Prevalence of epizootic lymphangitis and its associated risk factors in cart mules in Bahir Dar city, North Western Ethiopia

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Abstract

Epizootic lymphangitis (EL) is one of the most important diseases of equines in Ethiopia, causing a significant economic loss in cart-horse and mule producers. A cross-sectional study was carried out between April and June 2017 in Bahir Dar city to estimate the prevalence of epizootic lymphangitis (EL) and its associated risk factors. Study locations and cart mule owners were selected purposively based on the availability of cart mules. Whereas cart mules were sampled using a simple random sampling technique by a lottery system. A total of 384 cart mules were considered for this study. Both clinical and microbiological examinations were employed to identify EL in-cart mules. Among 384 sampled cart mules, which were subjected to clinical and microbiological examination, 88 (22.9%, 95% CI= [18.7, 22.1]) were positive for EL. Cutaneous form 69 (78.4%, 95% CI= [69.8, 87.0]) was the commonest form of the disease followed by mixed infection 10 (11.4%, 95%CI= [4.7,17.9]), ocular 6 (6.8%, 95% CI= [1.5,12.1]) and pulmonary 3 (3.4%, 95% CI= [0.4, 7.2]). Most EL lesions were observed on forelimbs 24 (27.3%, 95% CI= [17.9, 36.5]), followed by chest and forelimb 18 (20.5%, 95% CI= [12.0, 28.9]), hind limbs 16 (18.2%, 95% CI= [10.1, 26.2]), chest 11 (12.5%, 95% CI= [5.6, 19.4]), and head and forelimbs 8 (9%, 95% CI= [3.1,15.1]). The association between location, sex, and body condition score with the occurrence of EL was examined using the chi-square test. However, none of the examined risk factors considered were significantly associated with EL ($p>0.05$). In conclusion, a higher prevalence of EL was recorded in Bahir Dar city which could substantially affect the health, productivity, and

performance of cart mules. Further extensive mycological and epidemiological research on EL is suggested to develop evidence-based EL control and prevention measures in cart mules.

Keywords: Bahir Dar City; Epizootic Lymphangitis; Cart-Mules; Prevalence

Introduction

Ethiopia has the largest equine population in Africa and stands eighth in the world with its equine population (Endebu, 2000). The country owns about 12.7 million equine populations, of which mule accounts for about 461,665 and Amhara Region shares 45.2% of the country's mule population (CSA, 2018). Generally, equines play an important role in the transport of farm products, fodder, firewood, agricultural inputs, construction, and waste materials (Wilson, 1995; Garuma *et al.*, 2007). Although, Ethiopia has a huge number of equines; the production, productivity, and performance of these animals are lower due to infectious and non-infectious diseases (Abdisa, 2017). Major reported equine health problems in Ethiopia include harness-related wounds and sores, colic, Epizootic Lymphangitis, and African horse sickness (Kebede *et al.,* 2000; Abdisa, 2017).

Epizootic Lymphangitis is a contagious, chronic disease of horses, mules, and donkeys, which is characterized clinically by a suppurative, ulcerating, and spreading pyogranulomatous, multifocal dermatitis, and lymphangitis (OIE, 2008; Enbiyale *et al.,* 2018). The disease is common in Ethiopia, especially in cart horses, affecting an average of 18.8% of horses in warm, humid areas between 1500 and 2300 meters above sea level (Ameni and Terefe, 2004; Ameni, 2006; Ameni, 2007). The disease is currently endemic in regions of sub-Saharan Africa, and historically cases were reported in North Africa, some parts of Asia, India, Pakistan, Japan, and some countries bordering the Mediterranean Sea (Al-Ani *et al.,* 1986).

The causative agent of EL is Histoplasma capsulatum variety farciminosum (HCF). It is a thermally dimorphic fungal soil saprophyte. The mycelial form is present in the soil, while the yeast form is usually found in lesions. HCF is resistant to the effects of physical and chemical agents. It may survive for up to 10 weeks in non-sterile water at 26°C (Gabal and Hennager, 1983; Soliman *et al.,* 1986; OIE, 2008).

