

Research Article

Cytopathology of Superficial Lymph Nodes and Distended Lymphatic Nodules of Horses with Special Reference to Epizootic Lymphangitis in Hawassa City, Ethiopia

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Abstract

The study was conducted in drat-horses apparently recovered from Epizootic lymphangitis to evaluate the cytopathological findings in swollen superficial lymph nodes and lymphatic vessels, to identify the possible causative agents of such swellings and to assess the degree of resolution of the pathogen (*Histoplasma farciminosum*). Fine needle aspiration biopsy (FNAB) samples were collected from 126 cart horses with visible swollen superficial lymph nodes and/or lymphatic vessels (Group I) and 46 apparently recovered carthorses with linear scars on their sternum, face and limbs (Group II). Smears were prepared, stained with modified Giemsa and thoroughly examined for cellular responses and infectious agents. Cytological diagnosis revealed that 89.7% and 10.3% of the samples from the first group were positive for *Histoplasma farciminosum* and bacteria of unidentified species, respectively. On the other hand, examination of samples taken from group II drat-horses showed lymphadenitis without apparent causative agent in 15.2%, lymphadenitis along with *Histoplasma farciminosum* in 19.56% and absolutely normal lymph nodes in 65.2% of the horses. Further evaluation of lymphadenitis cases with out apparent causative agent (from group II) indicated that 42.86%, 42.86% and 14.3% of the cases were eosinophilic, neutrophilic, and lymphadenitis of immune stimulation in nature on the basis of dominant cellular reactions. All of the cases of lymphadenitis associated with *Histoplasma farciminosum* were pyogranulomatous in nature. In general, cytology was found to be a valuable tool in establishing a diagnosis, identifying the process and forming a prognosis. Horses apparently recovered from lymphangitis can harbor the agent in their superficial lymph nodes and act as a source of infection for naive horses. Humane destruction of severe cases and carrier horses should be practiced widely in the country to control epizootic lymphangitis effectively. Further study is required to isolate and characterize the bacterial causes of lymphangitis.

INTRODUCTION

Horses reared in different agro-ecological zones of Ethiopia, for the purpose of transportation and ploughing, face several diseases that impede their power generating ability [1]. Some of these diseases have the affinity to involve the lymphatic system directly or indirectly. Lymphatic vessels and lymph nodes are involved either as a primary target or secondarily during the attempt to eliminate pathogens from the surrounding wound. From the former group, Epizootic lymphangitis and ulcerative lymphangitis are two important health problems that mainly affect horses. Epizootic lymphangitis is a contagious, chronic disease of horses and other equines characterized clinically by a spreading, suppurative, ulcerative pyogranulomatous lymphangitis and lymphadenitis caused by *Histoplasma farciminosum* [2]. Fungal spores from infected animals are carried by direct contact or on inanimate objects such as grooming and harnessing equipment, bedding, saddle etc., biting flies and gain entry through skin abrasions [3].

Fine needle aspirate (FNA) smears are often diagnostic of either a process or disease condition that can be either primary or secondary in nature. Such smears can reveal the presence of infectious agents, reactive lymph nodes, spreading neoplastic cells and lymphoblastic transformations [4].

Except for a few studies conducted on the epidemiology of Epizootic lymphangitis [3,5], no research work has been done so far in Ethiopia to fully characterize the cytologic findings of swollen superficial lymph nodes associated with this disease and other infectious and non-infectious diseases. Moreover, previous studies were limited and clinically active lesions of epizootic lymphangitis. The present study was therefore planned to evaluate the cytopathological findings of swollen superficial lymph nodes and lymphatic vessels and to identify the possible causative agents of such swellings. Moreover, maximum effort was made to assess the degree of resolution of the pathogen (*Histoplasma farciminosum*) from apparently recovered horses.

MATERIALS AND METHODS

Study area

The study was conducted in Hawassa city. Hawassa, the capital of Southern Nations, Nationalities and People Regional State, is located at 275kms south of Addis Ababa. It is geographically situated at 7° 3' N latitude and 38° 28' E longitude, and at an elevation of 1739 m above sea level. The city receives an average annual rainfall of 955 mm with a mean annual temperature of 20 °C. The main rainy season in the area is from June to August the dry season lasts from November to February. Months between the wet and dry seasons have mild weather conditions [6].

