**TITLE: Molecular characterisation of human rabies in East Africa - a case series report and phylogenetic investigation.**

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**SUMMARY (For IDP case report, 228 words)**

**Background:** Rabies continues to represent a significant public health problem in low- and middle-income countries. However, the number of human rabies deaths laboratory-confirmed is relatively low, particularly in Africa. In this study, we use rabies virus sequence data to enhance investigations into a series of five human rabies deaths in East Africa and discuss the implications of these at the individual, healthcare, and societal levels.

**Case presentation:** The epidemiological context and care of these cases are markedly disparate. three of the bite victims did not receive any post-exposure vaccinations to prevent the fatal onset of disease, despite one attending a health facility on the day of exposure; one received only their first post-exposure vaccination; and the last received timely post-exposure vaccination but followed a vaccination timeline that is not recommended by the World Health Organization (WHO). These differences raise concerns about health-seeking behaviour, competency of healthcare professionals in handling rabies exposures and accessibility and effectiveness of post-exposure prophylaxis as it is administered in these settings.

**Result and conclusion:** Our investigation confirms dog-mediated rabies as the cause of each of these deaths. The viral genomic data highlight the transboundary circulation of rabies within domestic dog populations across the region. We conclude that urgent action is needed to improve awareness around the need for emergency post-exposure prophylaxis that should be accessible in local communities and administered appropriately, as well as investment in coordinated dog vaccination to control dog-mediated rabies, the underlying cause of these deaths.

**BACKGROUND**

Domestic dogs are the source of almost all of the 59,000 human rabies deaths that occur every year globally [[1,2]](https://www.zotero.org/google-docs/?0boMEm). In East Africa between 1500-2000 human deaths from dog-mediated rabies are estimated to occur annually [[1,3,4]](https://www.zotero.org/google-docs/?RAvcl2). Yet human rabies deaths are rarely ever confirmed in any country in East Africa [[5]](https://www.zotero.org/google-docs/?RN6SU3). The lack of verified statistics documenting the extent of the burden from this notifiable disease contributes to its continued neglect [[6]](https://www.zotero.org/google-docs/?1EL62l). Unlike in high-income countries, where dog vaccination has been used to eliminate rabies and the resulting risk to humans [[4,7]](https://www.zotero.org/google-docs/?k9b4KN), dog vaccination campaigns are still not conducted routinely or at scale across East Africa.

Post-exposure prophylaxis (PEP) is needed as an emergency measure for rabid bite victims to prevent the onset of this rapidly progressive fatal neurologic disease [[8]](https://www.zotero.org/google-docs/?QSu5gC). The rabies virus is highly neurotropic, moving along peripheral nerves from the virus inoculation site to the central nervous system, where it replicates and causes rabies [[4]](https://www.zotero.org/google-docs/?5NfY1h). In the absence of timely PEP, around one in five rabid bite victims will progress to rabies, depending on the site and severity of the bite(s) [[3]](https://www.zotero.org/google-docs/?e4FJtN). The WHO recommendations for PEP comprise thorough wound washing followed by a course of post-exposure vaccinations and, in the case of severe exposure, administration of rabies immunoglobulins (RIG) [[6]](https://www.zotero.org/google-docs/?jCe1gi). However, access to PEP and its timely use are far from universal. High costs and limited availability of rabies post-exposure vaccines, as well as a lack of awareness about the need for appropriate PEP lead to thousands of otherwise preventable deaths [[6,9,10]](https://www.zotero.org/google-docs/?9Be5jt).

New approaches for rabies diagnosis such as sensitive molecular methods and sequencing can improve the confirmation of human rabies and be used to characterise pathogen spread [[11]](https://www.zotero.org/google-docs/?0AfhNG). Sequencing approaches have the potential to enhance routine rabies surveillance and provide actionable information to inform elimination programmes, for example, to distinguish whether cases are due to continuous undetected local circulation or from new incursions and to identify the sources of such incursions [[12,13]](https://www.zotero.org/google-docs/?dvVlLD). More generally, sequencing could provide key insights into how rabies circulates within different populations and the processes responsible for its maintenance in specific geographic localities [[14]](https://www.zotero.org/google-docs/?qttR9c). In-country genome sequencing of rabies viruses from human rabies cases on the African continent has so far only been carried out in South Africa [[5,15–17]](https://www.zotero.org/google-docs/?y9y1z5), however, partial genome sequences are available from one human rabies case from Senegal [[18]](https://www.zotero.org/google-docs/?PHft71) and one human rabies case from Nigeria [[19]](https://www.zotero.org/google-docs/?G7ojyy). In this case series, we report rabies virus whole genomes sequenced from five human rabies cases in East Africa, investigating the factors leading to each death and how such deaths might be prevented in the future.

**CASE PRESENTATION**

All five deaths described in this report resulted from exposures by domestic dogs and a lack of, or inappropriate, PEP administration (Table 1). Four of the five patients were children who were ten years old or younger. Three of the children were bitten on the head or neck, sites that are at highest risk for progression to rabies in the absence of PEP [[20]](https://www.zotero.org/google-docs/?AWI1cW).

Case 1 was vaccinated against tetanus, but not advised rabies post-exposure vaccination despite attending a health facility the same day as being bitten multiple times by an unknown dog, including one bite to the forehead. Twenty-eight days after being bitten, case 1 started to show rabies symptoms. From symptoms onset the patient was treated for malaria, initially at home, then at a local hospital. The patient’s condition deteriorated rapidly, leading to their transfer to a major referral hospital where they died upon arrival. Although Case 2 had no bite history, the patient had killed his two dogs after they manifested signs of rabies one month prior to his death. After presenting to a nearby health facility with rabies symptoms the patient was transferred to a major referral hospital and pronounced dead 6 days later. Case 3 reported to a local hospital with bites to the lips from an unknown dog, and was vaccinated against rabies following an off-label intramuscular regimen (1mL on days 0, 7 and 18) that is not recommended by WHO. Rabies symptoms began 19 days later, i.e. one day after the third vaccination; and the patient died four days later following transfer to a major referral hospital. Case 4 started post-exposure vaccination, via the intramuscular route, one day after being bitten multiple times on the head and arm by an unknown dog, but did not receive further vaccinations as they were reportedly not advised to do so. After symptoms onset (69 days later) the patient was taken back to the health facility where they were initially vaccinated, then transferred to a major referral hospital where they died shortly thereafter. Case 5 was referred to a major hospital from a health facility where he presented with symptoms of rabies 19 days after exposure. The patient received traditional medicine after being bitten on the leg by his own dog, but otherwise did not receive health care after the bite. Palliative care was given until death 7 days after hospital admission. RIG was not administered to any of these patients, despite the site and severity of bites (multiple bites on the forehead and lips) for cases 1, 3 and 4. Details about each case are as follows:

**Case 1:** On 23rd February 2018, a 10-year-old boy presented with fever, headache, and general body weakness from Nyawara village, Gem sub-county, Siaya county, Kenya. Suspecting malaria infection, the boy was initially treated at home with a single dose of the antimalarial Coartem and observed. However, his condition worsened, and the next day he complained of headache, dizziness, body weakness and restlessness, vomiting several times and reportedly talking excessively and at times incoherently. The family took the boy to the local health facility where a rapid diagnostic test was positive for malaria. He was started on an intramuscular dose of the antimalarial Artesunate, with a repeat dose after four hours. The nurse observed the boy to be restless, aggressive to touch and having abnormal vocalisation. Upon inquiry, the nurse learnt that he had been bitten (three times on his left forearm and forehead) on his way from school by an unknown dog one month earlier, but had not received PEP. The boy’s parents described attending a local health facility on the day the boy was bitten where he was given painkillers (paracetamol), tetanus vaccination and his wound was cleaned with paraffin, but no advice or treatment was given to address the rabies risk. With rabies symptoms evident, the boy was referred to the nearest hospital, where he was given painkillers (Diclofenac). That night he was extremely restless, complaining of difficulty swallowing the oral medication, salivating uncontrollably, and extremely agitated at the sight of liquid. The next day, 25th February, his condition deteriorated and he was referred to the better equipped Siaya County Referral Hospital for palliative care where he was declared dead on arrival.

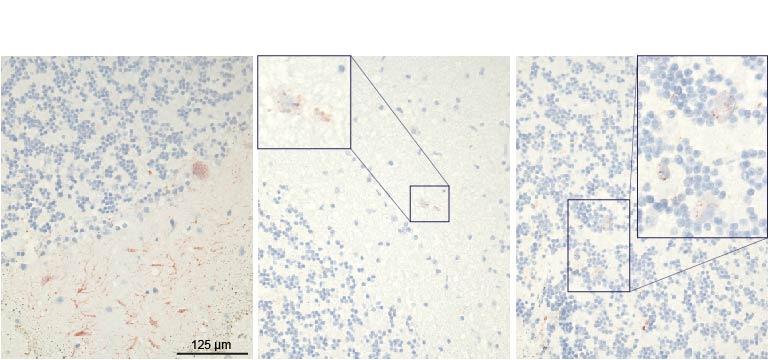
**Case 2:** On 8th July 2018, a 37-year-old man, from Rarieda village, Gem sub-county, Siaya county, Kenya, was reported dead due to suspected rabies. Family members were not aware of the man having been bitten by rabid animals before his death. However, his own two dogs had both manifested signs of rabies and the deceased had killed the animals after they changed their behaviour (one month and three weeks prior to his death respectively). Details of whether the dogs were vaccinated during the mass vaccination campaign conducted in Siaya in 2018 were not disclosed. On 3rd July 2018 the patient visited a local health facility, presenting with paralysis, abnormal vocalisation, and difficulty breathing. He was given paracetamol. His condition worsened on 4th July, whereupon he was taken to Siaya County Referral Hospital for further treatment. The exact treatment he received is unclear, and on 8th July 2018, he was pronounced dead.

**Case 3:** On 26th August 2019, a six-year-old girl from Tarakea-Rombo village, Moshi district, Kilimanjaro region, Tanzania, was bitten multiple times on the upper lip by an unknown dog that ran away after the bite. The patient reported immediately to her local health facility, received proper wound cleaning, and was administered a first dose of rabies vaccine on day 0 (26th August 2019) via the intramuscular route as well as an anti-tetanus injection. The girl returned for her second rabies vaccine dose on day 7 (2nd September 2019), followed by her third dose on day 18 (12th September). The family reported paying 30,000 Tanzania Shillings (Tsh) per vaccination, excluding transportation fees to get to the health facility with the vaccine (16 km away). The day after her third dose (13th September 2019), she was taken back to the same facility with a headache, was given painkillers (paracetamol) and discharged the same day. By the 15th of September, her condition had worsened with high fever, headache and hallucinations whereupon she was referred to Huruma district hospital and then on to Kilimanjaro Christian Medical Centre (KCMC) referral hospital in Moshi town the same day. She died the following day (16th September 2019, 22 days after the bite).

**Case 4:** On 22nd June 2022, a six-year-old boy fromAlara village, South West Sakwa ward, Bondo sub-county, Siaya county, Kenya, was bitten and scratched multiple times on the head and arm by an unknown dog while walking home. The dog was chased away by villagers. The boy received first aid at home, where his wound was washed with soap and water, and he was then rushed to the traditional herbalist where he received a concoction of herbs. On hearing this news, the community health worker advised the family to take the child to the hospital for PEP. The family took the boy to a local health facility, in West Sakwa, Bondo sub-county where he received the first dose of rabies vaccine on 23rd June 2022 via the intramuscular route. The family reported paying 1,000 Kenyan shillings (Ksh) for the vaccine, excluding transportation to the health facility (15 km away). They were given no further advice on the follow-up course of vaccination nor the severity of rabies. On 29th August 2022, the boy was taken to the same health facility complaining of fever, insomnia, abnormal vocalisation, difficulty breathing and swallowing, hallucinations and restlessness. He was given a normal saline IV and transferred to the referral hospital in Bondo after his condition worsened, where he was given palliative care and died four hours later.