EL has three forms: the cutaneous, the ocular, and the pulmonary depending on the route of entry (Radostits *et al.,* 1994; Ameni, 2007). The incubation period of the disease is from 3 weeks to 12 months. It causes considerable debility but low mortality (10% to 15%) (Jubb *et al.,* 2006). The mode of transmission is by direct or indirect contact of HCF with traumatized skin, by biting flies, by ticks, or by inhalation of HCF (Ameni and Terefe, 2004). The infection rate of EL varies with the geographic area and the age of the animal. Horses under six years of age are more susceptible (Al-ani, 1999).

Even though mules are resistant to EL theoretically (Al-Ani and Ali-Delaimi, 1986), except few studies (Ameni and Terefe, 2004) there is a paucity of information on the prevalence of EL in Ethiopia particularly in mules in Bahir Dar city, where mule cart transport is widely used. With the above background, this study was conducted in Bahir Dar city to estimate the prevalence of EL and its associated risk factors in cart mules.

Materials and methods

Study area description

The study was conducted at Bahir Dar city, northwest Ethiopia, where mule cart transport is widely used. Bahir Dar city is located 578 km away from Addis Ababa along the upper Blue Nile River basin. Geographically, Bahir Dar is situated at an altitude of 1840 m above sea level, located at a latitude and longitude of 11.36N, 37°23 E 11.6N, 37.38° E, respectively. The rainy season ranges from April to July, but 80% of the rain falls between May and September with a mean annual rainfall of 1035 mm. The city experiences a lower and higher average temperature of 10°C and 30°C. Bahir Dar city constitutes nine-sub-cities, with a total of 17 administrative kebeles (Bahir Dar City Agriculture Office, 2017).

Study population

Study populations were cart mules found in Bahir Dar city, kept mainly for cart pulling purposes. There were about 6678 equines in Bahir Dar and its environs, of which 2613 were mules (Bahir Dar city Agriculture Office, 2017). Of which, about 1058 cart pulling mules were registered in Bahir Dar city (Bahir Dar City Agriculture Office, 2017).

Study design, sampling technique, and sample size estimation

A cross-sectional study was employed to estimate the prevalence of EL in-cart mules between April and June 2017 in Bahir Dar city. Before the commencement of the study, a list of mule owners was obtained from the Bahir Dar city Agriculture Office. Among 17 administrative kebeles, three (kebele 14, Kebele 16, and Kebele 11) kebeles and owners were selected purposively based on the availability of cart mules. Cart mules were selected randomly by the lottery technique. As there was no previous report found on the prevalence of EL in Bahir Dar city during the planning of this study, 50% expected prevalence was used to estimate the required sample size. Thus, a total of 384 cart pulling mules were estimated as a minimum required sample size according to the formula described by Thrusfield (2005).

Methods of data collection

Clinical Examination

All sampled cart mules were clinically examined for EL by physical observation. All parts of the body including the nostrils and eyes were examined for the presence of nodules and ulcers. Emphasis was given to the lymphatic vessels, lymph nodes, and skin. Inspection and palpation were used during a clinical examination. Mules were classified as positive or negative based on clinical examination. Further confirmation was made by microscopic examinations on all suspicious/clinical cases. During clinical examination, sex, body condition score, and bodily distribution of EL lesions were recorded using a data recording sheet. The body condition score (BCS) of the mules was assessed and categorized as “poor”, “medium” and “good” according to Svendsen (1997).

Laboratory Examination

Clinically positive mules were subjected to further laboratory examination. Pus samples from un-ruptured nodules were used for direct microscopy. Before the collection of samples, the nodules were washed with soap and water, shaved, and disinfected with alcohol. Fine needle aspiration (FNA) was performed using a standard syringe (5 ml) and needle (22 and 23- gauge). The smears were fixed with methanol, stained with Giemsa stain for the identification of the yeast form of HCF. The examination was made using 40 x magnifi-

cation followed by oil immersion at 100 x magnification until the fungus was demonstrated in the clinical specimens. Samples with microscopic features of typical yeast-like, double-contoured oval or ovoid cells measuring 2.5-3.5 µm by 3-4 µm (Al-Ani et al., 1998; AL-Ani, 1999; Quinn et al., 2002), were considered as positive. The laboratory work was conducted at the Bahir Dar Animal Health Diagnostic Investigation Center.

