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CASE REPORT

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The role of plasma metagenomic sequencing in identification of *Balamuthia mandrillaris* encephalitis

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Abstract

Balamuthia mandrillaris is a rare, free-living amoeba (FLA) that causes granulomatous amoebic encephalitis, a disease with close to 90% mortality. The geographical ranges of many FLA are expanding, potentially increasing human exposure to *B. mandrillaris*. Here, we report a case of a 58-year-old woman with progressive neurological symptoms, ultimately diagnosed postmortem with *B. mandrillaris* encephalitis through plasma metagenomic next-generation sequencing (mNGS) despite negative results on both cerebrospinal fluid (CSF) mNGS and CSF PCR testing. Histologic analysis and real-time PCR (qPCR) studies on postmortem brain tissue confirmed *B. mandrillaris* infection with significant vascular clustering of trophozoites. Retrospective analysis of CSF mNGS data demonstrated subthreshold reads for *B. mandrillaris*, emphasizing the challenges of interpreting low-level pathogen signals. A systematic review of 159 published *B. mandrillaris* cases revealed only two reports of *B. mandrillaris* diagnosed using plasma mNGS, both of which also had diagnostic CSF studies. This case demonstrates the diagnostic challenges of *B. mandrillaris* infections, highlights its vascular tropism, and suggests that plasma mNGS may warrant evaluation as a diagnostic tool for *B. mandrillaris*.

Keywords *Balamuthia*, *Balamuthia mandrillaris*, Amoeba, Amoebic encephalitis, Plasma metagenomic sequencing, Free living amoeba, Granulomatous amoebic encephalitis

Introduction

Balamuthia mandrillaris is a free-living amoeba (FLA) that causes granulomatous amoebic encephalitis (GAE), a rare and highly fatal condition characterized pathologically by variable degrees of inflammation, granuloma formation, and tissue necrosis. Found in soil and water, *B. mandrillaris* infections typically arise from direct inoculation through skin wounds or inhalation [11]. GAE often presents with nonspecific neurological symptoms, including headache, fever, and focal deficits, which complicates diagnosis and can delay treatment [11]. Accurate diagnosis requires differentiation from *Acanthamoeba spp.*, another cause of GAE.

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The increasing prevalence of amoebic infections over recent decades has been associated with climate change [27], suggesting that the need for diagnostic tools for *B. mandrillaris* may increase. Traditional diagnostic methods such as cerebrospinal fluid (CSF) analysis and neuroimaging frequently fail to identify *B. mandrillaris* [92]. Recent advances in metagenomic next-generation sequencing (mNGS) have augmented diagnosis of many central nervous system infections [25, 29, 36, 104]. mNGS sequences all nucleic acids present in a sample at very high depth, allowing unbiased pathogen detection from diverse tissue samples [25, 29, 36, 104]. While mNGS has been applied successfully to CSF and brain tissue in many GAE cases, the diagnostic utility of plasma mNGS in GAE has not been comprehensively evaluated.

Here, we report a fatal case of *B. mandrillaris* GAE diagnosed via plasma mNGS, despite negative CSF mNGS results. Retrospective analysis of CSF mNGS revealed subthreshold reads for *B. mandrillaris*, emphasizing the challenges of interpreting low-level pathogen signals. This case and our review of the literature highlight the organism's pronounced vascular tropism, which we hypothesize could underlie a potential utility for plasma mNGS as a diagnostic tool for GAE.

Case presentation

A 58-year-old woman, with no significant past medical history, presented with 10 days of progressive neurological symptoms, including right facial numbness, left-sided hemiparesis, imbalance, and tinnitus. She presented with no visible skin lesions; however, she reported having sustained superficial scratches on her arms from gardening several weeks prior, which had resolved. Upon examination, no skin lesions were observed. Brain MRI revealed a 2.1 cm rim-enhancing lesion in the right pons with surrounding edema and mass effect (Fig. 1a). CSF analysis showed lymphocytic pleocytosis (132–203 cells/μL), increased opening pressure (27 cm H_2O), elevated protein (64 mg/dL), and normal glucose levels. Comprehensive testing for bacterial, fungal, mycobacterial, viral, and autoimmune etiologies yielded negative results (see Table 1). Flow cytometry indicated reactive T-cells without aberrancy.

A biopsy of the right middle cerebellar peduncle showed chronic inflammation with macrophage infiltration (CD68-positive) and necrosis, without identifiable organisms and with rare possible granulomas (Fig. 1b–d). Stains for acid-fast bacilli (AFB), fungal organisms (GMS), Gram-positive/negative bacteria, and other pathogens were negative. Cultures of blood, CSF, and biopsy tissue—including anaerobic, fungal, and mycobacterial screens—were negative. Despite broad-spectrum antimicrobials and methylprednisolone for suspected demyelination, the patient's condition deteriorated.

Follow up MRI, performed 28 days after the initial scan, demonstrated expansion of the initial lesion with new brainstem, cerebellar, cerebral, and leptomeningeal lesions (Fig. 2a–c), and multifocal vessel wall enhancement of the bilateral middle cerebral, internal carotid, and vertebral arteries (Fig. 4a–d). Cyclophosphamide was initiated for suspected primary CNS angiitis. Biopsies of the dura and cerebellum showed only chronic inflammation. mNGS of CSF was not reported to be positive for any organisms. The patient's disease progressed to status epilepticus, obstructive hydrocephalus, brainstem herniation, and death. Plasma mNGS, ordered prior to the patient's death, identified *B. mandrillaris* postmortem.

Autopsy revealed severe cerebral edema, cerebellar herniation, hemorrhage, and necrosis of the occipital lobes, brainstem, and cerebellum (Fig. 2d). Histopathological evaluation demonstrated trophozoites and cysts in necrotic brain tissue and perivascular spaces (Fig. 3a–c). There was marked perivascular inflammation with fibrinoid necrosis of vasculature (Fig. 4e).