**Case 5**: On 27th September 2022, a thirteen-year-old boy from Bulati village, Ngorongoro district, Arusha region, Tanzania, was admitted to Fame hospital after being referred from Bulati health facility on the same day. The boy was showing signs of rabies including excessive salivation, paralysis, abnormal vocalisation and restlessness. The boy had been bitten by his dog on the left leg on 8th September 2022 and did not report or receive any treatment from the health facility/hospital despite regularly attending the hospital for other medical treatment. The wound was washed with milk and a traditional practice for treatment was initiated (placing a coin on the wound to suck the poison). Upon reporting to the health facility, the medical staff inquiry found that the boy was bitten by the dog 20 days prior. He was referred to the major hospital on the same day where he received palliative care until he died on 3rd October 2022 (26 days after the bite).

The rabies incubation period varies; symptoms typically develop days to weeks after infection, but can take months depending on factors such as the bite location and severity [[1]](https://www.zotero.org/google-docs/?Q5ghIg). Three of the patients in this case series progressed to rabies within one month of exposure (the date of exposure was not possible to confirm for Case 2, although was recalled to be around one month before death), whereas the fifth patient developed symptoms more than two months later. Each patient displayed common clinical signs of rabies: fever, abnormal vocalisation, difficulty breathing and swallowing, hallucinations, paralysis, hydrophobia, aggressiveness, excessive salivation and restlessness. All patients except case 2 had a clear history of a dog bite making the clinical diagnosis straightforward. A history of close contact with two suspect rabid dogs assisted in reaching a diagnosis for case 2. Samples from four of the five cases were positive by a rapid diagnostic test. Case 3 had a negative test result; however the presence of rabies virus antigen was confirmed by immunohistochemistry, using the Streptavidin-biotin complex staining method (Fig 1).

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**Figure 1. Positive immunohistochemistry staining of case 3, at 40x magnification.** The red stain zoomed in with higher resolution magnification indicates the presence of rabies virus antigen detected with specific labelled antibodies (RABV-N, antibody 5DF12) and Streptavidin-biotin complex staining.

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|  | **Case 1** | **Case 2** | **Case 3** | **Case 4** | **Case 5** |
| **Sex/Age (y)** | M/10 | M/37 | F/6 | M/6 | M/13 |
| **Case location** | Nyawara village, Central Gem ward, Gem sub-county, Siaya county, Kenya | Rarieda village, Central Gem ward, Gem sub-county, Siaya county, Kenya | Tarakea-Rombo village, Moshi district, Tanzania | Alara village, South West Sakwa ward, Bondo sub-county, Siaya county, Kenya | Bulati village, Ngorongoro district, Tanzania |
| **Bite history** | Bites to the left arm & forehead by an unknown dog | No bite history - but killed his 2 dogs with suspect rabies | Multiple bites to upper lip by an unknown dog | Multiple bites to the head and arm by an unknown dog | Single bite to the left leg by own dog |
| **Exposure date** | 23 Jan 2018 | Unknown | 26 Aug 2019 | 22 June 2022 | 8 Sep 2022 |
| **Delay to attend health facility** | 0 days | After symptoms onset (8 Jul 2018) | 0 days | 1 day | After symptoms onset (19 days) |
| **PEP received** | Wound cleaning only | None | Wound cleaning; IM vaccine: d0, 7, 18, no RIG | Wound cleaning, IM vaccine: d0 only, no RIG | Wound cleaning only |
| **Other treatment at health facility** | Paracetamol, anti-tetanus inj | None | Paracetamol and anti-tetanus inj | None | None |
| **Why no/ inadequate PEP?** | Not advised | Reported after symptom onset | Received regimen that is not recommended | Not advised | Reported after symptom onset |
| **Incubation period till symptom onset** | 28 days | Approximately 1 month (family recall) | 19 days | 69 days | 19 days |
| **Days of illness (symptomatic)** | 3 days | 6 days | 4 days | 1 day | 7 days |
| **Diagnostic results** | RDT+ frozen brain | RDT+ frozen brain | RDT- frozen brain; IHT+ formalin-fixed brain tissue | RDT+ fresh brain | RDT+ fresh brain |
| **Viral lineages** | AF1b\_A2 | AF1a\_A1.1 | AF1b\_A1.1 | AF1a\_C1 | AF1b\_A1.1 |
| **Genome coverage (excluding masked sites)** | 76.75 | 51.12 | 97.49 | 94.24 | 97.63 |
| **Accession ID** | OR045959 | OR045960 | OR920212 | OR045927 | OR045947 |

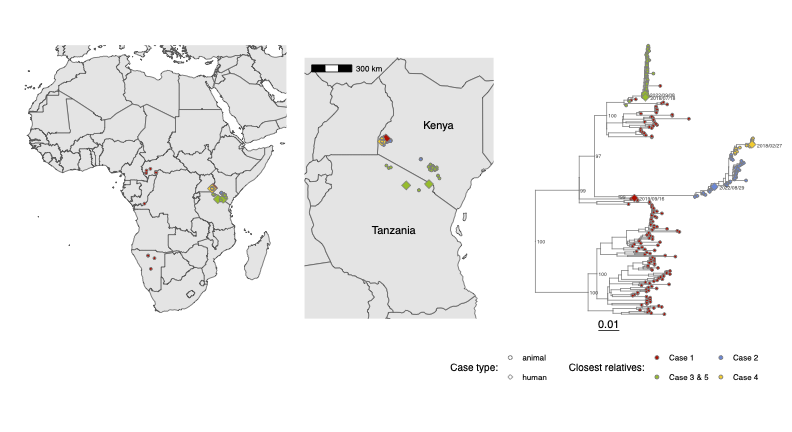
**Table 1. Summary of human rabies case histories, diagnostic results and viral genome characterization.** All the viruses belong to the Cosmopolitan major clade, and are classified here by minor clade and lineage. RDT = rapid diagnostic test. IHT = Immunohistochemical test.

