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Study on the Prevalence of Epizootic Lymphangitis in Equines in and Around Hawassa, Ethiopia

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Abstract: The current cross sectional study was carried out in and around Hawassa to determine the prevalence of epizootic lymphangitis (EL) in Equines and to assess some of the risk factors involved in the study area by clinical, microscopic and mycological examinations. Clinical examinations were conducted using 210 head of equine species (152 males and 58 female). An overall prevalence of 13.3% by both clinical and microscopic examination (gram staining) was recorded. There was statistically significant difference (P<0.05) in the prevalence of the disease among the different species. The infection rate between males and females showed that there was no statistically significant difference (P>0.05) in the prevalence of EL between sexes. The infection rate among age groups has also showed that there was no statistically significant difference (P>0.05) in the prevalence of EL among different age groups. The higher prevalence 15.99% and 15.21% in the age group of greater than or equal to 16 years and less than or equal to 6 years, respectively and lowest prevalence (11.11%) in age group of 11 to 15 years of age was recorded. Highest prevalence rate (16.8%) in moderate and lowest (10.41%) in fat body conditioned animals was observed. Furthermore, 16.17% prevalence was seen from months November to January (winter) and 8.1% prevalence in February (spring) which showed the presence of seasonal difference in the prevalence of EL. Generally, awareness should be created on the risk of the cart in occurrence of the disease and treating the infected animal as early as possible.

Key words: Epizootic Lymphangitis · Equine · Ethiopia · Hawassa · Prevalence

INTRODUCTION

Ethiopia is a country having 2.75 million horses, 5.02 million donkeys and 0.63 million mules. Equids are reared for a variety of purposes at various levels in different agro-ecological zones of Ethiopia [1]. Because of lack of infrastructure in the rural part of the country most of the transportation activities are performed by Equids [2]. However, equines of Ethiopia are exposed the several serious diseases including African Horse sickness, Equine Histoplasmosis (EH), dourine, Ulcerative lymphangitis, Mange, Anthrax, Rabies and Babesiosis [3]. Horse driven carts provide a means of survival for a significant number of the Ethiopian population as they are used to generate revenue. As with other equines, the working cart horses face several constraints that are threatening their survival, including EH [4].

Epizootic lymphangitis is a relativity common disease of horses and other equine in certain parts of the world [5]. It is a contagious chronic disease of horses and other equine and is as known as Equine Histoplasmosis [6, 7]. It is a debilitating fungal disease seen mainly in Equidae [8, 9].

Epizootic Lymphangitis is one of the major endemic diseases in Ethiopia known to cause a great economic loss and performance of equines due to the pain fullness of the ulcerating nodules with inflammation of lymphatic vessel and lymph nodes [10]. Previous epidemiology of

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the disease recorded the prevalence of EL in cart horses in 28 towns in different climatic zones of Ethiopia [2]. The overall prevalence was 18.8% (range 0-39%) [6]. The disease was detected to 1.0 to 19% of horses in Ethiopia [11].

Epizootic lymphangitis results from infection by a dimorphic fungus called *Histoplasma farciminosum* or *Histoplasma capsulatum var. farciminosum* [12]. *Histoplasma capsulatum* is a dimorphic fungus existing as mold at 25°C-30°C (saprophytic phase) and as yeast at 37°C. The mycelial form is present in sail and the yeast form is most commonly found in lesion [13, 14]. The disease is transmitted in several ways and route of entry may determine the form of the disease [15]. Direct contact with the infective materials through injured Akin or through continuous abrasions is the most common mode of infection. Biting flies in the genera Musca and Stomoxys are thought to spread the conjunctival form. Flies may also transmit the skin form mechanically when they feed on lesions and exudates [16]. The prevalence of epizootic lymphangitis is high when animals are kept in close contact together [11].

Diagnosis can be made based on clinical examination of characteristic lesions of EL, identification of the yeast form of HCF in smears of exudates or histological sections of lesion material, serological tests and skin hypersensitivity test [16, 15]. Laboratory confirmation is possible through gram stained direct smear of purulent materials and the organism appear as gram positive pleomorphic ovoid to globose structure [9].