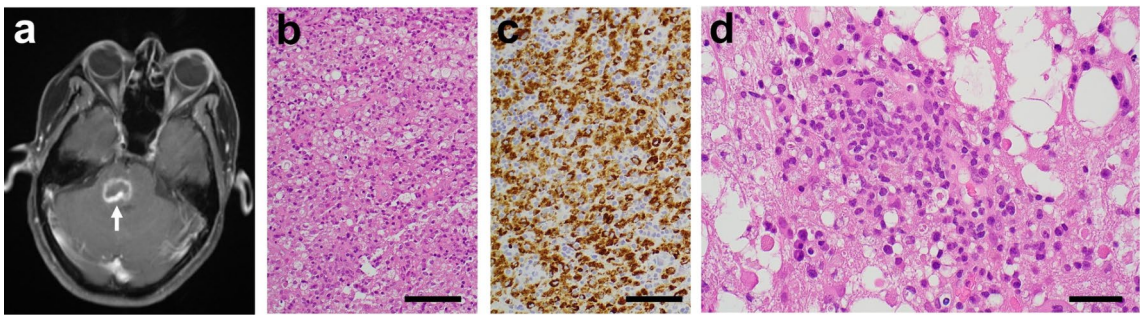

Fig. 1 Initial MRI and surgical biopsy. **a** Initial axial post-contrast T1-weighted MRI, performed the day after admission, showed a right pontine rim-enhancing lesion (arrow). Right middle cerebellar peduncle biopsy demonstrated **b** macrophage-rich chronic inflammation (H&E, 20x) with **c** CD68 stain highlighting numerous macrophages (CD68, 20x). **d** Rare possible granulomas were identified (H&E, 40x). Scale bars: b, c = 50 μm, d = 20 μm

Table 1 Laboratory test results

Test type	Test name	Result
Culture	Aerobic & Anaerobic	No growth
	AFB	No growth
Serology	Interferon-gamma Release Assay	Non-reactive
	HIV Ab/Ag screen	Non-reactive
	RPR Qualitative	Non-reactive
	Toxoplasmosis Ab IgG/IgM	Not Detected
	Aspergillus Ab	Not Detected
	CNS Demyelination Ab Panel	No Informative Antibodies Identified
	ANCA Ab	Not Detected
	Sjogren Ab	Not Detected
PCR	*E. coli KI*	Not Detected
	H. influenzae	Not Detected
	L. monocytogenes	Not Detected
	N. meningitidis	Not Detected
	S. agalactiae	Not Detected
	S. pneumoniae	Not Detected
	CMV	Not Detected
	HSV-1	Not Detected
	HSV-2	Not Detected
	VZV	Not Detected
	EBV	Not Detected
	HSV-6	Not Detected
	HPeV	Not Detected
	C. *neoformans/gattii*	Not Detected
	Coccidioides Ab	Not Detected
Other	CSF Cytology & Chemistry	Colorless & Clear, > 200 cells/mm^3, PMN 11%, Plasma 2%, Nucleated cell count 2%, Lymph 85%, Mono 60 mg/dL, Protein 64 mg/dL
	Flow Cytometry	No Abnormal T-cell Population
	Oligoclonal Bands	Absent

The trophozoites, many of which were necrotic, were round to ovoid with foamy cytoplasm; most had a single, round nucleus (Fig. 3b). The cyst forms appeared to show varying stages of development, with mature cysts characterized by thick, multilayered capsules enclosing vacuolated cytoplasm, and a nucleus (Fig. 3c). Prominent perivascular clustering of trophozoites and vascular invasion were observed, primarily by cyst forms (Figs. 4f and 5). Giemsa (Figs. 3a, 5d) and PAS (Fig. 5a and b) stains highlighted amoebae. Targeted qPCR on formalin-fixed paraffin-embedded (FFPE) brain tissue confirmed *B. mandrillaris* DNA in the brainstem and cerebellum (Fig. 3d). Retrospective analysis of CSF mNGS revealed subthreshold reads for *B. mandrillaris*. PCR for *B. mandrillaris* on this CSF sample was negative.

Discussion and conclusions

GAE is a rare but often fatal infection caused by free-living amoebae (FLA) such as *Acanthamoeba spp.* and *B. mandrillaris* (previously known as "*leptomyxid ameba*") [8]. Our autopsy findings included striking perivascular clustering of amoebae and vascular invasion (Figs. 4, 5). The organism's ability to cross the blood–brain barrier involves interactions with human brain microvascular endothelial cells (HBMECs) via carbohydrate moieties, facilitating CNS invasion [53]. This vascular affinity likely contributed to the multifocal vessel wall enhancement observed on imaging (Fig. 4a–d). We hypothesized that these characteristics facilitated the detectability of circulating trophozoites or DNA fragments in plasma, leading to the positive plasma mNGS result in this case.

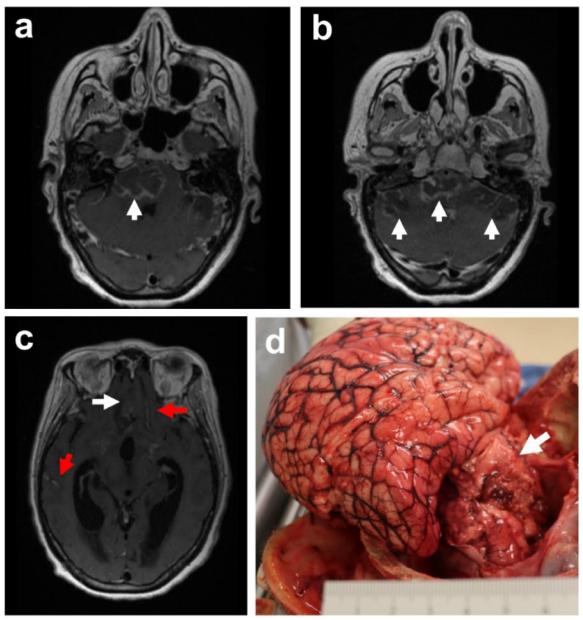

Fig. 2 Follow-up MRI findings and gross brain at postmortem examination. Axial post-contrast T1-weighted MRI performed on day 29 of hospitalization showed **a** expansion of the initial pontine lesion (arrow) with **b** new brainstem (arrow) and cerebellar lesions (arrows) as well as **c** cerebral (white arrow) and leptomeningeal (red arrows) lesions. **d** Brain at autopsy with cerebral edema, cerebellar herniation, hemorrhage, and brainstem necrosis (arrow)

