

ORIGINAL RESEARCH ARTICLE

DIAGNOSING RABIES CASES IN ANIMALS THROUGH COLLECTION OF BRAIN SAMPLE THROUGH FORAMEN MAGNUM IN SHIVAMOGGA DIST

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Introduction

Rabies is regarded as one of the most important zoonotic disease in the world. Commonly known as hydrophobia in man, it is a viral disease that affects the central nervous system (CNS) of humans and warm blooded animals.

World Health Organization (WHO) categorizes rabies as a Neglected Tropical Disease (NTD). India has highest burden of rabies with annual animal bite of 17 million cases and 20000 (1/3rd of global) human mortality. Globally, dog has been identified to cause 99 percent human deaths (one death every 15 minutes; 4 children of every 10 human mortality).

Rabies in livestock is always the result of bite by an infected animal of other species, usually of the canine, feline or vampire bat species. Animal bites introduce the virus into muscle and nerve ending rich tissues from which it penetrates into nerve cells where it replicates and progressively travels through the spinal cord to the brain. This process usually requires weeks or even months, depending upon the distance from the site of the bite to the brain.

A rabid dog biting the grazing livestock is common in India. Livestock are curious about strange animals and intend to smell. Thus, the great majority of livestock are bitten on the nostrils and face and rarely on legs. The nostrils are unprotected and well supplied by sensory nerve endings.

The onset of rabies in livestock is marked by prodromal symptoms which last from a few hours to one or two days. In this period, a clinical diagnosis of rabies is very difficult. There may be a temperature rise of one to three degrees and a general picture of malaise, anorexia and abrupt cessation of lactation. The affected animal usually show marked depression which may be readily confused with some intestinal disturbance. Rabid livestock are rarely belligerent and have a tendency to stay away from other animals. Therefore, a good history and the knowledge of prevalence of rabies in the community are invaluable which may save human exposure as well as subsequent treatment. Although there may not be visible salivation, the saliva of majority of rabid animals, at this stage and until death bears fully virulent rabies virus. In fact, the saliva may carry virus four to five days prior to the onset of symptoms. The vast majority of human exposures occur during this early stage when one is groping for a diagnosis. The resulting disease is usually a consequence of the failure of animal owners to vaccinate their animals.

The livestock production the major agriculture industry in almost all area in which rabies is known to exist in India. Livestock are particularly vulnerable to attack by carnivora and livestock losses from rabies correspond quite closely to the intensity of the infection in these animals. One species of animal vector cannot be incriminated to the exclusion of the other, because a rabid animal will bite any moving object, animate or inanimate which may

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come within its vision. If livestock are in the immediate vicinity, 12 to 15 may be bitten by one rabid animal, as evident by death of one after another in rapid succession.

The outbreaks of rabies in animals during the years 1996 to 2004 reported to the World Organisation for Animal Health (OIE, 2011) from India is 586 outbreaks among different species of animals of which 377 account for cattle this lead to the death of 2,463 animals, which comprising of 1542 cattle during this period. Subsequently, (OIE, 2013) 398 outbreaks of rabies in animals were reported during the period 2005 to 2011.

Disease Name	Species	Number of Outbreak	Number of Attack	Number of Death
Rabies	Bovine	28	258	258
	Buffalo	4	25	25
	Ovine/Caprine	10	41	41
	Canine	23	41	41
	Total	46	383	383
Source: Basic Animal Husbandry & Fisheries Statistics (2018-19), GOI				

Many areas of the country did not report any case during this long period of 7 years despite the endemic status of rabies there. The actual numbers, therefore, may be substantially higher considering the possibility of under reporting due to weak rabies surveillance and inadequate reporting mechanism in the country.

Rabies reporting is essentially based on laboratory confirmation of clinical cases. OIE recognized recognizes laboratory confirmed report of rabies animal. Absence of laboratory reports on animal rabies masks the magnitude of rabies, jeopardizing the strategies for its prevention and control in human population

Brain sample collection from rabies suspected animals for laboratory confirmation is tedious the zoonotic risk to the field veterinarians and public deters sample collection from most of the animals dead after showing rabid signs. Thus, many full blown cases went unreported owing to the lack of laboratory evidence.

Now, that the WHO has set the goal of global elimination of dog mediated human rabies by 2030, the wild life and field veterinarians of Karnataka are expected to contribute to the WHO campaign by strengthening rabies reporting in animals, based on OIE compliant laboratory evidence. Underreporting of rabies in animals has serious threat to livestock and human. Since Karnataka is bounded by 6 states, and possess a wild range of wildlife in the national, sanctuaries and western ghat area, understanding the ecology of rabies in parallel to laboratory confirmation will go a long way in control and prevention of rabies in animals. And, that's where the One Health steps in into the domain of rabies.

