



## Epidemiology of free-living amoebae infections in Africa: a review

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### ABSTRACT

FLA-related conditions are a rare medical occurrence. Despite their rarity, they are considered a public health concern for two reasons: the absence of a regular treatment regimen in the case of central nervous system infections and the fast progression of the symptoms leading to fatal outcomes. A total of 358 articles were retrieved from different databases (91 from PubMed, 26 from NCBI, 138 from Academia, 102 from Science Direct, and one from IJMED). 7 (46.6%) clinical cases came from Egypt, 2 (13.3%) cases of FLA infection came from Nigeria, 3 (20%) cases came from the Gambia, and 1 (6.6%) case was reported from African countries like Algeria, Tunisia, South Africa, and Zambia. Medical conditions caused by free-living amoeba are considered significant public health concerns. These ubiquitous organisms can cause both fatal and debilitating health conditions. Immediate diagnosis of cases and proper hygienic practices are necessary to provide direct medical intervention. They may be the key to reducing the morbidity and mortality rates from FLA-acquired infections. Although several government-led initiatives have been implemented to mitigate a plethora of parasitic diseases, the case of FLA-related conditions in African countries has yet to be realized.

### KEYWORDS

*Acanthamoeba*; Africa; encephalitis; free-living amoeba; *Naegleria*

## Introduction

Free-living amoebae (FLA) are ubiquitous organisms considered emerging zoonotic pathogens. FLAs invade the central nervous system (CNS), resulting in fatal meningitis-like conditions [1]. For this reason, the occurrence of FLAs in different environmental matrices has been identified as a public health risk for transmission [2]. Recent studies focusing on epidemiological cases involving FLA-related infections have been indispensable estimates for determining the extent of morbidity cases in some parts of the world [3,4]. Although existing literature is available on epidemiological data on FLAs, most of these studies focus on isolation cases in the environment rather than human cases [5–7]. There is a vacuity of information in the case of data that focuses on the human morbidity-mortality issues from FLAs.

The World Health Organization (WHO) has named four important genera under this group considered to be pathogenic: *Naegleria*, which causes primary amoebic meningoencephalitis (PAM), *Acanthamoeba* causing granulomatous amoebic encephalitis (GAE), *Balamuthia* causing *Balamuthia* amoebic encephalitis (BAE), and *Sappinia* which causes *Sappinia* amoebic encephalitis (SAE). *Acanthamoeba* spp., however, is known to cause non-fatal *Acanthamoeba* keratitis (AK)

infection [8]. The challenge for health practitioners in the case of FLA infection is the inability to provide a fast definitive diagnosis due to the rapid progression of the symptoms upon the onset of the disorder, and clinical features presented by FLA-caused meningitis are the same compared with bacterial and viral CNS infection. The Centers for Disease Control and Prevention (CDC) has declared that conditions from FLA-related infections as rare diseases, with only 34 documented cases from the last ten years (2010 to 2019) [9]. FLAs exist as free-living forms in the environment; it is highly pathogenic once it infects mammalian hosts. Despite its public health concerns: surveillance and epidemiologic report of cases have gone behind over the years in several countries [10,11].

The continent of Africa has seen significant economic progress over the last century [12]. Along with its progressive trend include rapid industrialization of its countries. This economic shift has led to several environmental concerns, such as pollution from factories set up by foreign investors, which, in turn, gives rise to health hazards [13]. Among these hazards include parasitic organisms that the population and visiting tourists may contract while visiting the continent. Africa has a tropical climate, and the continuous thermal pollution of its natural lakes and environment

paves the way for the emergence of zoonotic pathogens; among them are FLAs. Although current literature is available on parasitic organisms present on the continent, they focus on helminthic and selected protozoans only [14]. Also, the existing studies would only account for potential transmission, symptomatology, and treatment protocols of FLA-related conditions, and the exact number of documented cases is essentially not discussed [15]. This study aims to provide insight into the status of FLA infection in both human and animal reservoirs in Africa between 2010 to 2020.