***Phylogenetic Investigation***

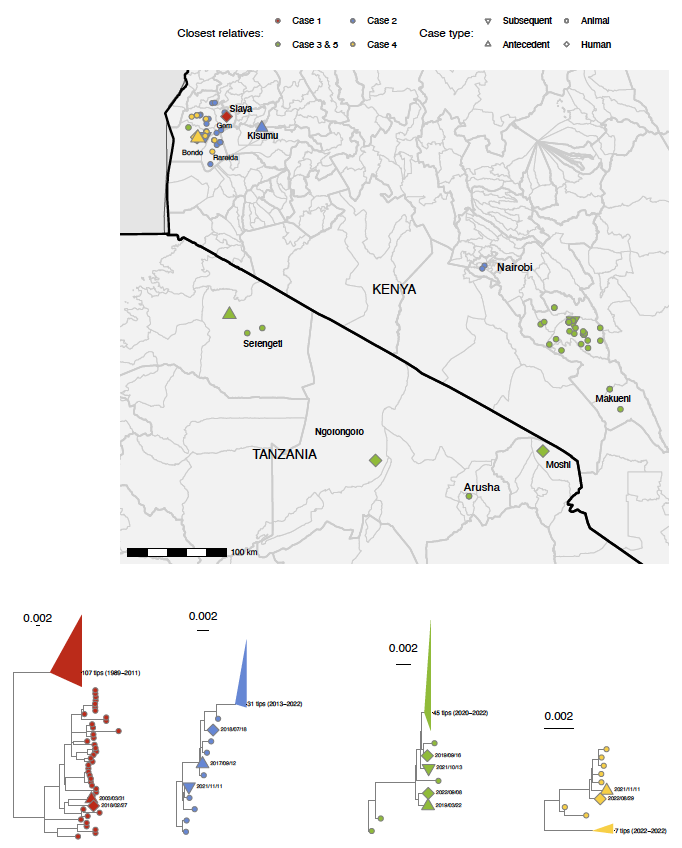
Amplicon-based sequencing was carried out to compare rabies viruses (RABVs) from the five human cases to those from recent animal rabies cases in the region. Details of the laboratory procedure are found in Supplementary File 1. Due to the poor sample conditions and primer mismatches related to the early primer set used (i.e., targeting RABV diversity in Tanzania from 2019-2020), a few of the sequences generated (14/98) had less than 90% genome coverage. Sequences from cases 1 and 2 from Kenya both had less than 90% genome coverage (Supplementary Table 1).

All the sequenced viruses were from the Cosmopolitan clade; cases 2 and 4 belonged to minor clade AF1a (both from Kenya), and cases 1, 3 and 5 belonged to minor clade AF1b (from Kenya, Tanzania and Tanzania respectively) (Fig 2). Cases 1, 2, 3 and 5 were from previously reported circulating lineages, with cases 3 and 5 from the same lineage (AF1b\_A1.1), while case 4 was from a newly designated lineage (AF1a\_C1). The most closely related antecedent and subsequent sequences to all the human cases were from domestic dogs, except for the subsequent sequence to case 2 which was from a cow, indicating likely spill over from lineages circulating in domestic dogs.

All cases except case 4 represent cross-border lineages, with lineage AF1b\_A2, widespread across Africa but only reported from East Africa in 2018 with this human death (case 1) in Kenya (Fig 3). The most closely related antecedent sequence to case 1 is from Bangui, CAR, where over 85% of cases in this lineage were also from (Fig 2A). The geographic distance and phylogenetic divergence between these cases indicate limited wider sampling of the lineage, which likely originated decades ago (supplementary Table 2) and is now widespread, though largely undetected, across Africa. In contrast, lineage AF1b\_A1.1 (cases 3 and 5) has been seen exclusively in East Africa; first in Burundi in 1990, Rwanda in 1994, Kenya in 2001, then Uganda in 2009 followed by Tanzania in 2011. After initial detection, the lineage was not detected in Kenya again until 2020, when there has been ongoing circulation and frequent detection, predominantly in Makueni county. Conversely, detection in Tanzania, has been sporadic and near the Kenyan border (Fig 3A), suggestive of cross-border spread. The closest antecedent sequence is the same for both cases 3 and 5 - a rabid dog from Serengeti District in Tanzania sampled in 2019 (Fig 3A). Lineage AF1a\_A1.1 (case 2), was originally detected in Ethiopia in 1987, then in Morocco in 1989 where it was seen frequently until 2008. There have also been infrequent detections of AF1a\_A1.1 in Algeria since 2000. Virus infections from this lineage were first detected in Kenya in 2013 with human cases in both Nairobi and Siaya (fig 3B). Lineage AF1a\_C1 (case 4) is newly designated and highly localised, found exclusively in Siaya County, Kenya since 2021. The detection of three lineages (corresponding to cases 1, 2 and 4) all within years or months of each other within Siaya County (Fig 3) highlight the apparently localised co-circulation of lineages.

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**Figure 2. Phylogeny of Rabies virus sequences from human cases in the context of relevant circulating lineages.** Maximum likelihood tree of lineages relevant to each case and map showing locations for each sequence (n=262). Sequences from the Arctic AL1a clade (GenBank accession AB699220, AY956319, EF437215, HE802675, HE802676, KF154996, KY775603, KY775604, LT909539, LT909541 and MG099711) were used as an outgroup (not shown) to root the tree. Tips and points are coloured by lineage, with diamonds denoting the human cases (OR045959, OR045960, OR920212, OR045927, OR045947). Scale in substitutions/site. Ultrafast bootstrap values of lineage-defining nodes shown.

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**Figure 3. Subtrees showing phylogenetic relationships between sequences from human and animal cases.** The map shows the locations of the sequenced human and animal cases from East Africa coloured by lineage. Phylogenetic subtrees are shown for case 1 from lineage AF1b\_A2 (n = 151) in red; case 2 from lineage AF1a\_A1.1 (n = 42) in blue; cases 3 and 5 from lineage AF1b\_A1.1 (n = 53) in green and case 4 from lineage AF1a\_C1 (n = 16) in yellow. Human cases denoted by diamonds (OR045959, OR045960, HB002 & OR045947, OR045927), and most closely related animal cases (antecedent and subsequent) as determined by minimum patristic distance by triangles (ID = KT119642 (Case 1), OR920307 & OR920256 (Case 2), OR045943 & OR920240 (Case 3 and 5), OR920236 (Case 4)). Relevant locations labelled. Scale in substitutions/site, and outgroup rooted with ordered nodes.