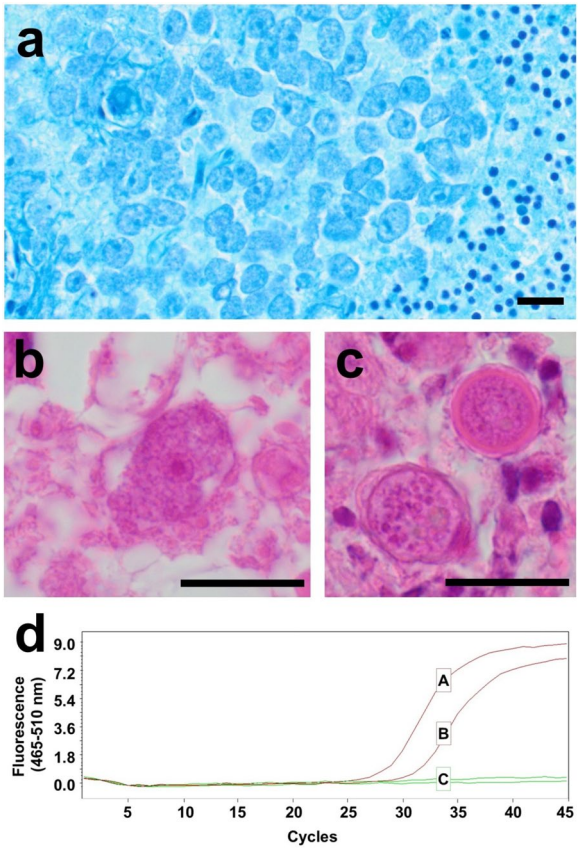

Fig. 3 *B. mandarallis* trophozoites and cysts, and free-living amoeba PCR. **a** Trophozoites adjacent to cerebellar granule cells (Giemsa, 40x). High-power views of **b** trophozoites (H&E, 100x) and **c** cysts (H&E, 100x). **d** PCR amplification curves of *B. mandrillaris* specific nucleic acid in: (A) *B. mandrillaris* positive control (Cp = 28.3) and (B) patient's cerebellar FFPE tissue (Cp = 31). (C) negative controls (*Naegleria fowleri* and Vero cell line controls). Scale bars: a = 200 µm; b, c = 10 µm

To further investigate diagnostic testing regimens and vascular association of *B. mandrillaris,* we performed a PubMed review with key words "*Balamuthia mandrillaris*" or "*leptomyxid ameba*" and identified 159 unique published cases of *B. mandrillaris* infection that had clear CNS or skin involvement and also reported patient demographics, outcome, and diagnostic methods (Table 2). This revealed 120 cases of *B. mandrillaris* diagnosed in brain tissue, 18 in skin, and 30 in CSF, with some cases diagnosed at more than one site. There was a high mortality rate of approximately 89%. The average patient age was 36 years, with cases spanning all age groups: children (< 18 years, 59 cases), adults (18–64 years, 72 cases), and older adults (65 + years, 28 cases). Gender distribution included 100 males and 58 females. One case lacked gender information. Within the United States, cases were geographically widespread, with the highest concentrations reported in Texas and California. Notably, the vascular tropism of *B. mandrillaris* observed in our case aligned with many prior studies. We identified both in vitro evidence for a predilection for perivascular invasion and vascular inflammation [53] as well as at least 10 cases with explicit mention of perivascular or vascular involvement on neuropathologic tissue analysis [24, 32, 33, 50, 56, 79, 81, 120–122].

First identified in 1989 in a pregnant mandrill baboon at the San Diego Zoo Safari Park though use of immunofluorescence assay (IFA) [87], *B. mandrillaris* was retrospectively identified in human cases dating back to 1974 [87]. Since then, diagnostic modalities have evolved and include immunohistochemistry, immunofluorescence, and molecular techniques such as PCR, qPCR, RT-PCR, Sanger sequencing, and enzyme-linked immunosorbent assay. These tools have been applied to various specimens, including FFPE tissue, amoebic cultures, blood, and CSF, with varying success. While PCR is a cornerstone of infectious disease diagnostics, its sensitivity for *B. mandrillaris* remains suboptimal, with reported detection rates as low as 6%, underscoring the limitations of traditional diagnostic methods [11].

In recent years, mNGS has emerged as a potential diagnostic tool for *B. mandrillaris* and has been applied to specimens including brain tissue, CSF, and, recently,

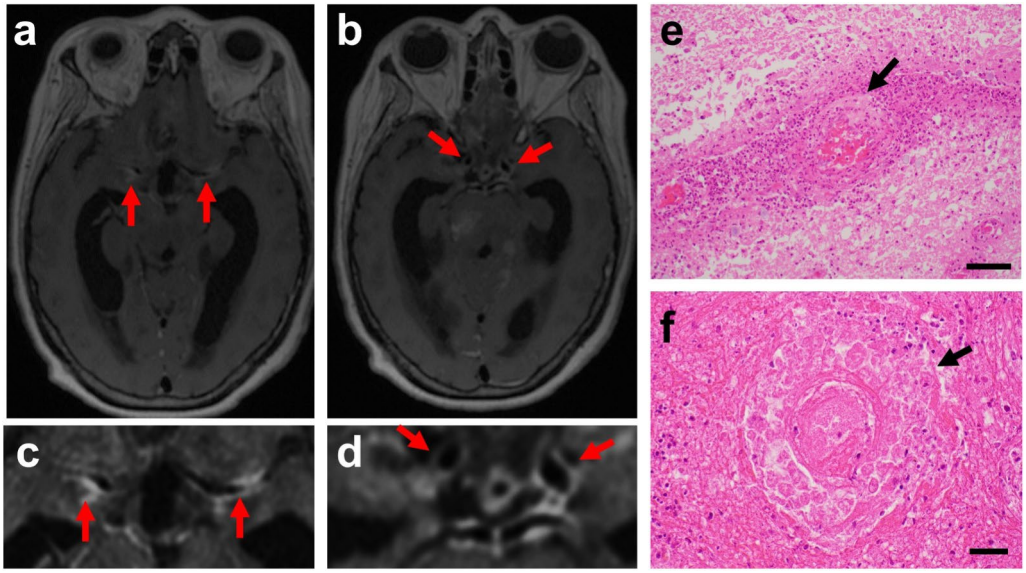

Fig. 4 Vessel wall enhancement on antemortem MRI and H&E stain of postmortem brain. Axial post-contrast T1-weighted MRI performed on day 29 of hospitalization showed vessel abnormalities, including **a** bilateral middle cerebral artery (MCA) and **b** bilateral internal carotid artery (ICA) wall enhancement (arrows). **c** and **d** provide closer views of vessel wall enhancement (arrows; brightness/contrast of image adjusted for visualization). **e** Fibrinoid necrosis of the vasculature (arrow, H&E, 20x). **f** Perivascular trophozoites in the cerebellum (arrow, H&E, 40x). Scale bars: e = 50 µm, f = 20 µm