## Methods

### Literature search strategy

The literature search was done in PubMed, NCBI, Academia, ScienceDirect, and Google Scholar databases. The search terms used were: 'Free-Living Amoeba', 'Primary Amoebic Meningoencephalitis', 'Granulomatous Amoebic Encephalitis', 'Balamuthia amoebic encephalitis', 'Sappinia amoebic encephalitis', 'Acanthamoeba', 'Balamuthia', 'Naegleria', 'Sappinia', 'Africa', 'Nigeria', 'Ethiopia', 'Democratic Republic of Congo', 'Egypt', 'South Africa', 'Tanzania', 'Kenya', 'Uganda', 'Algeria', 'Sudan', 'Morocco', 'Mozambique', 'Ghana', 'Angola', 'Somalia', 'Ivory Coast', 'Madagascar', 'Cameron', 'Burkina Faso', 'Niger', 'Malawi', 'Zambia', 'Mali', 'Senegal', 'Zimbabwe', 'Chad', 'Tunisia', 'Guinea', 'Rwanda', 'Benin', 'Burundi', 'South Sudan', 'Eritrea', 'Sierra Leone', 'Togo', 'Libya', 'Central African Republic', 'Mauritania', 'Republic of Congo', 'Liberia', 'Namibia', 'Botswana', 'Lesotho', 'Gambia', 'Gabon', 'Guinea-Bissau', 'Mauritius', 'Equatorial Guinea', 'Eswatini', 'Djibouti', 'Réunion', 'Comoros', 'Western Sahara', 'Cape Verde', 'Mayotte', 'São Tomé and Príncipe', 'Seychelles', and 'Saint Helena', 'Ascension', and 'Tristan da Cunha'. Titles and abstracts were screened using inclusion and exclusion criteria. Inclusion criteria consisted of (a) studies on the prevalence of FLA infection in humans and animals, (b) case reports, (c) retrospective studies, (d) prevalence studies conducted in different African countries on human and non-human infections, (e) studies conducted within the last ten years, and (f) although articles with full-text are of focus, articles without full-text were included provided that it includes statistical data on the prevalence of any of the FLAs were described in the abstract as this addressed the primary objective of the study. Exclusion criteria consisted of: 1. Studies were performed outside of the African continent, 2. Review papers, 3. Methods evaluation, 4. Modelling/simulation studies, 5. non-English language articles, 6. Studies conducted beyond ten years, and 7. Abstracts with incomplete data. This systematic review followed

preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines [16].

### Eligibility criteria

Studies published in English that report the prevalence of FLA infection in the African region are included in the study. Studies of the prevalence of pathogenic organisms not considered to be of FLA origin, effects of socio-behavioral patterns causing infection, reviews, and mini-reviews concerning FLA infection are excluded. Data from the collected studies, such as year of study, study site, sources or types of samples, clinical manifestations, isolated FLA, genotypes, prevalence, methods used for identification, title, and references, were extracted.

## Results

### Article search results and description of qualified articles

A total of 358 articles were retrieved from different databases (91 from PubMed, 26 from NCBI, 138 from Academia, 102 from Science Direct, and one from IJMED). Fifty-nine articles are duplicate titles and were removed from the study. A further 284 out of 299 articles were excluded from the review because they fall under the following exclusion criteria:

- Non-English text (2)
- Reviews (46)
- Studies beyond ten years (54)
- Non-African cases of FLA infection (54)
- Other types of analyses (100)
- Studies concerning the isolation of FLA from environmental sources (28)

After the exclusion screening, 15 articles were included in this study (Figure 1). Over the last decade, three (3) FLA-related conditions were documented in Africa. There is a total of 16 cases of reported FLA infections in Africa from 7 territories (Table 1) (Figure 2). Most of these cases are AK non-fatal conditions, totaling 194 (95.5%). Fatal cases from PAM and GAE account for only 1 (0.49%) case; respectively, three (3) subjects of fatal FLA-related conditions are documented from ruminants. At the same time, BAE, a rare FLA CNS infection, had 1 (0.49%) case in Africa (Table 2). There was a documented case of *Hartmannella* isolation in stool samples (1; 0.49%) (Figure 3). The potential risk factors causing AK showed that unhygienic practices in storing contact lenses are the most probable cause of infection (Table 3).

