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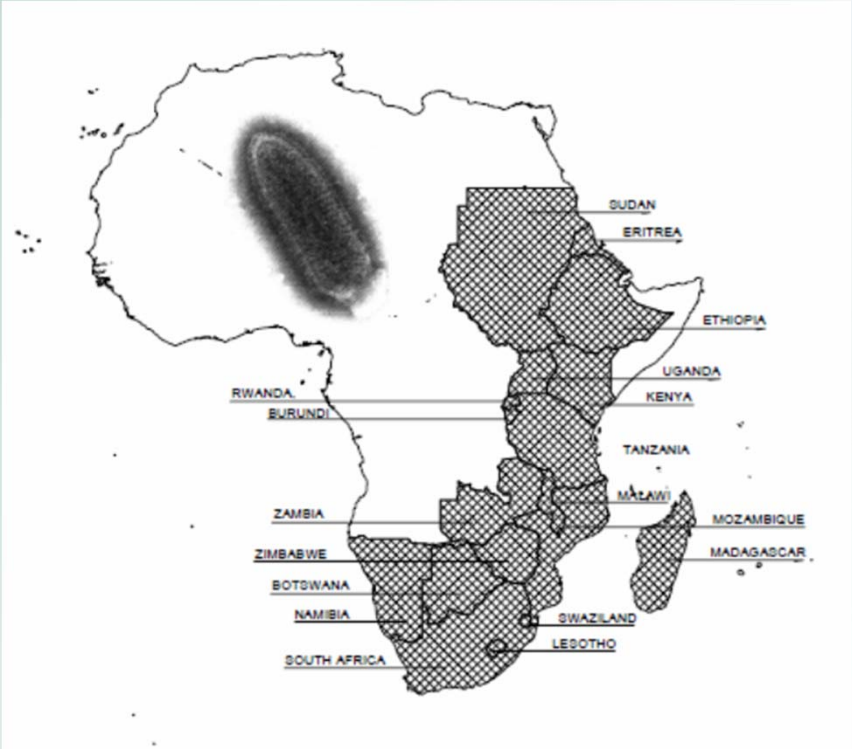
Laboratory proficiency testing for Rabies: an example of diagnostic support to national veterinary laboratories.

Claude Sabeta, 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



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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 ARCand SEARG contact point,

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Participants to the workshop……..

- 14 participants from all SADC countries (except Mauritius)

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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].

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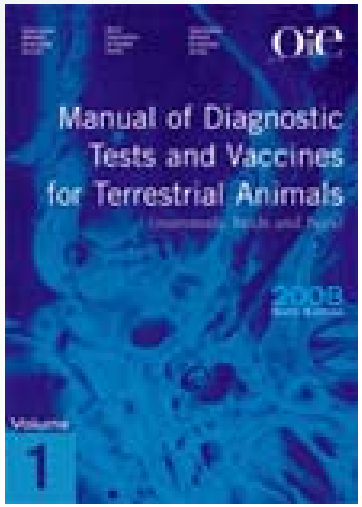
What did we learn from the training?

- 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



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The process of harmonisation of the FAT protocol.

- **Dates**: 26-28 August, 2010.
- **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)

