Diagnosis of Rabies in Humans

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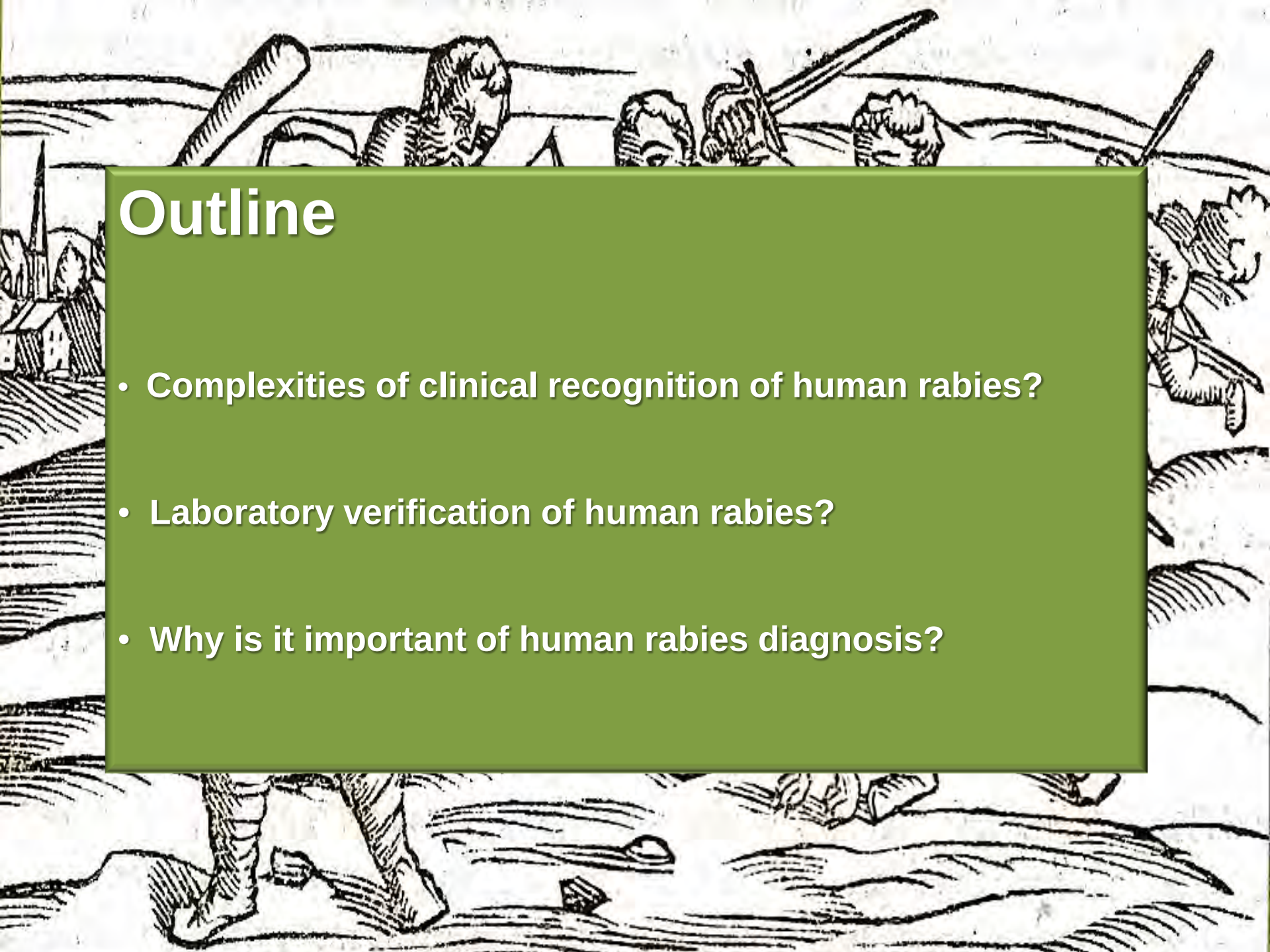
Center for Emerging and Zoonotic Diseases
National Institute for Communicable Diseases

PARaCoN MEETING, 9-11 JUNE 2015, SOUTH AFRICA

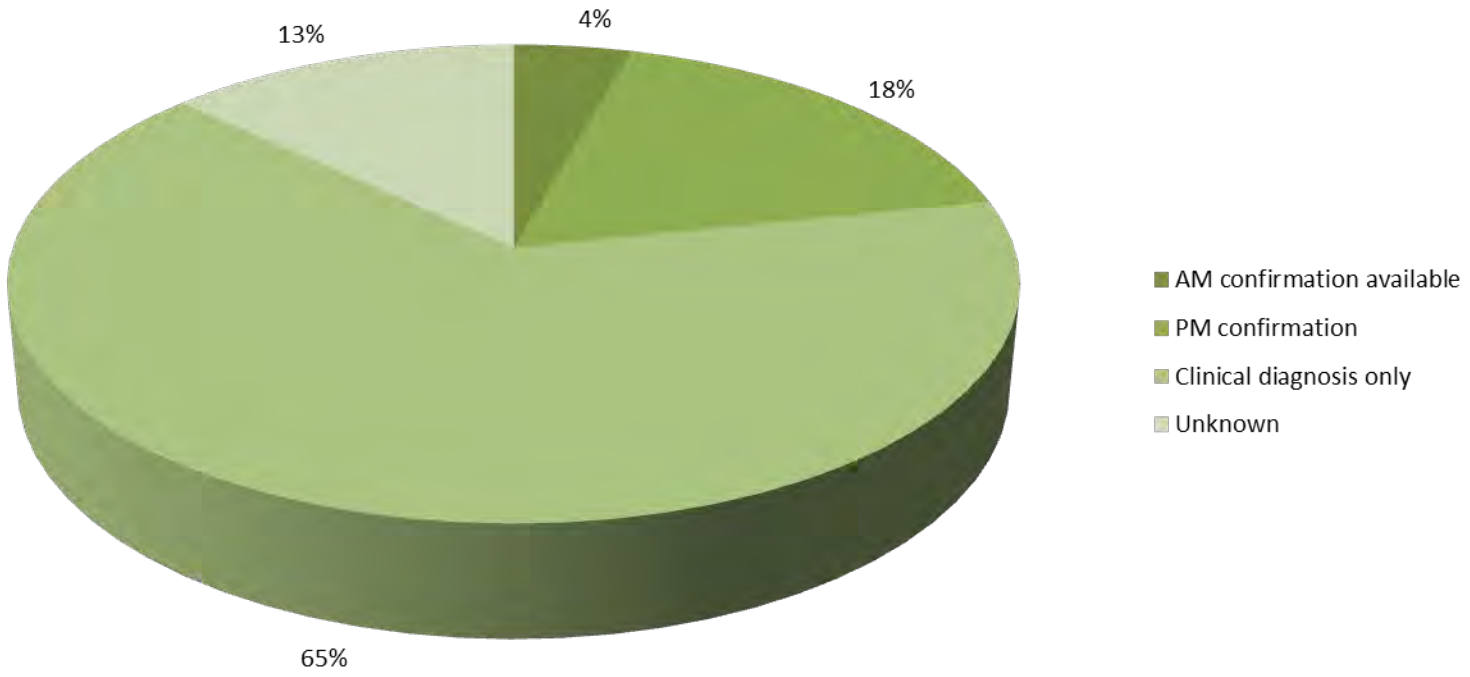
NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES
Division of the National Health Laboratory Service



Outline

- Complexities of clinical recognition of human rabies?
- Laboratory verification of human rabies?
- Why is it important of human rabies diagnosis?

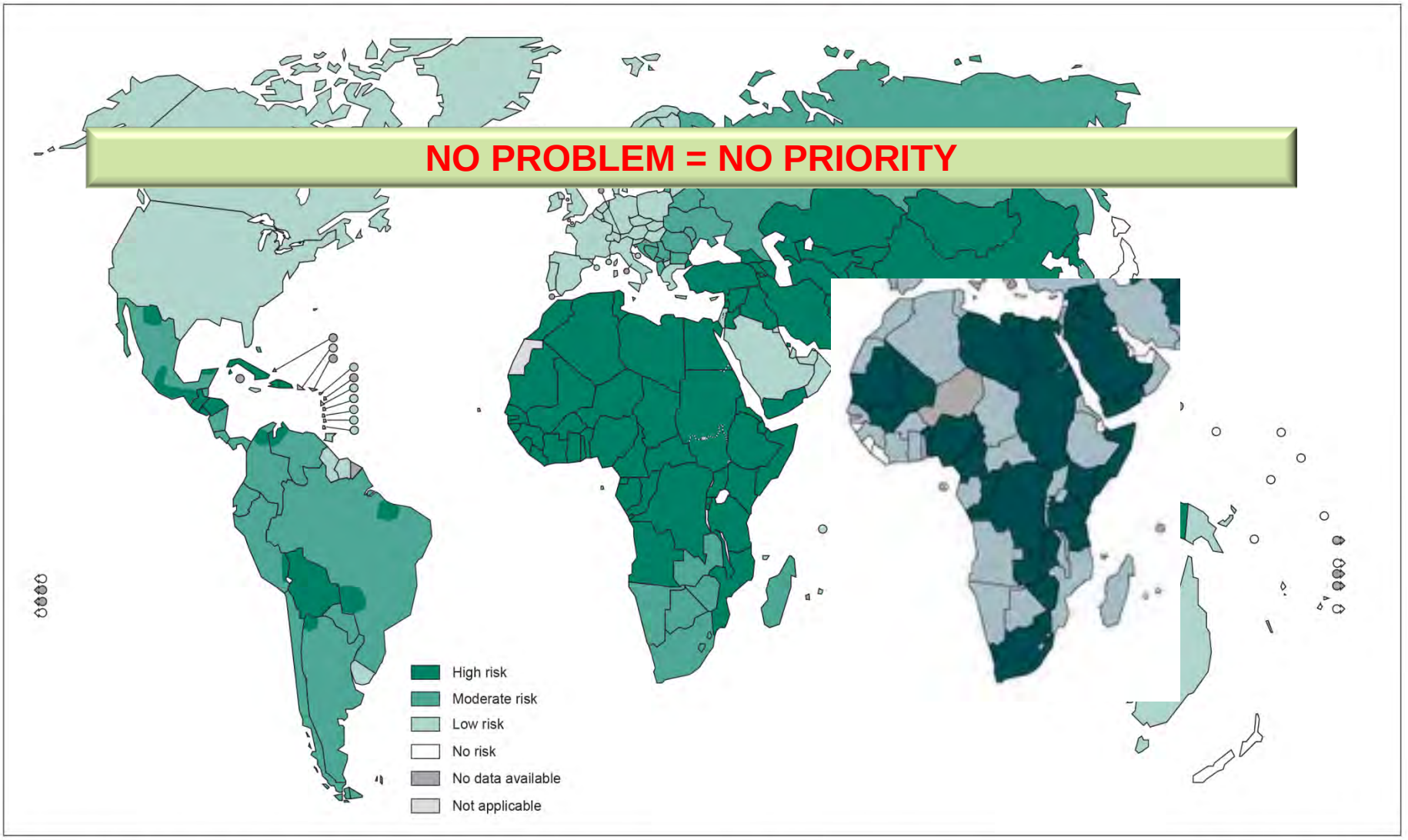
Laboratory capacity for human rabies confirmation in 23 African countries



Source: SEARG reports, 2011 and 2013

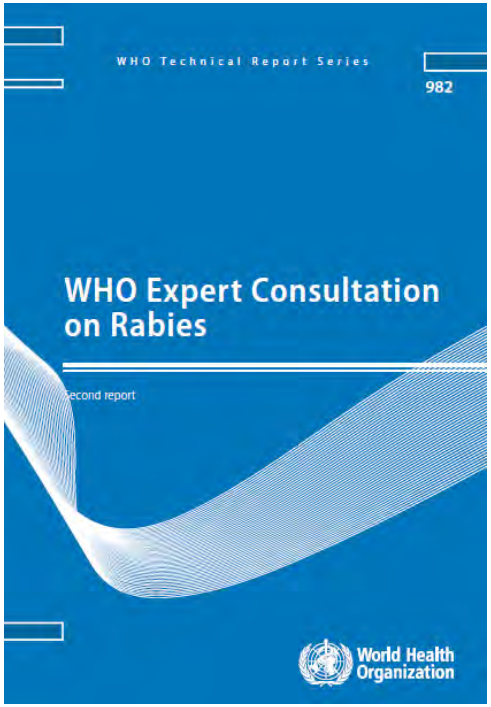
Distribution of risk levels for humans contacting rabies, worldwide, 2013