Treatment is often said to be unsuccessful. Although some cases may heal spontaneously a few weeks after the development of clinical signs [16], intravenous dosing of iodides was used [9]. Controlling EL depends up on elimination of the infection by culling infected horses and preventing spread by good hygienic practice [6]. Sever cases should be euthanized to reduce the source of infection to other [17]. Previous studies concluded that EL is prevalent in 28 towns of Ethiopia including Hawassa [2]. Thus, the present study was conducted with the following objectives: Previous studies indicated that the disease was prevalent in different parts of Ethiopia. However there is need on the current picture on the disease in the study area. Therefore, the objectives of this paper were to determine the prevalence and assessing the risk factors of epizootic lymphangitis in equine in and around Hawassa.

MATERIALS AND METHODS

Study Area: The study was conducted on randomly selected equine species in and around Hawassa town. Hawassa is the capital city of Sidama zone and southern nations, nationalities and people's regional state (SNNPRS) as well. It is located 275 km a way from Addis Ababa and 20km away from Shashamene on the shore of one of the rift valley lakes. It is situated at 1750 meters above sea level. It is geographically lies between 4°27° and 8°3°' latitude north and 34° 21' and 39°, East longitude (lat= 7.06, Lon =38.48). The total human population of Hawassa is estimated to be 123, 322. Out of which 62, 885 are males and 60, 437 are females. The town covers an area of 50km^2. The annual rainfall and temperature range of the town is 800-1000 mm and 20.1-25°C respectively. The total livestock population of Sidama zone (including Hawassa) is estimated to constitute 1.573, 318 cattle, 183, 462 goats, 221, 505 Sheep, 49, 150 horses, 48, 653 asses, 3, 959 mules, 1, 196, 506 poultry and 73, 479 bee hives [18].

Study Animal: The study animals are all equine species especially horses, donkeys and mules found in the randomly selected areas of Hawassa and its surroundings.

Study Type: A cross -sectional study was conducted in the study time for the determination of the prevalence of EL in Hawassa. Sampling was taken place monthly for 4 consecutive months in order to see the seasonal distribution of *H. farciminosum* infection.

Sampling Method and Sample Size Determination: The sampling method employed to select the study subject was simple random sampling. The approximate sample size required to estimate prevalence in a large population can be determined for a defined precisian and level of confidence. The limits of the associated intervals indicate the specified bounds with in which the estimate will with the defined level of confidence. The relevant formula for a 95% confidence interval is indicated below [19].

$$n = \frac{1.96^2 P\exp(1 - p\exp)}{d^2}$$

where n = required sample size
Pex*P* = expected prevalence
d = desired absolute precision

Using the above formula and with the expected prevalence of 12.3%, the total sample that should have been collected was 165 (where Pex*P*=12.3%=0.123 and d= 5%=0.05). However, the total sample was increased to 210 with the aim of getting accurate precision. So, during the study period 210 samples were collected from the study area.

Specimens Collection and Transportation: Before collection of pus and/ or swab sample, skin nodules were washed with soap and water. Then shaved and disinfected with denatured alcohol. Un-ruptured skin nodules were incised using sterile scalpel; and the contents were aspirated using syringe and needle and/ or collected using swab. These samples were placed in liquid nutrient medium containing chloramphenicol (antibiotics) prepared in test tubes [20]. They were kept refrigerated using ice box and transported to the laboratory and refrigerated at 4°C until culturing and gram staining were performed.

Study Methodology

Clinical Examinations: Horses, Mules and Donkeys were clinically examined using visual inspection and palpation for the characteristic lesion of EL, i.e., for the presence of nodules and /or ulcers. During clinical examination, clinically positive animals for EL are characterized by having spreading, suppurative, ulcerative and pyo-granulomatous dermatitis and lymphangitis [2].

Lesions were most commonly found on the skin of the face, fore limb and neck. Cutaneous lesions though mostly found on the legs, may also be found on the back, head and scrotum. The affected skin becomes hard and thick. Several purulent foci were apparent when the nodules were incised. Lymphatic vessels were distended and regional lymph nodes were swollen [7]. During clinical examinations, the body temperature and the general demeanor of the animal were not changed. In addition, history about previous occurrence of disease and nodules observed in other animal were taken.

Laboratory Confirmation (Gram Staining and Culturing): Nodules were washed with soap and water, shaved and disinfected with denatured alcohol and the contents aspirated using a needle and syringe [7] and/ or collected in to sterile swab prepared for sample collection. Smears were prepared on glass slide and fixed with methanol (2-3 minutes). Then, smears were stained with gram's stain and examined for the typical yeast form of HCF, which appears as a gram positive ovoid to globose that may occur singly or in group within macrophages or extracellulary [2].