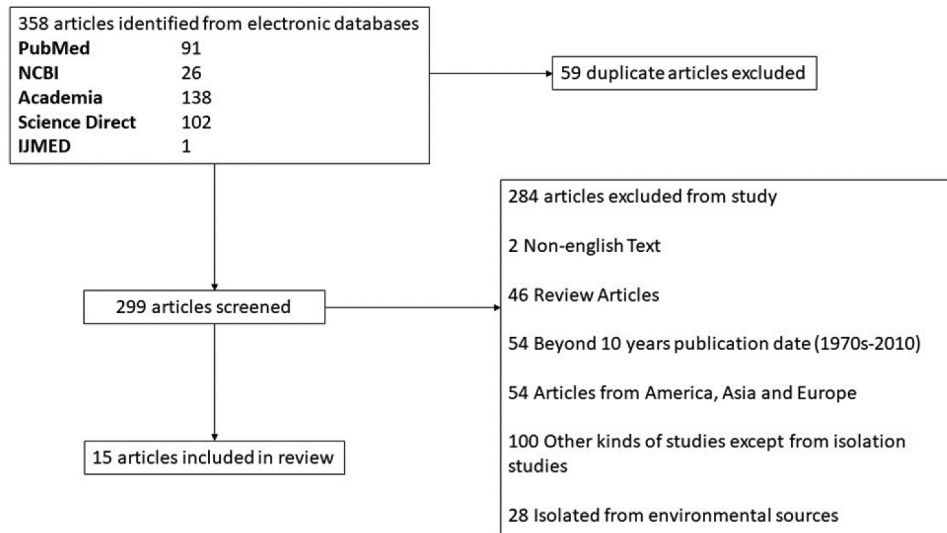


Figure 1. Flowchart of exclusion and inclusion process of articles qualified in study.

Table 1. Total number of FLA-related cases reported in the continent of Africa.

FLA case article count per African country	
African Country	Total reported cases
Algeria	1
Egypt	7
The Gambia	3
Nigeria	2
South Africa	1
Tunisia	1
Zambia	1
Total	16

### Discussion

#### Management of fatal encephalitis infections in Africa

The main challenge in treating and managing patients with FLA-related meningitis infection is the variability of symptoms that most often mirrors symptomatology presented by bacterial or viral meningitis. This includes severe headache, fever, nuchal rigidity, and Kernig’s sign [22]. This alone poses great difficulty in

