

# Country Updates on Rabies Diagnostics

**Dr. Shukes Chandra Badhy**

**Central Disease Investigation Laboratory**

**Bangladesh**

## Occipital Foramen approach for brain sampling

- Central Disease Investigation laboratory of Bangladesh uses the Occipital Foramen approach/Straw method of brain sampling in collecting brain samples from rabies suspected animals for rabies diagnosis since 2019.
- Since December 2019, a total of 110 samples found positive for rabies. Rabies have been detected in Cattle, goat, cat, dog and fox. Confirmation was done based on DFAT and few samples using qRT\_PCR. One human rabies have also been confirmed using qRT-PCR.
- Training on this method have not been extended to other laboratories but we have plan to be implemented. In this point technical support may be needed.
- Need Reference positive samples, although we use previously confirmed samples as positive control (Samples that were confirmed by DFAT and qRT-PCR)

## Lateral Flow Immunoassay (immunochromatographic assay)

- No commercially available Lateral Flow Immunoassays (LFA) or rapid diagnostic kits for animal rabies diagnosis are used in Bangladesh.
- Bangladesh is searching for the rapid diagnostic kits or lateral flow Immunoassay but not available to use.

## Direct Fluorescent Antibody test (DFA)

- CDIL of Bangladesh uses both Direct Fluorescent Antibody test (DFA) and qRT-PCR for routine animal rabies diagnosis. The conjugate is from Fujirebio Diagnostics.



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## Country Updates on Rabies Diagnostics

Since

OIE Virtual Training Series on Rabies Diagnosis  
for SAARC Region held on 5-6 November 2020

**Tshewang Gembo**

**Bhutan**

**OIE Virtual Training Series on Rabies Serology for  
SAARC Region**

11-13 October 2021, 2 PM Japan Time (GMT+9)

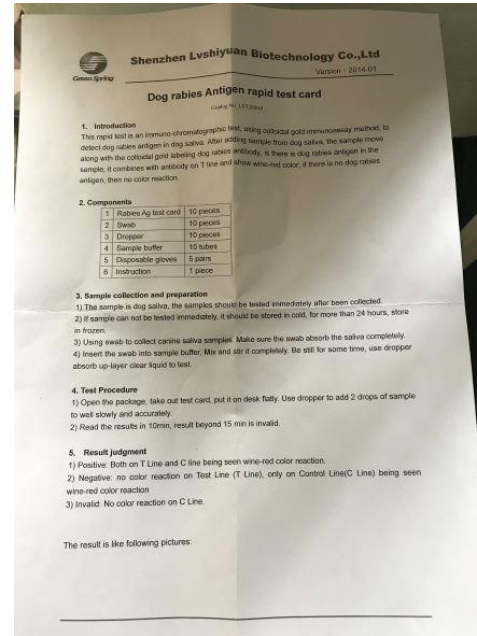
# Lateral Flow Immunoassay- Samples retrieval/collection and testing.



- The brain is exposed after having severed the skull with hacksaw.
- Tested and proper burial of the carcass.
- All procedures are carried out with full PPE.



