



## Case Report

# An autopsy case of granulomatous amebic encephalitis caused by *Balamuthia mandrillaris* involving prior amebic dermatitis

Tatsuro Maehara,<sup>1</sup> Tetsushi Mizuno,<sup>2</sup> Masaharu Tokoro,<sup>2</sup> Tatsuru Hara,<sup>3</sup>  Yui Tomita,<sup>4</sup> Kouki Makioka,<sup>5</sup> Sei-Ichiro Motegi,<sup>6</sup> Ayako Yamazaki,<sup>1</sup> Nozomi Matsumura,<sup>1</sup> Sumihito Nobusawa<sup>1</sup> and Hideaki Yokoo<sup>1</sup> 

Departments of <sup>1</sup>Human Pathology, <sup>4</sup>Diagnostic Radiology and Nuclear Medicine, <sup>5</sup>Neurology, <sup>6</sup>Dermatology, Gunma University Graduate School of Medicine, Maebashi, <sup>2</sup>Department of Global Infectious Diseases, Graduate School of Medical Sciences, Kanazawa University, Kanazawa and <sup>3</sup>Department of Parasitology, Kurume University School of Medicine, Kurume, Japan

**An 82-year-old man, who was healthy and had worked as a farmer, experienced worsening neurological symptoms over a seven-month period, which eventually caused his death. Multiple fluctuating brain lesions were detected radiographically. Clinically, sarcoidosis was ranked high among the differential diagnoses because of the presence of skin lesions showing granulomatous inflammation, confirmed by biopsy. The patient's cerebrospinal fluid was also examined, but no definitive diagnosis was made while he was alive. An autopsy revealed multiple granulomatous amebic encephalitis lesions in the brain. Genetic and immunohistochemical analyses identified *Balamuthia (B.) mandrillaris*, a free-living ameba, which resides in soil and fresh water, as the causative organism. A retrospective examination revealed *B. mandrillaris* in the biopsied skin as well as cerebrospinal fluid, strongly suggesting that the ameba had spread into the brain percutaneously. Few studies have detailed the cutaneous pathology of *B. mandrillaris* infections. In general, granulomatous amebic encephalitis is extremely difficult to diagnose without autopsy, but the present case provides a clue that could allow similar cases to be diagnosed earlier; that is, the presence of skin lesions.**

**Key words:** amebic dermatitis, autopsy, *Balamuthia mandrillaris*, free-living ameba, granulomatous amebic encephalitis.

## INTRODUCTION

*Balamuthia (B.) mandrillaris* is a free-living (more correctly, amphizoic) ameba, which can cause fatal granulomatous amebic encephalitis (GAE) in rare cases of humans and animals.<sup>1–5</sup> The number of studies of *B. mandrillaris*-induced GAE cases has gradually been increasing in recent years, but various issues regarding the exact incidence of the condition, its transmission routes, and diagnostic clues remain uncertain. Itoh *et al.* reviewed eight cases of *B. mandrillaris* infection in Japan up to 2015.<sup>6</sup> According to Hara *et al.*,<sup>7</sup> 18 cases of *B. mandrillaris* infection were reported in Japan up to 2018 (male to female ratio 9:9, age range 51–81 years, mean age 65.7 years). At present, the definitive diagnosis of amebic encephalitis is based on the detection of trophozoites and cysts within autopsied brain lesions. In previous studies, immunohistochemical analysis and PCR-based analysis with DNA extracted from formalin-fixed, paraffin-embedded tissue have successfully been used to detect *B. mandrillaris*.<sup>4,6,8,9</sup> However, it is difficult to make a clinical diagnosis of GAE while a patient is alive because the general condition of infected patients is usually too poor to allow brain biopsy. Even if a patient can be diagnosed as having GAE antemortem, there are only a few reports about its successful treatment with drugs.<sup>10</sup>

