

Case Report

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Case Report

# Transnasal Brain Sampling for Human Rabies Confirmation

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**Abstract:** Rabies remains a significant global threat, yet accurate estimations of its impact are hindered by the lack of confirmatory diagnoses. Postmortem confirmation of rabies traditionally involves invasive brain tissue testing, a process met with resistance from deceased patients' families, impeding consent. This paper presents and evaluates an innovative yet unpublished transnasal approach for postmortem brain tissue collection, offering a minimally invasive, easier, faster, and safer method. This method preserves the cadaver's integrity, potentially easing family reluctance towards autopsies. The limited testing of both human and animal rabies in Ghana highlights the challenges in diagnosing this fatal disease. Scarce diagnostic resources and the complexity of obtaining brain tissue samples exacerbate the issue. Cultural and religious beliefs surrounding autopsies contribute to familial hesitation, viewing these procedures as disruptive and disfiguring, further complicating consent. The transnasal technique involves approaching the brain tissue through the nostrils and cribriform plate without any superficial manipulation of the patient's head and face, thereby preserving aesthetics and natural features of the person. Technological advancements and seamless One Health collaboration among governmental, non-governmental, and research entities locally and globally culminated in Ghana's first confirmed rabies diagnosis using this method of brain tissue collection. This success emphasizes the efficiency and feasibility of transnasal brain collection approach and the invaluable role of the One Health approach and collaborative efforts in overcoming diagnostic challenges in rabies control.

**Keywords:** rabies; brain tissue; diagnosis; autopsy; transnasal

## 1. Introduction

Rabies, an infectious disease mainly caused by the rabies virus, presents a significant global health concern, leading to an estimated 59,000 preventable human deaths annually (1). This disease predominantly affects rural populations in Eastern Asian and African countries, including the most vulnerable demographic comprising children under 15 years of age (2,3). Human rabies manifests

with certainty of fatality upon the development of clinical signs and symptoms. However, it is crucial to underscore that rabies remains preventable through the timely administration of post-exposure prophylaxis (PEP). The overwhelming majority, approximately 99%, of human fatalities due to rabies stem from bites inflicted by rabid dogs (4). Research and previous experience indicate that a sustained intervention centered around canine vaccination stands as a crucial requisite for the successful elimination of rabies (5).

### *The Challenge of Human Rabies Confirmation*

The World Health Organization (WHO), World Organization for Animal Health (WOAH) and Food and Agriculture Organization of the United Nations (FAO) have established a milestone aiming to eliminate dog-mediated human rabies by 2030 employing the One Health approach—a synergistic collaboration between animal, human and environmental health system and aligning with the Sustainable Development Goals described by the United Nations (6). Achieving this target necessitates precise data for informed decision-making, efficient post-exposure prophylaxis, and rigorous monitoring mechanisms to comprehensively evaluate the progression of initiatives, such as the paramount mass vaccination of canines (5).

For the control of infectious diseases, a precise diagnosis is fundamental to accurately gauge the disease's impact and track intervention effectiveness. A significant challenge hindering progress in the global and regional rabies control activities is the scarcity of confirmed data regarding rabies-related fatalities, leading to a cycle of neglect of the disease. On the African continent, a region attributed for more than a third of all global human rabies deaths, countries are confronted with challenges when it comes to the accurate confirmation of human and animal rabies cases (7). In Ghana, the health service reported only a limited number of laboratory confirmed rabies cases (8,9). Otolorin and colleagues (10) discovered in Nigeria that among 78 reported cases of rabies-related deaths, none were confirmed through laboratory testing. Similarly, a retrospective study in Malawi observed that 11.5% (3 out of 26) cases initially diagnosed as cerebral malaria were, in fact, instances of human rabies (11).

## **2. Materials and Methods**

The Northeast Region, established in 2019 as one of Ghana's six newly created regions, has 2.2% (658,946) of the country's population comprising 322,149 males and 336,797 females, among which 296,448 are children under 15 years of age (12,13). The region has two municipalities, four districts, and a total of 128 health facilities under diverse ownership including government, mission, and private agencies.