NO PROBLEM = NO PRIORITY



Data Source: World Health Organization
Map Production: Control of Neglected Tropical Diseases (NTD)
World Health Organization

World Health Organization



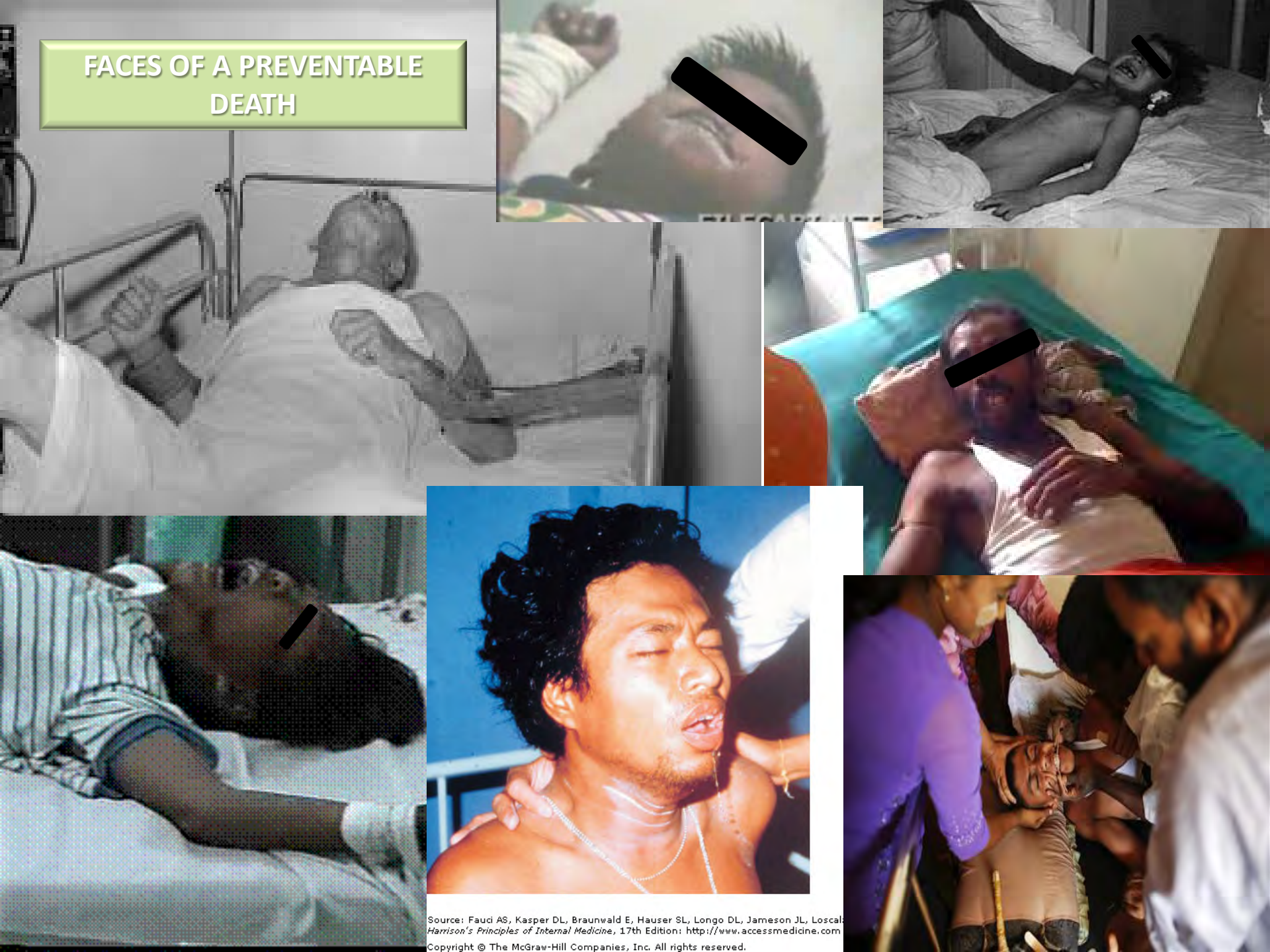
Case definition:

"A subject presenting with an acute neurological syndrome (i.e. encephalitis) dominated by forms of hyperactivity (i.e. furious rabies) or paralytic syndromes (i.e. dumb rabies) progressing towards coma and death, usually by cardiac or respiratory failure and typically within 7-10 days after first sign, if no intensive care is instituted.

Classification of rabies cases:

- Suspected: a case that is compatible with the clinical definition
- Probable: a suspected case plus reliable history of contact with suspected rabid animal
- Confirmed: a suspected or probable case that is laboratory confirmed.

FACES OF A PREVENTABLE DEATH



Source: Fauci AS, Kasper DL, Braunwald E, Hauser SL, Longo DL, Jameson JL, Loscal
Harrison's Principles of Internal Medicine, 17th Edition: http://www.accessmedicine.com

DIFFERENTIAL DIAGNOSIS IS VAST…

INFECTIOUS AETIOLOGIES:

- Cerebritis (Malaria/Trypanosomiasis)
- Bacterial meningitis
- Tetanus
- Other viral encephalitides, i.e. West Nile fever
- Poliomyelitis

NON- INFECTIOUS AETIOLOGIES:

- Poisoning
- Drug reactions
- ADEM

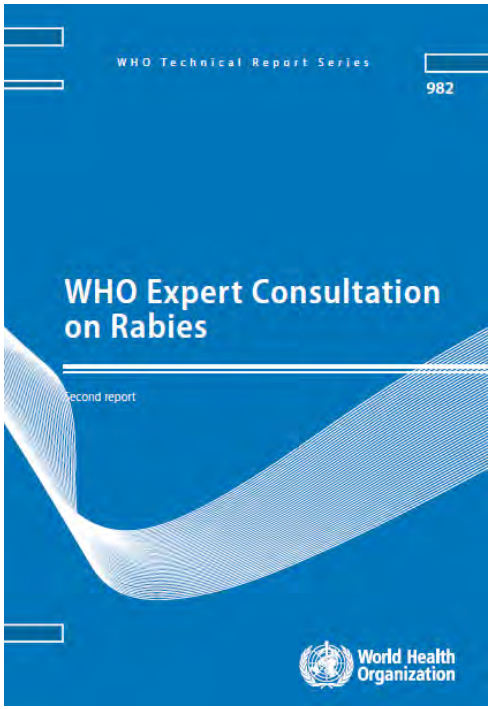
Rabies Encephalitis in Malaria-Endemic Area, Malawi, Africa

Macpherson Mallewa,*† Anthony R. Fooks,‡ Daniel Banda,† Patrick Chikungwa,§ Limangeni Mankhambo,† Elizabeth Molyneux,† Malcolm E. Molyneux,† and Tom Solomon*

In a malaria-endemic area of Africa, rabies was an important cause of fatal central nervous system infection, responsible for 14 (10.5%) of 133 cases. Four patients had unusual clinical manifestations, and rabies was only diagnosed postmortem. Three (11.5%) of 26 fatal cases originally attributed to cerebral malaria were due to rabies.

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 13, No. 1, January 2007

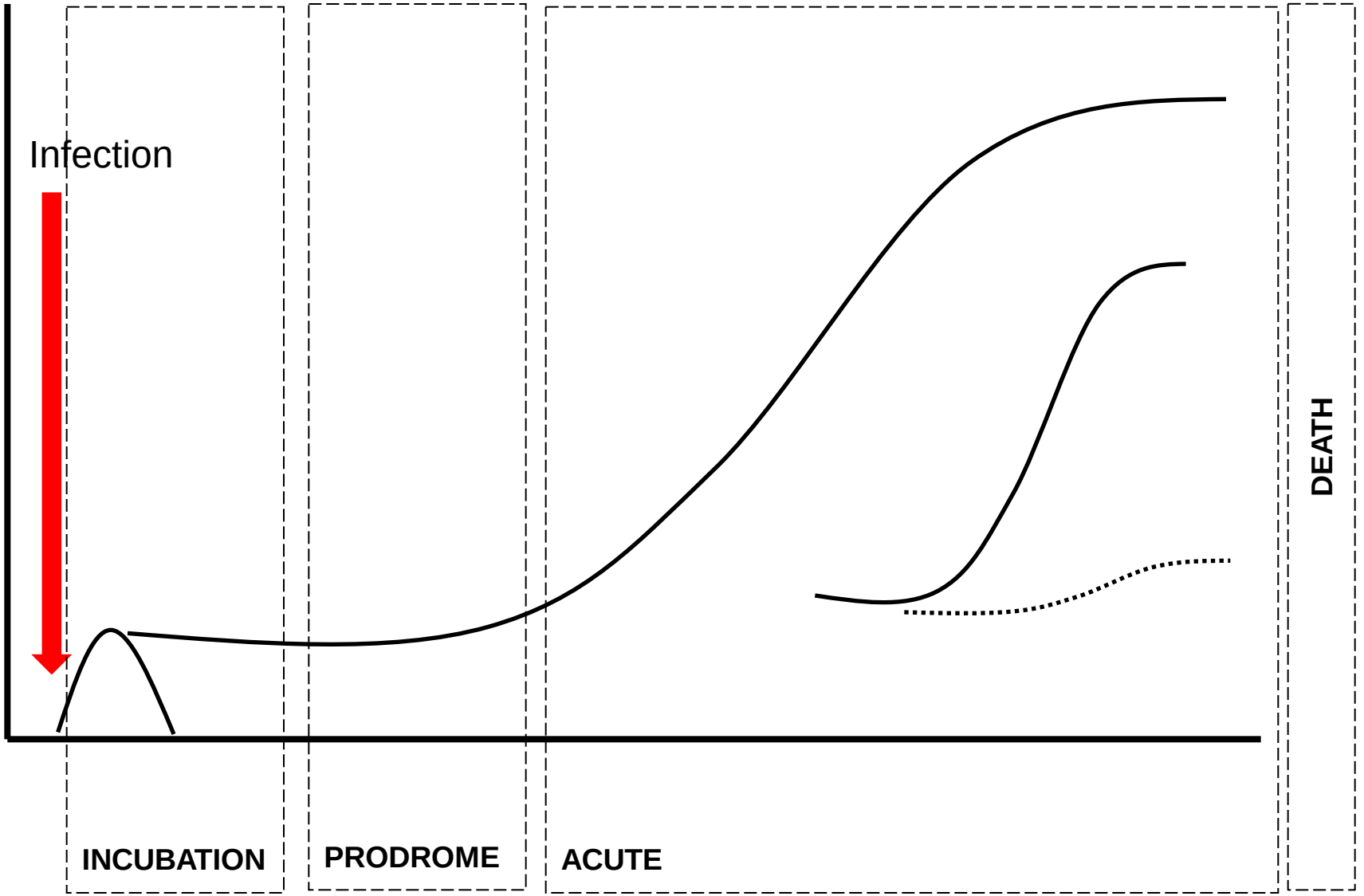
LABORATORY CONFIRMATION OF HUMAN RABIES CASES



Criteria for confirmation:

- Presence of viral antigens shown
- Isolation of live virus in cell culture or laboratory mice
- Presence of anti-rabies antibodies in cerebrospinal fluid or serum of unvaccinated person
- Presence of viral nucleic acid in saliva or other samples

KINETICS OF RABIES VIRUS INFECTION



WHAT ARE THE MINIMUM REQUIREMENTS FOR A LABORATORY TO PERFORM RABIES DIAGNOSIS?

- BSL2

  (BSL3 may be required in certain circumstances)

- Equipment? Reagents?