Study population and sample size

The study included two groups of purposively selected draft-horses found at Hawassa city. The first group (Group I) involved 126 draft-horses having clinical signs by swollen superficial lymph nodes and/or lymphatic vessels whereas the second group (Group II) encompassed 46 draft-horses apparently recovered from epizootic lymphangitis with linear scars on their sternum, face and limbs. Major draft-horse stations and outreach service sites of Society for the Protection of Animals Abroad (SPANA) were the preferred sites to get the horses for sample collection. These sites were preferred because they are the common areas used for loading and unloading of carts by the owners. SPANA provides veterinary services at these places. During the entire 6 months of study period, fine needle aspirate (FNA) samples were collected from a total of 172 draft-horses of the town.

Study methodology

History and clinical examination: History on the management, duration of illness (for lymphangitis), number horses involved per household, and response of treatment (if any) were collected from draft-horse owners or cart drivers. Cart-Horses were carefully examined for the presence of various abnormalities associated with the lymphatic system of the body. The entire body part of the horses, mainly the face, chest and extremities were clinically inspected and palpated for the presence of nodules and ulcers along the lymphatic vessels, swelling of superficial lymph node and wounds around the swollen lymphatic vessels and/or lymph nodes.

Cytological techniques: After thorough disinfection with denatured alcohol, fine needle aspirate (FNAB) samples were collected from un-ruptured nodules and superficial lymph nodes using sterile 22 gauge needles and 5 or 10ml syringes [7]. Depending on the size and consistency of the nodules and nodes, either aspiration or non-aspiration techniques were used to get sufficient aspirate at the hub of the needle. Squash preparation, blood smear techniques or "starfish" preparations were used to prepare smears depending on the nature of the aspirate obtained [8]. To increase the chance of getting diagnostic information, a minimum of three smears were prepared from each sample collected. The smears were then allowed to dry quickly at room temperature and subsequently stained with modified Giemsa (May-Grünwald-Giemsa, Merck KGaA, Darmstadt, Germany).

Examination of the smears: All the stained smears prepared for cytological examination, were scanned under 10x

magnification to check for staining intensity and cellularity and then thoroughly examined under oil immersion magnification for the presence of bacteria and yeast, both inside and outside the inflammatory cells Figure 1. The shape, arrangement, and staining properties of these organisms, if any, were considered during examination. The dominant inflammatory cells involved, the presence of degenerated and toxic neutrophils, and excessive fibrin were also recorded.

Data management and analysis: The data obtained from history, physical examination and cytologic examination were recorded and correlated to arrive at definitive diagnoses. Simple descriptive statistics was used to calculate the proportion of horses with or without *Histoplasma farciminosum* in both groups.

RESULTS

Cytological diagnosis of stained smears prepared from nodular lesions and swollen superficial lymph nodes of 126 clinically sick horses showed that 89.7% (n=113) of the cases were positive for the yeasts of *Histoplasma farciminosum*, whereas the smears from the remaining 10.3% (n=13) of cases showed the presence of bluish stained pleomorphic organisms in the cytoplasm of intact neutrophils and macrophages, which were tentatively diagnosed as bacterial infection. Examination of stained smears prepared from submandibular and prescapular lymph nodes of 46 horses which had linear scars on their sternum, face and limbs, and presumed to be recovered from epizootic lymphangitis, revealed lymphadenitis without an apparent causative agent in 15.2% (n=7), lymphadenitis along with *Histoplasma farciminosum* in 19.56% (n=9) and absolutely normal lymph nodes in 65.2% (n=30) Table 1, Fig 1a-f.

The yeasts of *Histoplasma farciminosum* were found as double layered, lemon shaped with one edge wider and the other bluntly pointed. The organisms were found both individually or in groups within (Fig 1a) or outside (Fig 1b) of white blood cells; high tendency of being in group and intracellularly. The cytoplasm of most of the yeasts was unstained.

The lymphadenitis cases with or without *Histoplasma farciminosum*, were further evaluated and classified as eosinophilic, neutrophilic and lymphadenitis of immune stimulation on the basis of the dominant cells observed per 100X (oil-immersion) objective field of the stained smears. Eosinophilic lymphadenitis, with ≥ 3 eosinophils eosinophils detected, was observed in 42.86% (n=3) cases of lymphadenitis without an apparent causative agent. Similarly, neutrophilic lymphadenitis (dominated by neutrophils) and lymphadenitis of immune stimulation (if plasma cells are ≥ 3 per field of vision) were observed in 42.86% and 14.3% of lymphadenitis cases without apparent causative agent, respectively. All of the cases of lymphadenitis associated with *Histoplasma farciminosum* (n=9) were pyogranulomatous in nature, in which the dominant cells were neutrophils and macrophages with few plasma cells and eosinophils. However in five of these cases (33.3%), the numbers of eosinophils per 100X (oil-immersion) objective field were ≥ 3 . When compared with clinically active cases, the yeast of *Histoplasma farciminosum* observed in this group of cases was found in the cytoplasm of very few macrophages and neutrophils. Moreover, the number of yeasts found in these cells varies from one to a maximum of six (Figure 1d).