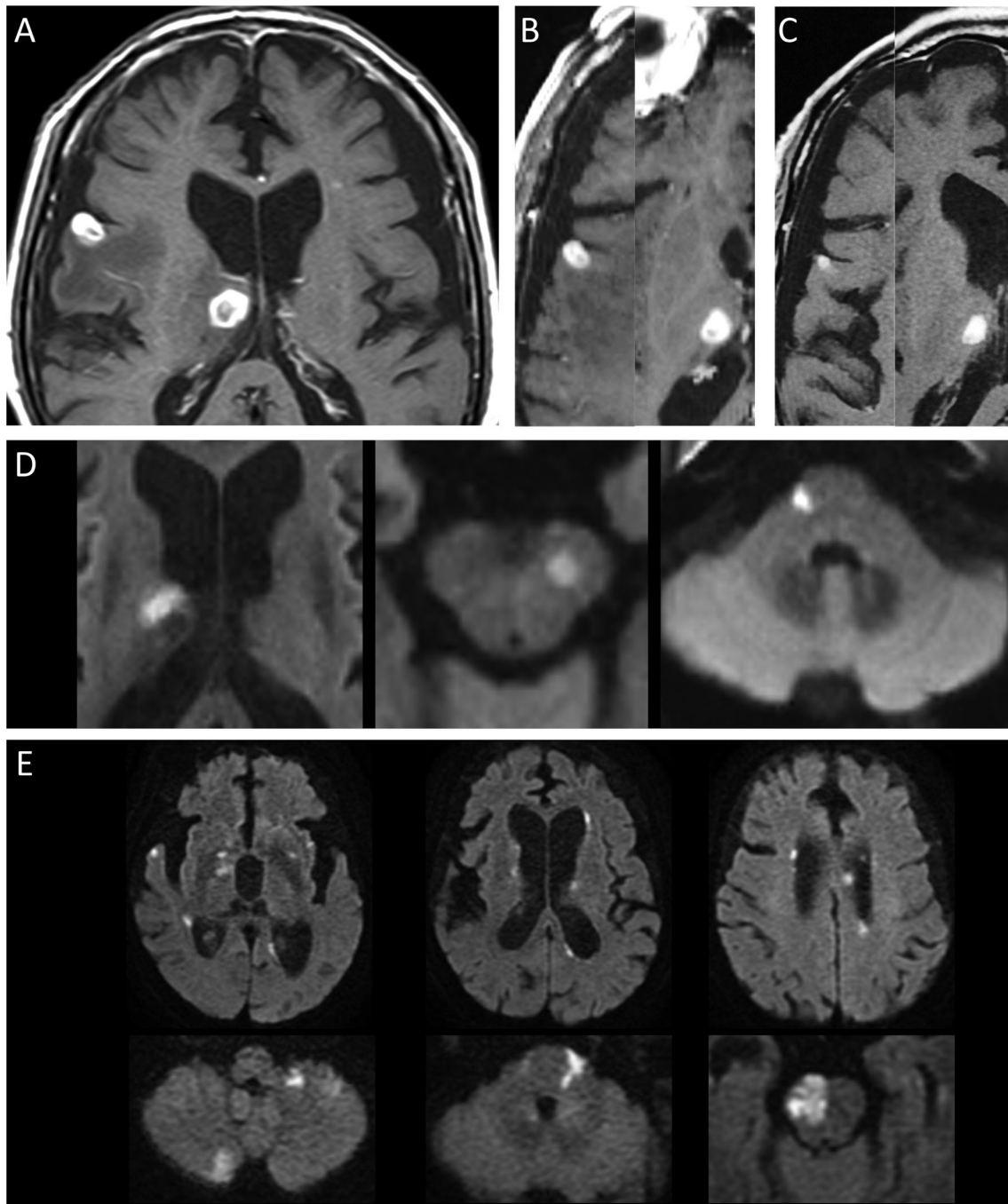
Herein, we report an autopsy case of GAE caused by *B. mandrillaris*. The treating clinicians had difficulty in diagnosing this case while the patient was alive, and sarcoidosis,

Correspondence: Hideaki Yokoo, MD, PhD, Department of Human Pathology, Gunma University Graduate School of Medicine, 3-39-22, Showa, Maebashi, Gunma, 371-8511, Japan. Email: [hyokoo@gunma-u.ac.jp](mailto:hyokoo@gunma-u.ac.jp)

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**Fig 1** MRI findings of the brain. (A) A T1-weighted image taken 69 days before death exhibits gadolinium-enhanced lesions with broad perifocal edema in the right thalamus and frontal cortex. (B, C) T1-weighted images taken 51 (B) and 29 (C) days before death display that both the lesions are shrinking despite not being treated. (D) On diffusion-weighted images taken 30 days before death, new lesions are emerging throughout the CNS. They are not enhanced by contrast medium and, hence, are considered to be ischemic lesions. (E) Diffusion-weighted images taken four days before death depict the lesions gradually increased in number. They tend to be distributed in areas in contact with CSF, such as the ventricular wall and subarachnoid space.

