

## Case Report

# From a suspected rabies death to definitive diagnosis: the crucial role of forensic medicine in a rural setting

Tushar Saini<sup>1</sup>, Bhanu Bhardwaj<sup>2</sup>, Omesh Kumar Bharti<sup>3</sup>, Sanjay Chavan<sup>4</sup>,  
Dimple Kasana<sup>4</sup>, Rajeev Goel<sup>5\*</sup>

<sup>1</sup>Department of Forensic Medicine, Civil Hospital, Nurpur, Himachal Pradesh, India

<sup>2</sup>Department of Pathology, Dr. R. P. Government Medical College, Kangra, Himachal Pradesh, India

<sup>3</sup>Zonal Hospital, Kangra at Dharamshala, Himachal Pradesh, India

<sup>4</sup>Rabies Research Centre, Central Research Institute, Kasauli, Himachal Pradesh, India

<sup>5</sup>Department of Biophysics, Dr. R. P. Government Medical College, Kangra, Himachal Pradesh, India

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### \*Correspondence:

Dr. Rajeev Goel,

E-mail: [rgoel302@yahoo.com](mailto:rgoel302@yahoo.com)

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## ABSTRACT

This case report highlights a rabies mortality confirmed through post-mortem analysis in a 43-year-old man in a rural area of Himachal Pradesh, India. The deceased didn't seek post-exposure prophylaxis following a dog scratch, on his right hand, which was ignored by him. The post-mortem analysis included different diagnostic tests for confirming rabies along with histopathology investigation. The case demonstrates a unique and critical convergence of forensic medicine and disease prevention, as the confirmed post-mortem diagnosis led to the timely public health action in terms of mass animal immunization. This particular case also highlights the importance of timely access to anti-rabies vaccine (ARV) and public health education in terms of information, education and communication (IEC). Concurrently, it signifies the critical medico-legal role of forensic medicine in facilitating the deceased's underprivileged family's eligibility for financial compensation under the ongoing government scheme for accidental deaths.

**Keywords:** Rabies, Negri body, Fluorescent antibody test, Mouse inoculation test, Forensic medicine, Public health, Mass animal immunization, Rural health care

## INTRODUCTION

Rabies is a zoonotic viral disease caused by the RNA virus (Genus *Lyssavirus*, Family *Rhabdoviridae*). It is most commonly transmitted through saliva of an infected animal, mainly through a bite or scratch.<sup>1</sup> Domestic dogs are the main cause of rabies in India and worldwide. However, any warm-blooded animal can carry and transmit the infection. The most common animals that spread rabies besides dogs are wild carnivores, bats and other domestic animals like cats, monkeys etc.<sup>2</sup> Rabies causes an estimated 59,000 human deaths globally each year, with Asia and Africa contributing about 95% of these deaths.<sup>3</sup>

The available data indicates that a striking 36% of rabies deaths happen in India alone, and with the majority attributable to dog bites.<sup>4</sup> The lack of awareness is the main cause of these avoidable fatalities despite the wide availability of life-saving anti-rabies vaccines and immune-globulins.<sup>5</sup> Rabies is entirely preventable through timely intervention of post-exposure prophylaxis (PEP) using anti-rabies vaccine (ARV) and rabies immunoglobulin (RIG).<sup>6</sup> It, however, is almost invariably fatal once clinical symptoms develop.<sup>7</sup> This article reports a fatal rabies case from a rural area in Himachal Pradesh, a hill state in India and outlines the forensic, diagnostic, and medico-legal processes that confirmed the death due to

rabies and facilitated financial compensation for the victim's family.

## CASE REPORT

The deceased, a 43-year-old man, reportedly sustained a dog scratch on the fingers of his right hand which was ignored by him, according to information provided by the police and family members. He did not receive any post-exposure anti-rabies prophylaxis or vaccination. He developed hydrophobia and agitation approximately twenty-four days after the alleged exposure, raising a clinical suspicion of rabies encephalitis. He was evaluated at multiple healthcare facilities including a tertiary advance care center. However, his condition progressively deteriorated despite medical attention. The patient died at home on the 27th day following the scratch. The next day, his body was brought to the Civil Hospital, Nurgpur, Himachal Pradesh, India, for medico-legal post-mortem examination.

