



## Research article

**Equine epizootic lymphangitis: A synopsis and current development**Ulrich Wernery<sup>1\*</sup>, Sunitha Joseph<sup>1</sup>, Jessika-M. Cavalleri<sup>2</sup> and Fatma G. Al Mheiri<sup>1,2</sup><sup>1</sup> Central Veterinary Research Laboratory, P.O. Box 597, Dubai, United Arab Emirates<sup>2</sup> University of Veterinary Medicine, Vienna, Austria**Article History:**

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**\*Corresponding author:**Ulrich Wernery  
cvrl@cvrl.ae**Abstract**

Equine Epizootic Lymphangitis (EEL) is a highly infectious and contagious systemic fungal disease caused by *Histoplasma capsulatum* var. *farciminosum*. It can affect all equids, but primarily horses and mules. Although the disease has been eradicated in many countries, it still exists in some Mediterranean countries, as well as in India, Pakistan, Japan, North Africa, and East Africa, notably Ethiopia. EEL is prevalent in rural, urban, and peri-urban areas where equids significantly contribute to the socioeconomic well-being of people who rely on these animals for their livelihood. The World Organisation for Animal Health (WOAH) classifies it as a listed disease due to its importance in economic and public health for countries involved in animal trade. Indeed, the disease can be transmitted through inhalation, ingestion, flies, fomites, and direct contact. The skin of equids typically becomes infected when the fungus enters through open wounds, which is the most common route of infection. Clinically, the disease presents in four forms: cutaneous, ocular (keratoconjunctivitis), respiratory (multifocal pulmonary lesions), and asymptomatic forms. The isolation is based on the isolation of *Histoplasma capsulatum* and serology; however, challenges arise due to the slow growth of the organism, which complicates isolation. Care must be taken to minimize contamination, as samples are primarily collected from abscesses, mucopurulent discharge, scabs, and tissues. This paper summarizes findings from various studies regarding the prevalence of EEL and its association with altitude and climate. In conclusion, implementing control measures is crucial for managing EEL. These measures include educating horse owners on the importance of regularly cleaning horse equipment, controlling flies, preventing wounds through proper harnessing, routine deworming, and protecting horses from feral dogs and hyena attacks.

**Keywords:** Equine Epizootic Lymphangitis, Fungal disease, *Histoplasma capsulatum* var. *farciminosum*, Epidemiology, Diagnosis, Prevention and control

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**Introduction**

The world population of equids is estimated to be 116 million (donkeys, horses, and mules), including approximately 57 million horses, 50.5 million donkeys, and 7.9 million mules (Merridale-Punter et al., 2024). In Africa, the estimated population of equids is around 25 million. Ethiopia has the second-largest equid population in the world, totaling 13.3 million equids. This includes the most significant number of donkeys globally, which is 10.7

million. These animals are used for transportation, as cart and riding animals, and play a significant role in agriculture (Asteraye et al., 2024). The majority of the world's working equids are found in low-income countries and have significant socioeconomic importance, significantly impacting the livelihoods of millions of families and individuals. They are crucial in reducing poverty, promoting gender equality, enhancing rural development, and ensuring food security. However, working equids are also

vulnerable to diseases recognized by the World Organization for Animal Health (WOAH, 2023), such as African horse sickness (AHS), glanders, and epizootic lymphangitis. Unfortunately, they are not typically included in overall livestock disease control programs, prevention policies, and strategies such as governmental vaccination programs, eradication campaigns, or legislation. Equine Epizootic Lymphangitis (EEL) is considered the most significant infectious disease affecting horses in many parts of Ethiopia (Ameni, 2006a; Guerin et al., 1992; Scantlebury et al., 2015; Stringer et al., 2017; Weeks et al., 1985). Ameni (2006a) stated that EEL is the second most prevalent disease in Ethiopian horses after AHS.