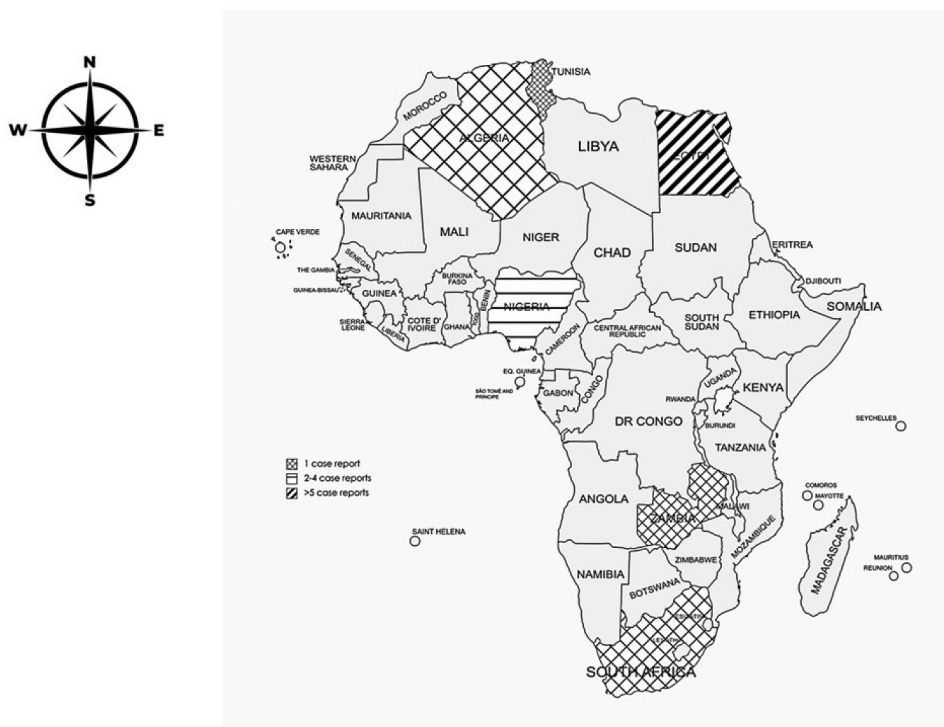
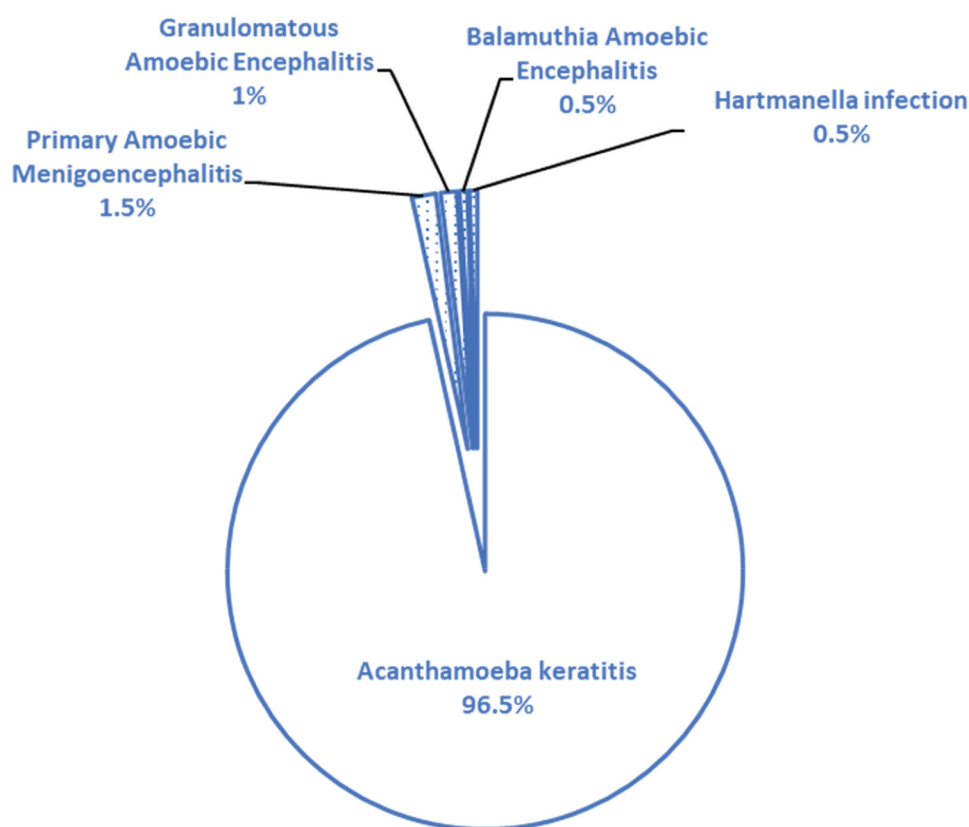


Figure 2. Geographical distribution of FLA cases in Africa from 7 territories. Reports include 1 case report from South Africa, Zambia, and Tunisia while only 2–3 cases are reported from Nigeria and the Gambia. There are more than 5 cases reported from Egypt.

**Table 2.** Summary of fatal cases of FLA-related infections in Africa between 2010–2020.

FLA	Disease Manifestation	Cases	Method of Identification	Host	Outcome	Reference
<i>Naegleria</i> spp.	Primary Amoebic Meningoencephalitis	2	Microscopy, Culture, PCR	Animal	Died	[17]
		1	Microscopy, PCR	Human	Died	[18]
<i>Balamuthia</i>	granulomatous hemorrhagic necrotic amoebic meningoencephalitis	1	Microscopy, PCR	Human	Died	[19]
<i>Acanthamoeba</i> spp.	Amoebic encephalitis	1	PCR	Human	Died	[20]
		1	Microscopy	Animal	Died	[21]

**Figure 3.** Breakdown of cases relative to FLA infection in Africa for the last 10 years (2010–2020).**Table 3.** List of risk factors in acquiring *Acanthamoeba keratitis* in Africa.

Country	Potential risk factors for AK cases in Africa	Reference
Tunisia	Cleaning of contact lenses with tap water Improper contact lens storage	[30]
Egypt	Use of stored water exposed to sun for cleaning	[64]
	Wearing contact lens while sleeping	
	Unhygienic cleaning of contact lenses	
	Swimming, showering, and cleansing the CL with tap water	
South Africa	Continuous wearing of CL for a week	[65]
	Use of disposable contact lenses	[66]

establishing an early definitive diagnosis for an effective treatment regimen. Here, we observed that all three fatal cases present the same symptoms, although in varying degrees, and it may have been because a different type of FLA caused each case.