# Lateral Flow Immunoassay- Rapid Test Kits.



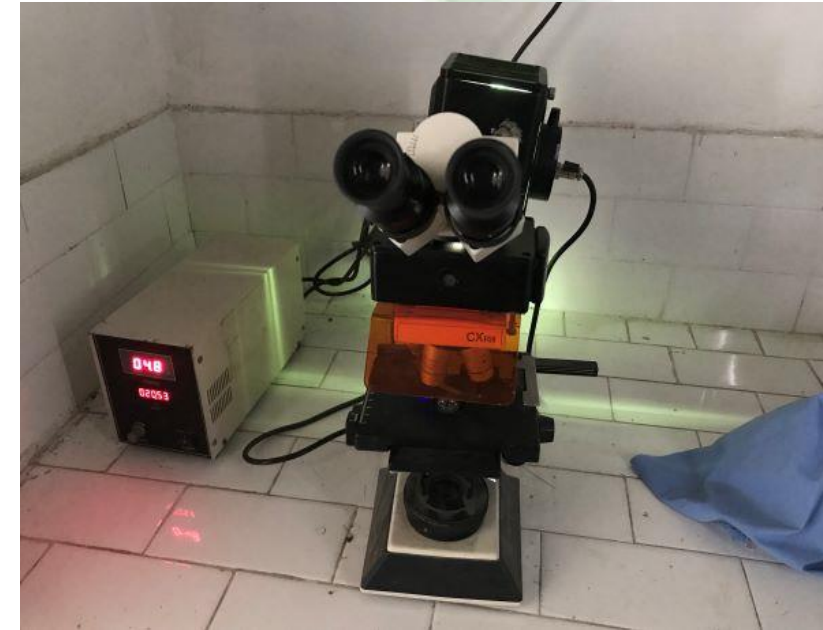
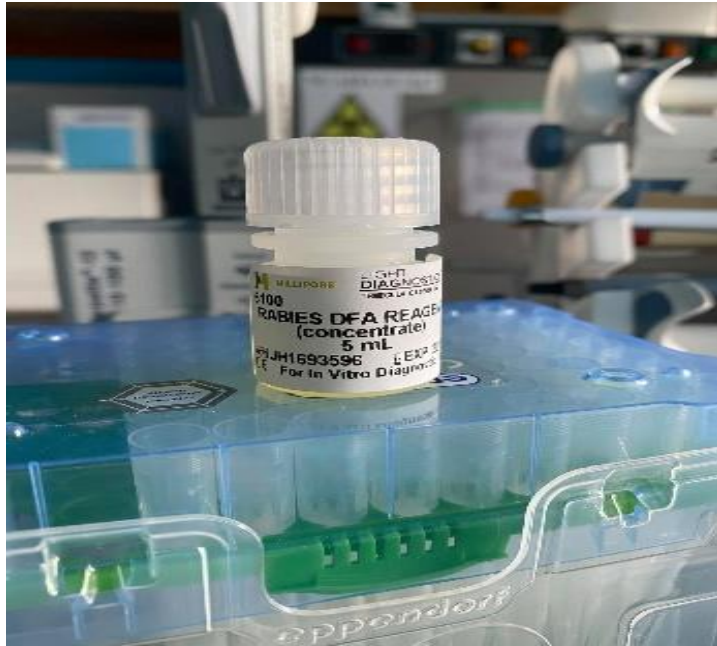
- The rapid test kit supplied depends on the suppliers.
- 10 kits per box along with instruction leaflet.
- Shenzhen Lvshiyuan Biotechnology Co. Ltd version : 2014-01, Green Spring, China.

# Lateral Flow Immunoassay- Rapid Test Kits.



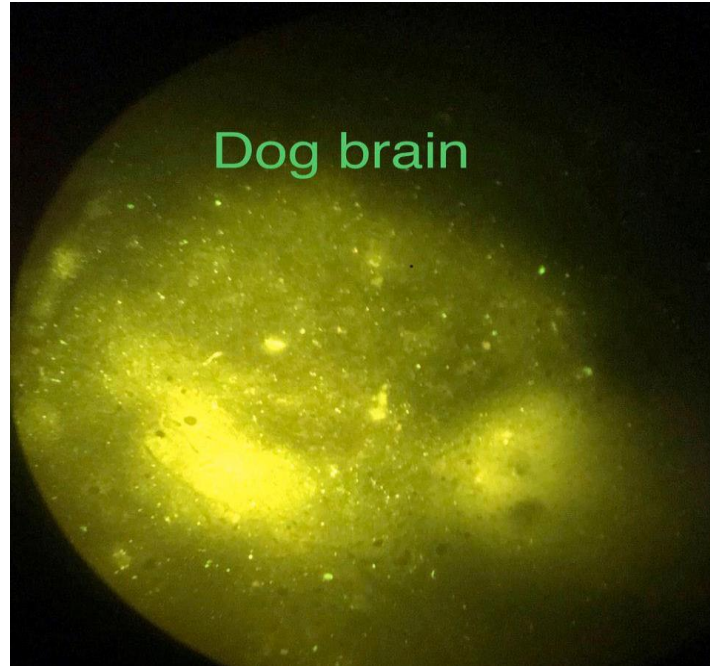
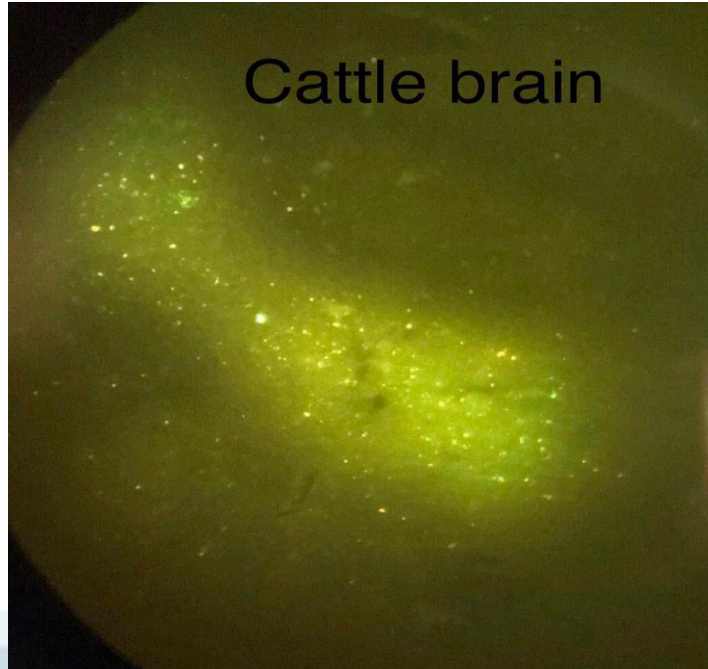
- BioNote Inc, 22 Samsung, Hwaseong-si, Republic of Korea
- 10 kits per box