- Pre-exposure vaccination of staff

- Immunity screening

Section 3.1.8 of Rabies Blueprint

Laboratory confirmation of human rabies →

- **Intensified dog vaccination campaign**
- ↑ Community awareness
- Health care worker training
- Provision of rabies vaccine and immunoglobulin

Concluding remarks

- Virtually no surveillance for human rabies in Africa = no appreciation for burden of disease
- Surveillance can be improved by training of health care workers; establishment of at least post mortem verification of cases
- Clinical confirmation of rabies is not reliable, but could supplement laboratory confirmed data
- Diagnosis of human rabies is helpful to trigger public health responses to prevent further cases

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Rabies Blueprint: Diagnosis

Claude Sabeta
OIE Reference Laboratory for Rabies
Onderstepoort

PaRaCon Conference, 9-11 June, 2015

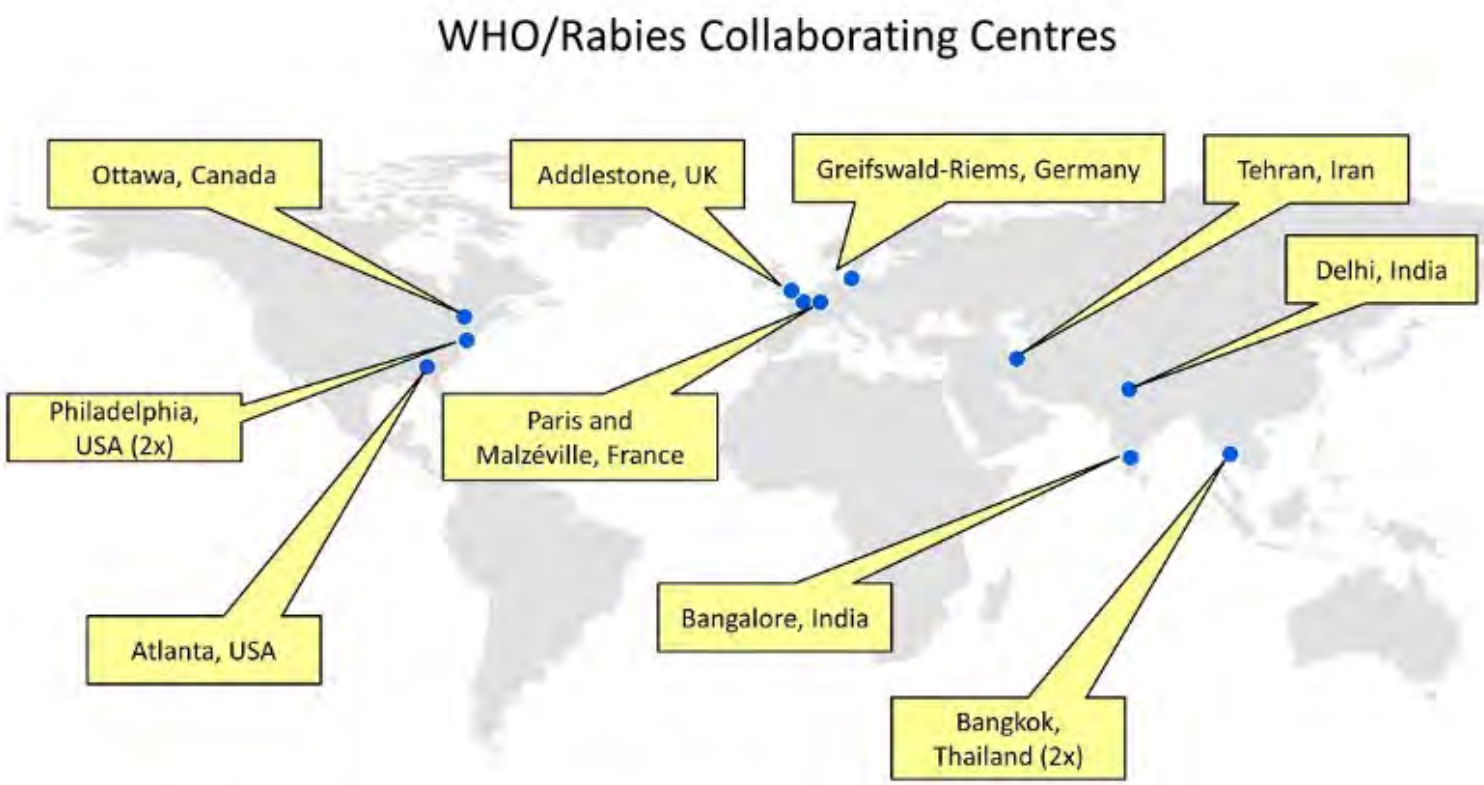
Outline

- Centralised and decentralized diagnostic approaches,
- Collaborating Centres for Rabies
  - WHO & OIE
- Laboratory tests for diagnosis,
  - Post mortem diagnosis,
  - Confirmatory tests (need and available tests)
- Characterising the virus
  - Antigenic and molecular characterisation
- Information required for surveillance

Centralised and decentralized diagnostics

- Each country should have a national reference laboratory (NRL)
  - Capacity for basic rabies diagnosis and confirmation using modern diagnostic techniques.
  - NRL for human and veterinary sectors.
- The decision whether to centralise/decentralize may depend on the **size of the country**.

WHO Collaborating Centres for Rabies ……..



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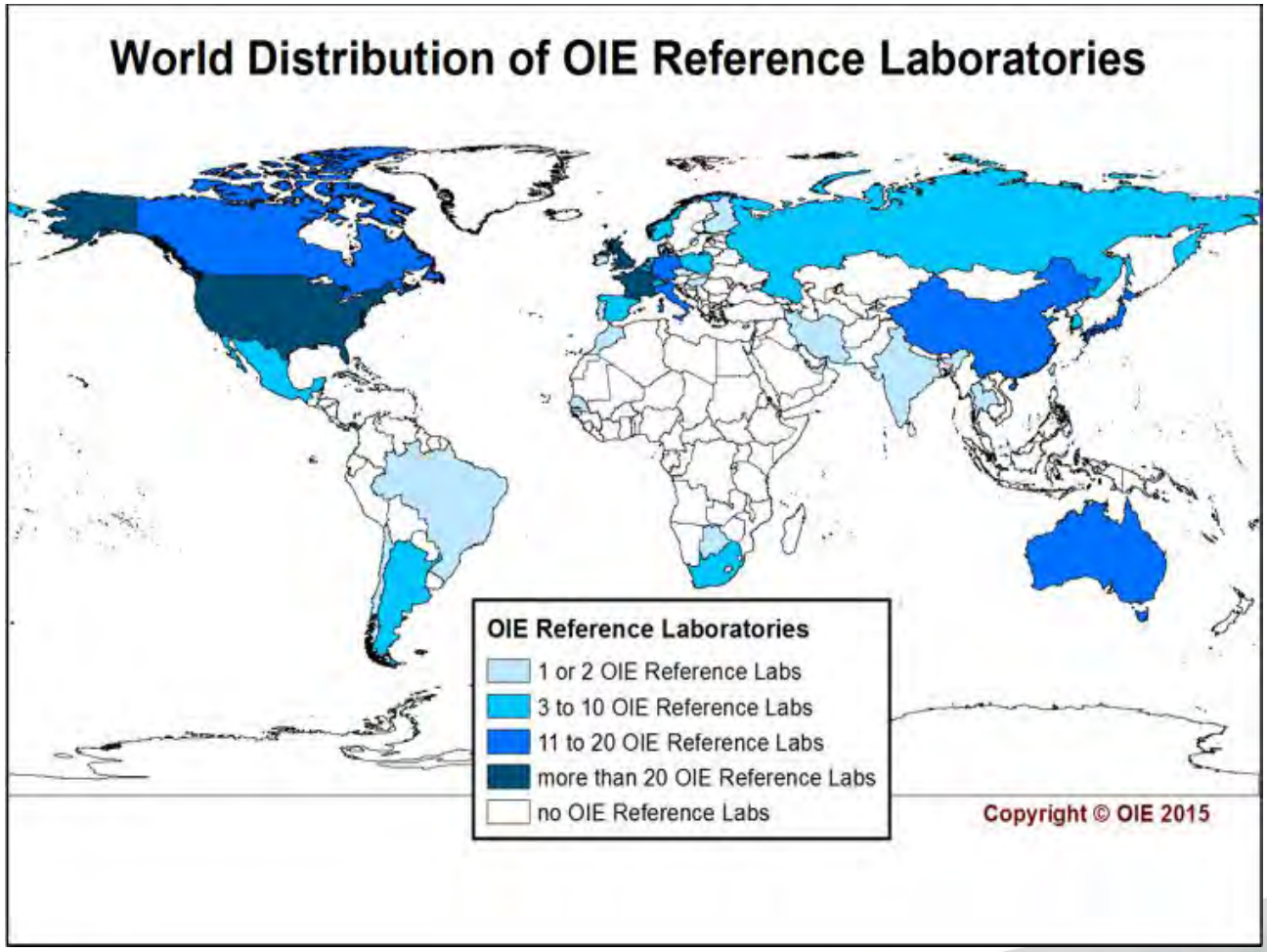
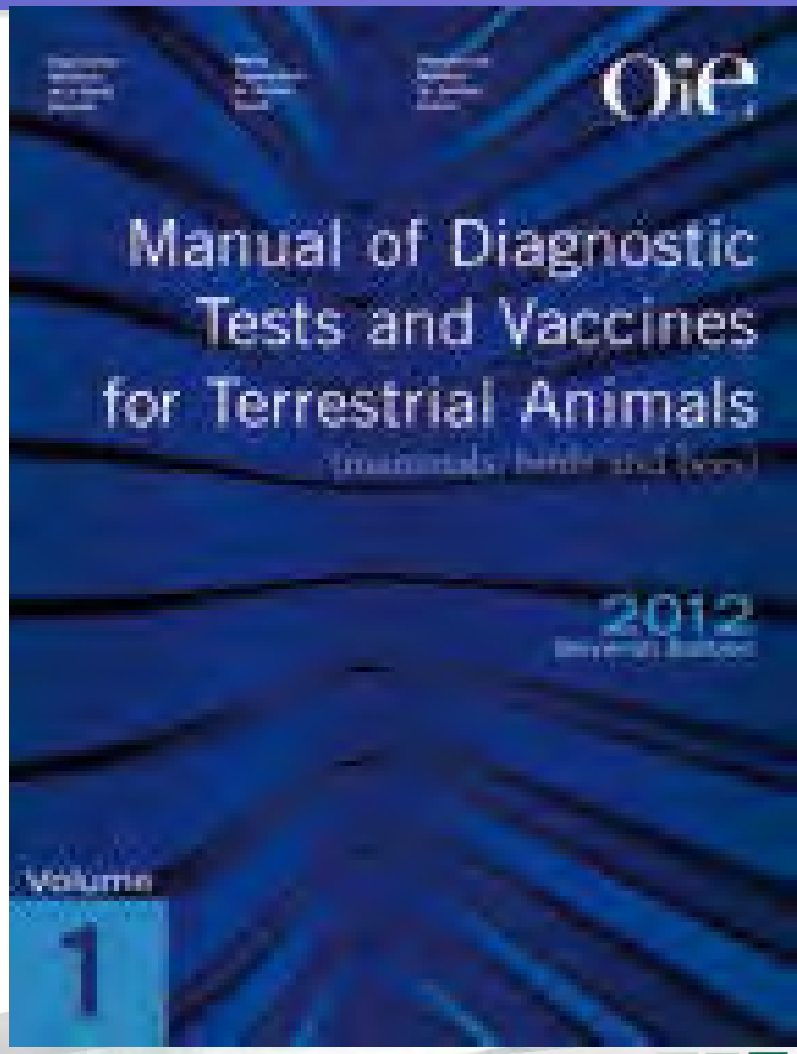
OIE Rabies Reference Laboratories



- Centre of Expertise for Rabies CFIA/ACIA (Canada)
- Centers for Disease Control and Prevention (USA)
- Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria (Mexico)
- Laboratoire de la faune sauvage de Nancy (France)
- Friedrich-Loeffler Institut (Germany)
- Animal and Plant Health Agency (UK)
- Ministry of Agriculture, Food and Rural Affairs (MAFRA) (South Korea)
- Changchun Veterinary Research Institute (CVRI) (China)
- Onderstepoort Veterinary Institute RSA)