Diagnosis of Rabies: The past and present

Diagnosis of rabies in the past was based on the post mortem demonstration of Negri bodies in brain tissue smeared by seller's stain technique and histopathology with H&E stain.

Hippocampus was the ideal sample collected by opening the skull of the rabies suspected animal. Considering the high zoonotic potential of rabies virus and associated limitations, wildlife and field veterinarians found it hazardous to collect appropriate sample for laboratory confirmation of clinically evident cases.

Of late, efficient and simple technique for collection of brain tissue from suspected cases by the Foramen Magnum approach and dispatching to the referral laboratory for confirmation by Direct fluorescent Antibody Assay (DFA) and Direct Rapid Immunohistochemistry (dRIT) (100% sensitivity) is being promoted by the OIE phasing out the Seller's technique (66% sensitivity), Further, the sample can be tested by Immunochromatography assay (Lateral

Flow assay) kit (Antigen detection) on the spot, facilitating adoption of immediate preventive measures for human and animal exposed to the Rabid case.

Objectives of the project

1. Collecting the brain sample through the Foramen Magnum approach by conducting portmortem of animals suspected to died of rabies and taken care of proper disposal of the carcass. Further, the sample can be tested by Immunochromatography assay (Lateral Flow assay) kit (Antigen detection) on the spot, facilitating adoption of immediate preventive measures for human and animal exposed to the Rabid case.
2. Sending the brain sample to OIE Reference Laboratory for Rabies, Department of Veterinary Microbiology, Veterinary College, Hebbal, Bengaluru. For further confirmation by DFA , dRIT and PCR
3. Creating awareness among public and school children by organizing seminars and visiting school, conducting essay, quiz, and poster competition activities
4. Organizing “World Rabies Day” for creating awareness among veterinarians about latest development in research on rabies, further all the veterinarians and animal handler of the dept Animal Husbandry are Pre exposure prophylactic vaccinated against Rabies.

Requirements for brain tissue collection from animals by foramen Magnum approach:

A. Personal Protective items:

1. Hand gloves
2. Full arm gloves (AI gloves)
3. Mask
4. Head cap
5. Goggles
6. Disposable apron/ Full length gynae apron

B. Sample collection- instruments

1. Disposable B.P Blade with handle
2. Tissue forceps
3. A I Sheath
4. 10 ml disposable syringe

C. Sample collection and transportation

1. Zip lock sample collection pouch
2. 50 ml sample/ specimen collection plastic bottle- screw –capped
3. Tissue paper- sufficient quantity
4. Thermocol box (for shipping to the referral laboratory) with biohazard symbol pasted on the sides
5. Ice packs- sufficient quantity
6. Gum tape(cello tape)
7. Marker pen

D. Additional requirements

1. History sheet of the case
 Particulars of the animals Pet/wild.....
 Species: Breed: Sex: Age:
 Location of the Animal- Farm / Field / Open range
 Address of the owner: Phone Number:
 Treatment given the animal, if any :
 Vaccination History:
 Duration illness:
 Any other relevant Information:
2. Letter of request to diagnose the sample, address to the
 Director
 OIE Reference Laboratory for Rabies
 Department of Veterinary Microbiology,
 Veterinary College, Hebbal. Bengaluru- 560024

**Procedure of brain sample collection and packing for submission to the rabies diagnostic Laboratory:
 The sample(s) must be packed in triple layers before shipping to the diagnostic laboratory:**

1. Primary layer:

Collect fresh brain tissue samples- cerebellum and brain stem in a leak proof plastic zip lock pouch. Do not add any buffer/ glycerol buffer/ NSS / PBS etc. just pack the brain sample as such and seal the zip by cello tape securely.

2. Second layer:

- a. Put the sealed zip locked sample pouch in to a screw- capped plastic specimen container with multiple layers of tissue paper to absorb any leakage from the sample pouch
- b. Close the screw- capped container tight and seal it with cello tape thoroughly
- c. Write the date of sample collection with wash proof on the body of the sample container.
- d. Put the history sheet in another plastic zip lock pouch and seal it with cello tape and fasten it on the body of the sample container.