# Direct Fluorescent Antibody Test( DFA)



- Rabies DFA reagent, Chemicon, Light Diagnostics, USA.
- Use 1:20 dilution of the above said FITC conjugate - approximately 100  $\mu$ l per impression.

# Direct Fluorescent Antibody Test( DFA)



- Observation under the dark field microscope.
- Appreciable fluorescence

## Samples subjected to Rapid Testing and DFA.

Sl. No.	Date received	Sub. No.	Owner	Address	Species	No.of sample	Result DFA	Rapid Test Kit
1	10/1/2021	771	Surjaman Mongar	Lower khaireni, Chukha	Bovine	1	Positive	Positive
2	25/2/21	801	Wangkha central jail	Chukha	Canine	1	Positive	Positive
3	25/2/21	802	Tading	Chukha	Canine	1	Negative	Negative
4	25/2/21	803	Norbugang	Chukha	Canine	1	Positive	Positive
5	25/2/21	804	Yoeseltse	Chukha	Canine	1	Positive	Positive
6	8/3/2021	838	Stray dog	Pasakha	Canine	1	Positive	Positive
7	9/3/2021	840	Stray dog	P/ling	Canine	1	Negative	Negative
8	10/3/2021	842	Stray dog	Dorpany, Samtse	Canine	1	Positive	Positive
9	18/3/2021	862	RBA, Damthang	Haa	Canine	1	Negative	Negative
10	19/3/21	866		P/ling, Chukha	Equine	1	Positive	Positive
11	24/3/21	874	Stray dog	Toorsa, Chukha	Canine	1	Positive	Positive
12	24/3/21	875	Stray dog	Drungkhag area, Chukha	Canine	1	Positive	Positive

- 12 samples were subjected to rapid rabies antigen detection test kit.
- Rapid testing carried out in the field.

# Capacity Building.



- Less than a week ( 12<sup>th</sup> -16<sup>th</sup> April 2021) hands on training on sample retrieval/collection, processing and to diagnose rabies.
- 17 participants , from National Veterinary Laboratory, National Veterinary Hospital, Regional Livestock Development centres, Dzongkhag Veterinary Laboratory.
- Conducted to enhance laboratory capacity and to gear towards achieving global goal of zero deaths from dog mediated rabies by 2030.



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# Country Updates on Rabies Diagnostics

Since

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for SAARC Region held on 5-6 November 2020

Dr. Debalina Mitra  
Assistant Commissioner  
Department of animal Husbandry & Dairying  
Ministry of Fisheries, Animal Husbandry & Dairying  
Government of India

**OIE Virtual Training Series on Rabies Serology for  
SAARC Region**

11-13 October 2021, 2 PM Japan Time (GMT+9)

# Laboratories Working in Rabies Diagnosis in India



# Occipital Foramen approach for brain sampling

- **YES**, following the virtual training in November 2020, India make use of the Occipital Foramen approach/Straw method of brain sampling in collecting brain samples from rabies suspected animals for rabies diagnosis.

