

## Occurrence of Cutaneous Epizootic Lymphangitis in Working Donkeys in Debre Zeit, Ethiopia

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**Received:** November 02, 2018; **Published:** April 29, 2019

### Abstract

Epizootic lymphangitis, caused by *Histoplasma farciminosum*, is the major life threatening fungal disease of equines in Ethiopia. The present investigation describes the cutaneous form of epizootic lymphangitis in two working donkeys, which were presented for diagnosis and treatment at the Donkey Clinic, Debre Zeit, Ethiopia. Both the donkeys (aged 6 to 7 years) had cutaneous lesions on the forelimbs. Cytological examination of Giemsa and Gram stained smears from the cutaneous lesions showed several oval to pear shaped yeast cells morphologically indistinguishable from *Histoplasma farciminosum*. The pathogen was also cultured from the pus of both the affected animals on Sabouraud dextrose agar with 2.5% glycerol at 25°C. The treatment with topical application of 4% tincture iodine solution was attempted in both the donkeys. The emphasis is given on the early diagnosis and therapy to mitigate the suffering of the diseased animals. Further research on the development of the potent, cheap, and easily available chemotherapeutic agent or vaccine to prevent this mycosis in equines will be rewarding. It is emphasized that cytological examination of exudates/pus by Gram or Giemsa technique can be used for diagnosing the disease in remote areas where laboratory facilities for cultural isolation are nonexistent.

**Keywords:** Cytological Examination; Donkey; Epizootic Lymphangitis; Fungal Disease; *Histoplasma farciminosum*; Skin Lesions

### Introduction

Ethiopia is basically an agricultural based country, where livestock plays a significant role in the nation's economy. The equine population in Ethiopia is estimated about 7.9 million, which include horses 2.03 million, donkeys 7.43 million, and mules 0.4 million [1]. Many infectious diseases are described in equines from developed as well as under developing countries of the world [2-5]. Among these, *Histoplasma farciminosum* (African farcey, epizootic lymphangitis, equine histoplasmosis, pseudo-farcy, pseudo-glanders) is an economically important contagious mycotic disease of equidae [3,6]. Epizootic lymphangitis has been eradicated from many areas of the world but is still a significant cause of morbidity and mortality in several countries, particularly in Africa and Asia [7]. The disease is caused by *Histoplasma capsulatum* var. *farciminosum*, a dimorphic fungus, which occurs in mycelium form in the environment or at 25°C and yeast form in tissue of the host or at 37°C [7]. The cutaneous form of epizootic lymphangitis should be differentiated from glanders, sporotrichosis, strangles, and ulcerative lymphangitis [3]. Maximum cases (> 90%) are recorded in horses, followed by mules and donkeys during winter and autumn [8]. Rarely, infection is also encountered in other animals such as camels, cattle, and dogs, besides humans [7,9]. Disease is endemic in several parts of Africa, Asia, and Middle East. Transmission is mainly by direct contact of the traumatized skin with the infected material or by indirect spread by contaminated fomites [3]. The role of arthropod vectors as mechanical transmitter of fungus is also

documented [3,10]. Disease usually occurs in sporadic form but outbreaks are also recorded in military horses, which are kept together in common sheds [3]. The morbidity is very high and mortality may reach to 10 to 15% [3]. The rarity of reports on epizootic lymphangitis in donkeys prompted the author to put on record two laboratory confirmed cases of cutaneous mycosis in donkeys due to *Histoplasma farciminosum* from Debre Zeit, Ethiopia.

### Materials and Methods

Two donkeys (one 6 years- old female and other 7-years-old male) presented at the Donkey Sanctuary Clinic at the College of Veterinary Medicine in Debre Zeit, Ethiopia, with cutaneous lesions, constituted the material for this investigation. The pus was aspirated from un-ruptured nodules after clipping and disinfecting the skin with 70% ethyl alcohol. The smears prepared from the aspirated pus were dried, fixed, and stained with Gram's stain and Giemsa for the identification of yeast form of the organism. The clinical samples were streaked on Sabouraud dextrose agar with 2.5% glycerol. The inoculated media were kept at 25°C for isolation of mycelial form, and for the conversion of mycelial form to the yeast phase, brain heart infusion agar with 5% horse blood was incubated at 37°C. The slants were examined periodically for the evidence of growth of fungus. The detailed morphology of isolates were studied in Narayan stain, which contained 4.0 ml of glycerin, 6.0 ml of dimethyl sulphoxide and 0.5 ml of 3% solution of methylene blue [11]. Topical application of 4% solution of tincture iodine was done on the excised nodules for about 6 to 8 weeks.

### Results

The clinical examination of both the donkeys showed nodules and ulcers on the skin and lymphatic vessels on the forelimbs. The number of lesions on the affected animals varied from 5 to 7. Microscopic examination of smears from the pus of both donkeys revealed Gram positive, pleomorphic, ovoid to globose yeasts cells (2 - 5 um in diameter) morphologically simulating to *H. capsulatum farciminosum*. Similar morphology of the yeast cells was observed in cytological smears by Giemsa technique. The colonies grew slowly and developed in 6 to 8 weeks at 25°C. Dry, granular, wrinkled and grayish-white colonies grew on Sabouraud medium at 25°C. However, the yeast phase of colonies on Brain heart infusion agar was whitish with a pasty consistency. The wet mount preparations of the mycelia growth in Narayan stain revealed branched hyphae, chlamydospores, and macroconidia. Both donkeys showed clinical improvement with topical iodine therapy after 6 weeks of therapy. However, no mycological follow up to assess the efficacy of drug was conducted.

### Discussion and Conclusion

Epizootic lymphangitis caused by dimorphic fungus *H. capsulatum va. farcimiosum*, is a debilitating fungal disease of equids, which is frequently reported from tropical and subtropical regions of the world [7]. In Ethiopia, the disease is endemic and commonly encountered in carthorses particularly in warm and humid areas with an altitude ranging from 1500 to 2300 meters above sea level [9]. One study in Ethiopia recorded the prevalence of 26.2% in carthorses [12]. The disease has a serious negative impact on the livelihood of carthorse owners in the affected areas, and also compromises the welfare of working horses [13]. The clinical presentation, direct microscopy, and cultural isolation conclusively proved that both the donkeys were suffering with cutaneous form of epizootic lymphangitis. In the current study, the presence of lesions on the forelimbs of both donkeys are in conformity with the observations of Powell and co-workers [14] who also described that lesions in donkeys most commonly affected the forelimbs. Histofarcin skin test was developed by Soliman and co-investigators [15] to diagnose the disease in horses in Egypt. Subsequently, others researchers also investigated the usefulness of Histofarcin test in the field on carthorses in Ethiopia [16,17]. However, it is emphasized that the efficacy of this skin test as a diagnostic tool should be assessed in donkeys. As epizootic lymphangitis is an important mycosis of equines, particularly in Ethiopia, sincere attempts should be made to develop a safe, potent, and cheap vaccine to control this devastating disease in equines. It is emphasized that Narayan stain, being cheap, can be widely used in diagnostic laboratories of poor nations for the detailed morphological studies of fungi, which are implicated in the etiology of human and animal mycoses.

## Acknowledgements

The author is thankful to his student for bringing the samples for this investigation. The thanks are also due to the staff of Donkey Project, Debre Zeit and Microbiology Laboratory for their kind help in collection of clinical samples, and technical assistance, respectively. The computer help of Anubha is gratefully acknowledged.

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**Volume 15 Issue 5 May 2019**

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