Diagnosis of Rabies in Humans

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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

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

INFECTION

INCUBATION

PRODROME

ACUTE

DEATH

NO TESTS

RT-PCR, SEROLOGY, VIRUS ISO

FA/dRIT/VI
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
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 ...
OIE Rabies Reference Laboratories

<table>
<thead>
<tr>
<th>World Distribution of OIE Reference Laboratories</th>
</tr>
</thead>
</table>

- 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

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

Trends of discrepant results (2011-2014)

- number of laboratories
- number of false negatives
- number of false positives

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

<table>
<thead>
<tr>
<th>Biotinylated Antibodies</th>
<th>FAT</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>True Positive</td>
<td>False Positive</td>
<td>True Negative</td>
<td>False Negative</td>
<td>Diagnostic Sensitivity*</td>
<td>Diagnostic Specificity*</td>
</tr>
<tr>
<td><strong>Polyclonal Antibody</strong></td>
<td>200</td>
<td>0</td>
<td>49</td>
<td>1</td>
<td>99,5% (97,25% -99,92%)</td>
<td>100% (92,68% -100%)</td>
</tr>
</tbody>
</table>

| 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)

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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.
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.
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
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
- Western and Central African Veterinary Laboratory Network for Avian Influenza and other transboundary disease (RESOLAB): Launched in 2007 thanks 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

- December 2010: the Rabies Subnetwork was created
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
Quarterly reports 2014 for 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

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of canine samples received</td>
<td>90</td>
<td>1 putrefied</td>
</tr>
<tr>
<td>Number of canine samples examined</td>
<td>89</td>
<td></td>
</tr>
<tr>
<td>Positive Cases</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>Negative Cases</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Other domestic animals samples received</td>
<td>7</td>
<td>2 putrefied</td>
</tr>
<tr>
<td>Number of other domestic animals samples examined</td>
<td>5</td>
<td>cats</td>
</tr>
<tr>
<td>Positive Cases</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Negative Cases</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Samples of wildlife received</td>
<td>1</td>
<td>Mouse</td>
</tr>
<tr>
<td>Samples of wildlife examined</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Positive Cases</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Negative Cases</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Total number of samples received</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>Total number of samples examined</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td>Total number of positive cases</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>Total number of negative cases</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>
RABIES DIAGNOSIS IN THE REGION
Rabies diagnostic capability

2010 - 2012

Laboratoire National Vétérinaire (LANAVET), Cameroon

- 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

2010

2012

Reagents, consumables
and equipment supply

2012

Laboratoire National Vétérinaire (LANAVET), Cameroon

(4-8 June and 1-3 August 2012)

National training:
- 13 participants from the Vet Lab
- 1 participant from public health (Pasteur Institute Garoua)

Regional training: 16 participants from the Vet Lab (Botswana, Mali, Burkina Faso, Senegal, Mozambique, Kenya, Tanzania, Ethiopia, Rwanda, Guinea equatorial, Uganda, Gabon, Central Africa Republic, Congo, Cameroon)

Laboratoire vétérinaire de Kinshasa, DRC

(21-25 May 2012)

14 participants from the Vet Lab
7 participants for the LDVB and 1 participant from the Laboratoire National de Santé Publique

Laboratoire de diagnostic vétérinaire de Brazzaville (LDVB), Congo

(3-7 June 2013)

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

Laboratoire National vétérinaire de Libreville (LNVL), Gabon

(10-14 February 2014)

- 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

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
2006 – 2014 trend
Animal suspected samples received from 2012 to 2014

Between 10 and 30 samples
Between 220 and 470 samples
Less than 10 samples
No sample received

Source: c. 2000 estimate data
- Cities over 1,000,000
- Cities 500,000 to 1,000,000

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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

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
Rabies outbreak suspected in Pointe-Noire

- June: Hands-on Training + Back-to-back Seminars
- August: Rabies outbreak suspected in Pointe-Noire
- Sept: 1 Death
- Oct: 2 Deaths
- Nov: 3° sample received in LNVB = Positive
- Dec: 2° sample received in LNVB = Positive
- Jan: Sequencing of the N gene (547 bp)
- Sept: Rabies diagnosis offered on a routine basis

Affected animals: Dogs

Species | Susceptible | Cases | Deaths | Destroyed | Slaughtered
--- | --- | --- | --- | --- | ---
Dogs | | | | | |

Affected population: A 5 month-old stray dog captured and showing signs of nervousness and aggressiveness.

Outbreak 1

Date of start of the outbreak: 15/11/2013
Outbreak status: Continuity (or date resolved not provided)

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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Katinka De Balogh – Senior Officer, Veterinary Public Health katinka.debalogh@fao.org

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
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
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)

<table>
<thead>
<tr>
<th>Total Number of samples tested</th>
<th>FAT Positive</th>
<th>FAT Negative</th>
<th>DRIT Positive</th>
<th>DRIT Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>140</td>
<td>66</td>
<td>74</td>
<td>65</td>
<td>75</td>
</tr>
</tbody>
</table>

- Of these samples:
  - Canine positive: 11
  - Kudu positive: 11
  - Bovine positive: 25
  - Other: 18
Positive cases per specie

number of positive species

<table>
<thead>
<tr>
<th>Specie</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine</td>
<td>20</td>
</tr>
<tr>
<td>Canine</td>
<td>10</td>
</tr>
<tr>
<td>Kudu</td>
<td>5</td>
</tr>
<tr>
<td>Other</td>
<td>3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>160</strong></td>
</tr>
</tbody>
</table>
Comparing FAT & DRIT

<table>
<thead>
<tr>
<th>FAT</th>
<th>DRIT</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Smears fixed in acetone in a freezer at -20°C&lt;br&gt;• Incubation at 37°C (require incubator)&lt;br&gt;• Use a fluorescent microscope to read slides&lt;br&gt;• Reading require good experience and patience</td>
<td>• Smears fixed in formalin at room temp.&lt;br&gt;• Incubation at room temperature&lt;br&gt;• Use normal light microscope to read the slides&lt;br&gt;• Reading require good experience and patience</td>
</tr>
</tbody>
</table>
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 inexperienced readers
- It works well even for less established labs
- It's good for doing second verification of result
Acknowledgements

- Prof. Louis H. Nel – Global Alliance for Rabies Control
- Andre Coetzer – Viral Zoonoses Research Group, University of Pretoria
- Dr S. Khaiseb – Acting DCVO, CVL – Windhoek
Thank you