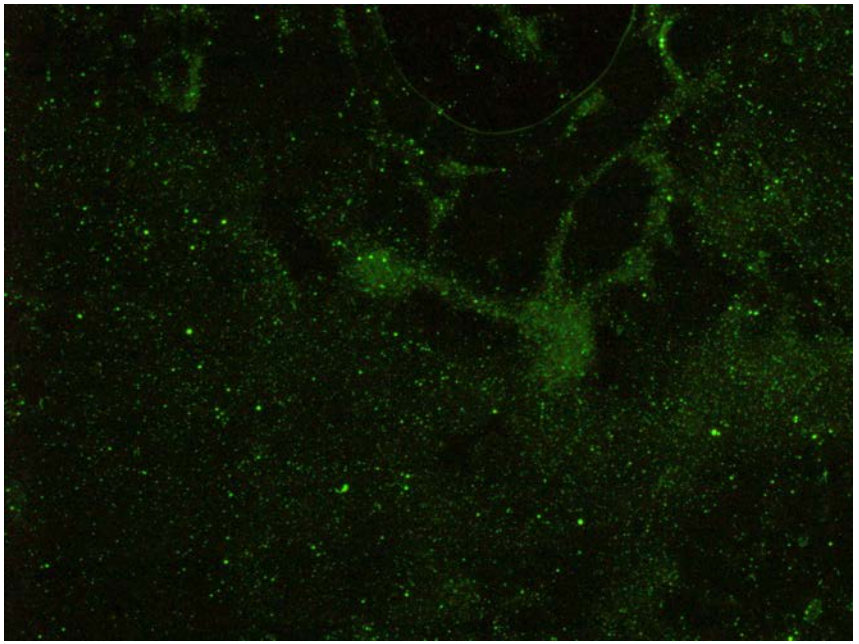
Laboratory tests for rabies diagnosis

- Variety of tests available for post-mortem diagnosis in both animals [OIE Terrestrial Manual – chapter 2.1.13]
  - Antigen detection using the fluorescent antibody test (FAT), recommended by both the WHO and OIE.
    - Used directly on a composite smear,
    - Confirm presence of antigen in rabies tissue culture isolation test (RTCIT), or on brain tissue of mice inoculated for diagnosis.
    - The FAT gives a reliable result within a few hours



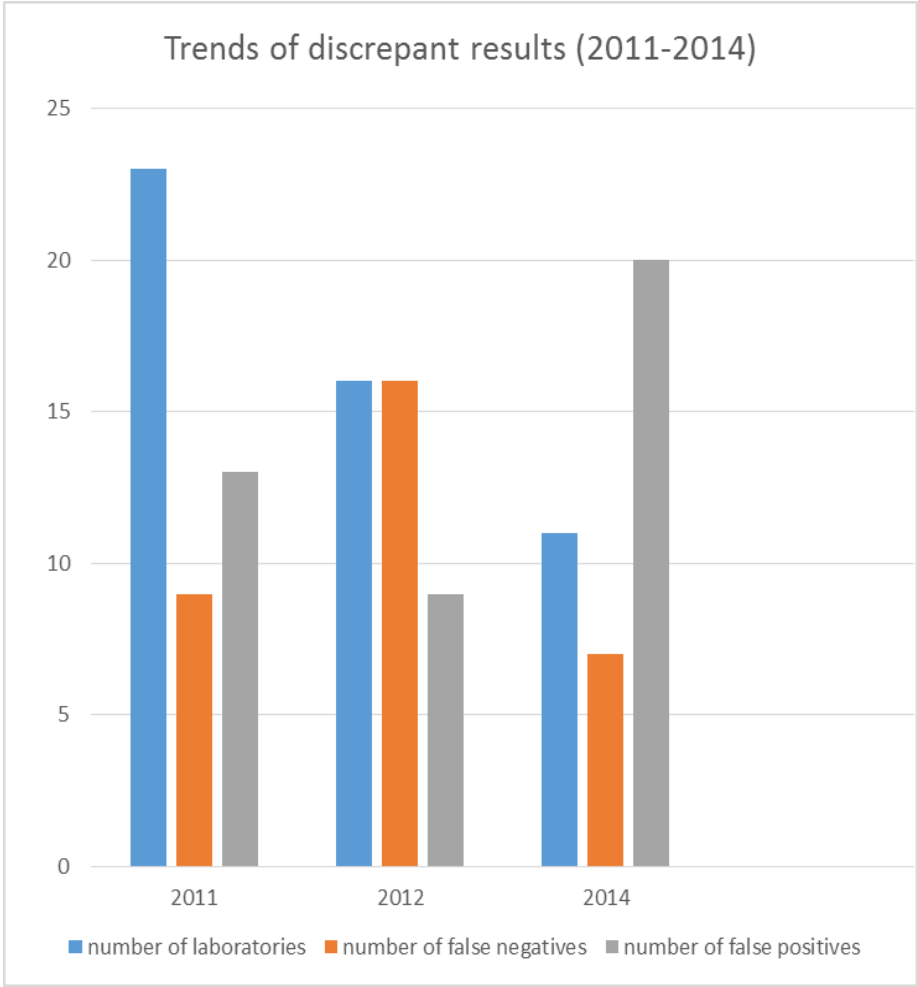
Laboratory tests for rabies diagnosis

- Harmonised protocols should be used within regional blocks,
- dFAT test undertaken on a specimen submitted to the OIE Rabies Reference Laboratory.
  - Stained with a polyclonal conjugate (Mok + ERA)
  - Species – canine
  - Locality: Phalaborwa
  - Contact type: Bite (1) Saliva (3) Handling (4)



Assessing for competence for rabies diagnosis

Sample#	Lab.reference #	Species of origin	Biotype	Dilution factor	Expected results
1	165/14	Bovine Bos taurus	Canid	Undiluted	Positive
2	268/14	Bovine Bos taurus	Mongoose	Undiluted	Positive
3	311/14	Domestic dog Canis familiaris	Canid	Undiluted	Positive
4	266/14	Domestic dog Canis familiaris	Canid	Undiluted	Positive
5	924/12 & 961/12	Domestic dog Canis familiaris	N/A	Undiluted	Negative
6	725/13 & 862/13	Domestic dog Canis familiaris	N/A	Undiluted	Negative
7	26/14	Feline Domestic cat	N/A	Undiluted	Negative
8	703/13	Waterbuck	N/A	Undiluted	Negative



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Alternative tests to dFAT

- The direct rapid immunohistochemical test (DRIT)
  - economical, low technology, real-time test particularly in countries **where laboratory infrastructure is lacking**.
  - **Enhanced surveillance** in North America where it has been used as a surveillance tool for wildlife rabies in support of vaccination campaigns,
  - the DRIT involves the examination of brain impressions, in comparison to the dFAT, **utilises light microscopy and biotin-labelled antibodies**,

Laboratory tests for rabies diagnosis - validation

Comparison of FAT and dRIT							
FAT							
Biotinylated Antibodies	**True Positive**	**False Positive**	**True Negative**	**False Negative**	**Diagnostic Sensitivity***	**Diagnostic Specificity***	**Kappa Value***
Polyclonal Antibody	200	0	49	1	**99,5%** (97,25% -99,92%)	**100%** (92,68% -100%)	---
dRIT							
Polyclonal Antibody	201	0	49	0	**100%** (98,16% -100%)	**100%** (92,68% -100%)	0.987 (0,963 - 1,000)
Monoclonal antibody #1	167	0	49	34	**83,08%** (77,17% - 87,99%)	**100%** (92,68% -100%)	0.649 (0,548 - 0,751)
Monoclonal antibody #2	182	1	48	19	**90,55%** (85,63% - 94,21%)	**97,96%** (89,10% - 99,66%)	0.767 (0,674 – 0,861)
* Value in brackets represented the 95% confidence interval (CI)							

Coetzer, A. 2012. MSc thesis, University of Pretoria.

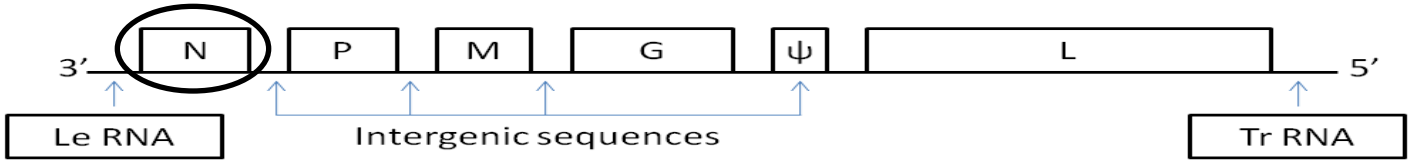
A variety of post-mortem tests are also available for rabies diagnosis

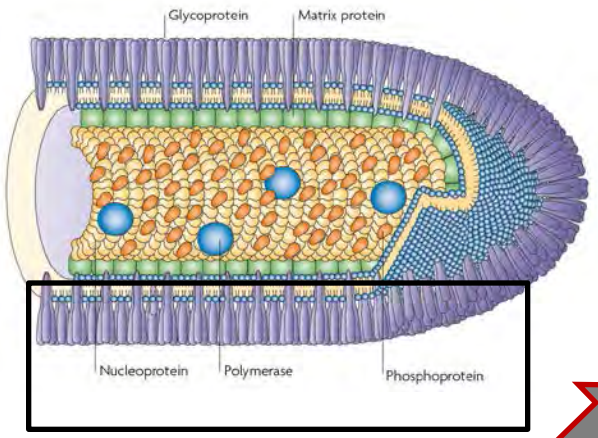
- A variety of other methods are available for the detection of rabies virus and lyssavirus antigens and viral RNA e.g. **lateral flow devices (LFDs)**.
- These tests are currently not recommended for routine laboratory use because:
  - (i) they lack **standardization** and **adequate validation** based on international standards
  - (ii) they require experience and strict quality control.

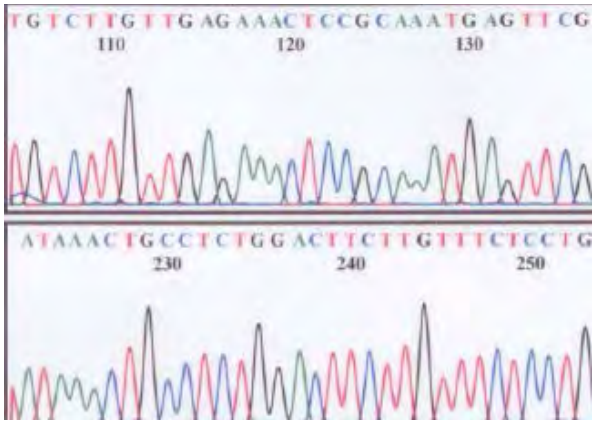
The need for confirmatory tests

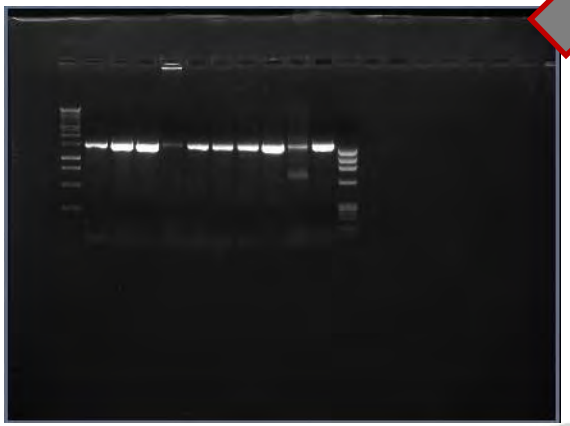
- In general, the sensitivity and specificity of the FAT and dRIT are very high, but may dependent on:
  - the quality of the specimen,
  - conjugate,
  - equipment and the skills and experience of the people involved in rabies diagnosis.
- In order to confirm FAT/dRIT inconclusive results, or FAT or dRIT negative results (in the case of human exposures), either **virus isolation** or **molecular techniques** can be used.
  - Reviewed in Fooks AR, Johnson N, Freuling CM, Wakeley PR, Banyard AC, et al. (2009) Emerging Technologies for the Detection of Rabies Virus: Challenges and Hopes in the 21st Century. PLoS Negl Trop Dis 3(9): e530. doi:10.1371/journal.pntd.0000530

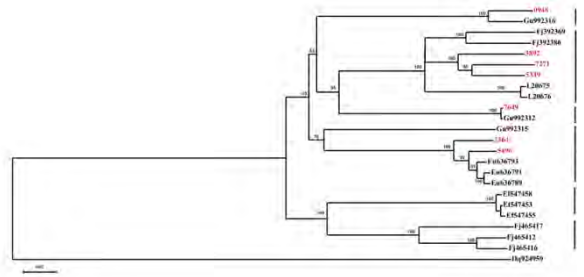
PCR not yet validated nor used for routine diagnostics ……..