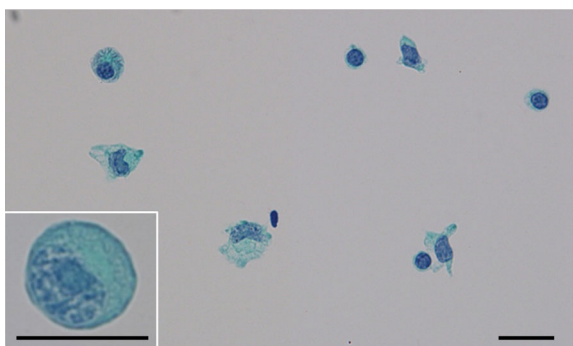
multiple brain infarctions, metastatic brain tumors, and malignant lymphoma of the central nervous system (CNS) were included as differential diagnoses. The patient also had erythematous skin lesions, which exhibited lymphocyte-rich granulomatous inflammation by biopsy. The entry route for *B. mandrillaris* infections is generally considered to be the

respiratory tract or skin, but this has rarely been proven in practice.<sup>10</sup> We noticed the presence of the pathogen in the patient's previously biopsied skin, and immunohistochemical and genetic analyses confirmed this. The significance of the early diagnosis of amebiasis using extracranial specimens is discussed.

## CLINICAL SUMMARY

The patient was a man who died at the age of 82 years. He was generally healthy and had previously engaged in farm work. Sixteen years before, he had undergone brachytherapy and anti-androgen drug (bicalutamide) treatment for early-stage prostate cancer, which was well-controlled. He had been gradually becoming forgetful for two years and was diagnosed as having dementia.

He was taken to a hospital by his family member two months before his death, as his amnesia had been progressing rapidly for seven months. Magnetic resonance imaging (MRI) revealed contrast-enhanced lesions with perifocal edema in the right thalamus and right frontal lobe, suggesting malignant lymphoma or metastatic brain tumors (Fig. 1A). In addition, computed tomography revealed multiple granular to nodular shadows in both the lungs. In the following month, the brain lesions spontaneously shrank, and the edematous changes improved (Fig. 1B, C), but the patient complained of a strong headache and so started taking 1 mg/day betamethasone for neuroprotection. However, he gradually became drowsy and unable to move. Head MRI revealed multiple abnormal signals in both the cerebral hemispheres, cerebellum, and brain stem (Fig. 1D, E). Based on the newly obtained findings, multiple brain infarctions, and sarcoidosis were additionally listed as differential diagnoses. Cerebrospinal fluid (CSF) tests produced the following results: initial pressure, 10 cm H<sub>2</sub>O; cell count, 39/ $\mu$ L (mononuclear cells, 94%); protein, 112 mg/dL; glucose, 38 mg/dL (simultaneous blood glucose: 90 mg/dL);  $\beta_2$  microglobulin, 6.60 U/dL; and soluble interleukin-2 receptor (IL-2R), 462 U/dL. CSF cytology revealed atypical cells of uncertain etiology (Fig. 2). Skin lesions were scrutinized under the clinical diagnosis of sarcoidosis. Another skin biopsy for erythema, which appeared on the skin of the upper right arm (Fig. 3A), was performed one month before



**Fig 2** Cytological features of CSF aspirated during the patient's clinical course, on Papanicolaou staining. The sample is cellular and contains a variety of inflammatory cells, such as lymphocytes, macrophages, and plasma cells. Inset shows an ameba noticed during a retrospective review. Scale bar: 20  $\mu$ m.