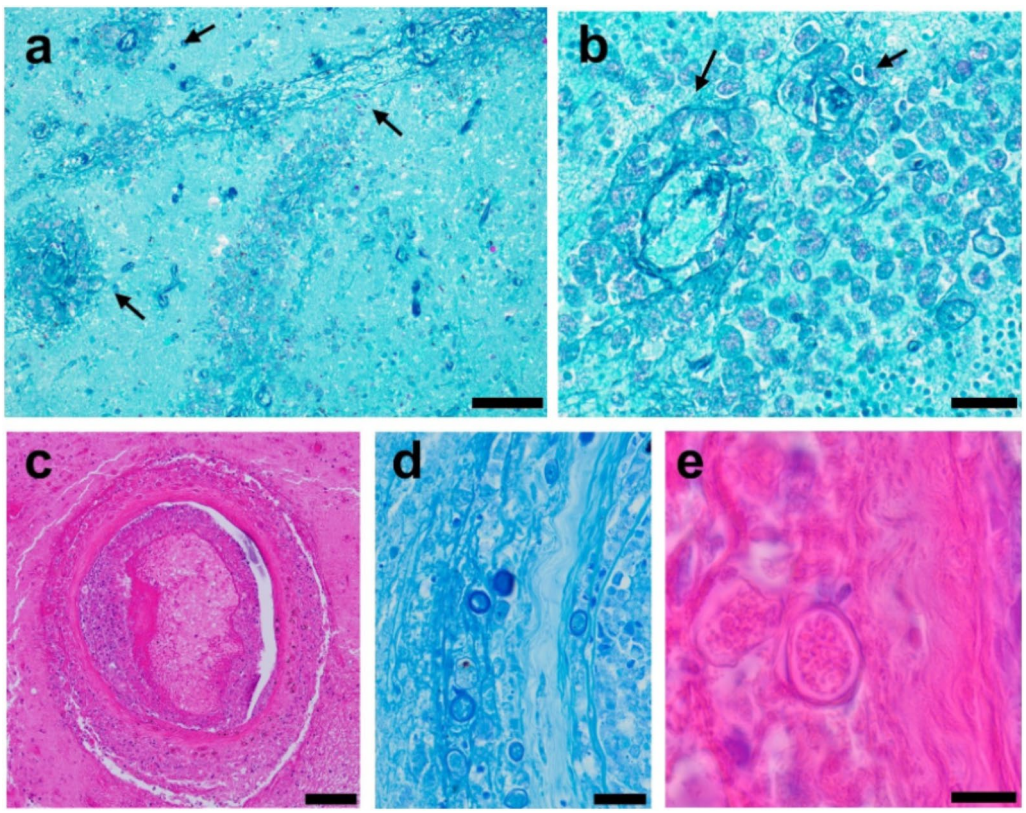

Fig. 5 Vascular tropism of *B. mandrillaris* observed at postmortem brain examination. **a**–**b** Perivascular trophozoites in the cerebellum (arrows) (PAS; a: 20x, b: 40x). **c**–**e** Invasion of the arterial wall, predominantly by cysts (c: H&E, 10x; d: Giemsa, 40x; e: H&E, 100x). Scale bars: a = 50 µm, b, d = 20 µm, c = 100 µm, e 10 µm

Table 2 A systematic review of 159 reported cases of *B. mandrillaris* infection: demographics, initial presentation, outcome, and diagnostic methods

Publication [References #]	Demographics (age in years, gender)	Clinical presentation	Outcome	Method of diagnosis	Diagnosed by plasma mNGS
Our case	58 F	Progressive facial numbness, hemiparesis, imbalance, and tinnitus	Deceased	mNGS (plasma), qPCR (brain), negative on PCR & mNGS (CSF)	Yes
Aboubechara et al. (2024) [120]	4 F	Facial droop, dysarthria, hemiparesis, upper respiratory tract symptoms, headache, fever	Deceased	mNGS (plasma), PCR (CSF)	Yes
Xiong et al. (2024) [119]	35 M	Fever, limb convulsions, progressive quadriparesis	Deceased	mNGS (brain)	No
Wang et al. (2024) [90]	49 F	Headache, dizziness, nausea, emesis, prior skin lesion(s)	Deceased	mNGS (brain)	No
Zheng et al. (2024) [102]	58 M	Headache, fever	Deceased	mNGS (CSF)	No
Szymanski et al. (2024) [80]	3 M	Fatigue and facial pain, previously healthy with baseline facial palsy with right lip droop	Deceased	Not specified	Not specified
Javed et al. (2024) [33]	33 F	Back pain, headache, emesis	Deceased	PCR & Sanger sequencing (specimen not specified)	No
Qin et al. (2024) [66]	47 M	Fever following allogenic renal transplant	Not specified	mNGS (specimen not specified)	Not specified
Xu et al. (2024) [94]	66 F	Headache	Deceased	mNGS (CSF)	No
Qin et al. (2024) [65]	52 M	Headache, difficulty walking, blunted affect	Deceased	mNGS (CSF), PCR (CSF)	No
Li et al. (2024) [46]	77 F	Dizziness, unsteady gait	Deceased	mNGS (CSF)	No
Ono et al. (2023) [56]	76 F	Headache, nausea	Deceased	PCR (brain), negative PCR (CSF)	No
Gramp et al. (2023) [24]	66 M	Skin lesion(s)	Deceased	PCR (skin & brain)	No
Yao et al. (2023) [97]	64 F	Headache, abdominal pain, dizziness, unsteady gait, cough, emesis, prior skin lesion(s)	Deceased	mNGS (CSF), PCR (CSF), qRT-PCR (CSF)	No
Spottiswoode et al. (2023) [105]	50s M	Seizure	Survived	mNGS (brain) & Sanger sequencing (brain), negative mNGS (CSF) & PCR(CSF)	No
Liu et al. (2023) [47]	61 M	Headache	Deceased	mNGS (CSF)	No
Sakusic et al. (2023) [71]	61 F	Headache, prior orthotopic heart transplant	Deceased	Broad-range PCR sequencing (brain)	No
Fan et al. (2023) [20]	56 M	Transient coma, nausea, emesis	Deceased	mNGS (CSF)	No
Xu et al. (2022) [108]	54 M	Headache, dizziness, skin lesion(s)	Deceased	mNGS (CSF)	No
Saffioti et al. (2022) [109]	17 M	Headache, weight loss, swollen nose	Deceased	qPCR (brain)	No
Levinson et al. (2022) [106]	80 M	Altered mental status, dizziness, vision loss	Survived	Multiplex RT-qPCR (CSF & brain)	No
Tootla et al. (2022) [85]	3(?)	Skin lesion(s)	Deceased	PCR (skin) with bidirectional Sanger sequencing	No
Wang et al. (2022) [89]	14 M	Skin lesion(s)	Survived	IHC (skin)	No
Wang et al. (2022) [89]	57 F	Skin lesion(s)	Deceased	IHC (skin)	No
Wang et al. (2022) [89]	9 M	Skin lesion(s)	Survived	IHC (skin)	No
Maehara et al. (2022) [50]	82 M	Amnesia, skin lesion(s)	Deceased	IHC (skin & brain), PCR and sequencing (skin & brain)	No
Zhang et al. (2021) [101]	7 M	Skin lesion(s)	Deceased	IHC (skin), PCR (skin), negative NGS (skin)	No