Data management and analysis

The data were checked, coded, and entered into Microsoft excel version 2016 and then the raw data was exported to STATA version 14.1 for analysis. Descriptive statistics were employed to estimate the prevalence of EL in cart pulling mules. Prevalence was defined as the proportion of the number of cart-mules positive for EL to the total number of cart mules selected in the study area. Chi-square (χ^2) test was used to measure the association between the prevalence of Epizootic Lymphangitis with sampling location, sex, and body condition of the animal. Statistical significance was set at $p<0.05$. In addition, a 95% confidence interval (CI) was used for evaluating statistical significance.

Results

Prevalence of EL

Of 384 sampled cart-mules in Bahir Dar city, 88 mules were positive for EL (Table 1), with an overall prevalence of 22.92% [95% CI=18.7, 22.12). All clinically identified cart mules were positive for EL using the microscopic examination. Relatively highest prevalence (29.8%, 95% CI= 20.5, 39.0).) of EL was recorded in Kebele-16 (Table 1).

Table 1. Prevalence of EL in Bahir Dar city across sampling locations

Sampling Location	Number of cart mules sampled	Result		Prevalence (%) [95% CI]
		Positive	Negative	
Kebele-16	94	28	66	29.8 [20.5, 39.0]
Kebele 14	141	31	110	21.9 [15.1, 28.8]
Kebele-11	149	29	120	19.4 [13.1, 25.8]
Total	384	88	296	22.9 [18.7, 22.1]

3.2. Forms of the disease

All clinically positive samples were positive for EL. The cutaneous form of the disease was most frequently encountered accounting for 78.4% [95%CI=69.8, 87.0] followed by mixed infection (11.4%, 95% CI= 4.7,17.9) (Table 2).

Table 2. Forms of EL observed during the study period in Bahir Dar city

Form of EL	Number of cases observed	Prevalence (%) [95% CI]
Cutaneous	69	78.4 [69.8, 87.0]
Mixed infection	10	11.4 [4.7,17.9]
Ocular	6	6.8 [1.5,12.1]
Pulmonary	3	3.4 [0.4, 7.2]
Total	88	100

Prevalence of EL=number of cases observed divided by the total number of cases recorded

3.3. Distribution and characteristics of lesions

From the clinical manifestation of EL, almost all of the cases encountered had one or more nodular lesions on different body parts including the fore and hind limb, scrotal, chest, and head regions. The forelimbs (27.3%, 95% CI=17.9, 36.5) were the most frequently affected body parts by nodular lesion followed by chest and forelimb (20.5%, 95% CI=12.0, 28.9) and hind limbs (18.2%, 95% CI= 10.1, 26.2) (Table 3).

Table 3. Distribution of EL nodular lesion on different body parts

Body parts affected	Number of cases observed	Prevalence (%) [95% CI]
Forelimb	24	27.3 [17.9, 36.5]
Chest and forelimb	18	20.5 [12.0, 28.9]
Hind limb	16	18.2 [10.1, 26.2]
Chest	11	12.5 [5.6, 19.4]
Head and forelimb	8	9 [3.1, 15.1]
Scrotal	6	6.8 [0.2.12.1]
Head	5	5.7 [0.8.10.5]
Total	88	100%

Prevalence of EL=number of cases observed divided by the total number of cases recorded

3.4. Risk factors of EL

Sampling location, sex, and body condition were considered during analysis (Tale 4). However, none of the examined risk factors were found significantly associated with EL in-cart mules (p>0.05).