EEL is caused by *Histoplasma*, a thermally dimorphic fungus found primarily in soil contaminated with bird droppings or excrements of bats. Although the fungus is known to exist worldwide, it is more common in tropical regions where it thrives in damp soil. The fungus has two forms: mycelial or mold form, which grows in soil or culture medium at an ambient temperature of 25°C, and the yeast form, which inhabits within the tissues of mammals and humans and grows in different culture media at a temperature of 37°C. The septate mycelia develop asexual spores such as tuberculate macro and microconidia. Animals and humans contract the disease by inhaling the spores, leading to a condition known as Darling's disease. The spores then transform into yeast form in animals and humans, reproducing through budding. *Histoplasma capsulatum* (HC) has three recognized varieties: *Histoplasma capsulatum* var. *capsulatum* (HCC), which is a pathogen found in the new world; *Histoplasma capsulatum* var. *duboisii* (HCD), an African pathogen affecting humans; and *Histoplasma capsulatum* var. *farciminosum* (HCF), a worldwide pathogen affecting equines (Markey et al., 2013). Interestingly, phylogenetic studies have identified at least eight different clades, indicating that the three recognized varieties are phylogenetically meaningless (Kasuga et al., 2003; Kasuga et al., 1999).

EEL is a highly infectious and contagious systemic disease caused by the fungus *Histoplasma capsulatum* var. *farciminosum* (HCF), which can affect all *Equidae*, but mainly horses and mules (Ameni, 2007). Donkeys can also be infected, but less, while the disease has

not been reported in zebras and other wild equids (Scantlebury and Reed, 2009; Aiello et al., 2016; Markey et al., 2013). Various animal species are susceptible to HCF, including bovines, bactrian and dromedary camels, canines, felines, and wildlife (Spesivtseva and Noskov, 1959; Dalling et al., 1966; Chandel and Kher, 1994; Purohit et al., 1985; Murata et al., 2007; Ueda et al., 2003).

Laboratory animal species such as guinea pigs, mice, and rabbits have also been experimentally infected (Herve et al., 1994; Singh, 1965). The primary route of infection in humans is through inhalation of the organism from the environment. Cave exploration is a known risk factor for histoplasmosis, sometimes called "cave fever". It occasionally occurs when people visit caves in tropical countries following the inhalation of aerosolized spores (Loebermann, 2023). Although interhuman transmission of this fungus is rare, it can affect immunocompromised individuals. However, HCF, the causative agent of EEL, is not considered a zoonotic fungus.

### Past and present

In 1903, Samuel Taylor Darling graduated from the College of Physicians and Surgeons of Baltimore in the US. In 1905, during his internship at Ancon Hospital in Panama (Chaves-Carballo, 2007), he performed autopsies when he first discovered *Histoplasma*. He believed it was a type of protozoan that he named *Histoplasma capsulatum* (Gossel, 2004) because it occupied the cytoplasm of histiocyte-like cells and was enveloped by a capsule. Since then, histoplasmosis has become known as "Darling's Disease" (Daniel and Baul, 2002). In 1912, Henrique da Rocha-Lima conducted further studies on tissues of Darling's Panama patients and suggested that the organism is associated with organisms of Leishmaniasis. However, later, he proved the similarity of this organism to *Cryptococcus farciminosum* (Rivolta), the cause of epizootic lymphangitis in horses (Lukela, 1961). He concluded that *Histoplasma capsulatum* was a yeast-like fungus rather than a protozoan (Gossel, 2004). In 1933, Dr. William A. De Monbreun successfully grew the first culture of the organism. Between the 1930s and 1950s, the Giemsa and Methenamine silver stains were introduced, which helped to diagnose cases that were mistakenly diagnosed as malaria, yellow fever, or tuberculosis correctly. In 1955, Amphotericin B, an effective fungicide, was

produced from the extract of *Streptomyces* bacterium by the Squibb Institute for Medical Research New Brunswick, Canada. From that time onward, it was used in clinics in 1958 to control and treat fungal diseases, including EEL (Lukela, 1961).