Table 2 (continued)

Publication [References #]	Demographics (age in years, gender)	Clinical presentation	Outcome	Method of diagnosis	Diagnosed by plasma mNGS
Cuoco et al. (2022) [14]	4 M	Seizure	Survived	multiplex RT-qPCR (brain)	No
Peng et al. (2022) [59]	54 M	Numbness and weakness of extremity	Survived	mNGS (CSF, brain, & serum)	No (serum)
Hu et al. (2022) [30]	37 F	Dizziness, skin lesion(s)	Deceased	mNGS (CSF)	No
Ai et al. (2022) [1]	15 M	Fever, altered mental status, previous skin lesion(s)	Deceased	mNGS (CSF), PCR (skin)	No
Hirakata et al. (2021) [29]	60 F	Homonymous hemianopsia	Deceased	mNGS (brain), PCR (brain & skin), IHC (brain & skin)	No
Kalyatanda (2020) [36]	51 M	Seizure, headache, altered mental status, disorientation, left-sided weakness, night sweats, diarrhea	Deceased	mNGS (plasma), multiplex qPCR (CSF), IHC (brain)	Yes
Yi et al. (2020) [98]	9 F	Emesis, loss of appetite, fever, headache, difficulty walking, blurred vision, altered mental status	Deceased	mNGS (CSF)	No
Mani et al. (2021) [51]	51 M	Blurry vision, headache, imbalance, altered mental status	Deceased	Multiplex qPCR (CSF)	No
Lee et al. (2021) [44]	50 M	Headache, dizziness, dysarthria, aphasia	Deceased	qPCR (brain)	No
Zwillman et al. (2021) [103]	65 M	Weakness, lethargy, lower extremity cellulitis, fever, encephalopathy	Deceased	qPCR (brain), IHC (brain)	No
Suyo-Prieto et al. (2020) [78]	21 M	Seizure, skin lesion(s), nausea, headache	Deceased	PCR and sequencing (skin)	No
Safavi et al. (2021) [70]	3 F	Altered mental status, emesis, dizziness, seizure, hemiparesis, ataxia	Deceased	PCR (brain)	No
Wu et al. (2020) [93]	13 F	Dizziness, emesis, and blurry vision, prior skin lesion(s)	Deceased	NGS (CSF), PCR (skin), immunostaining (serum & CSF)	No
Aoki et al. (2020) [3]	63 F	Dyspnea and left recurrent nerve paralysis	Deceased	PCR (brain), IHC (brain)	No
Pan et al. (2020) [58]	85 F	Confusion, weakness, slurred speech	Deceased	qPCR (brain), PCR (brain)	No
Yang et al. (2020) [96]	2 M	Fever	Deceased	NGS (CSF)	No
Suzuki et al. (2020) [79]	68 M	Seizures, transient right hemiparesis	Deceased	IHC (brain), PCR (brain)	No
Lee et al. (2020) [43]	45 M	Seizure	Deceased	qPCR (brain)	No
Kum et al. (2019) [110]	71 M	Muscle spasms	Deceased	Diagnosed based on histomorphology of brain tissue, no confirmatory testing	No
Yohannan et al. (2019) [99]	69 F	Altered mental status	Deceased	qPCR (brain), IHC (brain)	No
Hara et al. (2019) [116]	56 M	Seizure, hemiparalysis, fever, altered level of consciousness	Deceased	IHC (brain)	No
Hara et al. (2019) [116]	72 F	Lightheadedness, fever	Deceased	IHC (brain)	No
Hara et al. (2019) [116]	53 M	Headache, fever	Deceased	IHC (brain)	No
Hara et al. (2019) [116]	56 F	Fever, malaise	Deceased	IHC (brain)	No

Table 2 (continued)