**Discussion**

Human rabies deaths occur when rabies-exposed victims fail to receive timely or appropriate PEP. This case series highlights multiple challenges that bite victims face in obtaining PEP, including inappropriate advice from medical staff not fully aware of the dangers of rabies (case 1, 4 and 5) or not trained in correct PEP administration (case 3 and 4); high costs that act as obstacles to initiating (case 1) and completing PEP courses (case 4) as well as more general lack of understanding about rabies risks. We report how traditional yet ineffective healing practices are still commonplace (case 4 and 5), with two cases (2 and 5) only visiting hospitals after symptoms onset, when death is inevitable. Human rabies deaths in East Africa are typically not confirmed or reported and as a result rabies receives negligible investment for prevention and control. The clinical history of these five human cases together with use of rapid diagnostic tests and immunohistochemistry enabled their confirmation, while sequencing provided further epidemiological context as to their source of origin. This report demonstrates the feasibility of improving human rabies surveillance in East Africa (all 5 cases were identified via surveillance networks initiated by research projects), but also only begins to reveal the scale of the rabies burden. Most human rabies deaths within these communities are not confirmed, while the rabies virus sequences, we report indicate that circulating viral lineages remain largely uncharacterized.

**Improving rabies diagnosis and surveillance**

Human rabies diagnosis remains a challenge in Low- and Middle-Income Countries (LMICs) and cases are often misdiagnosed, for example, case 1 was considered malaria. If a history of rabies exposure is elicited, as for all five cases that we report (although atypical for case 2), clinical presentation of furious rabies is diagnostic, but paralytic rabies can be more difficult to identify[[7]](https://www.zotero.org/google-docs/?iMmX4T). Ante-mortem diagnostic tests include antigen detection, antibody assays and virus isolation, but all have limited success [[8]](https://www.zotero.org/google-docs/?V67KhJ). Post-mortem tests are rarely performed due to lack of personnel trained to collect samples, lack of accredited laboratories (Biosafety Level 2 or 3) for diagnosis and because many clinical cases return home in the absence of palliative care options and are subsequently not reported within surveillance networks which might allow the possibility of sample collection [[15]](https://www.zotero.org/google-docs/?YWR35S). The direct fluorescent antibody test (DFA) is the recommended “gold standard” for post-mortem diagnosis [[21]](https://www.zotero.org/google-docs/?rT7g60), but requires fluorescence microscopy which is expensive and limited in availability. The Direct Rapid Immunohistochemical Test which has similar sensitivity and specificity to the DFA and only requires light microscopy [[22]](https://www.zotero.org/google-docs/?ynUxpx) is based on a simplified version of the immunohistochemistry diagnostic assay [[23]](https://www.zotero.org/google-docs/?BsznGv). Immunohistochemistry was performed on one of the five cases reported here (case 3) at an overseas laboratory (Netherlands) through ongoing research, but is not carried out in East Africa. Rapid diagnostic tests show promise, being successfully used here to diagnose 4 cases *in situ*. The negative result for case 3 was likely due to sample storage (in formalin). More generally freeze-thaw cycles compromise the sensitivity of these tests which are recommended for use only on fresh brain samples. Though rapid diagnostic tests may increase human rabies diagnosis, they are not yet recommended by international organisations. Concerns remain about test sensitivity and quality control [[21,23]](https://www.zotero.org/google-docs/?Ra3ppb), particularly with respect to PEP decision-making. Laboratory comparisons of rapid test brands under different protocols have been inconsistent, with batch variability presenting an issue [[24,25]](https://www.zotero.org/google-docs/?UdDwfU). Nonetheless, the best performing test has been found to have high sensitivity on fresh samples [[26,27]](https://www.zotero.org/google-docs/?CGFSUh). In our situation, we found rapid tests provided a valuable diagnostic that was possible to quickly and easily perform in the absence of alternatives, and where the risk of rabies was already apparent.

Molecular techniques for diagnosis such as PCR and sequencing are promising and further help in understanding rabies virus biology, molecular epidemiology, pathogenesis and sources of transmission [[11]](https://www.zotero.org/google-docs/?33W6JC)*.* The viral genomic data associated with the cases presented here highlights the role of domestic dogs in maintaining rabies circulation and resultant risk to humans. These deaths could be avoided if the disease was eliminated from source populations of domestic dogs through mass vaccination, which remains the most cost‐effective measure for rabies prevention in endemic regions. Furthermore, the genomic data uncover population connectivity and frequent transboundary viral spread (cases 1, 3 and 5) indicating the need for regional planning and coordinated dog vaccination, as well as for much improved surveillance. Further interpretation is limited by the availability of sequences, though lineage assignment begins to reveal the extent to which under sampling is a problem. Our recent sequencing identified the lineage AF1b\_A2 for the first time in Kenya, and a new lineage, AF1a\_C1 only seen in Kenya. The new lineage designation and considerable divergence of the most closely related sequences to case 1 within lineage AF1b\_A2 (Table S1) illustrate the negligible sampling of circulating rabies viruses.