EEL was known around 1850 under several names, like African glanders, Blastomycosis, Farcin d' Afrique, *Mal de verme*, and Farcin de revière. The scientific name was lymphangitis epizootic. In 1883, Rivolta and Micellone detected the disease in Algeria and France. Rivolta recognized it in 1873 from horses' ulcers as a fungal yeast causing infections of lymph vessels. He differentiated it from glanders. In 1891, Nocard, then Tokishige in 1896, later Bierbaum in 1919, and Bouquet and Negre in 1920 investigated this disease more thoroughly (Hutyra et al., 1945).

EEL has been eradicated from many countries, but it still exists in some countries of the Mediterranean region, as well as India, Pakistan, Japan, North Africa, and East Africa, mainly Ethiopia (Spickler, 2019). There seem to be some endemic areas in the US, like Mississippi-Ohio, but this is disputed by Timoney (2021), who stated that EEL has not been reported in horses in the United States. EEL occurs much more frequently in tropical and subtropical areas than in temperate zones. The WOAHA classified it as a listed disease with economic and public health importance to countries involved in animal trade (WOAHA, 2023).

### **Endemic EEL status in Ethiopia**

EEL is often seen in different parts of Ethiopia in horses, mules, and donkeys. Several studies have been published about cases in the past two decades. The epicenter of the disease is in Debre Zeit (Bishoftu) and its surroundings, where Addis Ababa University (AAU), College of Veterinary Medicine and Agriculture (CVMA) is located. The college collaborates with the Society for the Protection of Animals Abroad (SPANNA), the equine hospital, and The Donkey Sanctuary, which runs the equine clinic and hospital under the administration of AAU. The equine clinic is conveniently located in the CVMA of AAU, which gave the authors an excellent opportunity to visit the faculty and clinic several times over the last few years to receive on-site information about EEL in this area. The last visit was in spring

2022, when samples were collected and returned to the Central Veterinary Research Laboratory (CVRL), Dubai, United Arab Emirates. Various studies on the prevalence of EEL in Ethiopia have been conducted, but none have been conducted in other countries. Ameni (2002) conducted a study on EEL highlighting its importance in cart horses, one of the main working equids activities in rural parts of Ethiopia, but also used as transportation in some towns; three towns, specifically Debre-Zeit, Mojo, and Nazareth, where mainly male equids are used for transportation. Ameni and Siyoum (2002) conducted a cross-sectional study on 19,000 cart horses in 28 Ethiopian towns and found an overall EEL prevalence of approximately 20%. He found an association between the altitude of the study towns and the prevalence of EEL. EEL cases were significantly higher in the low-altitude cities. The fungal disease was also more prevalent in humid and hot areas ranging from above 1500m sea level to 2300m altitude. EEL was low in cold, windy, and dry regions.

Interestingly, EEL in equines can be cured by moving horses to areas over 2300m. Infected horses rarely respond to antibiotics; antifungal drugs are not readily available in Ethiopia. Losses of cart horses, mules, or donkeys are often devastating to the owner and his family, as these animals are the only income source for an Ethiopian family. Horses so severely infected by the fungus that they can no longer work are abandoned by their owners in remote areas, where they die and are eaten by different vultures. Seeing these poor creatures is a significant health and welfare issue that the Ethiopian government should address.

### **Aetiology**

The aetiological agent of this disease is a dimorphic fungus called HCF, previously known as *Cryptococcus farciminosus*. It is considered a variant of HCC and HCD (Weeks et al., 1985; Kasuga et al., 1999) originating from South America. HCF infects equids and other animal species, including wildlife, whereas HCC can infect humans. It is essential to know that the fungus has two distinct phases: the mycelial form found in the environment and the yeast form growing in infected organs. This fungus forms a mycelium in the environment with hyphae, macro, and microconidia spores at the end of the hyphae, which spread into the air and infect the

host by inhalation. It can also be transmitted through infected fomites, infecting various tissues and organs of the host. The mycelial form can persist in soil for many months. Environmental contamination with the fungus may also occur through discharging open abscesses or nasal, lacrimal discharge. Survival of the HCC pathogen has so far only been investigated in vitro. Bardelli and Ademollo (1927) reported that the fungus remained viable after desiccation in a laboratory for 25 months.

