

## Study on Histoplasmosis (Epizootic Lymphangitis) in Cart-Horses in Ethiopia

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### ABSTRACT

A cross-sectional study was conducted to determine the prevalence of *Histoplasma farciminosum* in 2907 carthorses using clinical and microbiological examinations at three towns (Debre Zeit, Mojo and Nazareth), Central Ethiopia, between December 1999 and January 2001. An overall prevalence of 26.2% (762/2907) was recorded; the highest prevalence (39.1%) being recorded at Mojo whereas the lowest (21.1%) was recorded at Nazareth. The difference in prevalence among the three towns was highly significant ( $\chi^2 = 76.92$ ,  $P < 0.0001$ ). Carthorses found at Mojo [OR = 2.4, CI = (1.9-3.0),  $P < 0.0001$ ] and Debre Zeit [OR = 1.9, CI = (1.5-2.3),  $P < 0.0001$ ] were at higher risk of infection than those found at Nazareth. The mycelial and yeast forms of the *Histoplasma capsulatum* variety *farciminosum* were isolated on the Sabouraud's dextrose agar. The results of the present study showed the rampant occurrence of histoplasmosis farciminosi at the three towns and indicates the need for further nationwide investigation into the disease to design sound control strategy.

**Key words/phrases :** Ethiopia, carthorse, histoplasmosis *Histoplasma capsulatum* variety *farciminosum*, prevalence

### Introduction

Agriculture is the mainstay of the Ethiopian economy, and livestock production is an integral part of the country's agricultural system. Despite its importance to a large part of the population and the economy at large, the sub-sector has remained untapped (Asfaw, 1999). Of the major causes of economic losses and low productivity of livestock the prevalence of a large number of diseases in the country is considered to be the major one.

Ethiopia possesses 2.75 million horses, 5.02 million donkeys and 0.63 million mules (EARO, 1999). Of the total

horse population in the country, 63.33% are found in Oromiya Region. The three towns, Debre-Zeit, Mojo and Nazareth share 3056 of the 15060 carthorses in East Shoa Zone, almost all of which are males (EARO, 1999). Because of lack of infrastructure in the rural part the of Ethiopia, most of the transportation activities are performed by the use of equids. Moreover, as the topography of the country is not convenient for modern transportation technologies, the major means of transportation both for goods and man are equids. Besides, in certain parts of the country equids are used for ploughing.

Many diseases affect the power generating ability of carthorses. *Histoplasma farciminosum* (HF) (Selim et al., 1985; Weeks et al., 1985) known widely as epizootic lymphangitis, is a chronic contagious, afebrile, fungal infection of equids characterized by granuloma, suppurating ulcers of the lymphatic vessels and skin with possible extension to the associated lymph nodes (Buxton and Fraser, 1977). Infection may also lead to pneumonia and conjunctivitis (Ainsworth and Austwick, 1973). It is caused by *Histoplasma capsulatum* variety *farciminosum* (HCF) (Weeks et al., 1985) which until recently was known as *Histoplasma farciminosum*. Although the disease seems important, there is a shortage of information on the occurrence and magnitude of the disease. This study was, therefore, undertaken to determine the prevalence of HF in carthorses at three towns in Central Ethiopia.

### 2. Material And Methods

#### 2.1. Description of the study area

The fieldwork was conducted at three towns, Debre Zeit, Mojo and Nazareth where carthorses are used for the transportation of man and goods. Debre Zeit, Mojo and Nazareth are located at 44, 70 and 92 km's southeast of Addis Ababa, respectively. The altitudes of Debre Zeit, Mojo and Nazareth are 1900 m, 1870 m and 1622 m, respectively. The weather of Debre Zeit is hot and moist because of the presence of several lakes around Debre Zeit. Mojo is characterized by hot and humid weather condition whereas Nazareth has hot and dry weather condition. The laboratory work was carried out at the Institute of Pathobiology, a

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## 2.2. Study animals and sampling

Information regarding the number of carts in the towns was obtained from the municipality offices of each of the three towns. Usually two horses pull a cart with shift, one horse in the morning and the other in the afternoon. A total of 1528 carts were registered and thus making the number of cart horses to be 3056 out of which 2907 horses were included in this study. Major carthorse stations in the three towns were used as collection points of carts for loading and unloading. These stations (4 at Debre Zeit, 3 at Mojo and 12 at Nazareth) were used as sites for study. Each station was visited four times in two separate days: twice per day, in the morning and in the afternoon in order to examine the two shifts of horses of each cart.

## 2.3. Clinical examination

Horses were screened for the disease by clinical examination based on clinical signs of the HF. Whole parts of the body of horses were clinically examined for the presence of nodules, ulcers along the lymphatic vessels, on the skin and on the lymph nodes.