**Improving access to post-exposure prophylaxis**

WHO now recommends an intradermal post-exposure vaccination regimen that can be completed in one week, requiring visits only on day 0, day 3 and day 7 respectively [[1]](https://www.zotero.org/google-docs/?eqOsOA). This highly effective abridged regimen is both dose-sparing and more economical for patients and health providers [[28]](https://www.zotero.org/google-docs/?Ufr10D). Yet, rabies- endemic countries have been slow to adopt the updated WHO position. In parts of Tanzania a post-exposure vaccination regimen is used that is not recommended (case 3). Moreover, while advised for WHO category III exposures such as cases 1, 3 and 4, RIG has rarely been available in East Africa. Concern has been raised regarding recent human deaths in Tanzania attributed to confusion in post-exposure management, with RIG speculated to have been given and not vaccination. Hence there is an urgent need to update national guidelines to follow WHO guidance and ensure healthcare practitioners are trained and competent to manage rabies exposures appropriately. The high cost of vaccines remains a barrier for bite victims (typically costing around $10 per vaccination in East Africa, for example, case 3 paid 30,000 Tanzania shillings per dose, equivalent to $13 while case 4 paid 1000 Kenya shillings per dose, equivalent to $8) as well as for health providers, translating into inadequate supply and chronic stockouts. Meanwhile, indirect costs to patients (travel, lost income) also can be prohibitively high. The promise of investment in human rabies vaccines by Gavi, the Vaccine Alliance, offers a chance to address PEP access issues and radically redress inequalities underlying human rabies deaths [[29,30]](https://www.zotero.org/google-docs/?noR7gx). Estimates from modelling studies suggest improved PEP access would prevent over 1.3 million human rabies deaths by 2035 [[31]](https://www.zotero.org/google-docs/?0BKdQ2). But with global health priorities disrupted by the pandemic, Gavi support for human rabies vaccines has yet to begin and these deaths continue.

**Recommendations to address rabies from a One Health perspective**

Through this case series we highlight critical steps needed to combat the problem of rabies in East Africa. Thousands of people every year in the region still face challenges in accessing life-saving PEP. Medical practitioners urgently need training about the risk of rabies and to ensure effective post-exposure management [[32]](https://www.zotero.org/google-docs/?nVOgN6). However, the high cost of PEP remains the most immediate obstacle for rabies-exposed patients[[3]](https://www.zotero.org/google-docs/?UKmepF), compounded by structural factors leading to poor supply and frequent shortages in East Africa. If Gavi delivers on its proposed investment, it can address the market failure in access to lifesaving rabies vaccines [[31]](https://www.zotero.org/google-docs/?iDDeGp), catalysing progress on this One Health pathway. Unfortunately, human rabies cases are still rarely diagnosed. To improve the rate at which cases are diagnosed, we suggest rapid tests can be carefully deployed to confirm human cases, given the absence of decentralised laboratory capacity and highly trained personnel. We further present the first whole genome sequences from human rabies cases generated in the region. Viral genomic data support the role of domestic dogs in maintaining rabies circulation and resultant risk to humans. Research across large parts of Tanzania demonstrates that domestic dogs maintain rabies virus circulation [[33–35]](https://www.zotero.org/google-docs/?kp2pwN), in settings with abundant wildlife and even where wildlife cases are common [[36]](https://www.zotero.org/google-docs/?fywvWd). However, misperceptions of wildlife being responsible for rabies persistence, still act as a barrier to implementing rabies control in domestic dog populations [[37]](https://www.zotero.org/google-docs/?1CW2sE). A One Health approach is necessary to reduce the burden of rabies, comprising the scaling up of mass dog vaccination to interrupt transmission in reservoir populations, improving access to PEP while rabies continues to circulate, and public education to ensure participation in dog vaccination campaigns and improve health-seeking for PEP. As countries pursue the global ‘Zero by 30’ goal to eliminate human deaths from dog-mediated rabies [[38]](https://www.zotero.org/google-docs/?TdMCOz), genomic approaches have the potential to enhance rabies surveillance and provide actionable information, for example, distinguishing undetected local circulation from introductions that often set back progress towards elimination. We urge regional coordinated action towards this goal to prevent these tragic deaths.

**List of abbreviations**

DFA Direct Fluorescent Antibody

dRIT Direct Rapid Immunohistochemically Test

IHT Immunohistochemistry

KCRI Kilimanjaro Clinical Research Institute

LMICs Low- and middle-income countries

PCR Polymerase chain reaction

PEP Pre-Exposure Prophylaxis

RABV Rabies virus

RDT Rapid diagnostic tests

RIG Rabies Immunoglobulin

UNITID University of Nairobi Institute of Tropical and Infectious Diseases

WHO World Health Organisation

**DECLARATIONS**

**Ethics approval**

The study was approved by the Medical Research Coordinating Committee of the National Institute for Medical Research (NIMR/HQ/R.8a/vol.IX/2788), the Ministry of Regional Administration and Local Government (AB.81/288/01), and Ifakara Health Institute Institutional Review Board (IHI/IRB/No:22-2014) in Tanzania; and the University of Nairobi Institute of Tropical and Infectious Diseases (P947/11/2019) and the Kenya Medical Research Institute (KEMRI-SERU protocol No. 3268) in Kenya.

**Patient and Public Involvement**

Carers of family members were consulted to request permission for post-mortem sample collection and to describe diagnostic and investigative procedures. Following confirmation of results from investigations the families of these patients and their communities were visited to discuss the meaning of the investigation results and how findings can be used to advocate for improved rabies prevention and control, documenting discussions for sharing with practitioners and policymakers.

**Patient consent**

Attached SP2

**Data source and availability**

Data and code to reproduce the analyses and figures are available from our public repository https://github.com/Gurdeepjaswant/EA\_human\_rabies\_case\_series

**Conflicts of interest**

There are no conflicts of interest.

