Epizootic lymphangitis (EL) is a debilitating equine disease that in its classical form is characterized by chronic discharging cutaneous nodules. The disease has been reported to be endemic in Ethiopia, a country where equids provide a vital utility and source of income to many people in resource-poor settings. This study was conducted in Bahir Dar town, Northwest Ethiopia to investigate the prevalence, risk factors and bodily distribution of lesions of EL in cart-mules. To this effect, both clinical and microscopic examinations were made on a total of 402 mules and an overall prevalence of 32.84% (132/402) was recorded. The mules had nodular, ulcerative pyogranulomatous lesions following the lymphatic lines of the legs (79.6%), on their neck (9.85%), around the inguinal area (6.82%), on their back (2.27%) and on their perineum (1.52%). None of the risk factors considered (sex, body condition score, use of harness, and presence of wound prior to the disease) were noted to be significantly associated with the presence of EL on mules (p>0.05). Moreover, 34% of the cart-mule owners were reported not to allow mules affected with EL off-work even for a single day. In conclusion, the present study has revealed a high prevalence of EL in mules and it is the second work in Ethiopia disproving the assumption that mules are resistant to the disease. Thus, early detection of the disease and treatment with effective drugs and restriction of movement of affected animals is recommended to prevent the spread of the disease to unaffected equids. Also, future studies are required to find out the factors predisposing mules to EL.

**Materials and Methods**

**Description of study area**

The study was conducted in Bahir Dar town, Northwest of Ethiopia. The town is located at about 554 km away from Addis Ababa along the upper Blue Nile river basin. The area has an altitude ranging from 1600-1800 m.a.s.l. [12], receives an annual rainfall of 1500 mm and experiences a lower and higher average temperature of 10°C and 30°C, respectively. The major livestock population of the area consists of 7,839 cattle, 23,463 Goats and sheep, 12,528 equine and 62,012 poultry. The major farming system of the area is mixed crop livestock system [13].

**Study population and sampling**

The study populations were cart pulling mules found in Bahir Dar town. After identification of the gathering points of cart-mules (flour mills, construction sites, market places, cart stations), all animals found in the town (n=402) were included in the study. Cart stations were visited twice in a day, in the morning and afternoon, to examine the two mules that were used to pull a single cart in shifts. To avoid re-sampling, maximum effort was made to identify each mule by color, owner's name and cart number.

**Keywords**: Bahir Dar; Cart-mules; Ethiopia; Epizootic lymphangitis; Lesions; Prevalence
Clinical examination of mules

The disease was tentatively diagnosed by clinical examination including visual inspection and palpation for the characteristic lesions (presence of nodules and/or ulcers). Each mule was thoroughly examined for one or other forms of the disease with special emphasis on the cutaneous form. During clinical examination, sex, body condition score, working status, number of mules owned by the cart owner/driver and bodily distribution of EL lesions were recorded. The body condition score (BCS) of the mules was assessed and categorized as “poor”, “medium” and “good” according to Svendsen [14].

Microscopic examination

Clinically positive horses (horses with either nodules or ulcers of EP) were subjected to further laboratory examination. Depending on the size and consistency of the presented skin and subcutaneous nodules or proliferative lesions, fine needle aspiration (FNA) was performed using a standard syringe (5 ml) and needle (22 and 23-gauge) with aspiration technique. Briefly, after disinfecting the area (preferably intact nodules) with alcohol swab, the needle was inserted into the nodule and then redirected, while continuously applying negative pressure. The material collected was then transferred to a clean glass microscope slide and then blood smearing technique was used for smear preparation. The smears were allowed to dry quickly at room temperature. Dried smears were wrapped with clean and dry paper and transported to the Bahar Dar regional veterinary lab and stained with Gram’s and Giemsa stains. The stained smears were examined under oil immersion magnification for the presence of the organism [15,16].

Data management and analysis

The data collected during the study period was recorded into Microsoft Excel spreadsheet and encoded. Statistical analysis was carried out using STATA software version 11 (STATA corp., College Station, TX). The association between the prevalence of epizootic lymphangitis and the hypothesized risk factors was analyzed by χ²-independent test. In all the cases, 95% confidence level was set and p-value <0.05 was taken as significant.