### Different forms of equine epizootic lymphangitis

The EEL forms are well described by several scientists (Ameni, 2006a; Selim et al., 1985; Weeks et al., 1985; Ameni and Siyoum, 2002; Scantlebury and Reed, 2009). EEL is a chronic pyogranulomatous multifocal disease of the skin, lymph vessels, and lymph nodes of the limbs and neck of *Equidae*. One of the oldest descriptions of EEL was conducted in 1945 and displayed as a drawing. The clinical conditions are divided into four different forms: i) Cutaneous EEL, ii) Ocular (keratoconjunctivitis) EEL, iii) Respiratory (multifocal pulmonary lesions) EE, and iv) Asymptomatic EEL.

Clinical manifestations of EEL in equids have been reported by many researchers (Ajello, 1968; Gabal et al., 1983; Selim et al., 1985; Rippon, 1988; Al-Ani, 1999; Ameni and Siyoum, 2002; Ameni, 2006a).

### Cutaneous EEL

Cutaneous EEL is the most common and widely reported form, characterized by subcutaneous, freely transferrable nodules originating from infected superficial lymph nodes that progress along the lymphatic system. These alterations of the lymph nodes, skin, and lymphatic vessels are described by Buxton and Fraser (1977). The lesions develop within weeks up to 3 months to form. Nodules may appear in any body part, mainly in the lower limbs, neck, chest, and face (Figure 1). First, an intradermal swelling can be developed into a small lump, followed by several nodules along the lymphatics. These nodules rupture and discharge a thick pus, leaving ulcerated skin lesions. This process frequently undergoes alternating periods of discharge and ulceration. The skin covering these ulcers becomes thick and fuses with the subcutaneous tissues (Figure 1).



**Figure 1:** Clinical forms of equine epizootic lymphangitis (EEL). A) Chronic severe case of EEL showing skin lesions on the face and front limbs surrounded by innumerable flies, *Musca ssp.* B) EEL of an Ethiopian horse in Debre Zeit showing severe multiple ulcerated nodules on the right front leg. C) Skin ulceration caused by EEL.

The nodules are round pyogranulomatous with a thick fibrous capsule that contains thick pyogenous exudate. They eventually coalesce along the lymphatic vessels, and the pathogen migrates to the nearby lymph nodes, which become enlarged and contain pus, and the fungus disseminates to other areas of the body. The fungus can affect any body part but needs

entry through wounds. In cart horses, trauma through poor harnessing is one of the significant factors of chest, neck, and leg wounding, which facilitates the entrance of the organisms (Gabal and Hennager, 1983) and the spread to other parts of the body. Therefore, The Donkey Sanctuary in Debre Zeit (Bishoftu) established a simple, easy-to-understand guide to educate

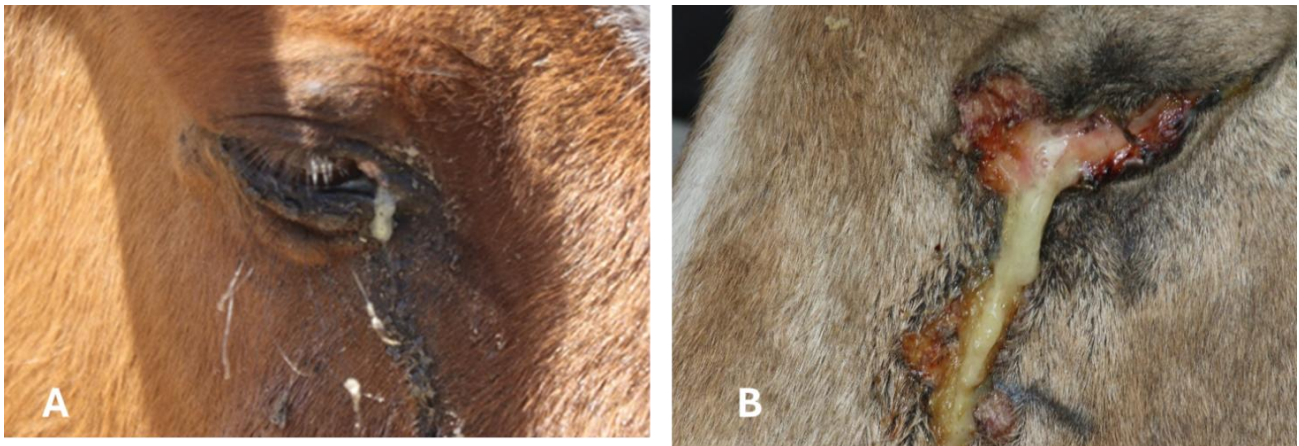
people on how harnesses should work correctly. A similar pattern of lesions, as described in horses, has also been observed in donkeys. The Donkey Health and Welfare Project (DHWP) in Ethiopia treated 34 cases of donkey EEL, predominantly in the cutaneous form (Anzuino, 2008). In some parts of Egypt, lacrimal histoplasmosis is a common manifestation, and chronic cases are sometimes seen with purulent nasal discharge from the fistulation of the lacrimal duct (Saleh, 1989; Heragy, 2003).