Pus samples as well as swabs collected from un-ruptured nodules and/or ulcerated skin lesions were kept cool and shipped as soon as possible [9]. Samples collected were placed on culture broth (liquid nutrient media) containing antibiotics (chloramphenicol) (Oxoid, England). Then, it was cultured on the following enriched fungal media.

Sabouraud's dextrose agar: 65 grams of Sabouraud's dextrose agar (with 2.5%glycerol) was measured and placed on clean flask; and 1000 ml of distilled water was poured in to the flask and thoroughly mixed using hot plate. Then it was allowed to cool using water bath. After the media was allowed to cool, 0.5 gm / liters chloramphenicol was added and the whole media was autoclaved using autoclave. After the pressure has dropped to 0, the media was taken out and poured into sterile Petri dishes (around 60) and placed in to refrigerator (Oxoid, England). However, because of wise use of media and small number of sample being collected per week, only half (32.5gm) of this proportion was prepared. So, for this proportion 0.25gm (1caps) chloramphenicol was added. The samples collected were cultured on SDA using sterile swabs prepared and/or syringe and needle. Swab samples were rolled small diameter over the center of the media and placed in the incubator adjusted at room temperature (25-26°C).

Identification of colony morphology: Culture results were examined periodically for the characteristic growth of the mycelial form of the organism and for the differentiation of other fast growing fungal colonies. Histoplasma capsulatum var. farciminosum grows on SDA with inhibitors (chloramphenicol and Cyclohexamide) [13]. Growth was relatively slow, most isolates required from 2- 8 weeks for the development of characteristic colonies [6]. Colonies appeared in 2-8 weeks as dry, gray-white, flaky and cerebriform [2]. So in this study some fungal colonies that can grow in this media were differentiated with by this characteristic colonial morphology of HCF. Conversion to the yeast phase can be demonstrated at 35-37°C by sub culturing the mycelium into brain heart infusion agar containing 5% Horse blood [20]. Because of materials shortage to collect blood and short research time conversion to the yeast phase was not carried out.

Data Analysis: The data was analyzed using SPSS version 17 statistical software. Pearson's chi-square(x^2) test has been used to measure statistical significance of results. In order to consider a result to be statistically significant 95%CI and *P* value less than 0.05 has been taken. The results are presented using frequency tables and prevalence was expressed as the proportion of positive animals (both clinical and Gram staining) over 210 animals examined.

RESULTS

Prevalence and Disease Risk Factors

Clinical Examinations: Out of 210 equine species (Horses, Donkeys and Mules) sampled, 13.33% (28/210) were clinically positive for EL. These clinical cases were 18.18 % (18/99) in Horses, 8.57 %(3/35) in Mules and 7.81 % (7/76) Donkeys. In addition, in this particular study cutaneous form is the only clinical form observed in which 21.42% in both limbs, 17.85% in both limbs and body, 21.42% in forelimbs, 10.71% in hind limb, 14.28% in body (back and armpit) 10.71% in both head and neck; and 3.57% lesion (nodule) in scrotum were recorded. Samples were collected from all clinically positive cases and were submitted to the laboratory for confirmation.

Laboratory Confirmation: Pus and/ or swab samples collected from all of the clinical cases (N=28) were subjected for gram staining and were confirmed as positive for typical yeast form of *Histoplasma capsulatum var. farciminosum* (HCF). Out of these 96.42% (27 out of 28) cases show typical growth o mycelial form of HCF by culturing on SDA after two to eight weeks. Thus for the calculation of prevalence, clinical cases that were positive for the typical yeast form of HCF on microscopic examination of gram stained smear were used. An overall prevalence of 13.33 %(28/210) was recorded in Equine species sampled from different kebeles of Hawassa.

Results of clinical examination and gram stained smears of samples in different seasons were recorded. The prevalence rate between November-January was 16.17% and 8.1% on February. Season was considered as the risk factor for the high prevalence of EL; and highest prevalence was recorded during winter (November- January) than spring (February) (8.10%). However, there was no statistically significant difference(x^2=2.700, *P*=0.73) in the prevalence of EL and season.

Results of clinical and gram staining of samples from different Equine species were recorded. The highest prevalence (18.18%) was recorded in Horses and the lowest prevalence (9.09%) was recorded in both Donkeys and Mules. There was statistically significant difference (x^2 =3.81, *P*=0.040) in the prevalence of EL among species.