Nevertheless, it is essential to note that the case in Nigeria presented a patient who was afebrile during the time of hospital admission [20]. This may have led

clinicians to provide the patient's wrong and delayed treatment regimen. Perhaps, the litmus test to differentiate a non-FLA caused meningitis from other forms is through the identification of the organism using microscopic methods, which will increase the chances of survival of the patient as compared to the case in India [23], Mexico [24], and the US [25]. A timely CSF wet smear microscopy for the presence of microorganisms may be crucial for a successful diagnosis and choice of the proper treatment regimen, which was overlooked as presented in two African cases [19,20]. Although this may be true, a successful therapeutic regimen and survival of the patient will still depend on the manifestation of motile organisms in CSF wet mounts, thus resulting in the earliest diagnosis. In the case of the patient in Zambia, identification of motile trophozoites was observed on the 8th day on which the patient died [18]; on the contrary, in the case of a 6-year-old boy in India, trophozoites were already observed on the first day after the onset of symptoms [26]. These two cases present a potential argument on

whether physiologic factors affect the immediate appearance of motile trophozoites in CSF fluid or whether it would be a matter of technical skill in the identification. Regardless of the reason, it needs to be placed in perspective for now. More importantly, patient survival from FLA-caused encephalitis is observed with a thorough history check on the patient's activities before symptoms. This has been followed in the successful treatment of FLA-caused encephalitis [23,26]. The incidence of fatal encephalitis in African animal hosts further provides evidence of the ability of FLAs such as *Acanthamoeba* spp. and *Naegleria* spp. to cause the same deadly effects on non-human mammalian hosts [17,21]. There are a total of only three fatal human cases of encephalitis caused by FLAs documented in Africa in the last decade; this accounts for only 20% of the total FLA-related infections in the entire continent. Although this is the case, the mortality rate presented from the gathered literature is a dismal 100%. We, therefore, hypothesize that there are cases elsewhere in the continent that may not have been documented due to a lack of affirmative protocols. It should be taken into significant consideration that two of the cases presented in this review were only given correct diagnosis postmortem, which further required consent from relatives.

### ***Acanthamoeba keratitis in Africa***

*Acanthamoeba keratitis* (AK) is considered the most common FLA-related infection and has been documented in several outbreaks in the past [27–29]. In this study, AK accounts for a huge percentage of FLA-related infections in Africa and is more prevalent in people who wear contact lenses (CLW) [30–32]. It is believed that it is acquired through unhygienic cleaning and storage of lenses [33]. However, this debilitating eye infection is also observed in non-contact lens wearers (NCLW) [34,35]. Infection of AK in NCLW in Africa is mainly caused by exposure of the eye through washing with contaminated tap water [36] or, to some extent, exposure to dust contaminated with *Acanthamoeba* infective cyst [34]. Most AK cases in Africa involved CLW, but a small fraction of NCLW is identified as having the infection [37]. To address the concerns in the growing number of AK cases in Africa, it is important to consider the potential causes and risk of infection, which can be grouped into either behavioral or non-behavioral. Risk factors considered behavioral include routine activities and practices like wearing contact lenses while sleeping, the continuous wearing of contact lenses for a week, and improper contact lens storage (Table 3). The potential infection can be avoided through behavior modification by establishing policy programs that promote good hygienic practices in handling contact lenses. On the other hand, non-behavioral causes involve CLW and

NCLW having contact with environmental sources contaminated with *Acanthamoeba* spp., such as using tap water to clean contact lenses and engaging in water recreational activities while wearing them. *Acanthamoeba* spp. is considered ubiquitous in the environment and isolated in different water sources [38–40]. Discouraging the use of tap water in cleaning contact lenses and observing proper water storage so as not to promote the growth of thermophilic FLAs are potential intervention methods that can be implemented to eliminate and avoid future infections. These intervention methods are important, especially with the emergence of non-*Acanthamoeba*-caused AK in the region, which is also the first report of human infection globally [41].