No. of brain samples Collected	animal species	locations	Test Performed	positivity rate
434	Canine/Bovine/Feline/ Caprine/Buffalo/ Bullock/Elephant/ Hog Dear/ Horse/ Jackal/ Pig/Pony/Bat	Karnataka/Manipur/ Assam/Gujarat/Kerala	DFA/LFA	324/434 = 75%

# Brain sample collection by field vets using foramen magnum method

– User friendly and simple approach

Fresh carcass:  
use scissor / scalpel



Old / preserved carcass:  
use syringe / AI sheath



# Impact of training on brain sampling (Occipital Foramen) -user friendly for trainees in the field



# Occipital Foramen approach for brain sampling

- No training of Occipital Foramen approach/Straw method of brain sampling has been done after November 2020.
- **Pros:**
  - This is the best method for brain sampling
  - This method is becoming popular in India
- **Cons:**
  - Biosafety issues are there in using at field level
  - certain amount of skill & expertise necessary,
  - The method is not equally efficient in all species.

# Lateral Flow Immunoassay (immunochromatographic assay)

- **YES** following the virtual training in November 2020, India make use of commercially available Lateral Flow Immunoassays (LFA) or rapid diagnostic kits for animal rabies diagnosis.
- Name and company of the LFA Kit used –Rabies Ag Test Kit by **Bionote**
- Brain samples tested, animal species, locations, the positivity rate

No. of brain samples tested	animal species	locations	positivity rate
337	Canine/Bovine/Caprine/Buffalo /Elephant/Hog Dear/ Horse/ Jackal/ Pig/Pony/Bat	Karnataka/Manipur/Assam	249/337 = 74%

# Lateral Flow Immunoassay (immunochromatographic assay)

- No National Training conducted. But over phone provided details with still pictures and Videos .
- State level training was conducted in Kerala
- Experience about Lateral Flow Immunoassay:
  - Most user friendly
  - Simple
  - Sensitive
  - specific
  - Rapid diagnostic tool at the field level
  - minimum skill requirement
  - Good correlation with FAT

# Direct Fluorescent Antibody test (DFA)

- **YES**, following the virtual training in November 2020, India make use of Direct Fluorescent Antibody test (DFA) for routine animal rabies diagnosis.
- Company of the conjugate used for DFA – **Light Diagnostics, Biorad & Millipore**
- No of brain samples tested, animal species, locations, the positivity rate

No. of brain samples tested	animal species	locations	positivity rate
434	Canine/Bovine/Feline /Caprine/Buffalo/Bull ock/Elephant/ Hog Dear/ Horse/ Jackal/ Pig/Pony/Bat	Karnataka/Manipur/A ssam/Gujarat	324/434 = 75%

# Direct Fluorescent Antibody test (DFA)

- No National level training was conducted
- State level training was conducted in Kerala
  
- Any other experiences in the use of Direct Fluorescent Antibody test (DFA)?
  - Putrefied brain samples are unfit to be tested by DFA
  - Very good test for brain sample, not equally good for ante-mortem samples, skill & expertise required, quality of conjugate important

# **Country Updates on Rabies Diagnostics**

Since

**OIE Virtual Training Series on Rabies Diagnosis  
for SAARC Region held on 5-6 November 2020**

Dr. Manju Maharjan

Veterinary Officer

Nepal

## Occipital Foramen approach for brain sampling

- We have been practicing the Occipital Foramen approach/Straw method of brain sampling before the virtual training in November 2020, but the training had added additional information in the procedure.
- 143 brain samples were collected mostly from canine(74), caprine (29), cattle (24), buffalo (11), rat (1), and swine (7)in Central Veterinary Laboratory and five Veterinary Laboratories. All the samples were tested by Lateral immunoassay and about 50% of the samples received by CVL were tested by Direct Fluorescent Antibody test.