Since October 2022, the region has been actively employing the One Health approach in managing incidents of dog bites through a close collaboration between the regional Ghana Health Service and the Directorate of Veterinary Services. This work is part of their interventions towards rabies control in the region. Samples from suspected human rabies cases undergo analysis at both the Accra Veterinary Laboratory and the Kumasi Centre for Collaborative Research (KCCR), research center affiliated with the Kwame Nkrumah University of Science and Technology.

### *2.1. Case Report*

The patient, a female aged 63 years reported to the outpatient department (OPD) of the Binde hospital, a community health facility in the district on the 4<sup>th</sup> of April 2023 with complaints of ear ache for one week. There was a history of dog bite on the 25<sup>th</sup> of March 2023, approximately 10 days prior to reporting. The dog had died four days after the bite, so the patient had approached Binde hospital for care. The patient did not receive PEP as she assumed she needed health insurance which had expired, although the vaccine was available free of cost. On the 9<sup>th</sup> of June 2023, 11 weeks following the bite, the patient reported to the Faith Community Hospital, a private health facility in the same district with complaints of vomiting, excessive sweating, inability to drink water and fear of water. A provisional diagnosis of rabies was made based on history and clinical signs. The patient

was referred to the Baptist Medical Centre, a larger health facility, for isolation and palliative care until death.

The scarcity of pathologists capable of performing the required autopsy, compounded by geographical barriers and funding limitations, posed significant challenges. The lead author of this report, on learning the news of a possible rabies death, attempted to bring the only willing pathologist in Ghana with the expertise of brain tissue collection who was approximately 900 kms away and would require 17 hours by road. Stymied, Dr. Moses Djimatey contacted collaborators who put him in touch with Dr. Anita Mahadevan, Professor and Head, Department of Neuropathology, National Institute of Mental Health, and Neurosciences, Bengaluru, India, who guided him with an alternate method of brain collection, the trans-nasal approach.

## 2.2. Autopsy

Dr. Moses Djimatey, along with three assistants, wore recommended personal protective gear appropriate for autopsies (4). Sterile tissue holding forceps were introduced into each nostril and gently advanced upwards and cranially until encountering resistance at the cribriform plate. Upon encountering this resistance, additional force was cautiously applied until a yielding point was discerned. The gradual manipulation of the tissue forceps resulted in the extraction of brain tissue, which was then carefully deposited into individual sample bottles from each nostril. The procedure was repeated 2-3 times to ensure adequate samples of brain tissue were obtained. Each nostril was then carefully packed with gauze soaked in alcohol to prevent leakage from the biopsy site and to serve to disinfect the site. All personnel handling the cadaver and brain tissue were previously immunized against rabies.

The execution of this technique was guided remotely by Dr. Anita Mahadevan via WhatsApp® call from Bangalore, India. Following the collection process, the samples underwent a triple packaging procedure and were placed on ice packs housed in cooler boxes. The samples were then transported to two different destinations: The Kumasi Centre for Collaborative Research in Tropical Medicine at the Kwame Nkrumah University of Science and Technology and the Accra Veterinary Laboratory located in the capital city of Accra for further testing.

## 2.3. Laboratory Diagnosis

The samples were diagnosed using RT-PCR at the Kumasi Centre for Collaborative Research using Rabies virus primers and probes and the method as described by Faye et al. (14). The sample was also tested at Accra Veterinary Laboratory using the One-step RT-PCR pyro method described by De Benedictis and colleagues (15). This protocol can be used for lyssavirus detection and typing in samples of both human and animal origin. This protocol is based on RabForPyro and RabRebPyro-*biot* primers. The results from both laboratories confirmed the brain tissue to be positive for rabies.