Publication [References #]	Demographics (age in years, gender)	Clinical presentation	Outcome	Method of diagnosis	Diagnosed by plasma mNGS
Hara et al. (2019) [116]	69 M	Altered level of consciousness	Deceased	IHC (brain)	No
Hara et al. (2019) [116]	72 M	Loss of appetite, cognitive decline	Deceased	CSF microscopy, no confirmatory testing	No
Hara et al. (2019) [116]	79 M	Skin lesion(s)	Deceased	IHC (brain), PCR (brain)	No
Hara et al. (2019) [116]	67 F	Diplopia, lightheadedness	Deceased	Not specified	No
Hara et al. (2019) [116]	61 F	Homonymous hemianopsia	Deceased	Whole genome sequencing (brain), PCR (brain), and IHC (brain)	No
Hara et al. (2019) [116]	55 M	Unknown	Deceased	IHC (brain), PCR (brain)	No
Piper et al. (2018) [62]	69 F	Skin lesion(s), seizures, prior chronic sinus infection	Deceased	qPCR (skin & brain), IFA (serum)	No
Tarai et al. (2018) [82]	57 M	Altered mental status, seizures	Deceased	PCR (brain)	No
Shehab et al. (2018) [112]	13 F	Hemiparesis, slurred speech	Deceased	Diagnosed based on histomorphology of brain tissue, no confirmatory testing	No
Joo et al. (2018) [34]	6 M	Headache, fever, emesis	Deceased	qPCR (CSF)	No
Takei et al. (2018) [81]	74 F	Somnolence	Deceased	IHC (brain), negative PCR (CSF, brain)	No
Cope et al. (2018) [12]	18 F	Headache, fever, lethargy	Deceased	qPCR (CSF)	No
Lehmer et al. (2017) [111]	84 M	Skin lesion(s)	Survived	IFA (brain & skin)	No
Mittal et al. (2017) [54]	32 M	Seizure	Not specified	IFA and PCR (specimen not specified)	No
Chang et al. (2016) [9]	91 F	Skin lesion(s)	Survived	IHC (skin), PCR (skin)	No
Vollmer et al. (2016) [88]	26 M	Visual disturbances, lightheadedness, headache, syncope	Survived	IFA (brain), PCR (brain)	No
Kobayashi et al. (2015) [40]	57 F	Headache	Deceased	IHC (brain), PCR (brain)	No
Ott et al. (2015) [113]	41 M	Encephalitis symptoms	Deceased	PCR (specimen not specified)	No
Roy et al. (2015) [69]	11 M	Nausea, emesis, lethargy, clumsiness, and right-sided weakness	Deceased	IFA (brain, lung), qPCR (brain, CSF), Sanger sequencing (brain, CSF), serology	No
Wilson et al. (2015) [92]	74 F	Altered mental status, fever	Deceased	mNGS (CSF), PCR (brain, CSF) Sanger sequencing (brain, CSF), serology, IHC (brain)	No
Schafer et al. (2015) [72]	82 M	Fever, chills, nausea, emesis, lethargy, altered mental status, prior skin lesion(s)	Deceased	qPCR (brain), IFA (skin)	No
van der Beek et al. (2015) [86]	61 F	Fever, headaches, myalgias, prior skin lesions	Deceased	qPCR (brain, CSF) with sequencing	No
Khurana et al. (2015) [39]	18 M	Headache, emesis, limb weakness, slurred speech, fever, altered mental status	Deceased	PCR (brain)	No
Khurana et al. (2015) [39]	18 M	Headache, diplopia, fever, emesis	Deceased	PCR (brain) with sequencing	No
Greninger et al. (2015) [115]	15 F	Limb weakness, headache, emesis, ataxia, confusion	Deceased	mNGS (CSF, brain), PCR (brain)	No
Itoh et al. (2015) [32]	81 M	Somnolence, disoriented, ataxic gait	Deceased	PCR (brain), IHC (brain)	No

Table 2 (continued)

Publication [References #]	Demographics (age in years, gender)	Clinical presentation	Outcome	Method of diagnosis	Diagnosed by plasma mNGS
Pindyck et al. (2014) [114]	56 M	Fever, hyponatremia	Deceased	IHC (brain), PCR (specimen not specified)	No
Lobo et al. (2013) [48]	48 F	Headache, neck pain, fever, diplopia, slurred speech, fever	Deceased	IFA (brain, serum)	No
Phillips et al. (2013) [61]	59 M	Headache, numbness, tingling	Deceased	PCR (brain)	No
Krasaelap et al. (2013) [42]	4 F	Headache, emesis	Deceased	PCR with sequencing (brain & CSF)	No
Moriarty et al. (2013) [117]	4 F	Headache, abnormal gait, seizures	Survived	PCR (brain), serology and IFA (brain)	No
Moriarty et al. (2013) [117]	11 F	Fever, headache, meningismus	Deceased	IFA (brain), PCR (brain)	No
Kato et al. (2013) [118]	72 M	Somnolence, altered level of consciousness, hemiparesis	Survived	Diagnosed based on CSF morphology, negative PCR (CSF) & IFA (brain)	No
Stidd et al. (2012) [77]	2 F	Initial fall followed by recurrent falls, ataxia, irritability, lethargy, photophobia, fever	Deceased	qPCR (brain), IFA (brain)	No
Hill et al. (2011) [28]	0 y 8 mo F	Lethargy, irritability, seizures, weakness	Deceased	IFA (brain)	No
Doyle et al. (2010) [18]	80 F	Skin lesion(s)	Survived	PCR (brain & skin)	No
Yamasaki et al. (2011) [95]	51 F	Seizure	Deceased	IHC (brain), ELISA (brain)	No
Silva et al. (2010) [75]	47 F	Headache	Deceased	IHC (brain), PCR (brain)	No
Centers for Disease Control and Prevention (CDC) (2010) [7]	27 M	Organ donor, presented with presumed stroke, prior skin lesion(s)	Deceased	Not specified	No
Centers for Disease Control and Prevention (CDC) (2010) [7]	56 M	Liver transplant recipient, presented with diplopia and difficulty walking	Deceased	qPCR (brain), IHC (brain & liver)	No
Centers for Disease Control and Prevention (CDC) (2010) [7]	24 M	Kidney and pancreas transplant recipient, presented with headache, nausea, emesis	Deceased	qPCR (brain), IHC (brain)	No
Centers for Disease Control and Prevention (CDC) (2010) [6]	4 M	Organ donor, presented with fever, personality changes, loss of appetite, muscle twitching, headache, seizure	Deceased	IHC (brain), IFA (brain), PCR (brain)	No
Centers for Disease Control and Prevention (CDC) (2010) [6]	31 F	Kidney transplant recipient, presented with paresthesias, muscle spasms, headache, nausea, altered mental status, seizure	Deceased	PCR (brain)	No
Centers for Disease Control and Prevention (CDC) (2010) [6]	27 M	Kidney transplant recipient, presented with headache, nausea, altered mental status, seizure	Survived	PCR (CSF)	No
Cary et al. (2010) [5]	2 M	Vomiting and diarrhea	Survived	IFA (brain)	No
Kansagra et al. (2009) [37]	43 M	Fever, headache, and nausea	Deceased	IFA (brain)	No
Prasad et al. (2008) [107]	22 M	Numbness, vertigo, difficulty swallowing, weakness, seizure, chronic suppurative otitis media	Deceased	PCR (brain)	No
Silva-Vergara et al. (2007) [76]	32 M	Headache, blurred vision, and fever	Deceased	IFA (brain)	No