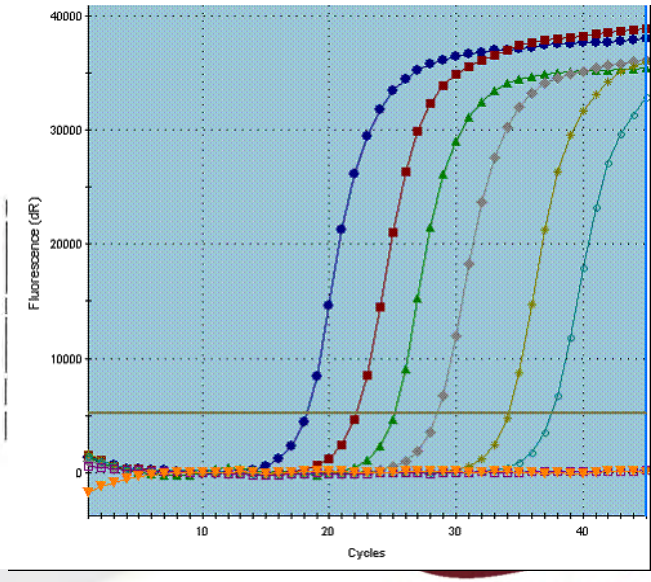












The need for confirmatory tests

- Virus isolation
  - detects replication competent viral particles can be performed on cells or upon intracranial inoculation of mice using the Rabies Tissue Culture Infection Test (RTCIT and the Mouse Inoculation Test (MIT). Whenever possible the RTCIT should replace the MIT.
  - For RTCIT at least three passages should be conducted to confirm a negative result.

Is it useful to characterize the virus?

- Virus identification using
  - monoclonal antibodies
  - molecular techniques
    - can provide valuable information for epidemiological. The epidemiological information is valuable for epidemiological analysis and can help identifying the source of infection.

Mab	canid	mongoose	Lagos bat	Mokola virus	Duv
1C5	-----	-----	-----	-----	-----
26AB7	+++	var	-----	-----	-----
26BE2	+++	var	-----	-----	-----
32GD12	var	var	-----	-----	-----
38HF2	+++	+++	+++	+++	+++
M612	-----	-----	+++	-----	-----
M837	-----	-----	-----	-----	+++
M850	-----	var	-----	-----	+++
M853	+++	-----	-----	-----	+++
M1001	-----	-----	-----	+++	-----
M1335	-----	var	-----	var	-----
M1386	-----	+++	-----	-----	-----
M1400	-----	var	-----	-----	-----
M1407	++	var	-----	-----	-----
M1412	++	var	-----	-----	-----

What minimum information are required for effective rabies surveillance?

- Species
- Location the animal was found / sample was taken
- Date of finding
- Date of submission
- Address of owner / finder
- Result of laboratory diagnosis and tests used

Acknowledgements

- Blueprint – GARC website
- Conference organisers
- Canadian Food Inspection Agency (CFIA)
- Agricultural Research Council

Thank you for listening

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Review of the WA/CA-RESOLAB rabies sub-network activities (2010-2014)

Angélique Angot & Paola De Benedictis on behalf of the WA/CA-RESOLAB rabies subnetwork

RESOLAB

- Western and Central African Veterinary Laboratory Network for Avian Influenza and other transboundary disease (**RESOLAB**) : Launched in 2007 thank to **FAO** and its **partners** (USDA-APHIS, World Organization for Animal Health (OIE), AU-IBAR)

- Network officially recognized by National authorities

- 23 National veterinary laboratories

- <u>December 2010: the Rabies Subnetwork was created</u>

RESOLAB rabies subnetwork main objectives

1. To identify priority gaps in rabies diagnosis/surveillance
2. To build diagnostic capacity
3. To promote involvement of national authorities
4. To improve interaction between the veterinary and public health counterparts
5. To promote awareness and education

UNITÉ RÉGIONALE FAO ECTAD

Afrique de l'Ouest et du Centre

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Quarterly reports 2014 for RESOLAB

RABIES SUB-NETWORK

QUARTERLY REPORT (January – March 2014)

Results were provided by the RESOLAB-WA/CA Laboratory members. It may be that, in some countries, rabies diagnosis is performed by other laboratories.

- **BENIN**

To date, no rabies diagnosis is being carried out. The activity is expected to start in the coming weeks.

- **BURKINA-FASO**

	Number	Observations
Number of canine samples received	90	1 putrefied
Number of canine samples examined	89	
Positive Cases	69	
Negative Cases	20	
Other domestic animals samples received	7	2 putrefied
Number of other domestic animals samples examined	5	cats
Positive Cases	5	
Negative Cases	0	
Samples of wildlife received	1	Mouse
Samples of wildlife examined	1	
Positive Cases	1	
Negative Cases	0	
Total number of samples received	98	
Total number of samples examined	95	
Total number of positive cases	75	
Total number of negative cases	20	

Map Search
ENGLISH | FRANÇAIS
« June 2015 »
M T W T F S S
1 2 3 4 5 6 7
8 9 10 11 12 13 14
15 16 17 18 19 20 21
22 23 24 25 26 27 28
29 30 1 2 3 4 5
RESOLAB RABIES SUB NETWORK
RESOLAB SOUS RÉSEAU PPR
RESOLAB SOUS RÉSEAU FMD
RESECOP FILIERES ANIMALES
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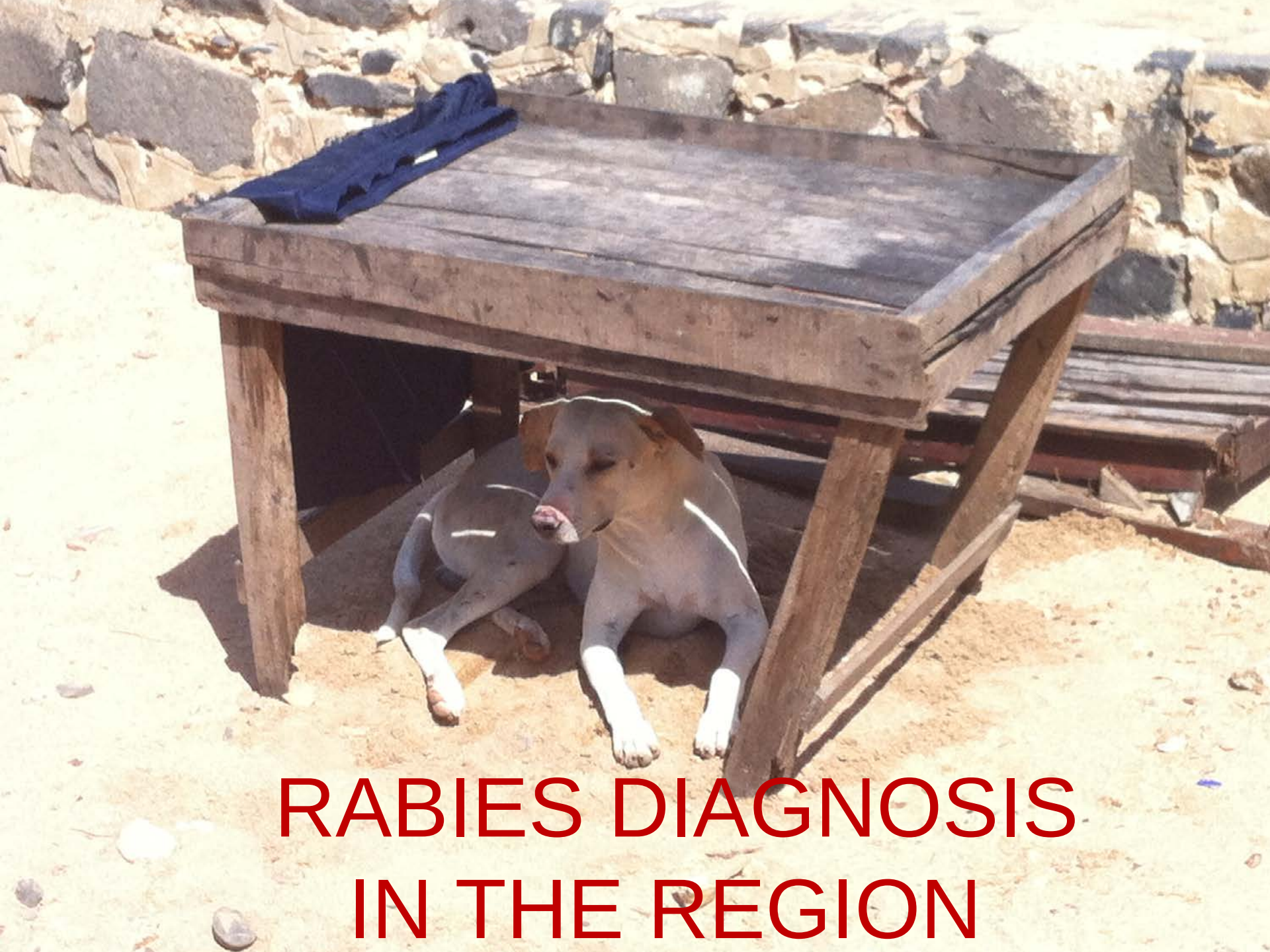
http://www.fao-ectad-bamako.org/fr/-Rabies-Sub-Network-?lang=en