## 3. Discussion

This is the first case of human rabies confirmed by laboratory diagnosis in postmortem brain tissue obtained via transnasal biopsy without the need to open the skull to reach brain tissue. Limited availability and accessibility of diagnostic facilities and tools pose a significant obstacle, compounded by the difficulty in obtaining brain tissue samples from deceased individuals. Cultural and religious beliefs strongly deter acceptance of autopsies, as they are perceived to disturb the peaceful rest of the deceased, cause disfigurement and fear of delayed funeral, leading to reluctance among family members to provide consent (16–18).

Introducing the transnasal approach for brain tissue collection emerges as a simple and effective solution, described here for the first time. This novel method circumvents disfigurement, leaving no visible alteration to the deceased and can be performed at the bedside. It offers a feasible and culturally sensitive alternative, potentially applicable globally. Physicians play a pivotal role in advocating for this method, emphasizing its necessity for disease confirmation while assuring families of its relatively non-invasive nature (19). Suitable training of medical professionals to

emphasize the importance of diagnosis and awareness of this simple bedside procedure will help in addressing the families' concerns and obtain consent.

Confirmation of a rabies diagnosis holds significant value for the formulation of effective post-exposure prophylaxis (PEP) strategies, catering not only to the affected family members but also to medical practitioners involved in patient care. Furthermore, the inherent simplicity, rapidity, and reduced risk of viral exposure associated with the diagnostic method emphasize its potential for extensive integration into global human rabies diagnosis protocols. This broader implementation not only facilitates PEP planning for other victims and offers solace to bereaved families but also serves as a valuable tool for gathering epidemiological data to establish the burden of the disease in the country which is crucial for the implementation of preventive and protective measures targeting both animal hosts and human populations.

The success of this first human brain collection in Ghana from a 63-year-old woman in June 2023 paved the way for another collection from a 4-year-old boy in August 2023 reiterating the feasibility and efficiency of the collection method.

#### 4. Conclusions

The transnasal approach stands as the recommended and optimal method for post-mortem brain tissue collection in human rabies diagnosis. Technological advancements, coupled with easy communication avenues and extensive intersectoral collaborations among governmental, non-governmental, and research entities—both local and international—led to the landmark achievement of the first confirmed rabies diagnosis in Ghana using this simple postmortem sample collection method. This milestone underscores the invaluable benefits derived from cross-sector cooperation and collaborative efforts, showcasing the profound impact of One Health and collaborative efforts in advancing disease diagnosis and control measures in rabies control.

**Author Contributions:** M. Djimatey\*<sup>1</sup> Lead investigator and initiator of this work. Involved in concept development, sample collection and manuscript development and writing. A. Abubakar<sup>1</sup> Biomedical scientist and laboratory consultant on the sample collection, packaging, and transportation. Contributed to concept development. A. Sylverken<sup>2</sup> Analyser of the sample at the Kumasi Centre for Tropical Medicine of the Kwame Nkrumah University of Science and Technology. Contributed to manuscript development. T. Odoom<sup>4</sup> Veterinary laboratory scientist and analyser of the sample at the Accra veterinary laboratory. Contributed to manuscript development. B. Abubakari<sup>1</sup> Supervisor of the Ghana team for the investigation team. Contributed to concept and manuscript development. J. Ohemeng<sup>5</sup> Member of the of the concept development team and contributed to manuscript development. Gowri Yale<sup>6</sup> Member of the concept development team, writing and reviewing of the of the manuscript. Frederic Lohr<sup>7</sup> Coordinator of the team outside Ghana and a member of the concept development team. Contributed to the manuscript writing and reviewing. Luke Gamble<sup>7</sup> Supervisor of the investigation team outside Ghana and part of the concept development team. Anita Mahadevan<sup>8</sup> Source of the methodology and instructor for undertaking the sample taking process. A member of the concept development team. Also contributed to manuscript development and writing.

**Informed Consent Statement:** Informed consent was obtained from the family of the deceased.

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**Conflicts of Interest:** The authors declare no conflict of interest and the funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results".

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