### Postmortem findings

**External injuries:** Superficial unscabbed reddish-brown abrasions likely due to falls during agitation were observed; General Condition: Rigor mortis was present, fixed postmortem livor mortis observed, no decomposition features were seen.

**Internal examination:** All the organs were congested and showed features of pulmonary edema. Food particles were present in the bronchus of both lungs which were suggestive of aspiration.

### Sample collection for confirmatory tests

Based on the clinical history of hydrophobia, agitation, and a reported dog scratch on the fingers of the right hand, rabies was considered in the differential diagnosis. However, no evidence of a dog bite or scratch was

identified during the post-mortem examination. Brain tissue samples were collected as a routine procedure after obtaining prior informed consent from a family member of the deceased. The samples were subsequently submitted for laboratory confirmation of suspected rabies infection. The brain tissue was preserved under sterile conditions while maintaining the cold chain. It was sent to Central Research Institute (CRI), Kasauli, Himachal Pradesh for the fluorescent antibody test (FAT), biological test (B.T.) and to the Pathology department of Government Medical College, Kangra at Tanda, Himachal Pradesh for histopathology analysis of Negri bodies formation if any, because the facilities for the same were not available at the civil hospital, Nurgpur. The FAT test is the global "gold standard" diagnostic test recommended by the World Health Organization (WHO). The Central Research Institute (CRI), Kasauli, Himachal Pradesh, is the main government-designated center in Himachal Pradesh, India for conducting the FAT test for definitive confirmation of rabies on brain samples. The traditional biological test (B.T.), also known as Mice Inoculation Test (MIT) was also conducted for the brain tissue of the deceased at CRI, Kasauli to validate the diagnosis of rabies.

### Diagnostic investigations

#### Histopathology studies

The Histo-Pathological sections of cerebellar tissue taken from the deceased person showed granular cell layer, Purkinje cell layer and the molecular layer (Figure 1). The Purkinje cell layer shows Negri bodies, which are classic diagnostic hallmark and pathognomonic feature of rabies virus infection. These are characterized by round to oval eosinophilic inclusions in Purkinje cells cytoplasm which are clearly visible, in the present case on hematoxylin & eosin (H & E) staining (Figure 1). The Negri bodies formation in different brain areas remains the gold standard for postmortem confirmation of rabies virus infection.<sup>8</sup>

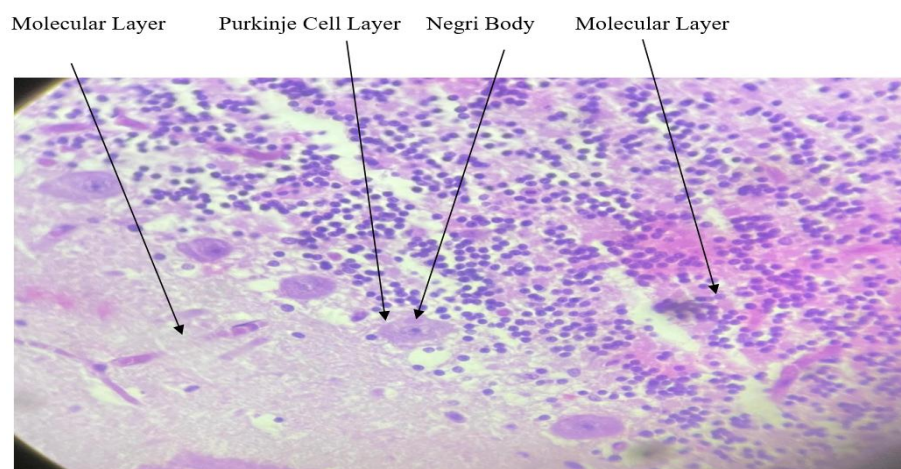


**Figure 1: Histo- Pathology section of cerebellar tissue showing Negri body formation in the Purkinje cell on hematoxylin & eosin staining.**