RABIES DIAGNOSIS IN THE REGION

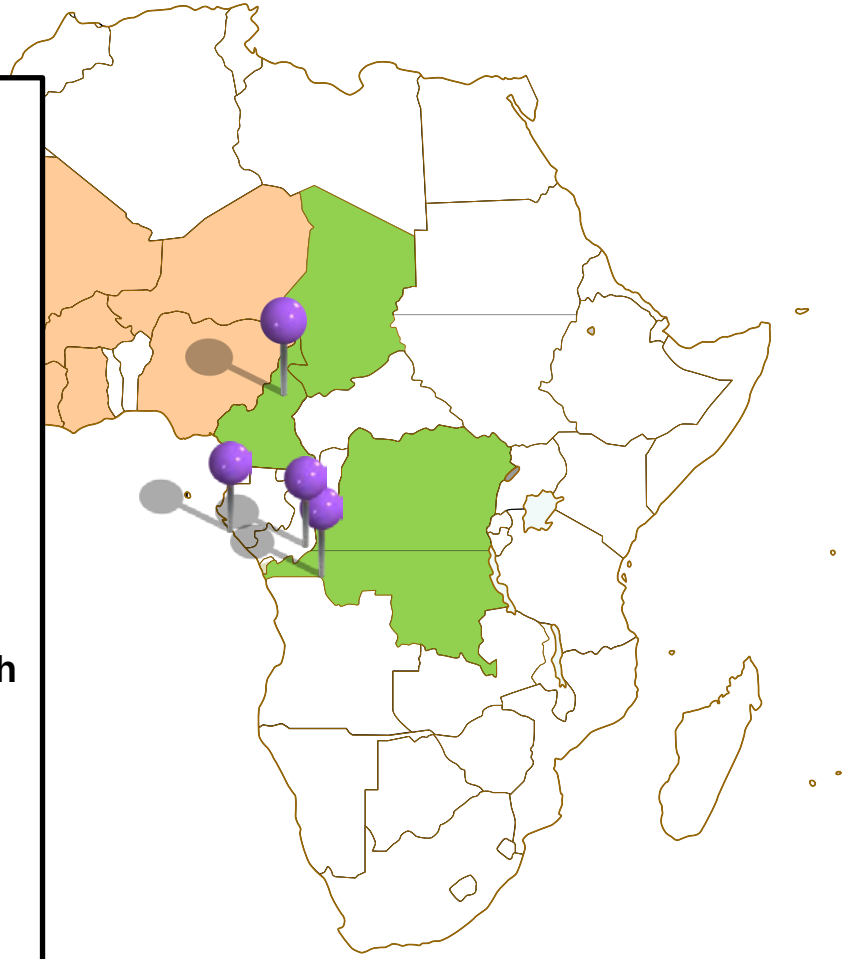
Rabies diagnostic capability

2010 2012

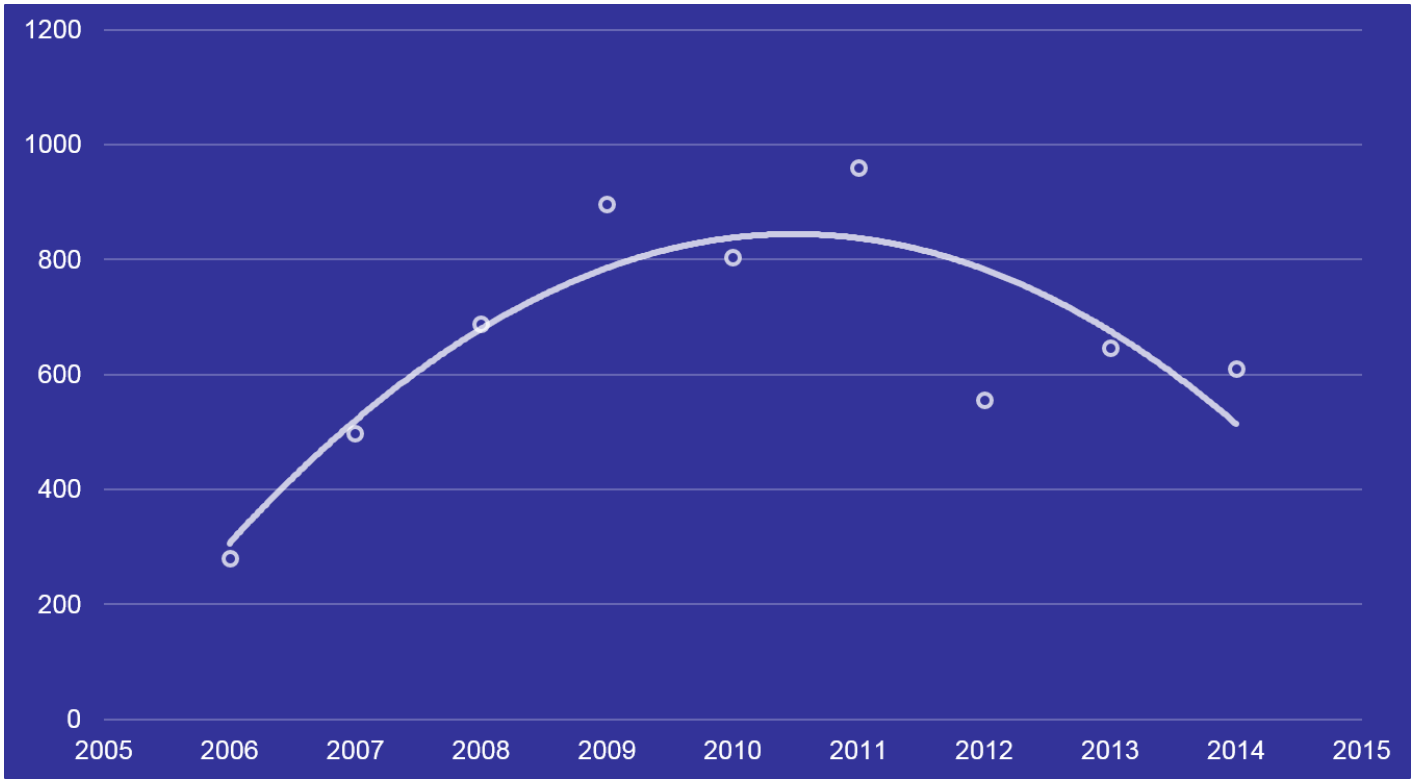
Laboratoire National Vétérinaire (LANAVET),

- Overall diagnostic capability : **11/23**
- Unique laboratory offering for animal rabies diagnosis at country level : **7/11**
- Lack of reagents and equipment were the constraints mostly claimed

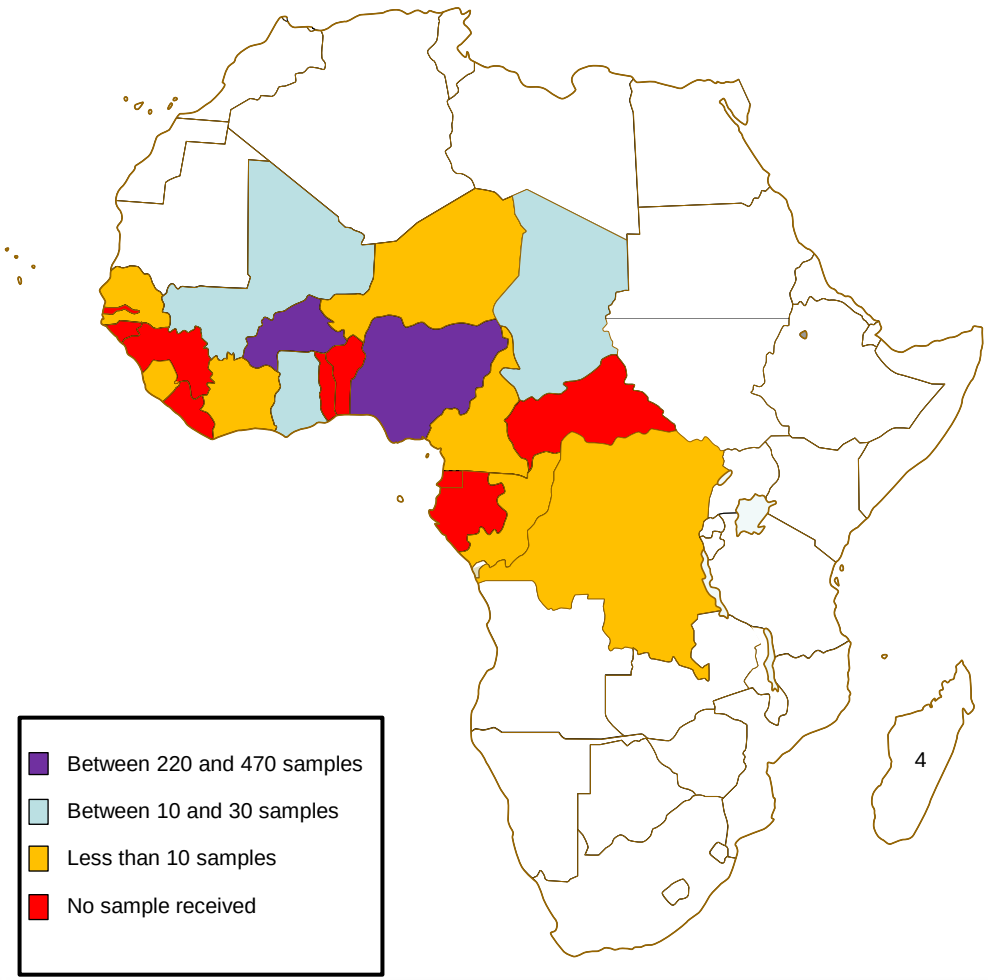
6 attendees from the **LNVL** and **2** trainees from the **Laboratoire National de Santé Publique** and 1 from the **Laboratory of Virology of Medicine University**

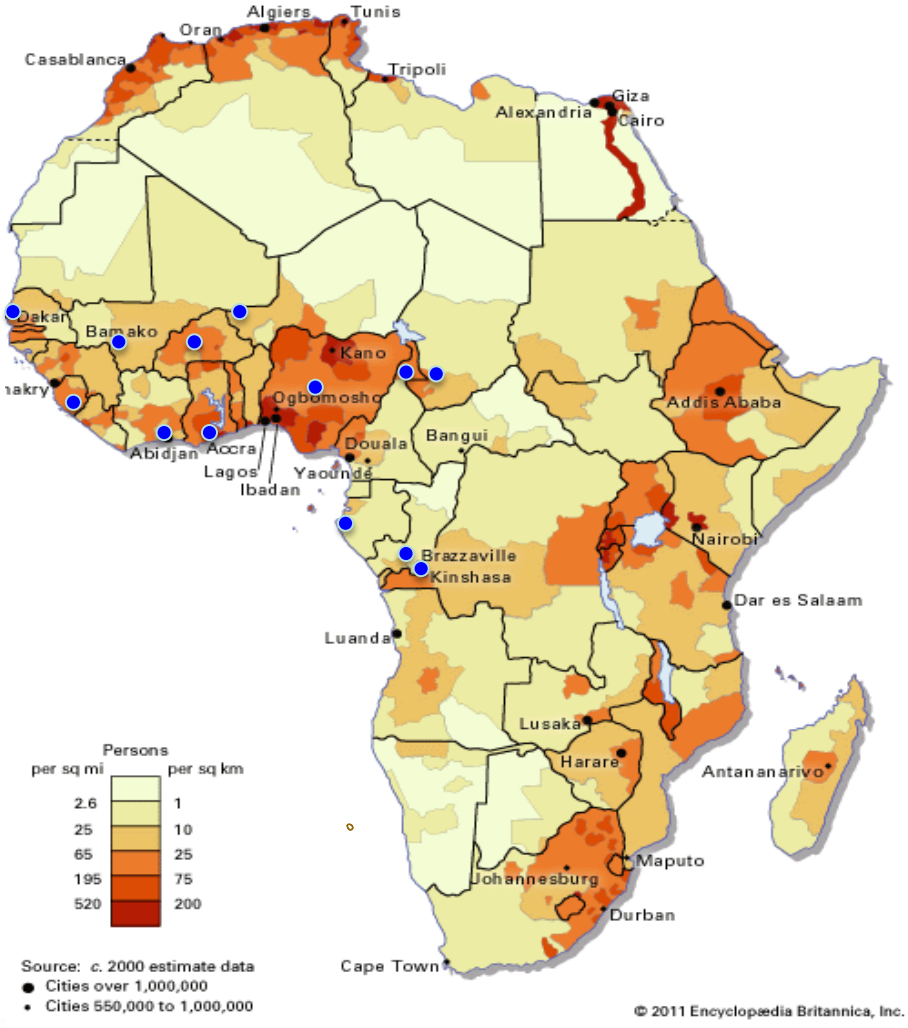


2006 – 2014 trend



Animal suspected samples received from 2012 to 2014





Samples analyzed between 2011 and 2014

Burkina Faso and Nigeria: @ 2,500 samples for @163M estimated population

A dog suspect case per @ 15,000 - 400,000 dogs, according to the estimated average human: dog population ratio

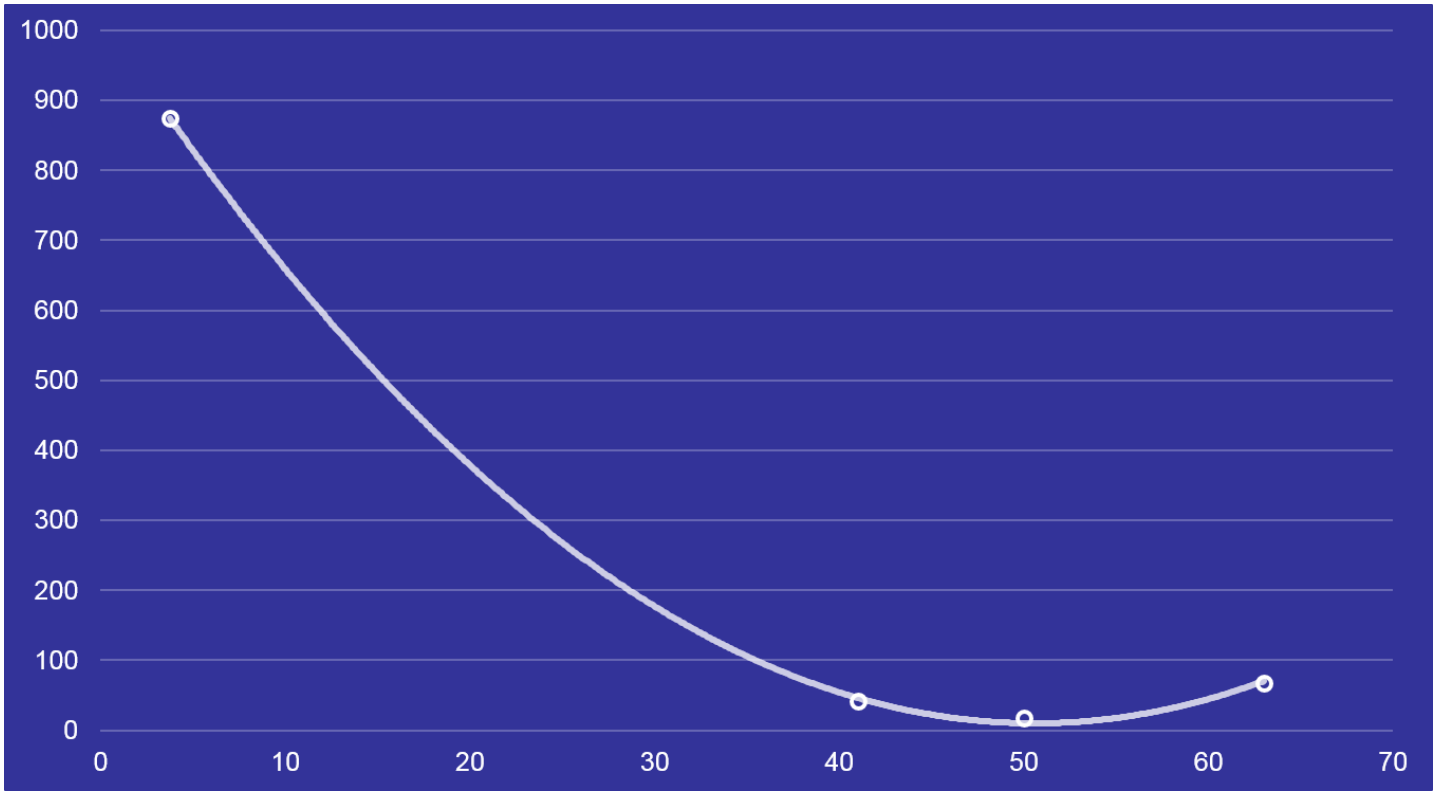
Remaini estimated

Is it a proper surveillance?