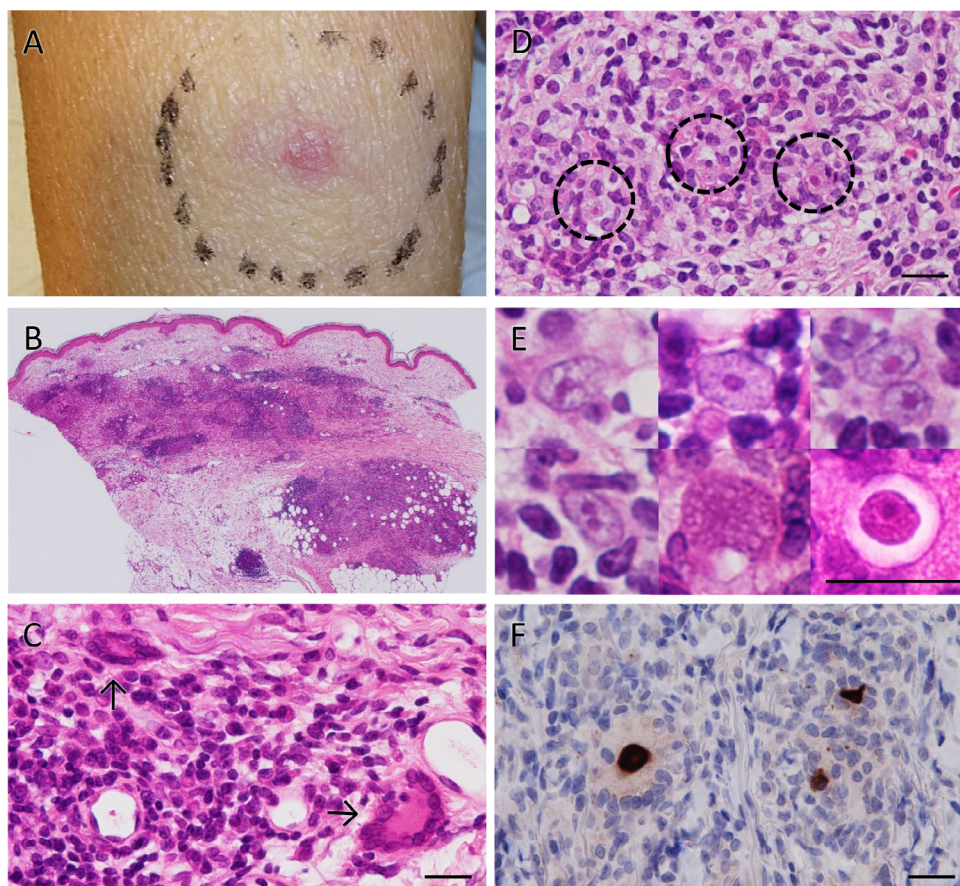
death, and it revealed multinodular, lymphocyte-enriched, noncaseous granulomatous inflammation localized in the dermis and subcutaneous tissue (Fig. 3B, C). The second MRI revealed new abnormal signals in the right cerebellum, left temporal lobe, and both the frontal cortices. Based on the laboratory and radiographical findings up to that point, mixed inflammatory and ischemic lesions associated with sarcoidosis were primarily considered. The steroid dose was increased, but the treatment response was poor, and the brain lesions worsened. The patient eventually died due to bronchopneumonia. Autopsy was approved by the patient's family member, and further investigation was conducted in accordance with the guidelines of an institutional ethics committee.

## PATHOLOGICAL FINDINGS

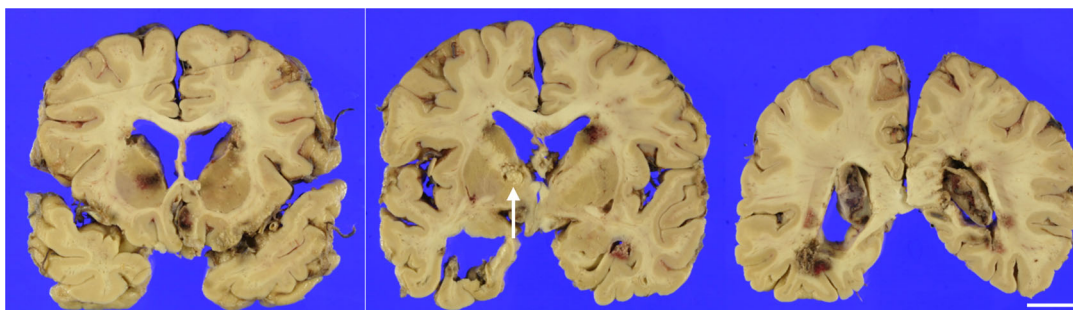
The autopsy was performed at 18 h postmortem. Externally, diffuse erythema was observed on the skin of the upper right limb and back. The brain weighed 1450 g. No turbidity of the arachnoid membrane was observed. After fixation, the coronal slices displayed multiple hemorrhagic and softening lesions measuring 5–30 mm in diameter, in both the cerebral hemispheres (Fig. 4). Larger lesions tended to be more common at sites that were in contact with CSF, especially the choroid plexuses of both the lateral ventricles, the basal ganglia, the inferior horns of the lateral ventricles, and the corpus callosum. The ventricular system was mildly dilated. Lesions with similar characteristics were distributed in the brain stem, the cerebellum, the fourth ventricle, and the subarachnoid space.