### Ocular keratoconjunctivitis EEL

The ocular form is characterized by serous to mucopurulent discharge, including a granulomatous reaction of the lacrimal duct (Figure 2). These lesions may reach the external skin and affect the canthus. Often, there is also swelling of the palpebrae. The fungal infection

may also affect the lacrimal ducts, leading to nodule development of the skin on the face. The ocular form has been reported mainly in donkeys in Egypt (Saleh, 1989) but has also been seen in horses and mules in Ethiopia. The severity of this infection can cause partial or complete loss of vision or eye in horses, as shown in Figure 2.

Flies are essential in transmitting the fungus to other parts of the animal or equid. The flies are such a nuisance that infected horses are often found in the middle of the road. The wind created by passing vehicles relieves them from this pest. Singh (1965) showed that the average horse flies, *Musca* and *Stomoxys* spp., could carry HCF in their digestive tract for 20 days and then transmit the disease. Ameni and Terefe (2004) believe that the tick *Boophilus* may also play a role.



**Figure 2:** Ocular form of equine epizootic lymphangitis (EEL); A) Ocular discharges, B) Severely affected eyes, resulting in eye loss.

### Respiratory EEL

The respiratory form causes multifocal pulmonary lesions, as described by Ainsworth and Austwick (1973), either through inhalation of the fungal spores from the environment or lesions of the nasolacrimal duct, nodules of the nasal mucosa, and external nares. It is rarely reported that badly infected horses are abandoned, and no necropsy is being performed.

Granulomas are present in the conchae, trachea, and lungs. Mucopurulent nasal discharge is associated with coughing, increased respiratory distress, and weakness. Respiratory EEL is a severe and debilitating disease involving the nose, conchae, trachea, and lungs. It resembles glanders, and therefore, proper laboratory investigations are necessary.

### Asymptomatic EEL

It is not entirely clear if asymptomatic EEL in equids exists, as there is a lack of information concerning immunological studies investigating the existence and role of these animals. However, Al-Ani (1999) reported this form. The fungus induces both humoral and cell-mediated immune responses (Gabal and Khalifa, 1983). Serological tests, like antibody ELISA, should be established to investigate asymptomatic carriers and antibody levels after treatment and eventual vaccination. As an alternative to testing antibodies, “histofarcin” similar to mallein (Soliman et al., 1985), may be used to diagnose EEL in equids. Ameni et al. (2006), who investigated the sensitivity and specificity of histofarcin produced in Ethiopia, concluded that

histofarcin could play a significant role in detecting early infection of EEL. However, standard test validation procedures are required before histofarcin can be safely applied to diagnose EEL, as 31% of false positives were reported in endemic districts (Ameni et al., 2006). In summary, the asymptomatic form is not well described; therefore, serological investigations are necessary to define the existence of this form. EEL should be differentiated from glanders caused by *Burkholderia mallei*, Strangles caused by *Streptococcus equi* var. *equi*, Sporotrichosis caused by *Sporothrix schenckii*, Melioidosis caused by *Burkholderia pseudomallei*, Caseous lymphangitis caused by *Corynebacterium pseudotuberculosis*, Cryptococcosis caused by *Cryptococcus neoformans*, and Ophthalmic and cutaneous Habronemiasis caused by *Habronema muscae*, *H. microstoma* and *Draschia megastoma*.