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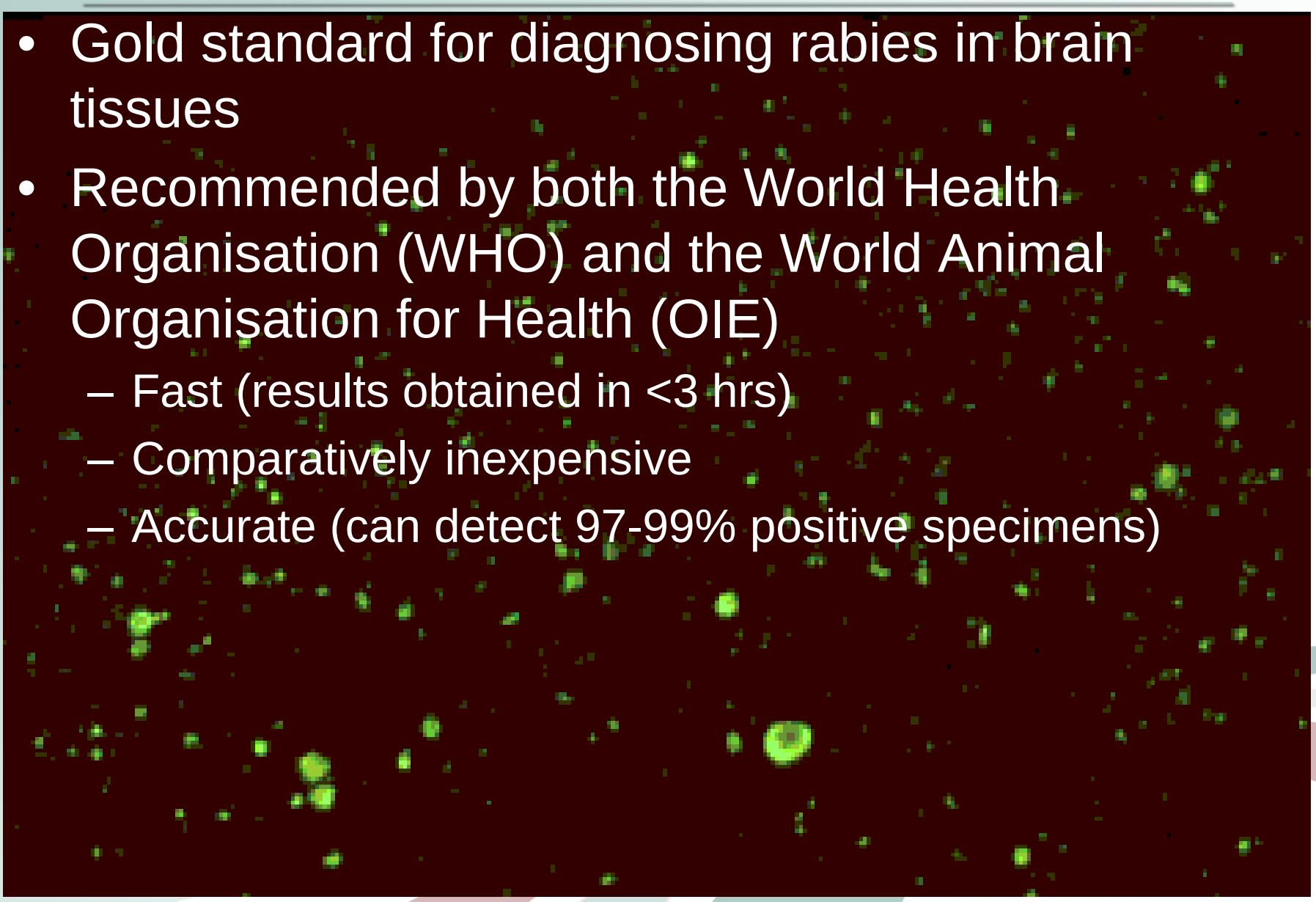
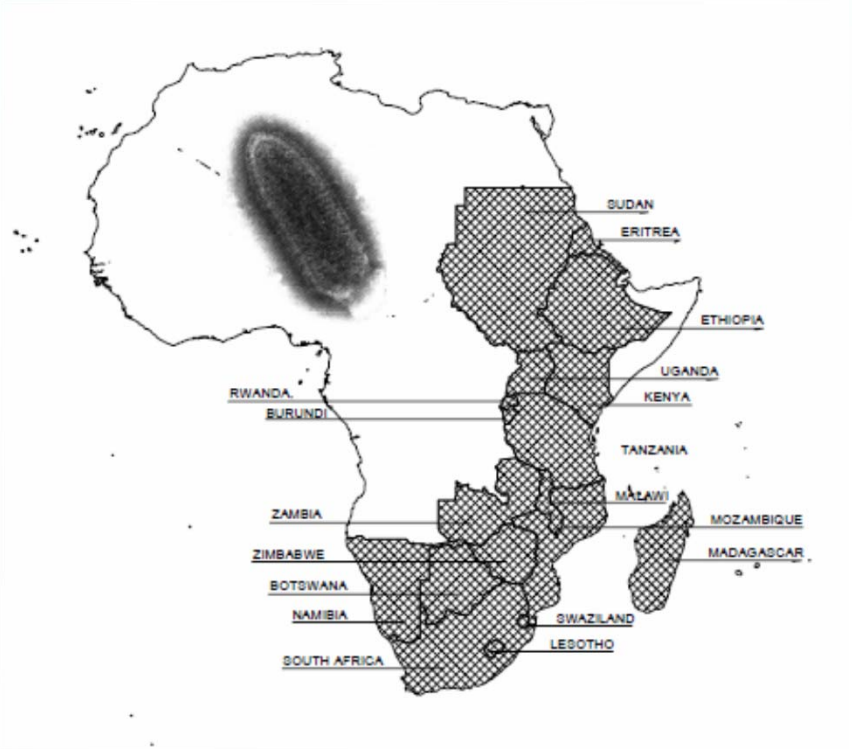
The FAT test