Laboratory accessibility

- Most laboratories perceive themselves as easily accessible
- Laboratories claiming to receive virtually all the suspected cases collected from the field operate in smaller countries (@ 278,400 skms)

Cost of testing



Outbreak 1	**Mpaka Area, Mairie de Tié-Tié, Pointe Noire, KOUILOU**
Date of start of the outbreak	15/11/2013
Outbreak status	Continuing (or date resolved not provided)
Epidemiological unit	Not applicable
Affected animals	**Species** / **Susceptible** / **Cases** / **Deaths** / **Destroyed** / **Slaughtered** Dogs / / 1 / 0 / 1 / 0
Affected population	A 5 month-old stray dog captured and showing signs of nervousness and aggressiveness.

June — August — Sept — Oct — Nov — Dec — Jan — Sept

Rabies outbreak suspected in Pointe-Noire

Rabies diagnosis offered on a routine basis

Hands-on Training + Back-to-back Seminars

1 Death

2 Death

1°sample received in LNVB **= Positive**

2°sample received in LNVB **= Positive**

3°sample received in LNVB **= Positive**

Sequencing of the N gene (547 bp)

LNVB: Laboratoire National vétérinaire de Brazzaville

Next steps ?

- Enlarge collaboration with national/international agencies working in the region

- Strengthen the network between focal points > implementing a sustainable support activity within the region

- Improve stakeholder’s recognition of their RESOLAB representatives as disease experts

Contacts

RESOLAB focal points

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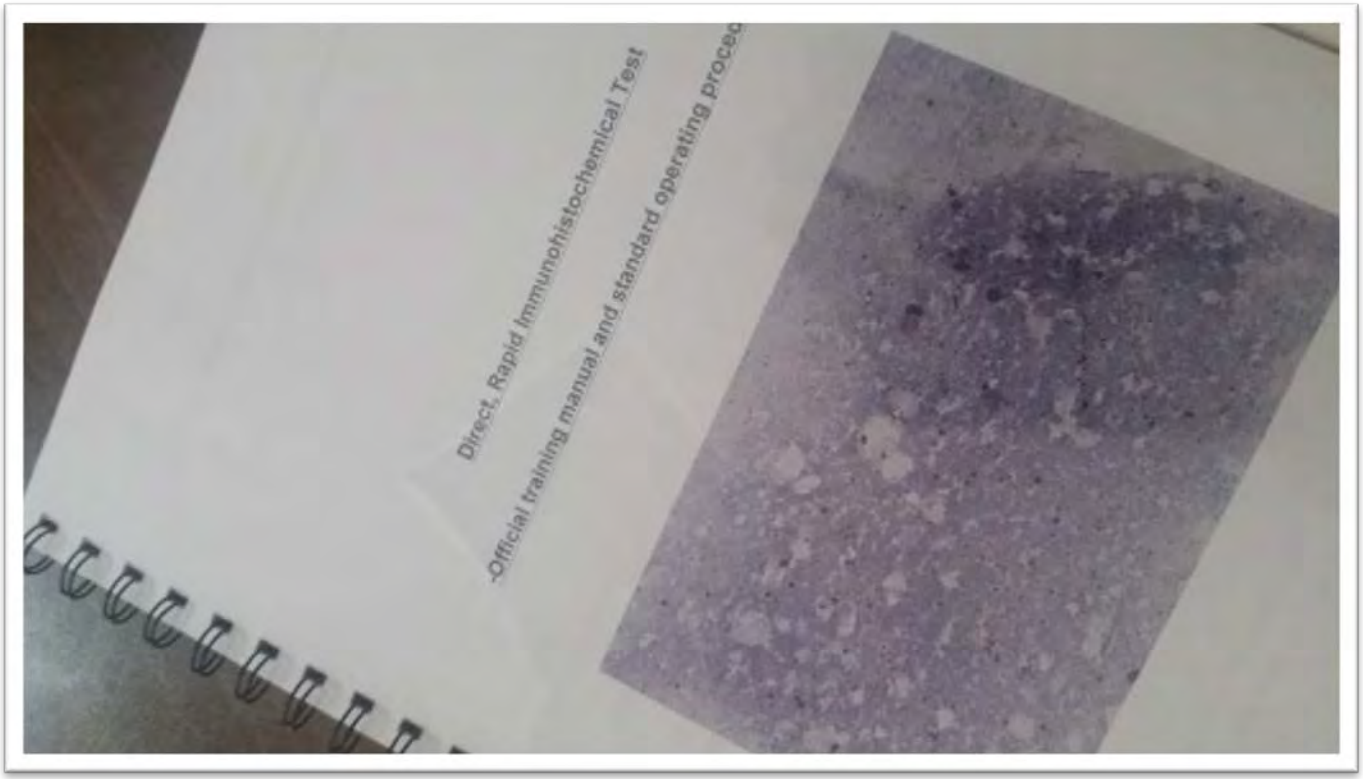
Namibia: DRIT case study



Juliet Kabajani, Central
Veterinary Laboratory (CVL)

Objectives of the study

- To assess the applicability of the DRIT assay as a supplementary test to the gold Standard FAT for the routine diagnosis of rabies.
- To see if this method could be validated and adopted as another suitable method for our local environments.

Where was it carried out?

CVL – Windhoek, Namibia

Training was 2 weeks , 7 people were trained

By Mr A.Coetzer

Test has been used For 15 weeks

DRIT training programme

- The DRIT training was done using the manual that is available on the Rabies Blueprint developed by GARC
- Training techniques used: lectures, practical demonstrations, physical application, duel microscope reading with a trained technician.

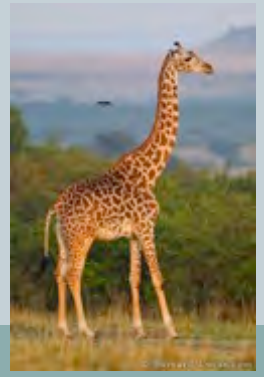





105 Samples screened
(84 positive samples)
From sample bank

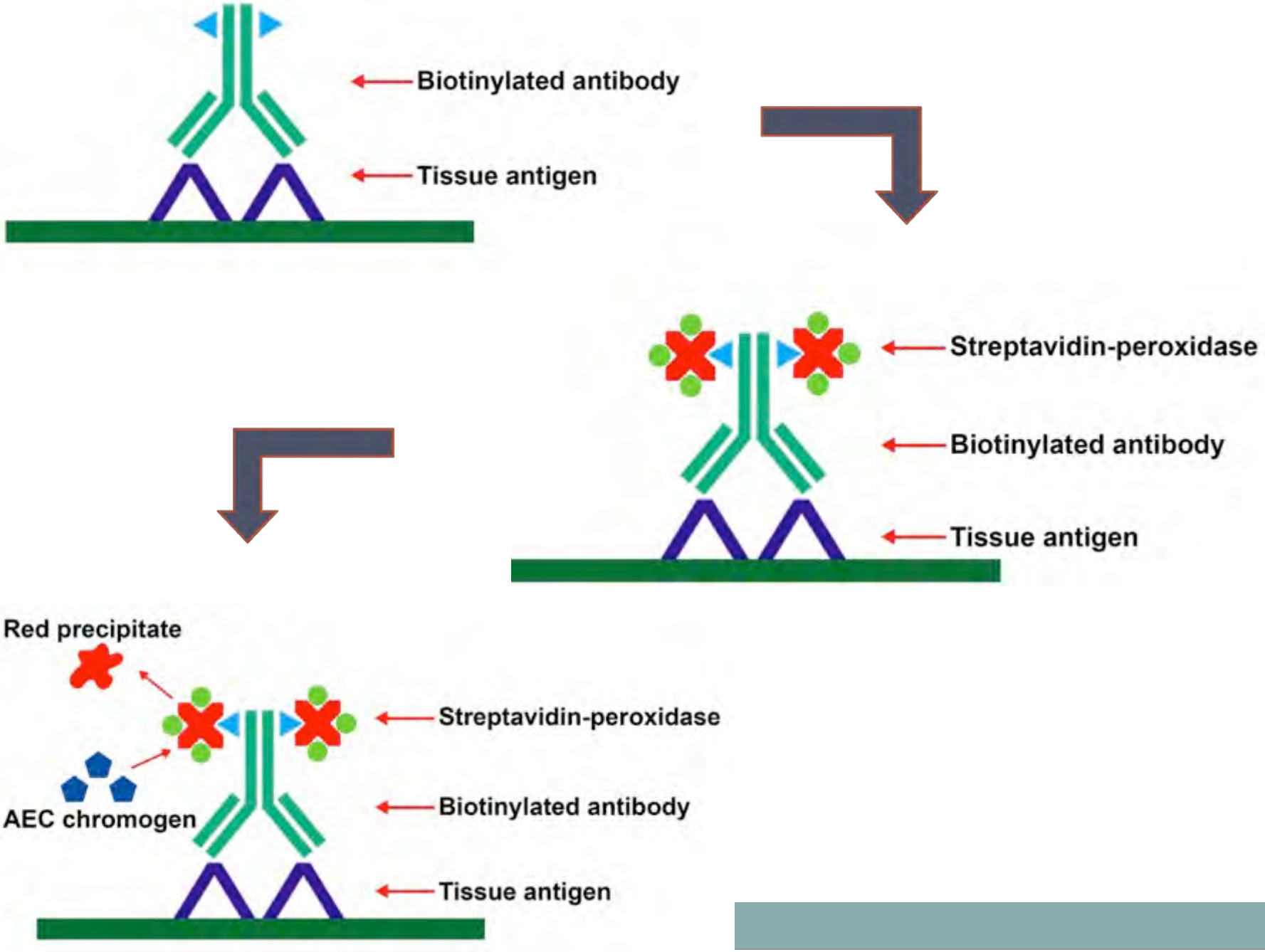




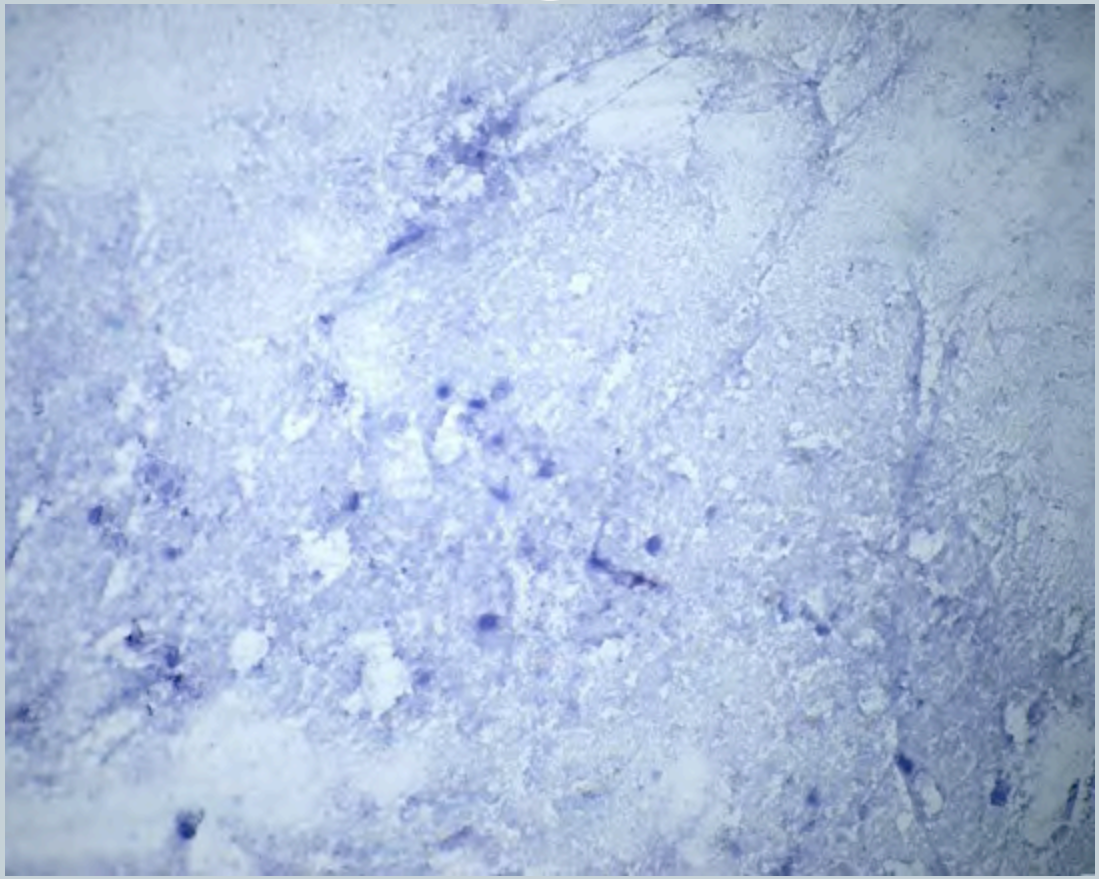




Materials/reagents

- Formalin
- Biotinylated antibody
- Streptavidin-peroxidase
- AEC Chromogen
- Hematoxylin stain
- Tween 80 buffer
- PBS
- 3% Hydrogen Peroxide
- Humid chamber
- Sterile distilled water