Out of 152 male animals examined (both clinically and microscopic), 24 (15.78%) were recorded as positive for EL and out of 58 female animals 4(6.89%) were recorded as positive for EL. The higher prevalence (15.78%) was recorded in males. There was no statistically significant difference(x^2= 2.873, *P*=0.066) in the prevalence of EL between the sexes.

Out of 210 animals examined, 15.21%, 12.12%, 11.11% and 15.99% positive cases were recorded at age groups of ≤ 6 years, 6-10 years, 11-15 years and ≥ 16 years, respectively. Higher prevalence was recorded in age groups of ≤ 6 years and ≥ 16 years of age. There was no statistically significant difference (x^2=0.709, *P*=0.871) in the prevalence of EL between sex.

Table 1: Prevalence of epizootic lymphangitis based on season

Season	Number of animal examined	Number of animals positive	Prevalence (%)
November- January	136	22	16.17
February	74	6	8.1
Total	210	28	13.33

(x^2=2.700, *P*=0.73)

Table 2: Prevalence of epizootic lymphangitis based on species

Species	Number of animals examined	Number of animals positive	Prevalence (%)
Horses	99	18	18.18
Donkeys and Mules	111	10	9.09
Total	210	28	13.33

(x^2=3.81, *P*=0.040)

Table 3: Prevalence of epizootic lymphangitis based on sex

Sex	Number of animals examined	Number of animals positive	Prevalence (%)
Male	152	24	15.78
Female	58	42	6.89
Total	210	28	13.3

(x^2=2.87, P=0.066)

Table 4: Prevalence of epizootic lymphangitis Based on age

Age (year)	Number of animals examined	Number of animals positive	Prevalence (%)
≤ 6	46	7	15.21
6-10	66	8	12.12
11-15	54	6	11.11
≥ 16	44	7	15.99
Total	210	28	13.30

(x^2=0.709, P=0.871)

Table 5: Prevalence of epizootic lymphangitis based on body condition score

Body condition score (2-4)	Number of animals examined	Number of animals positive	Prevalence (%)
2 (moderate)	77	13	16.8
3 (good)	85	10	11.76
4 (fat)	48	5	10.41
Total	210	28	13.30

(x^2=1.37, P=0.503)

Table 6: Prevalence of epizootic lymphangitis based on purpose

Purpose	Number of animals examined	Number of animals positive	Prevalence (%)
Cart	196	26	13.26
Pack	14	2	14.29
Total	210	28	13.30

(x^2=0.12, P=0.583)

Out of 210 animals examined 16.8%, 11.76% and 10. 41% prevalence's were recorded in animals with body condition score (BCS) of moderate, good and fat respectively, the highest prevalence (16.8%) was recorded in animals with BCS of 2(moderate). There was no statistically significant difference (x^2=1.37, P=0.503) in the prevalence of epizootic lymphangitis in animals with different body condition score.

Cart (purpose of animals used for) was considered as the risk for the higher prevalence of epizootic lymphangitis in the study area. However, there was no statistical significant difference(x^2=0.12, P=0.583) in the prevalence of EL based on purpose.

DISCUSSION

Epizootic lymphangitis is commonly known as "Niddift" in the study area. Previously it was considered as only horses are affected but clinically positive cases were observed in both Donkeys and Mules. Although clinical signs and distribution of lesions were similar in all species, the frequency of occurrence of EL was greater in horses. Hawassa is located 1750 m.a.s.l. (hot and subtropical). From the current study it has been demonstrated that EL is still prevalent in this environment, which agrees with reports from [2]. An overall prevalence of 13.33% was recorded in Hawassa by both clinical examination and microscopic examination of gram stained smears of pus samples. The common sites that lesions (nodules) observed were body and limbs (extremities) (89.28%) where as only 10.71% have lesions on head and neck. This result agrees with a previous report that extremities were the most affected with EL than other parts [10].

The presently recorded prevalence of 13.3% has indicated that the overall prevalence of EL in Hawassa is in line with reports from a previous study (12.3%) by Ameni [2]. As it can be observed from the result, majority of the equines are used for cart. Carters can again use more than one horse, mule or donkey for the same cart by shift. So, there was increased number of cart horses. As a result slight (only 1%) difference in the prevalence might be associated with increased number of animals. In addition, once animals (Equines) infected by EL, the

owners think that it will not be cured and loss its body condition and no further management is taken because of being less efficient to work. Thus, cases with chronic disease (EL) form are sometimes left outside of home near Lake Hawassa and Chafe area, which may act as source of transmission to other healthy animals (equines). Even though there was no statistically significant difference (P>0.05) in the prevalence of EL between purposes in the current study, majority of carts designed for draft purpose were causing injury to animals as it was being traditional and higher prevalence of the disease was observed in cart horses. This was because of increased access to the disease by direct contact with equipments that were used previously and great chance for the agent to enter through wound. So the result of the current finding is in line with a previous report that cart horses face several constraints that are threatening their survival, including epizootic Lymphangitis [3, 10].