- 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



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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

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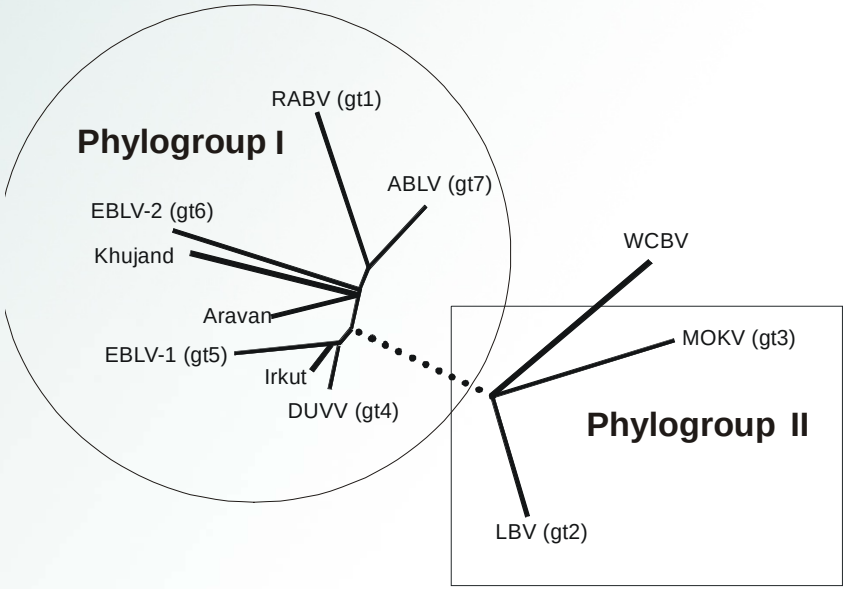
Staining of brain smears …..

- 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)



Reading test results …..

- 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)

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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^{o}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).

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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)

