivity and specificity of a technique (calculated on the basis of random sampling).
Results from labs …..

• Most labs produced satisfactory results, although collectively false negative (n=23) and false positive results (n=9) were a concern.

• 1:400 (diluted sample) gave many laboratories problems.
  – Microscopy [equipment] problem?
  – Inexperienced readers?
  – Adhering to protocol? E.g. use of EVANS blue in the test.

• For this proficiency test, the specificity and sensitivity (65.4% & 80%)[100% & 99.2% for the FAT and for an Anses PT exercise, n=3 (4.6% of negative samples, and n=7 [8% of positive samples]
Recommendations & improvements

• Provide larger amounts of testing material,
• Follow up questionnaire to establish areas of improvement,
• Backstopping visits,
• Assessment of the QMS in each of the laboratories,
• Use of good controls recommended.