Results

Out of the total of 402 cart mules examined, 132 (32.84%) were found to have epizootic lymphangitis. The prevalence of EL did not have a significant association (p>0.05) with any of the variables considered as risk factors (sex, BCS, use of harness, and presence of wound before the development of EL) (Table 1).

| Risk factors | No of mules examined | No of EP cases | Prevalence (%) | 95% CI | χ²    | p     |
|--------------|----------------------|----------------|----------------|--------|--------|-------|
| Sex          |                      |                |                |        |        |       |
| Female       | 111                  | 38             | 34.2           | 25.3-43.1 | 0.14   | 0.71  |
| Male         | 291                  | 94             | 32.3           | 26.9-37.7 | 0.46   | 0.03  |
| BCS          |                      |                |                |        |        |       |
| Poor         | 174                  | 50             | 28.7           | 21.9-35.5 | 0.32   | 0.27  |
| Medium       | 146                  | 53             | 36.3           | 28.5-44.2 | 1.63   | 0.20  |

Table 1: Prevalence of epizootic lymphangitis in cart-mules and association with different risk factors. EP=Epizootic lymphangitis.

The characteristic lesion of EL (nodular, ulcerative pyogranulomatous which undergo alternating eruption and granulation) were found distributed on different parts of the body. However, the lesions were detected more frequently on the fore and hind legs following the lymphatic lines (Table 2).

| Body parts                          | Frequency | Percentage |
|-------------------------------------|-----------|------------|
| Perineum                            | 2         | 1.52       |
| Back                                | 3         | 2.27       |
| Inguinal area                       | 9         | 6.82       |
| Neck region                         | 13        | 9.85       |
| Lymphatic lines of the legs         | 105       | 79.6       |
| Total                               | 132       | 100        |

Table 2: Bodily distribution of epizootic lymphangitis lesions in the affected mules.

During the course of the study the cart-mules owners/drivers were asked if they give rest for mules affected with EL. Accordingly, about 52% of the cart owners indicated that they allowed mules to get rest for one day, 10% for two days and 4% for three or more days per week following the development of the characteristic lesion of the disease. However, 34% of the cart owners responded that they didn't allow the sick mule to get rest even for a single day. Moreover, 23 respondents mentioned that they abandoned chronically affected mules outdoors for scavengers because of lack of promising treatment and offensive odor arising from the lesion.

Discussion

There is paucity of information on the prevalence of EL in mules both locally and globally. Perhaps this may be due to the fact that mules are considered resistant or less susceptible to the disease [17]. However, observation of a high prevalence (32.8%) of EL in mules in Ethiopia that disproved the assumption that mules are resistant to the disease.
There is only a single EL study in mules in the country [11] in which a prevalence of 21% reported in cart-mules in Bako and Ejaji towns, Western Ethiopia. The prevalence documented in the current study is considerably higher than the previous report. This variation between the two studies could be attributed to differences in the climatic condition, season of the study and level of attention given for the disease. The current finding is also higher than average prevalence reports in horses from different parts of Ethiopia which range from 18.8% to 26.2% [6,7,10,18]. Regarding to the distribution of EL in lines, and less frequently on the neck, inguinal, back and perineum areas. Moreover, they provide the only means of living for most of the families in the area and are probably the only means of living for most. The wide distribution of EL in lines, and less frequently on the neck, inguinal, back and perineum areas could be attributed to the widely used harnesses, which have rigid and rough edges, increase the friction and wounding of the body of horses and mules [6,20]. The clinical presentation and distribution of EL lesions observed in the current study is in agreement with previous reports in horses and mules [4,7,10,11,21,22].

In the present study, the characteristic lesions of EL were detected more frequently on the front and hind limbs following the lymphatic lines, and less frequently on the neck, inguinal, back and perineum areas in that order. Frequent exposure to injury through chaffing of legs to each other and trauma caused by harnessing may act as predisposing factor. The widely used harnesses, which have rigid and rough edges, increase the friction and wounding of the body of horses or mules [6,20]. The clinical presentation and distribution of EL lesions observed in the current study is in agreement with previous reports in horses and mules [4,7,10,11,21,22].

The current study indicated that a considerable proportion (34%) of cart-mule owners do not let mules affected with EL off work. This condition may weaken the immunity of the animals and worsen the severity of the lesions. Moreover, the practice of abandoning chronically infected animals outdoor by some of the cart-mule owners may lead to spread of the disease to unaffected mules and horses through direct contact and flies.