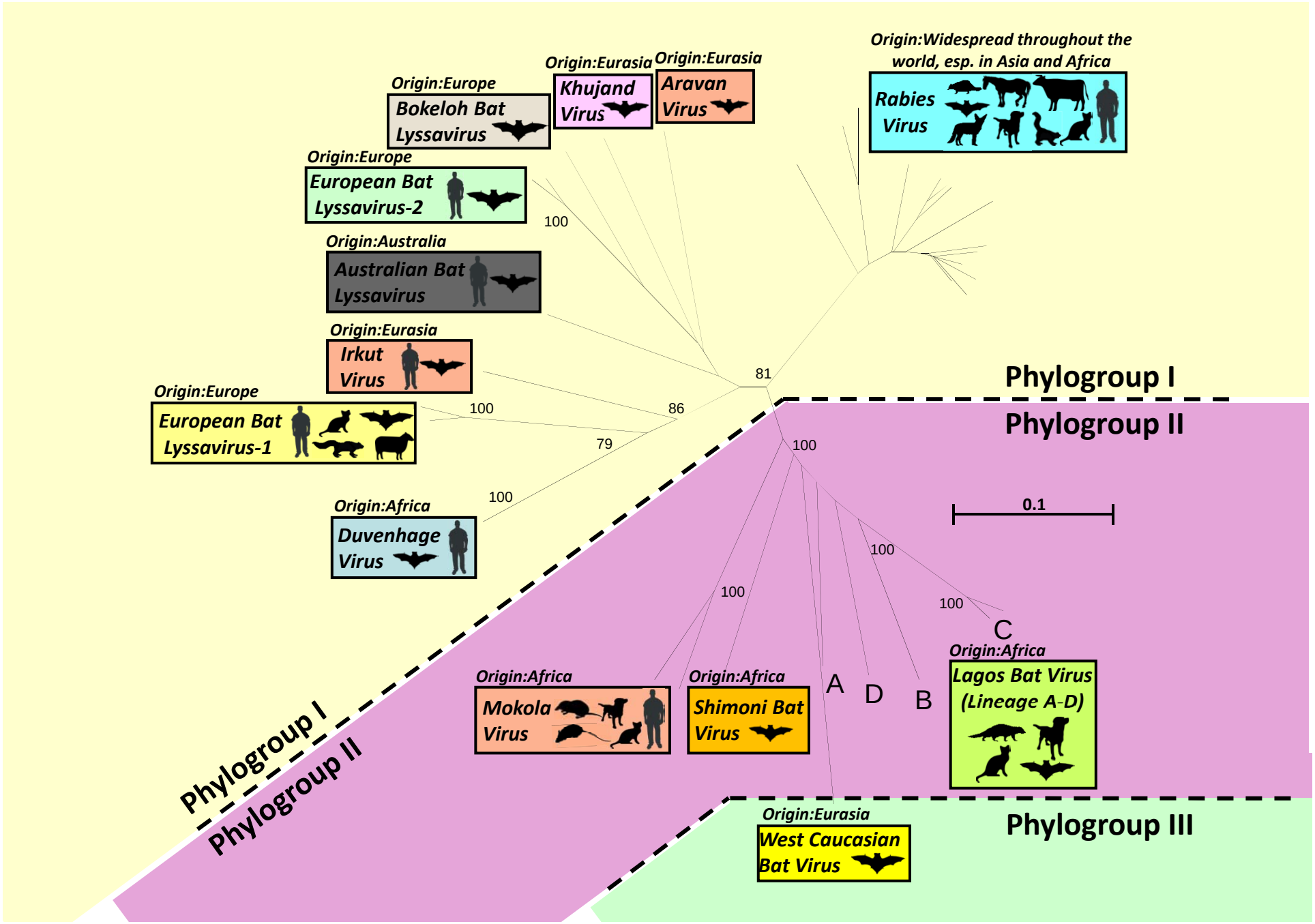
Participating countries…

Country	Code	Participated
Angola	L01	N
Botswana	L02	Y
Democratic Republic of Congo	L03	Y
Lesotho	L04	Y
Madagascar	L05	N
Malawi	L06	Y
Mauritius	L07	N
Mozambique	L08	Y
Namibia	L09	Y
Seychelles	L10	N
South Africa	L11 & L12	Y
Swaziland	L13	Y
Tanzania	L14	Y
Zambia	L15	Y
Zimbabwe	L16	Y

Panel of samples ….

Virus material	Laboratory reference no.	Genotype	Dilution
Lagos bat virus	RA390	Genotype 2	Undiluted
Mokola virus	173/06	Genotype 3	Diluted
Duvenhage virus	SA06	Genotype 4	Undiluted
Mongoose rabies virus	1164/10	Genotype 1	Undiluted
Negative (bovine)	366/11	N/A	Undiluted
Positive A	341/11	Genotype 1 (mongoose)	Undiluted
Positive B	341/11	Genotype 1 (dog)	Undiluted
Positive C	343/11	Genotype 1 (dog)	1:5
Positive D	173/06	Genotype 3	1:400
Positive E	351/11	Genotype 1	1:100
Negative	367/11	N/A	Undiluted