### Transmission

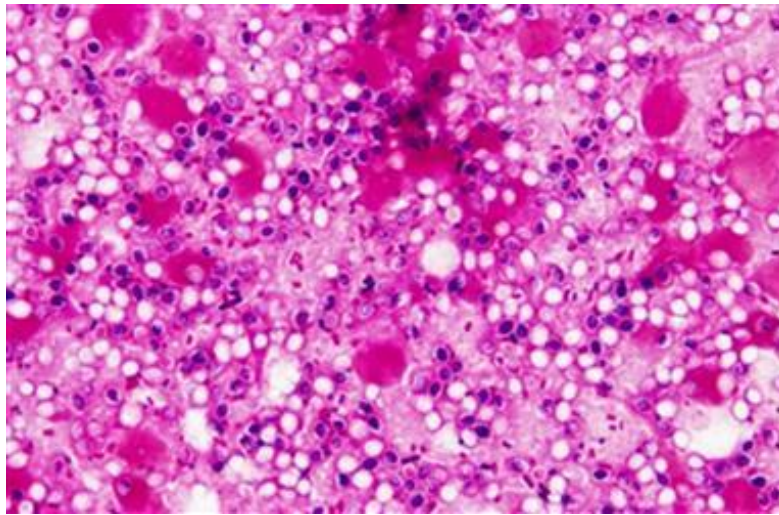
EEL zoonotic capability is not yet fully understood, but human infection has been described (Al-Ani, 1999; Chandler et al., 1980; Guerin et al., 1992; Murata et al., 2007). Animal-to-animal transmission can occur through inhalation, ingestion, flies, fomites, and direct contact. The skin of equids gets infected by the fungus through direct contact with an open wound, which is the most common route of infection. This way of transmission was confirmed by Singh (1965) and Singh (1966) through experimental infection experiments in India. The organism requires a point of trauma to gain access through the skin. Ameni (2006b) reproduced EEL experimentally in two naïve horses; one horse was injected with pus aspirated from an unruptured abscess of an EEL horse into the prescapular and femoral lymph nodes, 0.2 mL each. Additionally, 0.2 mL of pus was brushed on the scarified skin of the inner hind legs and nasal membrane and dropped onto the conjunctiva. The second horse was infected with a mycelial form of HCF grown *in vitro*. HCF, which was grown *in vitro*, was injected into the same lymph nodes, brushed on the same scarified skin, and dropped onto the conjunctiva as performed in the first case. Typical EEL lesions appeared after 4 weeks on many sides of the first horse's body, infected with the yeast form of the fungus, whereas similar lesions appeared after three months, only at the infected

lymph nodes and scarified areas. HCF was re-isolated from both horses, confirming Koch's postulate. This experiment has opened the way for vaccine production.

The disease spreads frequently when large populations of equids are stabled together in a small area. It is reported that the fungus can stay viable in stable dust for a month (Scantlebury et al., 2015). Poor hygiene and poor stable management reinforce the spread of the fungus. Flies are considered vectors for EEL and HCF and have been isolated from the alimentary tract of biting flies that had fed on open lesions. The disease developed in horses three miles from the nearest cases (Saunders, 1948; White and Jordan, 1963). EEL lesions were also found on the genitals of mares, and therefore, it is believed that the disease can be transmitted venereally (Jubb and Kennedy, 1970). EEL can be transmitted during mounting from the stallion to the mare, and the mare's milk may infect suckling foals (Mekonnen et al., 2012).