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**References**

[1. Rabies vaccines: WHO position paper – April 2018. [cited 18 Oct 2022]. Available: https://www.who.int/publications/i/item/who-wer9316](https://www.zotero.org/google-docs/?MLQDWh)

[2. Hampson K, Coudeville L, Lembo T, Sambo M, Kieffer A, Attlan M, et al. Estimating the Global Burden of Endemic Canine Rabies. PLoS Negl Trop Dis. 2015;9: 1–20. doi:10.1371/journal.pntd.0003709](https://www.zotero.org/google-docs/?MLQDWh)

[3. Changalucha J, Steenson R, Grieve E, Cleaveland S, Lembo T, Lushasi K, et al. The need to improve access to rabies post-exposure vaccines: Lessons from Tanzania. Vaccine. 2019;37: A45–A53. doi:10.1016/j.vaccine.2018.08.086](https://www.zotero.org/google-docs/?MLQDWh)

[4. Fooks AR, Banyard AC, Horton DL, Johnson N, McElhinney LM, Jackson AC. Current status of rabies and prospects for elimination. Lancet Lond Engl. 2014;384: 1389–1399. doi:10.1016/S0140-6736(13)62707-5](https://www.zotero.org/google-docs/?MLQDWh)

[5. Thiptara A, Atwill ER, Kongkaew W, Chomel BB. Epidemiologic trends of rabies in domestic animals in southern Thailand, 1994-2008. Am J Trop Med Hyg. 2011;85: 138–145. doi:10.4269/ajtmh.2011.10-0535](https://www.zotero.org/google-docs/?MLQDWh)

[6. Nel LH. Discrepancies in Data Reporting for Rabies, Africa - Volume 19, Number 4—April 2013 - Emerging Infectious Diseases journal - CDC. [cited 17 Feb 2023]. doi:10.3201/eid1904.120185](https://www.zotero.org/google-docs/?MLQDWh)

[7. Rasooli A, Pourhossein B, Bashar R, Shirzadi MR, Amiri B, Kheiri EV, et al. Investigating Possible Etiologies of Post-Exposure Prophylaxis Failure and Deaths From Rabies Infection: Case Reports. Int J Med Toxicol Forensic Med. 2020;10: 27378–27378. doi:10.32598/ijmtfm.v10i3.27378](https://www.zotero.org/google-docs/?MLQDWh)

[8. Warrell MJ, Warrell DA. Rabies and other lyssavirus diseases. The Lancet. 2004;363: 959–969. doi:10.1016/S0140-6736(04)15792-9](https://www.zotero.org/google-docs/?MLQDWh)

[9. Soun VV, Eidson M, Wallace BJ, Drabkin PD, Jones G, Leach R, et al. Antemortem Diagnosis of New York Human Rabies Case and Review of U.S. Cases. Int J Biomed Sci IJBS. 2006;2: 434–445.](https://www.zotero.org/google-docs/?MLQDWh)

[10. Madhusudana SN, Sukumaran SM. Antemortem diagnosis and prevention of human rabies. Ann Indian Acad Neurol. 2008;11: 3–12. doi:10.4103/0972-2327.40219](https://www.zotero.org/google-docs/?MLQDWh)

[11. Talbi C, Lemey P, Suchard MA, Abdelatif E, Elharrak M, Jalal N, et al. Phylodynamics and Human-Mediated Dispersal of a Zoonotic Virus. PLOS Pathog. 2010;6: e1001166. doi:10.1371/journal.ppat.1001166](https://www.zotero.org/google-docs/?MLQDWh)

[12. Trewby H, Nadin-Davis SA, Real LA, Biek R. Processes Underlying Rabies Virus Incursions across US–Canada Border as Revealed by Whole-Genome Phylogeography. Emerg Infect Dis. 2017;23: 1454–1461. doi:10.3201/eid2309.170325](https://www.zotero.org/google-docs/?MLQDWh)

[13. Lushasi K, Brunker K, Rajeev M, Ferguson EA, Jaswant G, Baker LL, et al. Integrating contact tracing and whole-genome sequencing to track the elimination of dog-mediated rabies: An observational and genomic study. eLife. 2023;12: e85262. doi:10.7554/eLife.85262](https://www.zotero.org/google-docs/?MLQDWh)

[14. Layan M, Dellicour S, Baele G, Cauchemez S, Bourhy H. Mathematical modelling and phylodynamics for the study of dog rabies dynamics and control: A scoping review. PLoS Negl Trop Dis. 2021;15: e0009449. doi:10.1371/journal.pntd.0009449](https://www.zotero.org/google-docs/?MLQDWh)

[15. McElhinney LM, Marston DA, Golding M, Nadin-Davis SA. Chapter 12 - Laboratory diagnosis of rabies. In: Fooks AR, Jackson AC, editors. Rabies (Fourth Edition). Boston: Academic Press; 2020. pp. 401–444. doi:10.1016/B978-0-12-818705-0.00012-1](https://www.zotero.org/google-docs/?MLQDWh)

[16. Mollentze N, Weyer J, Markotter W, le Roux K, Nel LH. Dog rabies in southern Africa: regional surveillance and phylogeographical analyses are an important component of control and elimination strategies. Virus Genes. 2013;47: 569–573. doi:10.1007/s11262-013-0974-3](https://www.zotero.org/google-docs/?MLQDWh)

[17. Coetzee P, Weyer J, Paweska JT, Burt FJ, Markotter W, Nel LH. Use of a molecular epidemiological database to track human rabies case histories in South Africa. Epidemiol Infect. 2008;136: 1270–1276. doi:10.1017/S0950268807009582](https://www.zotero.org/google-docs/?MLQDWh)

[18. Faye M, Faye O, Paola ND, Ndione MHD, Diagne MM, Diagne CT, et al. Rabies surveillance in Senegal 2001 to 2015 uncovers first infection of a honey-badger. Transbound Emerg Dis. 2022;69: e1350–e1364. doi:10.1111/tbed.14465](https://www.zotero.org/google-docs/?MLQDWh)

[19. Ogo MF, Nel LH, Sabeta CT. Phylogenetic Evidence of the Public and Veterinary Health Threat of Dog Rabies in Nigeria. Niger Vet J. 2011;32. doi:10.4314/nvj.v32i1.68996](https://www.zotero.org/google-docs/?MLQDWh)

[20. Hampson K, Dobson A, Kaare M, Dushoff J, Magoto M, Sindoya E, et al. Rabies Exposures, Post-Exposure Prophylaxis and Deaths in a Region of Endemic Canine Rabies. PLoS Negl Trop Dis. 2008;2: e339. doi:10.1371/journal.pntd.0000339](https://www.zotero.org/google-docs/?MLQDWh)

[21. Rupprecht CE, Fooks AR, Abela-Ridder B. Laboratory techniques in rabies, volume 2. 5th ed. Geneva: World Health Organization; 2019. Available: https://iris.who.int/handle/10665/310837](https://www.zotero.org/google-docs/?MLQDWh)