Table 4. Prevalence of EL across location, sex, and body condition of the mule in Bahir Dar city using Chi-square (χ^2) test

Risk factor	Examined	Positive	Prevalence (%) [95% CI]	Sig.
Location				Ns
Kebele -16	94	28	29.8[20.5, 39.0]	
Kebele 14	141	31	21.8[15.1, 28.8]	
Kebele -11	149	29	19.4[13.1, 25.8]	
Total	384	88	22.9[18.7, 22.1]	
Sex				Ns
Male	143	32	22.3[15.4, 29.2]	
Female	241	56	23.2 [17.9, 28.5]	
Total	384	88	22.9[18.7, 22.1]	
Body condition				Ns
Good	39	9	23.1[0.9, 36.3]	
Medium	253	58	22.9[17.7, 28.1]	
Poor	92	21	22.8 [14.1, 31.4]	
Total	384	88	22.9 [18.7, 22.1]	

Sig.=Significant level, Ns = not significant (P>0.05)

Discussion

The overall prevalence (22.9%) of EL in cart-mules in Bahir Dar city reported in the present study was found relatively lower as compared to the previous studies conducted by Dressie *et al.* (2017) and Meselu *et al.* (2018), who reported 33.7% and 32.8% prevalence of EL in-cart mules in Bahir Dar city, respectively. The discrepancy among the current and previous studies in the same study area might be attributed to variations in sampling season, sample size and sampling locations. Nevertheless, the present finding was found consistent with the reports of Ameni and Terefe (2004), who reported 21% prevalence of EL in cart-pulling mules in Bako and Ejaji towns of Western Ethiopia. Besides, the present finding was also found comparable with the previous reports in cart horses (Ameni and Seyoum, 2002; Ameni, 2006; Asfaw *et al.,*

2012) from different parts of Ethiopia. The current and previous studies on EL in cart-mules in Ethiopia suggested that cart-mules are susceptible to EL which is contradictory to earlier reports (Al-Ani and Ali-Declaim, 1986), which assumed that mules are resistant to EL. This suggests that EL is becoming a very important disease of cart pulling mules that needs due attention (Meselu *et al.,* 2018).

The comparable prevalence of EL in cart pulling mules as to cart horses, in the present study area, might be attributed to the level of humidity and fly abundance in Bahir Dar city. Humidity and fly burden can favor the survival of the environmental form of the HCF and facilitate the transmission of the disease (Gabal *et al*., 1983; Gabal and Hennager, 1983). The humidity in Bahir Dar is relatively higher because the presence of rivers and lakes in the city could favor the survival of HCF and the breeding of flies. According to Gilbert (1998), warm and moist environmental conditions allow HCF to persist for many months in the soil.

In the present study, the characteristic lesions of EL were observed more frequently on the chest and forelimbs following the lymphatic lines, and less frequently on the scrotal, and head. The distribution and clinical presentation of EL lesions observed in cart mules in the present study were consistent with those reported by previous studies in horses and mules (Ameni, 2006; Asfaw *et al.,* 2012; Meselu *et al.,* 2018) as the aforementioned body parts are affected because of their frequent exposure to wound inflictions. However, this finding contradicts the findings of Ameni and Siyoum (2002), who reported a higher frequency of lesions in the axial and scrotal anatomical sites of mules in western Ethiopia.

Cutaneous was the most frequent form of EL in the present study area. This finding was also found consistent with other previous reports (Ajello, 1968; Gabal *et al*., 1983; Salim *et al*., 1985; Sewell and Brocklebsy, 1990; Al-Ani, 1999; Ameni and Fasika, 2002). The cutaneous form is most common, causing chronic, suppurative, ulcerating pyogranulomatous dermatitis and lymphangitis (Abdisa, 2017) as the causative fungus can easily access the cutaneous part of the body through wounds. None of the potential risk factors considered (location, sex, and body condition score) were significantly associated with EL. A consistent finding by Meselu *et al.,* (2018) was reported in cart mules in Ba-

hir Dar city. However, the limited variables (presumed risk factors) and lack of geographical disparity among study locations could result in such insignificant associations.

Conclusions

The present study revealed that nearly a quarter of examined cart pulling mules in Bahir Dar city were affected by Epizootic Lymphangitis (EL). Such high prevalence could have a substantial impact on the health, productivity, and performance of cart mules in the study area. Thus, control of EL is warranted and can be achieved through proper harnessing and strict hygienic practices along with timely treatment of early cases and culling of the chronic ones. Further extensive mycological and epidemiological research on the nature, dynamics, and risk factors of EL is suggested to develop evidence-based EL control and prevention measures in cart mules.

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