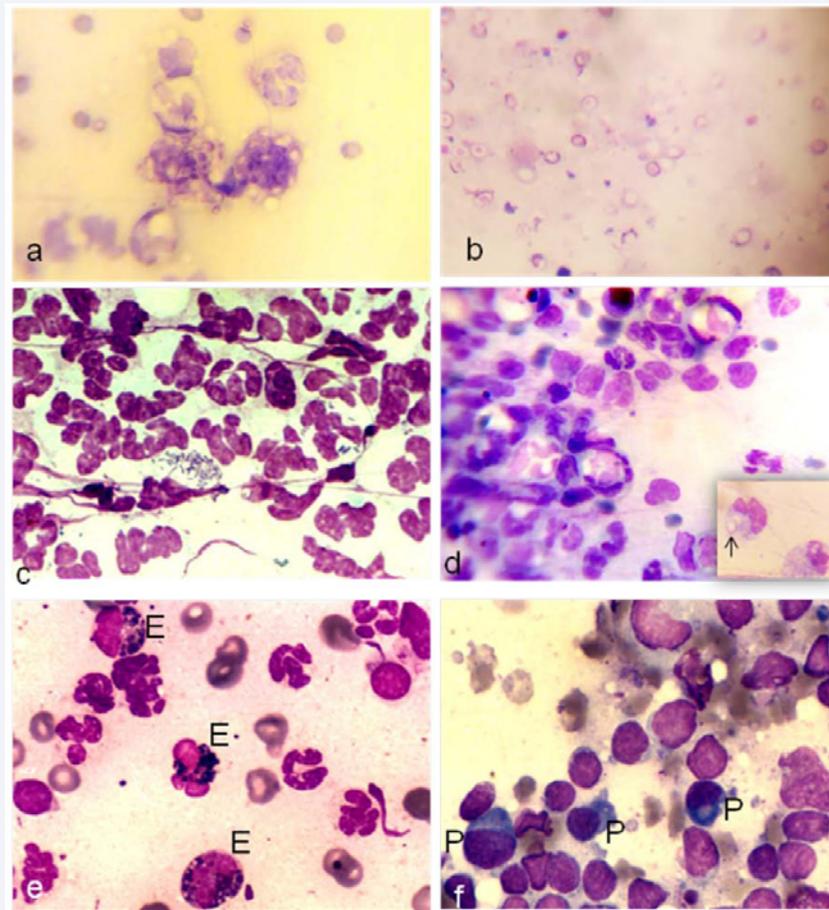


Figure 1 FNA smears stained with modified Giemsa: a. Clinically active EL case: pyogranulomatous reactions with several *Histoplasma farciminosum* yeasts within white blood cells; b. Several *H. farciminosum* yeasts outside white blood cells; c. Lymphangitis with unidentified bacterial: smear made from nodule showing several degenerated neutrophils and pleomorphic bacteria; d. Smear from apparently recovered case showing few number of yeasts in few macrophages (arrow on insert) and neutrophils; e. Lymphadenitis of immune stimulation: Smear made from reactive lymph node showing increased number of plasma cells. E - eosinophils, P- plasma cells. All under oil-immersion.

DISCUSSION

The clinical manifestations of epizootic lymphangitis in horses in the present study were similar to previous reports [3, 8-11]. In most of the cases, the lesions were nodular and granulomatous in nature and distributed on the face, chest, limbs and sternum with a linear pattern. The pathogen, *Histoplasma farciminosum*, enters through a wound and spreads locally by invasion and then via the lymphatic system with the formation of purulent nodules. Obliteration of the lymphatic vessels associated with inflammatory reaction can cause delayed lymph drainage and hence swelling of the affected area.