[22. Lembo T, Niezgoda M, Velasco-Villa A, Cleaveland S, Ernest E, Rupprecht CE. Evaluation of a Direct, Rapid Immunohistochemical Test for Rabies Diagnosis. Emerg Infect Dis. 2006;12: 310–313. doi:10.3201/eid1202.050812](https://www.zotero.org/google-docs/?MLQDWh)

[23. Coetzer A, Nel LH, Taylor L. Direct, Rapid Immunohistochemical Test (DRIT). Global Alliance for Rabies Control; 2017.](https://www.zotero.org/google-docs/?MLQDWh)

[24. Klein A, Fahrion A, Finke S, Eyngor M, Novak S, Yakobson B, et al. Further Evidence of Inadequate Quality in Lateral Flow Devices Commercially Offered for the Diagnosis of Rabies. Trop Med Infect Dis. 2020;5: 13. doi:10.3390/tropicalmed5010013](https://www.zotero.org/google-docs/?MLQDWh)

[25. Eggerbauer E, de Benedictis P, Hoffmann B, Mettenleiter TC, Schlottau K, Ngoepe EC, et al. Evaluation of Six Commercially Available Rapid Immunochromatographic Tests for the Diagnosis of Rabies in Brain Material. PLoS Negl Trop Dis. 2016;10: e0004776. doi:10.1371/journal.pntd.0004776](https://www.zotero.org/google-docs/?MLQDWh)

[26. Le M, Zinsstag J, Dacheux L, Alfaroukh O. Validation of a Rapid Rabies Diagnostic Tool for Field Surveillance in Developing Countries. 2016; 1–16. doi:10.1371/journal.pntd.0005010](https://www.zotero.org/google-docs/?MLQDWh)

[27. Mauti S, Léchenne M, Naïssengar S, Traoré A, Kallo V, Kouakou C, et al. Field Postmortem Rabies Rapid Immunochromatographic Diagnostic Test for Resource-Limited Settings with Further Molecular Applications. JoVE J Vis Exp. 2020; e60008. doi:10.3791/60008](https://www.zotero.org/google-docs/?MLQDWh)

[28. Cantaert T, Borand L, Kergoat L, Leng C, Ung S, In S, et al. A 1-week intradermal dose-sparing regimen for rabies post-exposure prophylaxis (RESIST-2): an observational cohort study. Lancet Infect Dis. 2019;19: 1355–1362. doi:10.1016/S1473-3099(19)30311-1](https://www.zotero.org/google-docs/?MLQDWh)

[29. Thumbi SM, Blumberg L, le Roux K, Salahuddin N, Abela B. A call to accelerate an end to human rabies deaths. Lancet Lond Engl. 2023;400: 2261–2264. doi:10.1016/S0140-6736(22)02487-4](https://www.zotero.org/google-docs/?MLQDWh)

[30. Wentworth D, Hampson K, Thumbi SM, Mwatondo A, Wambura G, Rui N. A social justice perspective on access to human rabies vaccines. Vaccine. 2019; 8–10. doi:10.1016/j.vaccine.2019.01.065](https://www.zotero.org/google-docs/?MLQDWh)

[31. Hampson K, Ventura F, Steenson R, Mancy R, Trotter C, Cooper L, et al. The potential effect of improved provision of rabies post-exposure prophylaxis in Gavi-eligible countries: a modelling study. Lancet Infect Dis. 2019;19: 102–111. doi:10.1016/S1473-3099(18)30512-7](https://www.zotero.org/google-docs/?MLQDWh)

[32. Audu SW, Mshelbwala PP, Jahun BM, Bouaddi K, Weese JS. Two fatal cases of rabies in humans who did not receive rabies postexposure prophylaxis in Nigeria. Clin Case Rep. 2019;7: 749–752. doi:10.1002/ccr3.1972](https://www.zotero.org/google-docs/?MLQDWh)

[33. Lembo T, Hampson K, Haydon DT, Craft M, Dobson A, Dushoff J, et al. Exploring reservoir dynamics: a case study of rabies in the Serengeti ecosystem. J Appl Ecol. 2008;45: 1246–1257. doi:10.1111/j.1365-2664.2008.01468.x](https://www.zotero.org/google-docs/?MLQDWh)

[34. Mancy R, Rajeev M, Lugelo A, Brunker K, Cleaveland S, Ferguson EA, et al. Rabies shows how scale of transmission can enable acute infections to persist at low prevalence. Science. 2022;376: 512–516. doi:10.1126/science.abn0713](https://www.zotero.org/google-docs/?MLQDWh)

[35. Hampson K, Dushoff J, Cleaveland S, Haydon DT, Kaare M, Packer C, et al. Transmission dynamics and prospects for the elimination of canine Rabies. PLoS Biol. 2009;7: 0462–0471. doi:10.1371/journal.pbio.1000053](https://www.zotero.org/google-docs/?MLQDWh)

[36. Lushasi K, Hayes S, Ferguson EA, Changalucha J, Cleaveland S, Govella NJ, et al. Reservoir dynamics of rabies in south-east Tanzania and the roles of cross-species transmission and domestic dog vaccination. J Appl Ecol. 2021;58: 2673–2685. doi:10.1111/1365-2664.13983](https://www.zotero.org/google-docs/?MLQDWh)

[37. Lembo T, Hampson K, Kaare MT, Ernest E, Knobel D, Kazwala RR, et al. The Feasibility of Canine Rabies Elimination in Africa: Dispelling Doubts with Data. PLoS Negl Trop Dis. 2010;4: e626. doi:10.1371/journal.pntd.0000626](https://www.zotero.org/google-docs/?MLQDWh)

[38. Minghui R, Stone M, Semedo MH, Nel L. New global strategic plan to eliminate dog-mediated rabies by 2030. Lancet Glob Health. 2018;6: e828–e829. doi:10.1016/S2214-109X(18)30302-4](https://www.zotero.org/google-docs/?MLQDWh)