Histopathological examination was performed on formalin-fixed, paraffin-embedded sections from various regions, including the visceral organs and brain. Sections were deparaffinized, rehydrated, and stained with hematoxylin and eosin (HE), periodic acid-Schiff (PAS), and Grocott. Consistent with the macroscopic lesions, many circular amebic trophozoites were observed, chiefly around the blood vessels (Fig. 5A). Inflammatory cell infiltrates, such as lymphocytes, plasma cells, macrophages, and neutrophils, were seen around these organisms, indicative of GAE. The amebae were negative on staining with PAS and Grocott. Cysts with thick walls were scattered within the lesions and were not always limited to the regions around blood vessels (Fig. 5B). Larger lesions tended to show more marked necrosis and bleeding, and few viable amebae were located within them. Apart from the GAE lesions, many amyloid  $\beta$ -immunoreactive senile plaques were widely distributed throughout the cerebral cortex.

The left and right lungs weighed 830 g and 680 g, respectively. They showed purulent bronchopneumonia, which was considered the direct cause of death. No



**Fig 3** Gross (A), histological (B-E), and immunohistochemical (F) features of the skin lesions. (A) An erythematous nodule, measuring approximately 2 cm in diameter, is observed on the skin of the right upper arm shown. (B) A low magnification of the biopsied skin, multinodular inflammatory lesions are found in the dermis and subcutaneous tissue. (C) The predominant cellular components are lymphocytes, plasma cells, and macrophages, whereas a limited number of multinucleated giant cells (arrow) are scattered within and around the inflammatory foci. No necrosis is observed. The diagnosis at the time of the biopsy is granulomatous inflammation. (D) On a retrospective examination, unfamiliar, somewhat large cells with characteristic nuclear features are embedded in the inflamed foci, indicative of amebic trophozoites (depicted by circles). (E) At a higher magnification, each ameba has an oval nucleus and a distinct nucleolus, surrounded by relatively clear, occasionally vacuolated, amorphous cytoplasm (upper right). Some of the cell bodies of the amebae are narrow (upper middle). Most amebae are distributed separately, but sometimes two or more are found together (upper right). Some amebae with two nucleoli (lower left) or unclear nucleoli (lower middle) are also observed. An ameba engulfed by a macrophage is also found (lower right). (F) Immunohistochemistry with the antiserum against *B. mandrillaris* identifies immunoreactive organisms, indicative of the parasite infection. Scale bars: 20  $\mu$ m.