## 2.4. Microscopic and mycological examinations

Pus samples from un-ruptured nodules were used for culturing and direct microscopy. The nodule was shaved, washed with soap and water, and disinfected with denatured alcohol. The content of the nodule was aspirated using a sterile needle and syringe, and inoculated immediately onto sabouraud's dextrose agar (SDA, Difco, Detroit, MI). Paralleling, smears were made on glass slide and subjected to fixatives for Gram's stain (Carter, 1984).

**Mycelium:** -aseptically aspirated loop-full of pus from un-ruptured nodules was streaked or inoculated in universal bottles containing SDA (OIE, 1996). The media were incubated at 26 °C aerobically. Sub-culturing was also made on SDA.

**Yeast:** - SDA slants of universal bottles were used for the isolation of the yeast form. A similar method described above was used for the isolation of the yeast form except the

difference in incubation temperature, which is 37 °C in this case.

## 2.5. Statistical analysis

Prevalence was defined as the proportion of the number of horses positive for epizootic lymphangitis by clinical and microscopic examinations to the total number of horses examined. It was generated by frequency (FREQ) procedures of the Statistical Analysis System (SAS, 1994) and was expressed in percent. Variations of prevalence between towns were analyzed by the chi-square ( $\chi^2$ ) test. Odds ratio (OR) was computed by FREQ procedures with the option of Cochran-Mantel-Haenszel (CMH) statistics for Statistical Analysis System (SAS, 1994) to estimate the level of risk of epizootic lymphangitis by explanatory variable (town).

## 3. Results

### 3.1. Prevalence

Table 1 shows the overall prevalence of HF at the three towns. The overall prevalence of HF in carthorses in the three towns was 26.2% (762/2907). The highest prevalence (39.1%) was recorded at Mojo while the lowest (21.1%) was at Nazareth. The difference in prevalence among the three towns was highly significant ( $\chi^2 = 76.92, P < 0.0001$ ). The odds ratio comparisons for prevalence of HF among the different towns are indicated in Table 2. Carthorses found at Mojo [OR=2.4, CI=(1.9-3.0),  $P < 0.0001$ ] and Debre Zeit [OR=1.9, CI=(1.5-2.3),  $P < 0.0001$ ] were at higher risk than those found in Nazareth.

### 3.2. Distribution and characteristics of lesions.

The higher percentage of the lesion was found on the fore (33.18%) and hind (29.44%) limbs as compared to other body parts. Almost all of the cases encountered during the study period had one or more nodular lesions on different body parts. The nodules were varied in size from 3-5 cm in diameter. Recently erupted nodules were scattered, upon palpation, they were found to be firm and freely moving. When they occurred in a line following a lymphatic vessel they appeared as a rigid knotted-rope (Fig. 1). Aged and un-ruptured nodules were usually flabby with no hair and smooth tip indicating the site of rupture. In advanced cases, ruptured nodules were observed to be arranged in line and

**Table 1.** Prevalence of *Histoplasma farciminosum* in Central Ethiopia on the basis microscopic examination

Name of towm	No. of horses examined	Positive	Negative	Prevalence
Debre Zeit	590	198	329	33.6%
Mojo	412	161	251	39.1%
Nazerath	1905	403	1502	21.1%
<b>Total</b>	<b>2907</b>	<b>762</b>	<b>2145</b>	<b>26.2%</b>

( $\chi^2 = 76.92, P < 0.0001$ )

**Table 2.** Comparison between towns for risk from epizootic lymphangitis

Comparison	Odds ratio for MARD	95% confidence interval	CMH value	P value
Debre Zeit vs. Mojo	0.79	(0.61, 1.02)	3.21	NS
Debre Zeit vs. Nazerath	1.88	(1.54, 2.31)	37.89	****
Mojo vs. Nazerath	2.39	(1.90, 3.00)	59.06	****

\*\*\*\*,  $P < 0.00001$ ; NS, not significant; CMH, Cochran-Mantel-Haenszel Statistics

discharging white to yellow pus. In such cases, usually lymph nodes were swollen, reaching 7-12 cm in diameter, which discharge massive amount of thick creamy white pus on puncture or excision. In more advanced cases, the ulcerated lesions formed of a firm granulation tissue were observed. A few cases of pneumonic HF with nasal pus discharge and unilateral conjunctivitis were observed (Fig. 2).



**Fig. 1.** Cutaneous form of histoplasmosis

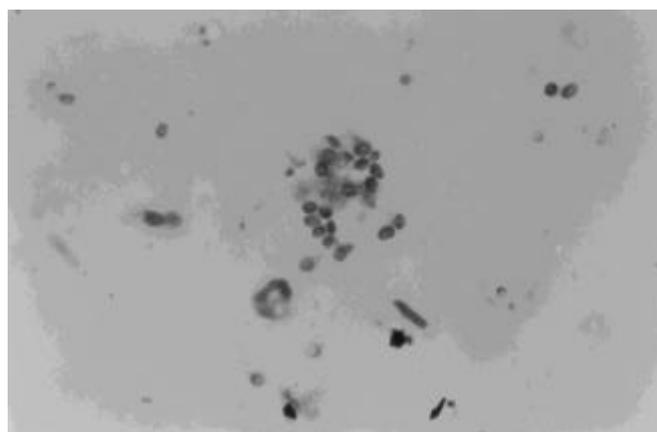


**Fig. 2.** Respiratory and ocular forms of histoplasmosis

### 3.3. Direct microscopic examination

Gram stained smears from pus/swabs revealed a Gram-positive yeast forms with capsule-like unstained structure around them. The yeast forms were lemon-shaped with one edge wider and the other bluntly pointed (Fig. 3). They could occur individually or in groups either free or 1-7