## Lateral Flow Immunoassay (immunochromatographic assay)

- We have been using Rabies Ag test kit from Bionote as a commercial kit for Lateral Flow Immunoassays (LFA).
- 143 brain samples were from canine(74), caprine (29), cattle (24), buffalo (11), rat (1), and swine (7) were tested by Lateral and the positivity rate was 33.5%.



## Direct Fluorescent Antibody test (DFA)

- We have been using Lyophilised adsorbed anti-rabies nucleocapsid conjugate from Biorad for Direct Fluorescent Antibody test (DFA) for routine animal rabies diagnosis.
- 20 samples were tested by DFA.



# Training

- A 3 days training on “Strengthening capacity towards rabies elimination in Asia” was conducted at Central Veterinary Laboratory in collaboration with TCP/RAS/3708 from 4<sup>th</sup> to 6<sup>th</sup> February 2021.
- 10 participants participated in the programme.
- The objective of the training was to capacitate veterinary officers from different Veterinary Laboratories in brain sample collection and diagnostic procedures of rabies (LFA, DFAT and Histopathological test).

# Training photos



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Dr. Muhammad Numan

Pakistan

## Occipital Foramen approach for brain sampling

- Following the virtual training in November 2020, did your country make use of the Occipital Foramen approach/Straw method of brain sampling in collecting brain samples from rabies suspected animals for rabies diagnosis?.....YES
- If yes to above question, please elaborate in terms of how many brain samples collected, species, locations, what tests done and what was the positivity rate? Provide still pictures/video of the same if available.....01 BRAIN SAMPLE WAS COLLECTED FROM CATTLE IN FIELD BY HEAD DECAPITATION APPROACH, PUNJAB PROVINCE, SENT FOR RT-PCR TESTING, SAMPLE WAS POSITIVE FOR RABIES. PICTURES ARE APPENDED IN NEXT SLIDES.
- Did you provide further training on above methodology of brain sampling to other staff at national/regional/state laboratories in your country? Please elaborate if any training done.....ONE VETERINARY OFFICER AND ONE VETERINARY ASSISTANT WERE TRAINED IN ABOVE CASE WHO LATER ON COLLECTED BRAIN SAMPLE FROM ANOTHER 01-ANIMAL LATER ON WHICH WAS ALSO DECLARED AS POSITIVE FOR RABIES.
- Any other experiences in the use of above sampling method.....NIL
- If your country did not use the above method, please state the reasons .....NOT APPLICABLE

## Lateral Flow Immunoassay (immunochromatographic assay)

- Following the virtual training in November 2020, did your country make use of any commercially available Lateral Flow Immunoassays (LFA) or rapid diagnostic kits for animal rabies diagnosis? .....NIL
- If yes to above question, please elaborate in terms of name and company of the LFA used, how many brain samples tested, animal species, locations, the positivity rate? Provide still pictures/video of the same if available.
- Did you provide further training on above methodology of rabies diagnosis to other staff at national/regional/state laboratories of your country? Please elaborate if any training done.....NIL
- Any other experiences in the use of above diagnostic tool? .....NIL
- If your country did not use the above diagnostic tool, please state the reasons. ....NIL

## Direct Fluorescent Antibody test (DFA)

- Following the virtual training in November 2020, did your country make use of Direct Fluorescent Antibody test (DFA) for routine animal rabies diagnosis? .....NIL
- If yes to above question, please elaborate in terms of company of the conjugate used for DFA, no of brain samples tested, animal species, locations, the positivity rate? Provide pictures of the same if available.
- Did you provide further training on above methodology of rabies diagnosis to other staff at national/regional/state laboratories of your country? Please elaborate if any training done .....NIL
- Any other experiences in the use of above diagnostic tool? .....NIL
- If your country did not use the above tool, please state the reasons .....NIL