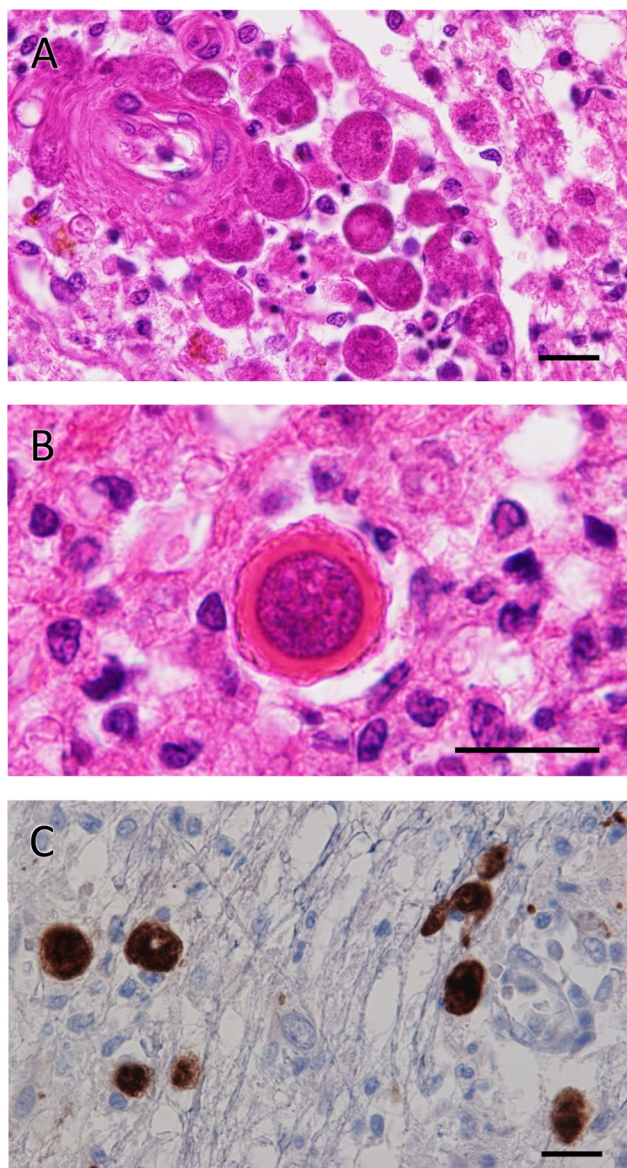
**Fig 4** Macroscopic findings of coronal slices of the autopsied brain. There are many dark red spotty lesions, indicating hemorrhagic or necrosis, on the cut surface. As pointed out on MRI, lesions are common in areas in contact with the ventricular wall or the brain surface. At autopsy, the choroid plexuses in the lateral ventricles are found to be severely affected. The lesion in the right thalamus (arrow), as detected at an earlier stage on MRI, is mostly necrotic and looks like a less active, obsolete lesion. Scale bar: 2 cm.

amebic lesions were found in the lungs. A small amount of cancer remained within the prostate. Otherwise, no significant changes were observed in the other major organs.

Based on the results of the autopsy, the CSF obtained antemortem was retrospectively examined in detail, and a few floating amebae were identified (Fig. 2). The biopsied skin tissue was also reviewed, and large ovoid cells with prominent nucleoli, which were consistent with amebic trophozoites, were scattered within dense inflammatory

cell infiltrates, containing lymphocytes, plasma cells, macrophages, and neutrophils (Fig. 3D, E).

Immunohistochemical staining with an antiserum against *B. mandrillaris* revealed positive reactions in the skin (Fig. 3F) and brain (Fig. 5C), consistent with the presence of the parasite. The antiserum was obtained by immunizing mice with cultured *B. mandrillaris*. It immunolabeled the organisms in both the culture conditions and in formalin-fixed paraffin embedded cell blocks (data not shown). For further information about the antiserum production, please contact one of the authors (TH).



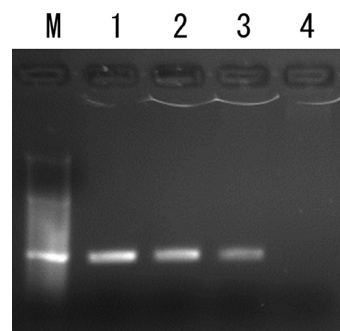
**Fig 5** Histological findings of the autopsied brain. (A) Amebae are readily found in a smaller brain lesion. The amebae tend to reside around blood vessels and invade or compress the walls of the blood vessels, possibly responsible for bleeding and circulatory disorders. (B) A cyst of ameba with a prominent thick shell, is shown. (C) The antiserum against *B. mandrillaris* clearly immunolabels the amebae within the brain lesions. Scale bars: 20  $\mu$ m.

## GENETIC FINDINGS OF AMOEBAE

Total DNA was extracted from the formalin-fixed, paraffin-embedded brain and skin lesions, and polymerase chain reaction (PCR) targeting the mitochondrial 16S rRNA gene locus of *B. mandrillaris* was performed. The cultured *B. mandrillaris* used to produce the antiserum and a human brain biopsy sample that was unaffected by the ameba were employed as positive and negative controls, respectively. The primer sequences were as follows: 5'-CGAGTGAATGCTAGCGAAAG (forward), 5'-CCAACTGCCTAATTATGTAT (reverse). As a result of the PCR, amplicons were detected at a predicted size of 178 bp (Fig. 6), and DNA sequencing confirmed that the results matched the nucleotide sequence of *B. mandrillaris* (GenBank: KT175740). The results of PCR analysis targeting *Entamoeba histolytica*, *Acanthamoeba* spp., and *Naegleria fowleri* were all negative (data not shown).