### **Direct fluorescent antibody test**

Direct fluorescent antibody (DFA) test also known as FAT (Fluorescent Antibody Test) is another gold-standard test for rabies diagnosis.<sup>9</sup> It detects rabies virus nucleoprotein antigen in fresh or frozen brain tissue- specifically targeting brainstem and cerebellum- using fluorescein isothiocyanate (FITC)-conjugated anti-rabies antibodies.<sup>10</sup> The positive antigen-antibody binding, under fluorescence

microscopy, is identified by characteristic intense apple-green fluorescence.<sup>10</sup> The post-mortem cerebellar tissue, in the present case, harvested from the deceased was processed for the DFA test as per the standard protocol.<sup>9</sup> The fluorescence microscopy, in the present case, demonstrated discrete, intracytoplasmic apple-green intense fluorescence of aggregated nucleocapsids within the cerebellar tissue, consistent with the presence of rabies virus antigen and confirming the diagnosis of rabies (Figure 2).

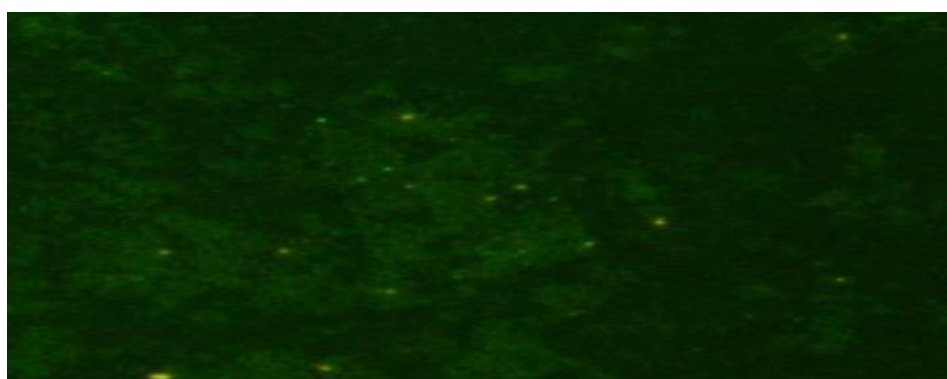


**Figure 2: The brain impression smear obtained from the deceased showing apple- green fluorescence.**

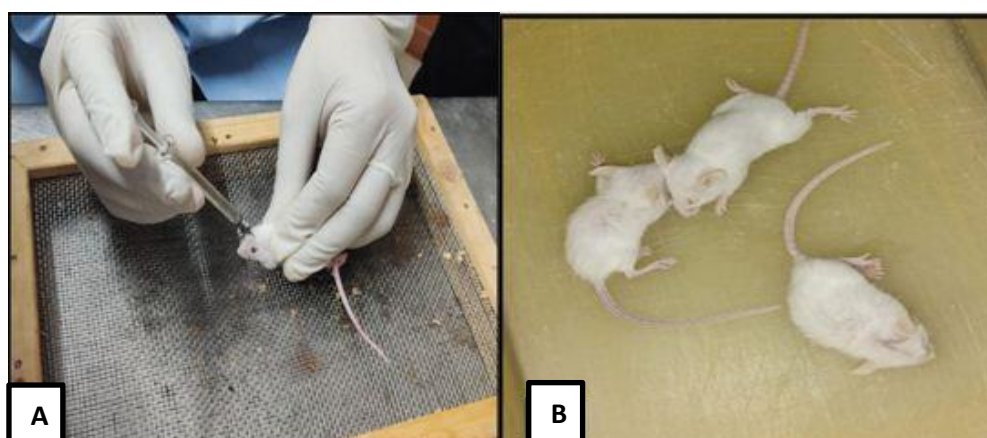


**Figure 3: (A) Intra-cerebral inoculation of mice with the diseased brain sample; (B) mice exhibiting clinical signs of rabies following intra-cerebral inoculation.**