### ***Potential sources of FLA infection in Africa***

The use of FLA-contaminated tap/stored water is considered the main reason for most AK cases in Africa. Groundwater is considered one of the major sources of freshwater in Africa for the purpose of drinking and domestic use [68]. Although there has been no direct evidence of FLAs causing disease by consumption of contaminated water, the possibility of contracting infection such as AK by using contaminated tap water to wash the face should be greatly considered as documented by some cases [69]. This enables FLAs to initiate infection upon contact with exposed membranes of the eye causing AK. In the case of fatal brain infections caused by FLAs, swimming in contaminated freshwaters such as rivers and lakes is the primary reason for infection. Although as argued, other forms of transmission routes may occur and are yet to be documented. Due to these reasons, it is important to consider the inclusion of survey studies of FLAs in different freshwater sources in Africa.

### ***Non-anthropogenic impacts of free-living amoebae infections in Africa***

Here, we presented from the gathered literature two fatal cases of FLA-related infections in ruminants [17,21]. Interestingly, these cases were caused by two different species of FLA, causing fatal health outcomes in humans. The mechanism of infection is believed to be due when ruminants drink in freshwater sources enabling FLAs to access the olfactory route. Livestock parasitosis has been considered a vital livestock husbandry problem that may reduce animal productivity and quality [42]. Although several African countries are now industrialized, many territories are still considered highly agricultural and dependent on livestock for livelihood [43,44]. While there has been an established surveillance monitoring for known parasitic organisms in humans, such as those belonging to taxonomic group order Kinetoplastida and phylum Apicomplexa



[45], helminths [46–48], and other types of parasitic organisms [47,49,50]; there appears to be none for FLAs in both human and animal reservoir in the continent. Therefore, it is imperative to consider including FLAs in surveillance studies of natural freshwater sources and mortality cases in humans and livestock. The two separate cases involving cattle and ruminants in this study may potentially elucidate new transmission routes of FLAs, isolation of new pathogenic species from non-human hosts, and establishment of new protocols in veterinary medicine for the management of future cases. The current data for FLA occurrence in different environmental matrices in Africa is considered fragmented and are yet to be explored. This translates further to a low turnout of cases from the continent compared to other territories with an established surveillance and monitoring system [3,32,51].

### Future perspectives of FLA research in Africa

The continent of Africa is an amalgam of 59 countries, each having various ethnic, geographic, and cultural differences [52]. The continent is home to the great lakes, considered vital sources of fresh water and food for its population [53]. Although several studies concerning these aquatic matrices have been conducted [54,55], a survey on the biodiversity of FLAs in great African lakes has yet to be realized. It has been postulated that aquatic matrices are an essential point source for the initial infection of FLAs, as several studies have argued [2,5,40,56,57]. The importance of environmental surveys for FLA has been proven vital not only for identifying sources of infection, as with the Zambian adult who swam in the Kafue River [18] but also for identifying new pathogenic species, as it was with the case in Thailand [58]. This evidence and other studies conducted on freshwater matrices worldwide have provided factual data on the biodiversity of FLAs in different aquatic sources [58–62]. It is crucial to consider investigating the vast natural rivers and lakes found on the continent for the possibility of identifying new species of FLAs.

Opportunities to further enhance the identification of FLAs using modern methods may need to be implemented in several African health facilities. Although 9 out of 15 (60%) gathered cases in this study have confirmed the presence of FLA DNA from the samples, only one proceeded with sequencing for a definitive identification [30]. Modernizing molecular diagnostic methods for pathogen identification is essential to health care delivery. As argued by studies, several African countries are not only faced with a high burden of various infectious and noninfectious diseases. Still, they are also challenged in terms of resources [63]. The use of molecular methods not only increases the accuracy of diagnosis but also speeds up the delivery of proper therapeutic intervention for patients suffering

from FLA-related fatal infections. As we stated in our previous work, the lapses on the part of healthcare diagnostics directly translate to two possible outcomes: underreporting and misdiagnosis of cases [3].

### Conclusions

Regardless of the disease, epidemiological data are essential for implementing public health programs to safeguard the population's health and safety. The continent of Africa, along with the countries within it, are considered hotspots for development not only economically but also in the aspect of health. Although several government-led initiatives have been implemented to mitigate a plethora of parasitic diseases, the case of FLA-related infections should become a subject of investigations. As indicated in this review, managing fatal FLA-related conditions in the continent has been unsuccessful, considering the three deaths from the human cases reported. In the case of AK infections, the growing number of NCLW cases reflects hygiene and potential contamination of water sources used for daily use. Finally, the non/human cases of FLA infection open a new door of opportunities to investigate further the potential negative health impacts of FLA in livestock in several countries in Africa and around the world.

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