## DISCUSSION

Free-living amebae are present in soil and water, and they are widely distributed throughout the world. GAE may



**Fig 6** Results of PCR targeting the mitochondrial 16S rRNA gene locus of *B. mandrillaris*. Amplicons are detected at a predicted size of 178 bp on lanes 1-3 but not lane 4. M, marker; 1, positive control (cultured *B. mandrillaris* used to produce the antiserum); 2, brain lesion sample obtained at autopsy from the present case; 3, skin lesion sample obtained at by biopsy from the present case; 4, negative control (human brain sample obtained at biopsy from a case unaffected with amebae).

occur as either an opportunistic or non-opportunistic infection. The present patient was a farmer, who had been in daily contact with soil. Although he was relatively old, he was basically healthy and not in an immunocompromised state.

*Entamoeba (E.) histolytica* is the most common pathogenic amoeba encountered by pathologists. When we first saw the brain lesions of the current case, knowledge of the enteritis caused by *E. histolytica* was helpful. The findings on HE-stained sections indicated that this disease was an amoebic infection, and we were then able to proceed with further analyses. We histopathologically diagnosed this case as having GAE and predicted that the pathogen was likely to be *Acanthamoeba* spp. or *B. mandrillaris*; it was unlikely to be *N. fowleri*. However, that was the limit of the conclusions we could draw from the histopathological investigation.

PCR analysis was effective at furthering the identification of the causative amoeba. PCR of the mitochondrial 16S rRNA gene locus has been reported to be a useful approach to detecting amoebae, and the present case was proved to be infected with this organism. One of the authors (MT) is an expert in the genetic identification of protozoans and is available for analytical consultations (<https://www.parasitology.jp/>).

Although it was virtually impossible to diagnose GAE based on an open brain biopsy while the patient was alive, in several studies, *B. mandrillaris* has been detected in CSF as an alternative method.<sup>7,11</sup> However, once the organism migrates through blood vessels and spreads into the brain, treatment becomes extremely difficult. The identification of the pathogen in CSF may be useful for antemortem diagnosis, but it is uncertain whether it also helps to improve patient care.

The skin is presumed to be an entry point of the pathogen; however, a limited number of cases in which skin lesions harbored *B. mandrillaris* have been described.<sup>5,7,10,12,13</sup> The description of skin lesions in past studies was mainly focused on macroscopic findings and histological identification of the pathogen, and there have been few studies detailing the cutaneous pathology of *B. mandrillaris* infections. In fact, the histopathology of the biopsied skin in the current case was quite similar to that produced by non-specific granulomatous inflammation. Since the results of staining with PAS and Grocott on sections of the skin biopsy specimens were negative, a correct diagnosis could not be made. After a re-examination of the skin biopsy specimens, we noticed the presence of unusual organisms scattered within inflammatory infiltrates. Based on the observations of the patient's brain lesions, we were eventually convinced that the pathognomonic organisms were amoebic trophozoites, and additional immunohistochemical and genetic analyses confirmed the diagnosis.

It has been reported that the appearance of antiserum against *B. mandrillaris* let us know unexpectedly high prevalence of the amoebic infections in the general healthy population, suggesting that there are many opportunities for subclinical infections in daily life.<sup>14</sup> Thus, *B. mandrillaris* infections may require more attention than was previously thought. These serological data, together with the findings of the present case, suggest that a significant number of cases of amoebic dermatitis have been overlooked.

The neuropathological findings of GAE obtained at autopsy are definitive, and the recognition of amoebae during postmortem examinations is not difficult. However, it is important for healthcare workers to be able to accurately diagnose such infections while treatment is still possible. To achieve this, proper understanding of the disease by clinicians, radiologists, and pathologists is necessary, as is appropriate support by parasitologists. If such condition can be diagnosed at an early stage, treatment can be started promptly. Unfortunately, our patient could not be saved, but the identification of the organism in the skin may facilitate the early diagnosis and appropriate treatment of this tragic disease and improve its prognosis. We hope that our experience will aid the recognition of similar cases in future.

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## DISCLOSURE

The authors have no conflicts of interest to declare regarding this article.

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