yeast forms together phagocitized in macrophages. The staining reaction and granulation of the yeast forms showed either (1) whole unstained transparent lemon-shaped spaces, (2) granules concentrated more at the wide end and little in the center, (3) granules arranged inside wall of the yeast, (4) granules almost filling the cell, (5) whole stained yeast forms filled with granules or (6) granules sparsely dispersed in the yeast. Of the above forms of the yeast, the second form was quite common. Monocytes and lymphocytes were observed in most of the smears.



**Fig. 3.** Gram-stained yeast form of *H. capsulatum* var. *farciminosum* (oil immersion)

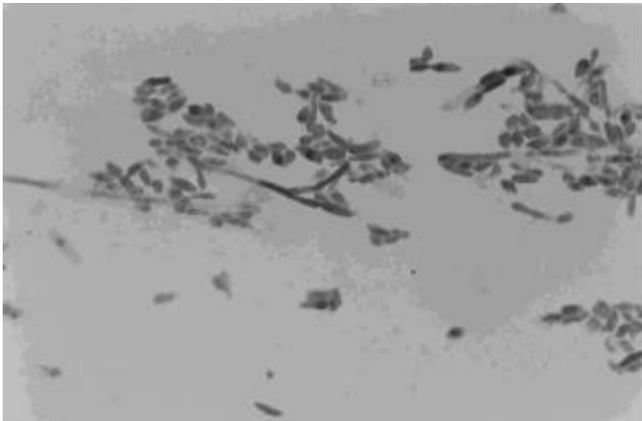
### 3.4. Isolation

**Mycelium:** The mycelial forms grew within 8 weeks as white to grayish white, folded, raised cerebriform colonies on SDA (Fig. 4). The colonies were adherent to the medium becoming brownish on aging. A direct microscopic examination using Gram's stain preparations revealed hyaline septate branched hyphae (Fig. 5).

**Yeast:** The colonies of the yeast form appeared flat, raised, wrinkled, and white to grayish white in color and pasty in consistency. On a microscopic examination, the yeast cells from the colonies appeared larger than the same cells from lesion. Moreover, the number of granules and budding cells were observed to increase in smears made from the colonies as compared to the same cells from the lesion.



**Fig. 4.** Colonies of mycelial form of *H. capsulatum* var. *farciminosum* grown at 26



**Fig. 5.** Gram-stained mycelial form of *H. capsulatum* var. *farciminosum* (oil immersion)

#### 4. Discussion

The overall prevalence of the disease at the three towns was 26.2%; highest being at Mojo (39.1%) followed by Debre-Zeit (33.6%) and Nazareth (21.1%). Variation in prevalence among the study towns could be due to the difference in climatic conditions of the towns such as difference in relative humidity, which influences the survival of *HCF* and the breeding of flies (Gabal, 1982; Radostatis et al., 1994). According to OIE (1996) the ocular form of HF is spread by biting flies of the *Musca* and *Stomoxys* genera. Endebu (1996) reported an overall prevalence of 10.4% of HF at Akaki and Debre-Zeit towns. From the indicated figures, the increase in prevalence of the HF at Debre Zeit could be due to the fact that there were no control/intervention methods in the country.

Clinical manifestations of HF observed by the present study were in agreement with the previous reports (Ajello, 1968; Gabal et al., 1983; Selim et al., 1985; Rippon, 1988; OIE, 1996; Al-Ani, 1999). HF was observed to affect any part of the body including the lips, scrotum and eye (conjunctiva). Nevertheless, it was frequently observed on

the front and hind limbs, neck, chest and armpit. Frequent exposure to injury through chaffing of legs to each other and trauma caused by harnessing may act as predisposing factor. The widely used rubber harnesses, which have rigid and rough edge, increase the friction and wounding of the body of horses. Especially, the harness that passes across the chest pulls heavy load, so there is a high friction and frequent wounding of the chest, which facilitates the entrance of the organism (Gabal, 1982) and the spread of the lesion to the forelimb, neck, armpit and chest.

The gross appearance of the colonies of both the mycelial and yeast forms and the microscopic appearance of the mycelial and yeast forms observed by the present study were consistent with the reports by Gabal et al. (1983) and Selim et al. (1985).

HF is widespread and rampant in carthorses at the three study towns. Nationwide epidemiological and socioeconomic studies are, therefore, recommended to estimate its impact on the nation and then devise applicable control strategy.

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