In this study, none of the risk factors considered were found to have a significant effect on the prevalence of the disease. Nonetheless, large-scale epidemiological cohort studies are required to provide further evidence on these.

Conclusion

The present study has revealed a high prevalence of EL in cart-mules. Carts are a means of survival for a significant number of families in the area and are probably the only means of living for most. Moreover, they provide the only affordable transportation service for goods to and from construction sites, flour mills and market places. With this high prevalence, it is clear that the disease can have a devastating impact on the income of poor families due to morbidity and mortality of abandoned mules. Thus, early detection of the disease and treatment with the available drugs, and restriction of movement of positive cases is recommended. Furthermore, the use of less traumatic harness and raising the awareness of equine owners towards the disease should be considered. Also, future studies are required to find out the factors predisposing mules to EL.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

DM collected the data and did the laboratory work. BM designed the study, supervised data collection and lab works, and drafted the manuscript. RA analyzed the data and critically and substantially revised the manuscript. All authors read and approved the final manuscript.

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References

1. Radosits MO, Gay CC, Hinchcliff KW, Constable PD (2007) Veterinary Medicine. A text book of the disease of cattle, horses, sheep, pigs and goats. 10th edn. Spain, Saunders Ltd., p: 1479.
2. Scantlebury C, Reed K (2009) Epizootic Lymphangitis. In: Mair TS, Hutchinson RE (eds.), Infectious diseases of the horse. FVJ Ltd, Fordham, Cambridgeshire, United Kingdom, pp: 397-406.
3. Jones K (2006) Epizootic lymphangitis: The impact on subsistence economies and animal welfare. Vet J 172: 402-404.
4. Ali-Ani KT (1999) Epizootic lymphangitis in horses: a review of the literature. Rev Sci Tech 18: 691-699.
5. Singh T (1965) Studies on epizootic lymphangitis I. Mode of infection and transmission of equine histoplasmosis (epizootic lymphangitis). Indian J Vet Sci 35: 102-110.
6. Ameni G (2006) Preliminary trial on the reproductibility of epizootic lymphangitis through experimental infection of two horses. Vet J 172: 553-555.
7. Ameni G, Siyoum F (2002) Study on histoplasmosis (epizootic lymphangitis) in cart-horses in Ethiopia. J Vet Sci 3: 135-139.
8. Ameni G, Tilahun G (2003) Preliminary laboratory and field evaluation of Endod for treatment of epizootic lymphangitis. Bull Anim Health Prod Afr 51: 153-162.
9. Endebu B, Roger F (2003) Comparative studies on the occurrence and, distribution of epizootic lymphangitis and ulcerative lymphangitis in Ethiopia. Int J App R Vet Med 1: 1-8.
10. Asfaw R, Pal M, Ameni G (2012) Prevalence of Epizootic Lymphangitis in Cart Horses in Southwest Shewa of Oromia Region, Ethiopia. Int J Livest Res 2: 146-151.
11. Ameni G, Terefe W (2004) A cross-sectional study of epizootic lymphangitis in cart- mules in western Ethiopia. Prev Vet Med 66: 93-99.
12. Solomon W, Abebe W (2007) Prevalence study of ruminant fasciolosis in Ethiopia. Am J Trop Med Hyg 75: 892-898.
13. Carter GR, Chengappa MM, William G (1991) Essential of Veterinary bacteriology and mycology. 4th edn. Lea and Febinger, Philadelphia, p: 264.
14. Quinn PJ, Carter ME, Markey B, Carter GR (2002) Clinical Veterinary Microbiology. Mosby International Ltd, London, pp: 456-458.
15. Al-Ani FK, Al-Delaimi AK (1986) Epizootic lymphangitis. Pakistan Vet J 6: 96-100.
18. Jagema T, Jarso D (2016) Study on Epidemiology and Socioeconomic Impact of Epizootic Lymphangitis in Carthorses in Southwestern Shoa. J Vet Sci Res 1: 000114.

19. Gilbert RO (1998) Foreign Animal Disease. United States Animal Health Association, Richmond, Virginia, pp: 201-206.

20. Gabal MA, Hennager S (1982) Study on the survival of *Histoplasma farciminosum* in the environment. Mykosen 26: 481-487.

21. Rippon JW (1988) Medical Mycology. 3rd edn. WB Saunders Company, Philadelphia, p: 417.

22. World Animal Health Organization (OIE) (2008) Epizootic lymphangitis. In OIE Terrestrial Manual.