Phylogenetic analysis of lyssavirus genus using partial N-gene sequence



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

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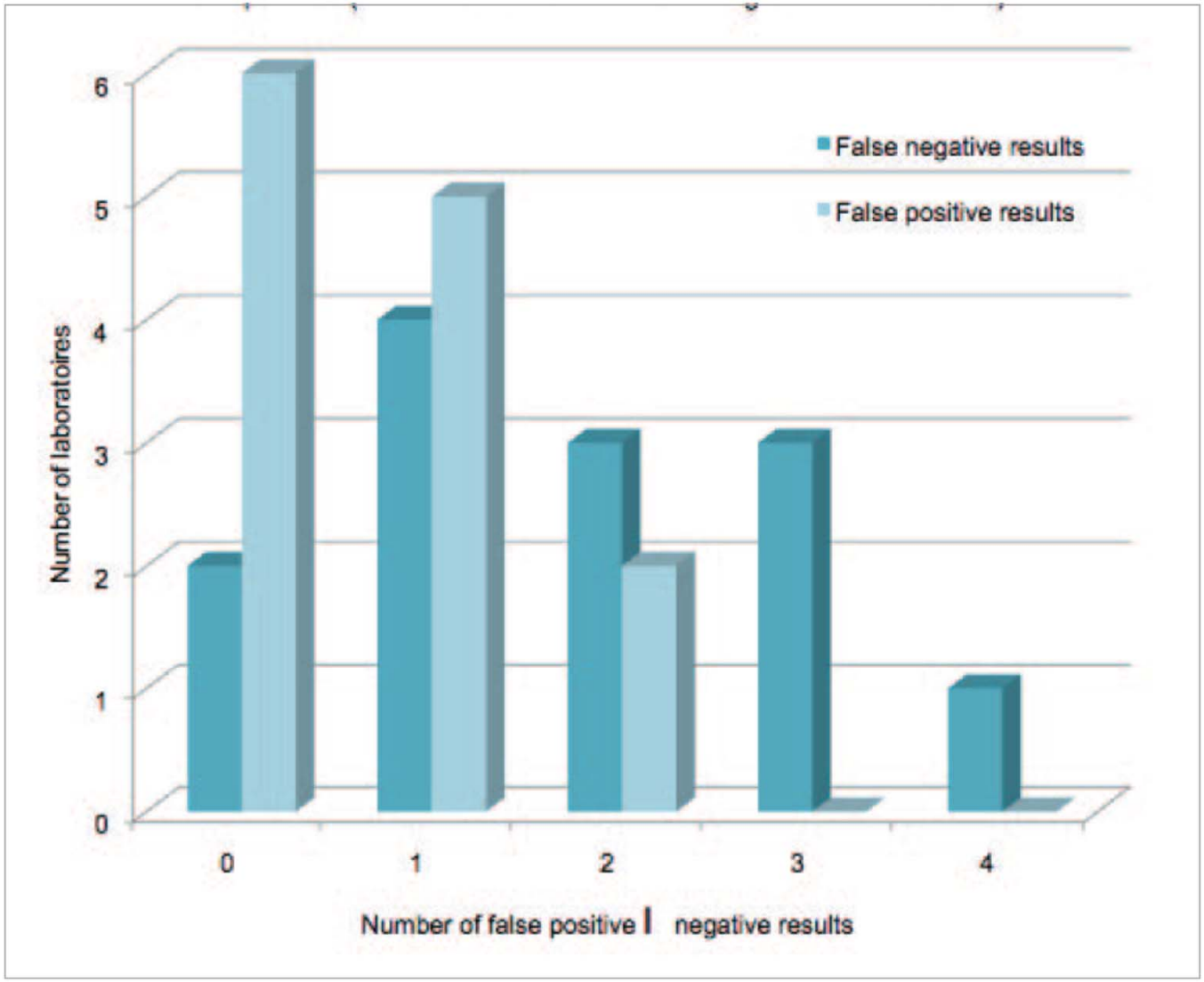
Results interpretation ….

- **Discrepancy**: result given by a laboratory different from the expected result (positive or negative)., also include false positive/false negative.
- **Sensitivity**: [No. of true pos. samples found by labs/Total number of pos samples (True pos. + false neg)] x 100
- **Specificity**: [No. of true neg samples found by labs/Total no. of negative samples (true neg + false pos] x 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]


False negative results
False positive results
Number of laboratoires
0
1
2
3
4
5
6
0
1
2
3
4
Number of false positive I negative results

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.

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World Organisation for Animal Health

SADC REGIONAL INTER-LABORATORY PROFICIENCY TESTING EXERCISE FOR **RABIES DIAGNOSIS**

29 NOVEMBER 2011

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