Morphological and staining features of *Histoplasma farciminosum* observed in this study were diagnostic. Such morphological features were also reported so far by previous studies [5,12-14]. Unlike the frequently used Gram stain, additional merits were noticed from the modified Giemsa stain used in the present study. This stain is cheaper, has very few procedures, time evaluate and much more appreciate to appreciate cellular reactions. In this regard, examination of the stained smears revealed a pyogranulomatous reaction for all

Histoplasma farciminosum positive cases. Mycotic infections are often pyogranulomatous in nature and characterized by macrophages, multinucleated giant cells, neutrophils, lymphocytes, and varying number of eosinophils, plasma cells, and mast cells [4,15]. The presence of increased eosinophils in 5 epizootic lymphangitis cases may be attributed to possible concurrent parasitism, allergic conditions and fly larvae (maggot) deposition on the lesions.

In addition to the cutaneous form of epizootic lymphangitis found in this research, a few cases (10.3%) of unidentified bacterial lymphangitis were observed in this study. Though isolation and characterization of the agent is believed to be the gold standard, the presence of several pleomorphic organisms in the cytoplasm of neutrophils along with the suggestive clinical signs was diagnostic enough for ulcerative lymphangitis. The bacterial causes, some presumed to be *Corynebacterium pseudotuberculosis*, were detected only from the nodules. Cytologically, the dominant inflammatory cells detected from these nodules were neutrophils. In line with this, Shelly [15] stated that FNA from nodular lesions induced by bacteria, especially simple forms of bacteria, usually revealed predominately neutrophils. As stated

Table 1: Proportion of epizootic and ulcerative lymphangitis in both groups of drat-horses examined.

Group	No horses examined	Etiologic diagnosis							
		Epizootic lymphangitis		Bacterial lymphangitis		Lymphadenitis without causative agent		Normal lymph nodes	
		N	Prop (%)	N	Prop (%)	N	Prop (%)	N	Prop (%)
Group I	126	113	89.7	13	10.3	-	-	-	-
Group II	46	9	19.6	-	-	7	15.2	30	65.2

Group I: Clinical active cases of lymphadenitis and lymphangitis
Group II: Apparently recovered cases with linear scars

in most [1,16], the nearest superficial lymph nodes were swollen but not abscessed. Cytological findings from these lymph nodes however revealed several plasma cells, which are indicative of reactive lymph nodes for the processed and presented antigens from the surrounding tissue [4].

The detection of nine (19.56%) *Histoplasma farciminosum* positive cases from 46 drat-horses having linear scars on their sternum, face and limbs clearly showed that even if the horses seemed apparently recovered from the disease, the pathogen can be sequestered in the lymph nodes. The disease in such horses can relapse at times of stress and immunosuppression and even spread to other horses. In the study area, we had a chance to see traditional practices like hot iron application and topical application of plant extracts on the characteristic lesions of diseased drat-horses. These traditional 'treatments' might have contributed by the partial healing and scar tissue formation on affected skin. In advanced cases, however, such traditional practices will fail to cure the disease and horses are usually abandoned outdoors by their owners.

In addition to the above 2 diseases, the regional lymph nodes can be involved when and wherever there is a lesion in the surrounding tissue. In this regard, chaffing of the legs together, faulty shoeing, improper harnessing and mechanical damage on the different body parts are the common problems observed on drat-horses of the country. In these conditions, pathogens or their antigens reach the nearest lymph nodes and induce lymphadenitis of neutrophilic, eosinophilic or immune stimulation type. Over 40% of the lymphadenitis cases were neutrophilic in nature which is believed to be caused by ubiquitous lower forms of bacteria like *Staphylococcus* and *Streptococcus* species. Whereas 33.3% of the lymphadenitis cases were eosinophilic in nature and were probably associated with fly larvae deposited on the damaged sites and /or because of internal parasitism.

Horses apparently recovered from lymphangitis may have the agent in their superficial lymph nodes and act as source of infection for naive horses. The trend of using one harness for two or more horses can help to spread the condition further. Humane destruction of severe cases and carrier horses should be practiced widely in the country to effectively control epizootic lymphangitis. In a nutshell, cytology was found to be a valuable tool in establishing a diagnosis, identifying the process and forming a prognosis. Therefore, cytological examination should be practiced in the veterinary clinics in diagnosing different disease conditions of large animals.

CONCLUSION

It is necessary to check that resolution of the pathogen

[*Histoplasma farciminosum*] from the lymph nodes and lymphatic vessels complete for the implementation of effective disease control measures. As this is the first study, at least in Ethiopia, conducted to evaluate the cytological findings of superficial lymph node and lymphatic vessel pathology, further research are needed to substantiate the present findings. Moreover, further study on the microbiological characteristics and antibiogram of the bacterial agents detected during cytological examinations is recommended.

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