### Diagnosis

Diagnostics of the fungus, including staining, culture, skin tests, and serology, are well described by Scantlebury and Reed (2009). Here, we can confirm that the culture of HCF is a challenge as the organism grows very slowly despite using different fungal agars. Care must also be taken to reduce overgrowth by contaminants, as samples originate mainly from abscesses, mucopurulent discharge, scabs, and tissues. It is also evident that the mycelia grown on culture media can be converted into the yeast phase in the laboratory by altering the culture conditions (Bullen, 1949; Al-Ani, 1999). When testing samples from equids or laboratory-infected animals, the yeast form is isolated only as it grows at temperatures above 30°C. Therefore, the yeast form of HCF is found in histology samples obtained from abscesses or biopsies (Figure 3). The mycelial or mold form lives in moist soil and beddings at lower temperatures, for example, in horse stables where spores are produced. These spores may be inhaled, producing the pneumonic EEL form, or may enter skin wounds when animals roll on the ground. In lungs and skin wounds, the fungus converts into yeast form.



**Figure 3:** Gram-stained smear of a pus sample from an Equine Epizootic Lymphangitis (EEL) horse abscess, characterized as Gram-positive pleomorphic ovoid globose structures, 2-5  $\mu\text{m}$  in diameter with a “halo” appearance due to unstained capsule, typical for HCF yeast, magnification 100x (oil immersion).

### Treatment and prevention

No utterly satisfactory treatment is known yet. Surgical excision of lesions combined with antifungal drugs could be used, but these treatments are limited due to financial constraints and the availability of the drug. SPANA, Ethiopia, has developed a protocol for the treatment of infected equids and also advises horse owners on how to react to control the disease. The details of these strategies are laid down by [Scantlebury and Reed \(2009\)](#). In Ethiopia and other African countries where the disease occurs, disease control measures were laid down by SPANA, which include educating horse owners to regularly clean horse equipment, fly control, preventing wounds by proper harnessing, routine deworming, and protecting their horses from attacks of feral dogs and hyenas. Horses are often housed on mud flooring, the primary contamination source. Therefore, floors must be regularly cleaned and, if possible, disinfected.

The [WOAH \(2023\)](#) has also recommended how to control EEL, but particularly in developing countries, these recommendations are often impracticable as most equid owners are very poor and cannot afford any financial restraint. Many treatment types have been tried with disappointing results ([Mekonnen et al., 2012](#)). Differences in response to treatment between cases in early and advanced stages were also reported ([Endebu, 1996](#); [Siyoun, 2001](#); [Hadush et al., 2008](#)). N-butanol extracts from berries of *Phytolacca dodecandra* were also used

and showed antifungal effects in laboratory experiments ([Mekonnen et al., 2012](#)). [Hadush et al. \(2008\)](#) evaluated the efficacy of sodium iodide (NaI), potassium iodide (KI), and phytochemical (Endod) extract as well as Pen-Strep for horses with EEL. They found a statistically significant difference in the therapeutic effect of this treatment.

Two vaccines that are not commercially available have been developed some time ago. A live attenuated vaccine may protect 75% of horses vaccinated ([Zhang et al., 1986](#)). The fungus for the vaccine production was isolated from an infected horse in Manchuria, Mongolia, and named T21. More than 70 subcultures were made using 14 different temperature changes for growth. However, the best results were achieved with passage 71 of strain T21 with the protection of 75.5% immunized horses, subcutaneously administered with a dose of 3 mL.

The second vaccine, which is also not commercially available, was produced by [Al-Ani \(1999\)](#). It is a formalized inactivated vaccine prepared from the yeast form of the fungus. It was administered subcutaneously at a dose of 5 mL once a year with good results. No information was given on how this vaccine was produced.

### Conclusions

EEL is a fungal, chronic pyogranulomatous multifocal disease of the skin, lymph vessels, and lymph nodes of limbs and neck of *Equidae*, caused by a dimorphic fungus *Histoplasma capsulatum var. farciminosum*. EEL is a disease prevalent in areas with high humidity and

temperature and is very difficult to control. Apart from being a significant health issue for horses, it has a severe socioeconomic impact on many Ethiopian families living in the lowlands, who rely solely on cart horses as their source of income. Implementing preventive measures and educating horse owners on the importance of regularly cleaning horse equipment, controlling flies, preventing wounds through proper harnessing, routine deworming, and protecting horses from feral dogs and hyena attacks is crucial.

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**Authors contribution.** All authors contributed equally to the work.

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