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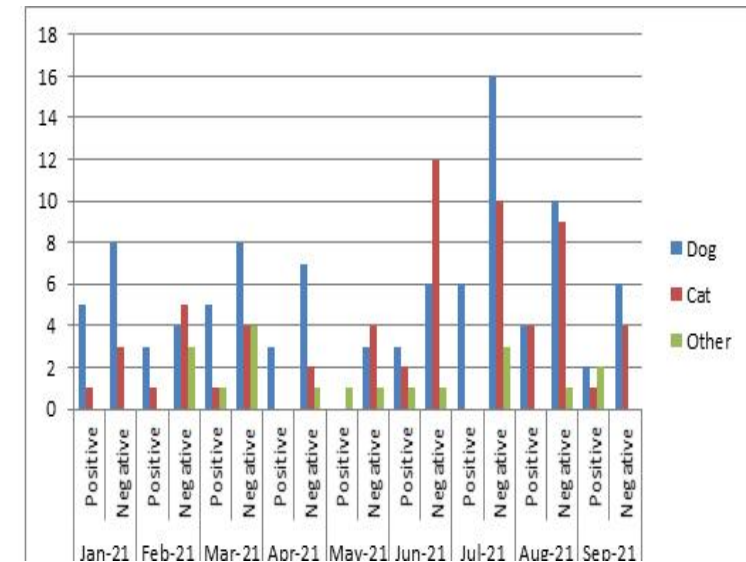
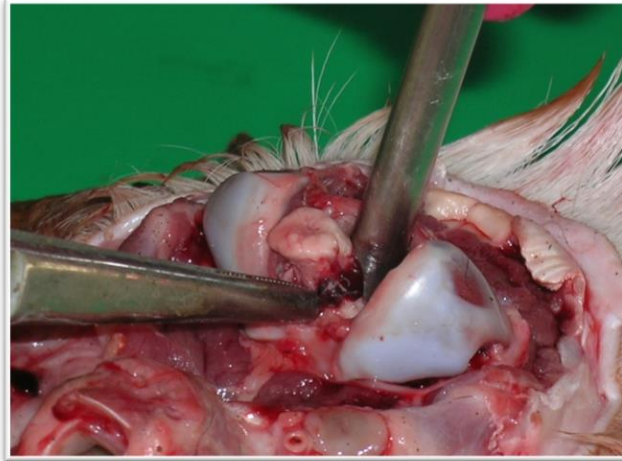
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for SAARC Region held on 5-6 November 2020

**Professor Panduka Gunawardena**

**Sri Lanka**

# Occipital Foramen approach for brain sampling

- Since 2009, we have been practicing occipital foramen (*foramen magnum*) approach using brain scoop, drinking straws and various pipettes for collection of brain samples for rabies diagnosis.
- We have trained some veterinary investigating officers and field veterinary surgeons on this technique.
- However, the field level use of this technique is limited and practiced only for a few large animal species such as cattle and goats.
- Recently (after November 2020) we have not received any samples collected using this technique.
- One probable reason is most of the laboratory submissions are of dog and cat samples sent by the general public.
- **Reference:** Gunawardena, G.S.P. de S. (2010) Brain spoon technique: a simple technique for the collection and dispatch of brain samples for rabies diagnosis. Sri Lanka Veterinary Journal, Vol. 54&55, 17-19.
- <https://vet.pdn.ac.lk/rdu/rcu.html>



# Lateral Flow Immunoassay (immunochromatographic assay)

- We have been using the Antigen rapid rabies Ag test kit (BioNote, Republic of Korea) since 2015 for diagnosis of dog and cat rabies.
- We have found 100% sensitivity and specificity in this kit when used for diagnosis of rabies in dogs.
- We have trained some veterinary investigating officers on using this kit.
- However we do not use them at present due to unavailability of the kits .
- **Reference:** Samarasinghe, D.V.S.M., Asiriwardena, M.U., Jayaweera, W.R., Obayashi, Y., Hiko, T. and Gunawardena, G.S.P. de S. (2016) Sensitivity and specificity of the Anigen Rapid Rabies Antigen Test Kit in the diagnosis of rabies in dogs. Book of Abstracts, Forth Conference on Sri Lanka-Japan Collaborative Research, 19-21 August 2016, University of Peradeniya, 35.



# Direct Fluorescent Antibody test (DFA)

- We have been using Direct Fluorescent Antibody test (DFA) for routine animal rabies diagnosis since 2007.
- We use FITC Anti-Rabies Monoclonal Globulin (FUJIREBIO Diagnostics, Inc. USA) for the DFA.
- We have trained some veterinary investigating officers on this technique.
- More information on animal rabies in Sri Lanka can be found in our Rabies Diagnostic Unit website <https://vet.pdn.ac.lk/rdu/rcu.html>