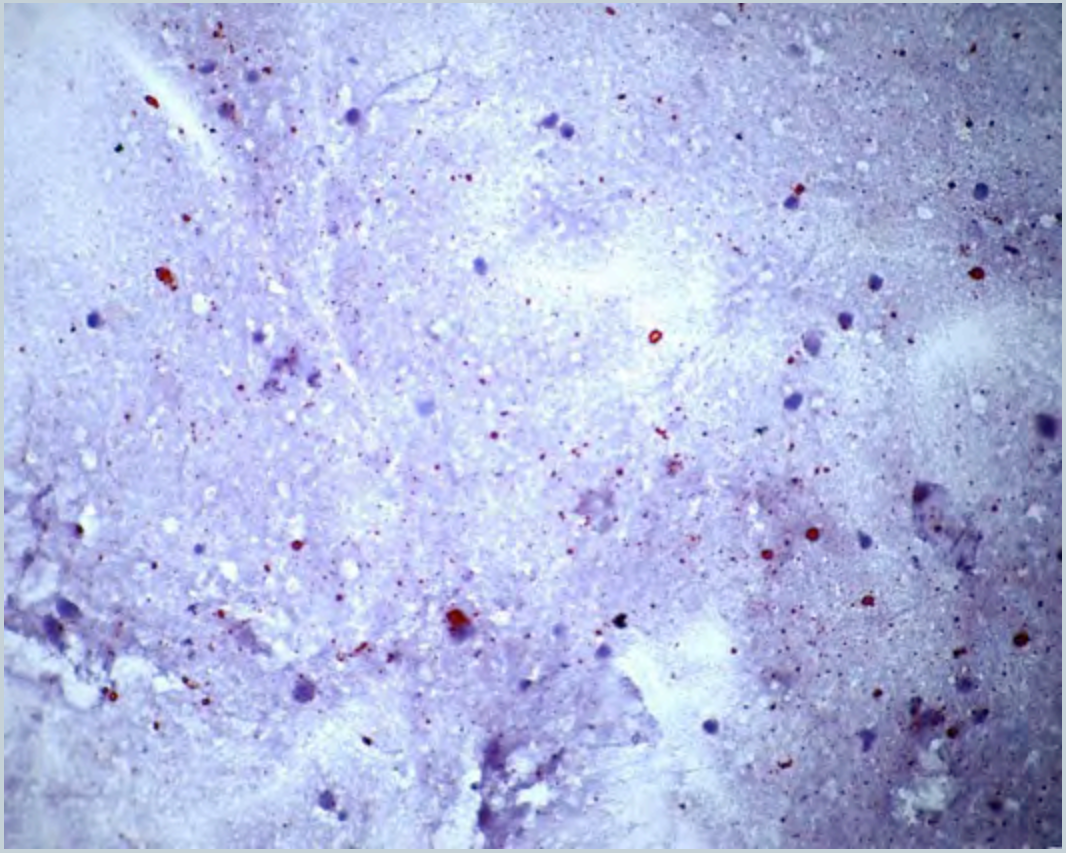

Biotinylated antibody
Tissue antigen
Streptavidin-peroxidase
Biotinylated antibody
Tissue antigen
Red precipitate
AEC chromogen
Streptavidin-peroxidase
Biotinylated antibody
Tissue antigen

DRIT Negative Result



No red insoluble inclusions on the blue background

DRIT Positive Result



Rabies antigen appear as red insoluble inclusions on a blue background

Samples required?

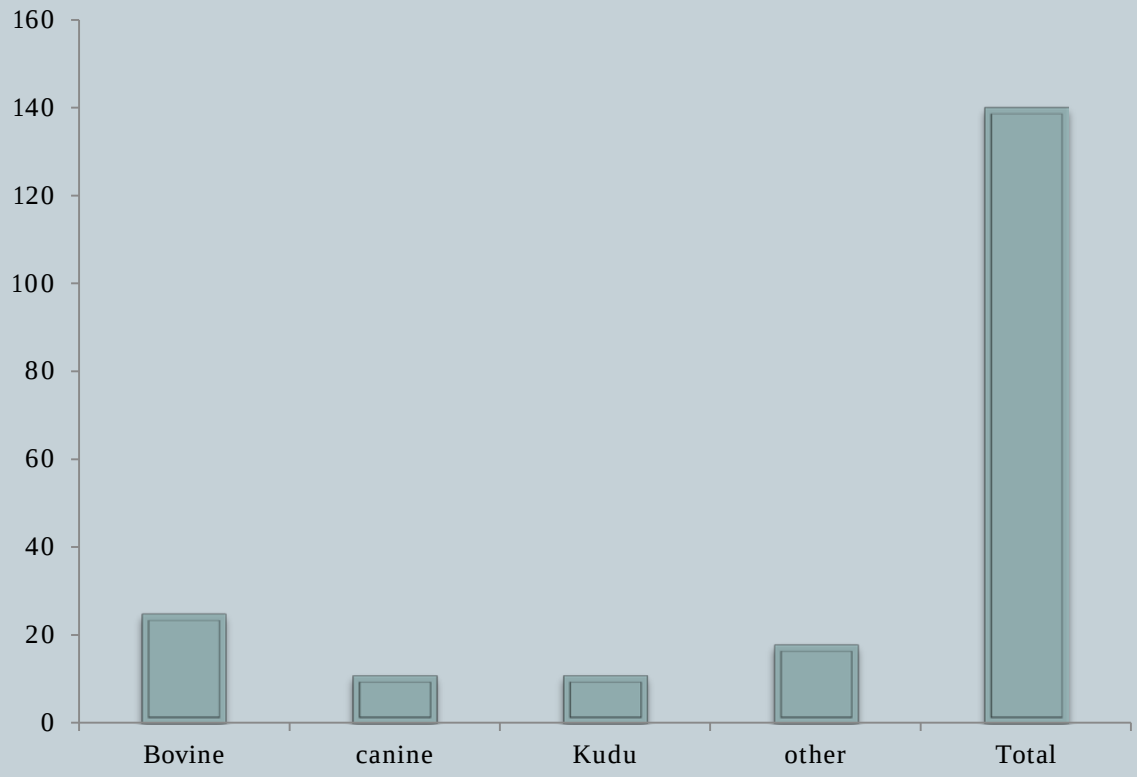
- Fresh brain samples
- Samples preserved in GS
- Frozen samples
- Not formalin preserved samples

Number of samples routinely tested since the training period (September 2014)

Total Number of samples tested	FAT Positive	FAT Negative	DRIT Positive	DRIT Negative
140	66	74	65	75

- Of these samples:
  - Canine positive: 11
  - Kudu positive: 11
  - Bovine positive: 25
  - Other: 18

Positive cases per specie

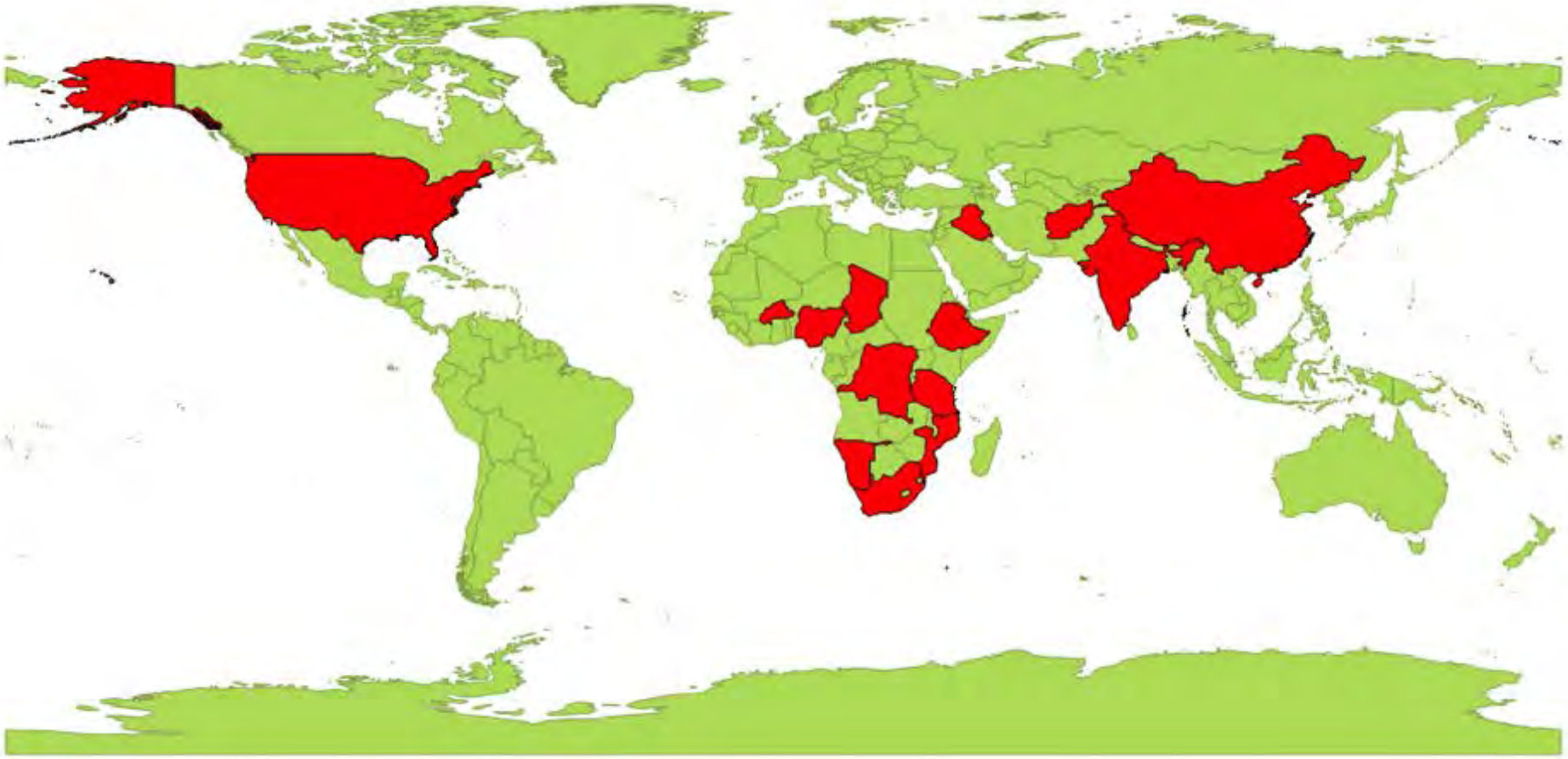


Comparing FAT & DRIT

FAT	DRIT
• Smears fixed in acetone in a freezer at -20°c • Incubation at 37°C (require incubator) • Use a fluorescent microscope to read slides • Reading require good experience and patience	• Smears fixed in formalin at room temp. • Incubation at room temperature • Use normal light microscope to read the slides • Reading require good experience and patience

DRIT advantages

- Excellent diagnostic efficacy
  - Equal to that of the FAT in all studies done to date
- Cost effective
  - Despite throughput, DRIT remains cheaper
- Quicker to perform each diagnostic run
  - 60min
- Influenced less by glycerol preservation
  - Tested numerous times
- Easier to interpret by inexperienced readers



Conclusion

- In the hands of the Namibian staff the DRIT, works just as well as the FAT
- Good training required for implementation by a diagnostician
- Accurate reading can be done by inexperience readers
- It works well even for less established labs
- Its good for doing second verification of result

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Thank you