As indicated in Table 2, highest prevalence was recorded in winter (November-January) than spring (February). Which is in line with a previous report by Jones [6] in that high prevalence was seen in months November-January. However, there was no statistically significant difference (P>0.05) in the prevalence of EL in different seasons, which contradicts with a previous study that showed statistically significant association between prevalence and average annual temperature but not with mean annual rain fall [2]. The reason for this might be similar seasonal temperature profile in both seasons during the current study. For example, maximum extreme and minimum temperature recorded in one of the three months (November-January) was 34.5°C and 8.0°C respectively, while maximum monthly extreme and minimum temperature recorded in February was 31.5°C and 9.5°C respectively.

The present study has indicated that there was statistically significant difference (P<0.05) in the prevalence of EL among species. The higher prevalence in horses and lower prevalence in both donkeys and mules was recorded. It has an agreement with a previous study that donkeys and mules are resistant [7, 10]. This also has an agreement with Quinn *et al*. [21] (about 90% of EL cases occur in Horses). In addition, Horses are preferred species used for cart transportation to donkeys and mules by the society. However, it has been observed that there was no as such veterinary care as compared to donkeys and mules for the reason yet unknown. Almost 70% of horses have wound on their body which is created by improper harness type. So, they may be predisposed to EL easily. On the other hand, the Donkey Sanctuary Hawassa project has been providing good service to donkey and mules in and around the town. So donkeys and mules are well managed and harnesses provided were suitable. As a result, lesser wound rate was observed.

The higher prevalence in males as compared to females; and highest prevalence in age groups of greater than or equals to 16 years of age followed by higher prevalence in age groups of less than or equals to 6 years of age was recorded. However, there was no statistically significant difference (P>0.05) in the prevalence of EL between sex; and also there was no statistically significant difference (P>0.05) in the prevalence of EL among age groups. Thus, this result agrees with a previous review that there is no predilection of age or sex in EL cases.

The higher prevalence's recorded in age groups of ≤6 years and ≥ 16 years; and in males might be due to increased number of matured and male animals that are frequently used for cart. In addition, most of cart horses as well as donkeys and mules with body condition score of 2 (moderate) and above are used for cart. The association in the prevalence of EL and BCS were also compared. There was no statistically significant difference (P>0.05) in the prevalence of EL between animals with different body condition score. The highest prevalence was recorded in animals with body condition score of 2. This might be because of the fact that most of cart horses as well as donkeys and mules with body condition score of 2 (moderate) and above are frequently used for draft purpose. Additional explanation might be due to the fact that carters have a tendency to exploit the power of infected animals (Equines) for they have a perception that they cannot be cured and want to exploit and abandon the animal. The body condition of an animal is one of the indicators about the well being of an individual. Thus, the present study has indicated that high prevalence of the disease was observed in animals with body condition score of 2 and this shows that affected animals are losing body condition due to EL. The reason for this is inhumane handling of animals when they are sick in that no good management such as veterinary care and feeding were provided. This is in consistence with the finding of Ameni [2] that most of (≥95%) of cases do not recover as a result owners consider that looking after such animal is simply waste of money and energy. Hence the impact of EL on animal welfare needs to be considered as highly important and there is a need to asses the extent of welfare compromise.

CONCLUSSION

This cross-sectional study that was conducted on epizootic lymphangitis in Equines in Hawassa which revealed the overall prevalence of 13.33%. The present prevalence was calculated by both clinical examination and microscopic examination of gram stained smears. Different risk factors were considered for the high prevalence of epizootic lymphangitis. Off the two seasons, winter was found to be the most common that high prevalence of EL seen. Out of the three species, Horses were found to be more infected and highest prevalence was recorded. Majority of animals especially cart horses infected with EL were found to loss body condition and their power were exploited by the carters. Diseased animals suffer from painful lesions. Many of these animals are owned by poor people and work in harsh environmental condition. So it has a great economic loss in this poor society. There fore, awareness should be created on the risk of improper use of cart and the economic impact of the disease in order to reduce and control the disease distribution.

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