### **Biological test for rabies diagnosis**

Historically, the mouse inoculation test (MIT) was considered a gold-standard bioassay for rabies diagnosis. The test involves intracerebral inoculation of clinical specimens into 3–4-week-old Swiss albino mice, followed by daily monitoring for neurological manifestations and subsequent confirmatory examination of brain tissue from affected animals.<sup>11,12</sup> In the present case, brain tissue homogenate was inoculated intracerebrally into a group of six mice, which were observed for up to 21 days following inoculation as per the standard protocol.<sup>13</sup> Animals developing clinical features suggestive of rabies, including tremors, ruffled fur, ataxia, and paralysis, were humanely euthanized, and brain tissue was collected for confirmatory

fluorescent antibody testing (FAT).<sup>11</sup> Deaths occurring within the first 5 days post-inoculation were considered nonspecific, whereas clinical signs consistent with rabies were first observed from day 6 onwards. The affected mice subsequently developed progressive neurological manifestations, including tremors, ruffled fur, ataxia, and paralysis, as illustrated in Figure 3.

### **DISCUSSION**

The case illustrates the crucial collaboration between forensic medicine, pathology, public health authorities, and reference laboratories. The confirmation of the cause of death in this case was possible only because of the integrated approach. The confirmation was legally

essential for death certification, epidemiological tracking, and accessing compensation under government scheme for accidental deaths.

The confirmation of rabies through the confirmatory tests such as DFA, B.T. and Negri bodies formation in the brain tissue of the deceased finally led to a significant public health response. It illustrates how a single confirmed rabies death can become a catalyst for a community-wide preventive action in a rural area. The local administration, following the post-mortem confirmed cause of death as rabies to health authorities, initiated mass vaccination campaign in the concerned rural block. The veterinary teams from the state department of animal husbandry carried the vaccination campaign, targeting stray and pet dogs of the area. Besides this, the awareness drives on dog-bite prevention, wound washing, school and village-level IEC sessions and the importance of timely administration of anti-rabies vaccine were promptly undertaken in the concerned rural block. Further, the laboratory confirmation of rabies through post-mortem investigations provided objective scientific evidence linking the death directly to an animal exposure. Such confirmation eliminated diagnostic uncertainty and facilitated accurate certification of the cause of death, which is required by the insurance providers and for the government accidental death compensatory scheme. It, thus, strengthened the legal basis for compensation claims by the deceased's family.<sup>12,13</sup>

This case also highlights the importance of public health messaging and the critical role of Information, Education and Communication (IEC) strategies in rabies prevention. The deceased's lack of awareness regarding wound washing, first aid measures, and anti-rabies vaccination (ARV) resulted in failure to seek timely post-exposure medical care despite having access to free of cost ARV and rabies immunoglobulin (RIG) at health centres. The robust IEC strategies like regular school and community-level awareness campaigns, involvement of grass root accredited social health activists (ASHA) and Panchayati Raj Institutions (rural local self-government bodies) in India, training of health workers in counseling dog-bite victims, posters, local radio/TV announcements and dog-bite reporting protocols should therefore be initiated and implemented to prevent avoidable rabies deaths, in rural areas of India and South Asia, which bear a substantial portion of global rabies burden

## CONCLUSION

This case represents a confirmed rabies death because of non-compliance with post-exposure prophylaxis (PEP). It highlights the medico-legal role of a forensic medicine in establishing the transition from a suspicious to a confirmed cause of death due to rabies through the initiation of appropriate laboratory investigations such as histopathology, DFA and biological testing. Further, the post-mortem rabies confirmation could ultimately support the compensation claims of the deceased's family. Beyond it, the case also underscores the urgent need for robust

information, education, and communication (IEC) initiatives, coupled with inter collaboration between healthcare providers, forensic experts, and diagnostic laboratories. Such coordinated efforts are essential to improve rabies surveillance, timely PEP intervention and reducing preventable rabies deaths in rural areas in India and South Asia.

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