Table 2 (continued)

Publication [References #]	Demographics (age in years, gender)	Clinical presentation	Outcome	Method of diagnosis	Diagnosed by plasma mNGS
Perez et al. (2007) [60]	40 M	Seizure	Deceased	IFA (brain)	No
Cuevas et al. (2006) [13]	5 F	Skin lesion(s)	Deceased	Diagnosed based on histomorphology of brain tissue, no confirmatory testing	No
Tavares et al. (2006) [84]	8 M	Headache, medial esotropia, diplopia, signs of intracranial hypertension	Deceased	IFA (brain), PCR (brain)	No
Li et al. (2005) [45]	6 F	Headache and stiff neck	Deceased	IFA (brain)	No
White et al. (2004) [91]	32 M	Confusion and focal neurological signs, prior skin lesion(s)	Deceased	IFA (serum)	No
Intalapaporn et al. (2004) [31]	23 M	Skin lesion(s)	Deceased	IFA (brain)	No
Jung et al. (2004) [35]	72 F	Seizure, visual loss, aphasia	Survived	IFA (brain)	No
Pritzker et al. (2004) [64]	89 M	Skin lesion(s)	Deceased	IFA (skin)	No
Deetz et al. (2003) [15]	64 M	Hemiparesis, speech difficulty, seizure	Survived	IFA (brain)	No
Deetz et al. (2003) [15]	5 F	Seizure, fever	Survived	IFA (brain)	No
Bakardjiev et al. (2003) [4] & Schuster et al. (2003) [73]	3 F	Fever, fatigue, otitis media, dehydration,seizure, emesis	Deceased	IFA (serum, brain), PCR (brain) with sequencing	No
Bakardjiev et al. (2003) [4]	2 F	Fever, headache, lateral eye deviation, facial palsy, hemiparesis, otitis media	Deceased	IFA (brain)	No
Bakardjiev et al. (2003) [4]	7 M	Fever, chills, seizure, wide based gait, cranial nerve palsy (VI), abdominal pain personality change	Deceased	Elevated serum titers	No
Bakardjiev et al. (2003) [4]	2.5 M	Fever, emesis, ataxia	Deceased	IFA (brain), titers (serum, CSF)	No
Shirabe et al. (2002) [74]	78 F	Fever	Deceased	IFA (brain)	No
Galarza et al. (2002) [21]	12 M	Skin lesion(s)	Deceased	IFA (brain)	No
Galarza et al. (2002) [21]	5 M	Skin lesion(s), seizure, loss of consciousness	Deceased	IFA (brain)	No
Galarza et al. (2002) [21]	3 F	Seizure, loss of consciousness, hemiparesis	Deceased	IFA (brain)	No
Galarza et al. (2002) [21]	6 M	Skin lesion(s), hemiparesis, loss of consciousness	Deceased	IFA (brain)	No
Katz et al. (2000) [38]	52 F	Seizure, prior skin lesion(s)	Deceased	IFA (brain)	No
Deol et al. (2000) [17]	38 M	Skin lesion(s), weight loss, seizures	Deceased	IFA (brain)	No
Recavarren-Arce et al. (1999) [122]	22 M	Skin lesion(s), neurological symptoms	Deceased	IFA (brain)	No
Recavarren-Arce et al. (1999) [122]	5 M	Skin lesion(s), neurological symptoms	Deceased	IFA (brain)	No
Recavarren-Arce et al. (1999) [122]	9 M	Skin lesion(s), neurological symptoms	Deceased	IFA (brain)	No
Recavarren-Arce et al. (1999) [122]	10 M	Skin lesion(s), neurological symptoms	Deceased	IFA (brain)	No
Recavarren-Arce et al. (1999) [122]	12 M	Skin lesion(s), neurological symptoms	Deceased	IFA (brain)	No
Recavarren-Arce et al. (1999) [122]	50 F	Skin lesion(s), neurological symptoms	Deceased	IFA (brain)	No

Table 2 (continued)

Publication [References #]	Demographics (age in years, gender)	Clinical presentation	Outcome	Method of diagnosis	Diagnosed by plasma mNGS
Recavarren-Arce et al. (1999) [122]	34 M	Skin lesion(s), neurological symptoms	Deceased	IFA (brain)	No
Recavarren-Arce et al. (1999) [122]	14 M	Skin lesion(s), neurological symptoms	Deceased	IFA (brain)	No
Recavarren-Arce et al. (1999) [122]	28 M	Skin lesion(s), neurological symptoms	Deceased	IFA (brain)	No
Kodet et al. (1998) [41]	3 M	Lethargy, seizure	Deceased	IFA (brain)	No
Denney et al. (1997) [16]	32 M	Nausea, emesis, headache	Deceased	IFA (brain)	No
Reed et al. (1997) [67]	5 M	Skin lesion(s)	Deceased	IFA (brain)	No
Duke et al. (1997) [19]	3 M	Pharyngitis, fever, chills, emesis, irritability, somnolence	Deceased	Not specified	No
Rowen et al. (1995) [68]	5 M	Extremity weakness, slurred speech	Deceased	IFA (brain)	No
Rowen et al. (1995) [68]	15 M	Headache, emesis, abdominal pain	Deceased	IFA (brain)	No
Martinez et al. (1994) [52]	14 M	Seizure, limb weakness, headache	Deceased	IFA (brain)	No
Zagardo et al. (1997) [100]	34 F	Seizure, fever, cranial nerve palsy, pronator drift	Deceased	IFA (brain)	No
Lowichik et al. (1995) [49]	0 y 11 mo F	Internal strabismus, unsteady crawling, difficulty grasping objects, cranial nerve palsy	Deceased	IFA (brain)	No
Griesemer et al. (1994) [26]	2 M	Otitis media, hemiparesis, aphasia, emesis, lethargy, fever, instability	Deceased	IFA (brain)	No
Griesemer et al. (1994) [26]	13 F	Headache, diplopia, hemiparesis, left facial weakness, emesis	Deceased	IFA (brain)	No
Neafie et al. (1993) [55]	1 M	Hemiplegia, lethargy	Deceased	IFA (brain)	No
Gordon et al. (1992) [23]	52 M	Skin lesion(s), headache, fever, chills	Deceased	IFA (brain & skin)	No
Popek et al. (1992) [63]	0 y 5 mo F	Sore throat, otitis media, seizures, fever	Deceased	IFA (brain)	No
Chimelli et al. (1992) [10]	47 F	Weakness, myalgia, fever, weight loss, headache	Deceased	IFA (brain)	No
González-Alfonzo et al. (1991) [22]	7 M	Pain, swelling, serous eye discharge	Deceased	IFA (brain)	No
Taratuto et al. (1991) [83]	12 M	Skin lesion(s), hyperthermia, headache, conjugate gaze deviation, facial palsy, dysarthria, hemiparesis	Deceased	IFA (brain)	No
Anzil et al. (1991) [2]	36 M	Headache, fever	Deceased	IFA (brain)	No
Visvesvara et al. (1990) [87]	2 F	Headache	Deceased	IFA (brain)	No
Visvesvara et al. (1990) [87]	0 y 3 mo M	Diarrhea, dehydration, fever, pneumonia	Deceased	IFA (brain)	No
Visvesvara et al. (1990) [87]	2.5 M	Neurologic dysfunction, headache, emesis	Deceased	IFA (brain)	No
Visvesvara et al. (1990) [87]	72 M	Chronic renal failure	Deceased	IFA (brain)	No
Visvesvara et al. (1990) [87]	0 y 9 mo F	Diarrhea, cough, fever, cranial nerve palsy	Deceased	IFA (brain)	No
Visvesvara et al. (1990) [87]	61 M	Altered mental status, prior skin lesion(s)	Deceased	IFA (brain)	No