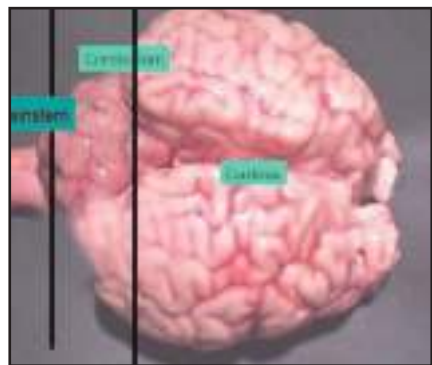
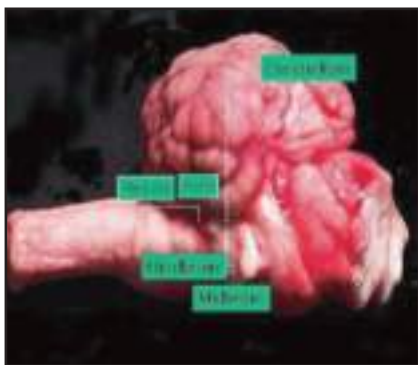
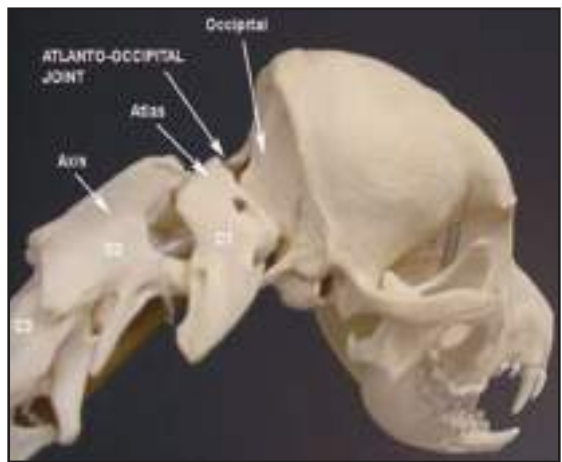
3. Tertiary layers (Shipping package)

- a. Put the sample container and the history sheet in a sturdy thermocol box with ice or frozen gel packs all around the sample and seal it immediately with multiple layers of cello tape.
- b. Past the address of the sender and receiver with biohazard symbols prominently on the shipping package.

Immunochromatography assay (Lateral Flow assay)

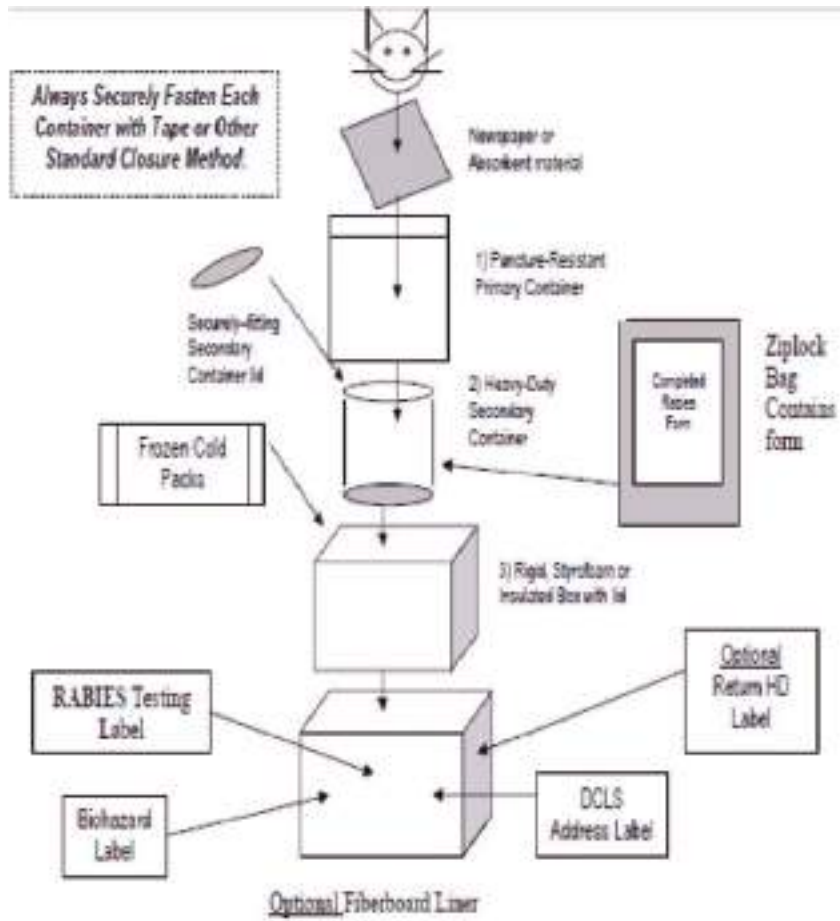
This is a rapid immunodiagnostic test, a useful method for rabies diagnosis through detection of RABV antigen in post mortem samples without the need for laboratory equipment on the spot using the Antigen Rapid Rabies Ag Test Kit of BIONOTE, Korea, as per manufactures instruction Protocol:

- The brain tissue be mixed with equal quantity of the assay diluents in a micro centrifuge tube, A negative control be also simultaneously tested
- The test device be placed on horizontal surface and four drops of virus diluents mixed be added to the sample well
- The result are read within 5-15 min
- Presence of two bands in the result window at position “T” (Test sample) and “C” (Control) indicate the presence of virus



Brain sample collection :
Conventional V/s Occipital
foramen approach

Lateral Flow assay – WHO support



Basic requirements for packing brain sample



Packing brain tissue in Primary and Secondary containers



Placing brain tissue in a zip lock bag (Primary container) and sealing
Placing sealed zip lock bag in secondary container
Placing absorbent material in secondary container



Sealing secondary container and enclosing Submission Form in a ziplock and taped outside



Zip lock bag with submission form taped outside secondary container



Secondary container placed inside the shipping container along with sufficient frozen cool packs
Sealing the shipping container having Biohazard symbol

Sealing the shipping container having Biohazard symbol

Expected result

1. In due course, the reporting of the Rabies disease will increase so the better surveillance and monitoring will leads to better understanding of the disease epidemiology helps in planning of prevention and control measures. Aid in archiving zero death of Rabies by 2030
2. Community involvement helps in responsible ownership of pet and livestock
3. Reduces dog bite victims in children

Expected result

Estimated budget for one district range and expected of 100 rabies cases per year

1 Materials for collection of brain sample and transportation to OIE Reference Laboratory for Rabies

a. Personal Protective items:

- | | |
|--|------------------|
| 1. Hand gloves , (10 pares per case) | Rs 50*10= 500=00 |
| 2. Full arm gloves (AI gloves) (10 pares per case) | Rs 5*10 =50=00 |
| 3. Mask(10 per case) | Rs 15*10=150=00 |
| 4. Head cap (10 per case) | Rs 15*10=150=00 |
| 5. Goggles (10 per case) | Rs 15*10=150=00 |
| 6. Disposable apron/ Full length gynae apron | Rs 25*10=250=00 |

b. Sample collection- instruments

- | | |
|---|----------------|
| 1. Disposable B.P Blade with handle | Rs 50*4=200=00 |
| 2. Tissue forceps | Rs 50*4=200=00 |
| 3. A I Sheath (available in Veterinary Hospitals) | |
| 4. 10 ml disposable syringe (available in Veterinary Hospitals) | |
| 5. Sanitizer (70% alcohol) | Rs100=00 |

c. Sample collection and transportation

- | | |
|---|-----------|
| 1. Zip lock sample collection pouch | Rs 10=00 |
| 2. 50 ml sample/ specimen collection plastic bottle- screw –capped | Rs 50=00 |
| 3. Tissue paper- sufficient quantity | Rs50=00 |
| 4. Thermocol box | Rs 100=00 |
| 5. Ice packs- sufficient quantity (available in Veterinary Hospitals) | |
| 6. Gum tape(cello tape) | Rs 50=00 |
| 7. Marker pen | Rs 20=00 |

- d. Disposal of the carcasses including transportation of Dead animal to graveyard, digging of pit and spraying of sodium hypochloride solution for large animals **Rs 4000=00**
for small animals and dog **Rs 2000=00**

- e. Purchase of LFA kit **Rs 1000=00**

- f. Courier charges **Rs 500=00**

Total Rs 7500=00

For 100 cases 6000*100 Rs 750000=00

2. For conducting seminars/ classes for public and school children

a. Purchase of projector with screen	Rs 300000=00
b. Purchase of laptop	Rs50000=00
c. Purchase of mic and speaker	Rs 50000=00
d. Organizing cost (tea& snacks)	Rs 10000=00
Total	Rs 410000=00

3. Organizing “World Rabies Day”and PreEP for veterinarians and Animal handlers

a. Organizing cost (study materials, tea, snacks with lunch)	Rs 20000=00
b. Cost of vaccination for 200 persons (1st year 2 dose on day 0 and 7th day route of injection by Intra dermal 2-0-2-0-0. Second and subsequent years 2-0-0-0)Rs 100*200person*2 times =	Rs 40000=00
c. Cost of insulin syringe Rs 10/- per, for 800 syringe =	Rs 8000=00
d. Cost of surgical sprit, cotton and miscellaneous cost	Rs 1000=00
e. Cost of remuneration to medical staff for vaccination (for 2 times)	Rs 5000=00
Total	Rs 74000=00

4. Travel and miscellaneous charges

Rs 60000=00

Total cost of the project for 1year in one district 1294000=00