Table 2 (continued)

Publication [References #]	Demographics (age in years, gender)	Clinical presentation	Outcome	Method of diagnosis	Diagnosed by plasma mNGS
Visvesvara et al. (1990) [87]	22 M	Head injury, headache, seizure	Deceased	IFA (brain)	No
Visvesvara et al. (1990) [87]	11 F	Seizure, headache, fever vomiting	Deceased	IFA (brain)	No
Visvesvara et al. (1990) [87]	39 M	Seizure, cranial nerve deficits, coma	Deceased	IFA (brain)	No
Visvesvara et al. (1990) [87]	22 M	Headache, seizure, nasal mass	Deceased	IFA (brain)	No
Visvesvara et al. (1990) [87]	30 M	Headache, nausea, emesis, dizziness, diplopia	Deceased	IFA (brain)	No
Visvesvara et al. (1990) [87]	60 M	Skin lesion(s)	Deceased	IFA (brain)	No
Visvesvara et al. (1990) [87]	60 M	Seizure, hemiparesis	Deceased	IFA (brain)	No

blood. We identified 20 cases reporting positive *B. mandrillaris* mNGS testing results, with 6 positive tests performed on brain tissue, 14 on CSF, 2 on plasma, 1 on serum, and 1 that did not identify the specimen (see Table 2), with some testing more than one tissue type. One report identified *B. mandrillaris* using mNGS on serum, also with positive mNGS on CSF and brain [59]. One case reported negative mNGS on CSF in the setting of positive mNGS on brain biopsy in a patient who survived [105]. Our analysis found only two other reported instance of *B. mandrillaris* detected via plasma mNGS. In both of those cases, CSF was also positive via mNGS and/or PCR [36, 120]. In contrast, in our case, plasma mNGS successfully identified the pathogen, while CSF *B. mandrillaris* PCR and CSF mNGS were both reported negative.

Upon retrospective review of the CSF mNGS raw data for this case, two reads mapping to *B. mandrillaris* were identified—far below the assay's reporting threshold of 50 reads. PCR on this sample was also negative. Our observation of subthreshold reads highlights the challenges of interpreting mNGS data and may reflect several factors, including pathogen distribution between compartments and timing of sample collections. We note that CSF was collected 7 days prior to plasma and thus mNGS of CSF sampled concurrently with plasma may have yielded a positive result. While mNGS of CSF shows utility for many organisms, its sensitivity for *B. mandrillaris* is unknown. A comprehensive study of nearly 5,000 CSF mNGS results from patients with suspected CNS infections detected subthreshold *B. mandrillaris* reads in CSF in 2 of 3 identified cases [104]. Detection of *B. mandrillaris* DNA in plasma but not in CSF raises plasma mNGS as a possible diagnostic tool if CSF testing is inconclusive. However, the sensitivity and specificity of plasma mNGS for the diagnosis of *B. mandrillaris* GAE are not established. In the absence of these data, results should be interpreted with caution. Diagnostic laboratories offering mNGS testing on CSF could potentially consider reporting rare and difficult-to-diagnose pathogens like *B. mandrillaris* even if below the usual threshold for reporting.

Abbreviations

AFB	Acid-Fast Bacilli
B. mandrillaris	*Balamuthia mandrillaris*
CD	Cluster of Differentiation
CNS	Central Nervous System
Cp	Crossing Point (used in PCR)
CSF	Cerebrospinal Fluid
DNA	Deoxyribonucleic Acid
ELISA	Enzyme-linked immunosorbent assay
FFPE	Formalin-Fixed, Paraffin-Embedded
GAE	Granulomatous Amoebic Encephalitis
GMS	Gomori Methenamine Silver
H&E	Hematoxylin and Eosin
HBMECs	Human Brain Microvascular Endothelial Cells
IFA	Immunofluorescence Assay
IHC	Immunohistochemistry
mNGS	Metagenomic Next-Generation Sequencing
MRI	Magnetic Resonance Imaging
PAS	Periodic Acid-Schiff
PCR	Polymerase Chain Reaction
qPCR	Real-time Polymerase Chain Reaction

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Author contributions
S.Y.E., A.H., and S.B.W. wrote the main manuscript text. S.Y.E and A.H. prepared all figures and Table 2. R.W.R and A.H. prepared Table 1. R.W.R., A.P.N., K.M.H., and D.C. edited the manuscript text. A.P.N. also performed the free-living amoeba PCR and interpretation. M.S.S. provided radiologic interpretation. C.K. and J.H.B. performed the autopsy. A.J.M. and K.M.H. provided surgical biopsy interpretation. All authors reviewed the manuscript.

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Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
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Competing interests
The authors declare that they have no competing interests.

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