REVIEW ON EPIZOOTIC LYPHANGITIS

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Abstract: Histoplasmosis is an important systemic mycosis in the most countries. Increasingly cases are recognized in non-endemic areas. Proper management requires recognition of the clinical syndromes caused by *Histoplasma capsulatum var*. *fircinumum* infection, the disease commonly develops into a chronic debilitating condition that can manifest itself in one of three clinical forms: cutaneous, ocular and respiratory forms. Epizootic lymphangitis is a relatively common infectious disease of horses and other equids in certain parts of the world. Epizootic lymphangitis is second only to African Horse Sickness as a most important disease of horses in Ethiopia. The wounds caused by harness are reported as major predisposing factors of Epizootic lymphangitis in carthorses in Ethiopia.Diagnosis is possible by direct visualization of the yeast form of the fungus in pus from infected lymphatic nodules and by culture or histopathologic examination of tissues from clinically affected cases. It is also possible to visualize the organism in stained histological sections of matured or developing lesions. The infection rate of Epizootic lymphangitis varies with the geographic area and the age of the animal. This review will address these issues with the goal of providing physicians in non-endemic areas sufficient information to suspect, diagnose, and treat patients with histoplasmosis. Therapeutic effects of Sodium Iodide (NaI), Potassium Iodide (KI), ground berries of "Endod" (Phytolacca dodecandra) and Pen strip are used in for equine hitoplasmosis (EH). Response to each treatment was assessed using clinical examination of the lesions. Statistically significant difference, in therapeutic effect was observed among the different remedies. Cases treated either with a combination of NaI and Penstrip or "Endod".
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1. INTRODUCTION

Epizootic lymphangitis is a contagious, chronic disease of horses, mules and donkeys. The disease is characterized clinically by suppuration, ulcerating and spreading pyogranulomatous, multifocal dermatitis and lymphangitis. It is seen most commonly in the extremities, chest wall and the neck; but it can also be present as an ulcerating conjunctivitis of the palpebral conjunctiva, or rarely as a multifocal pneumonia (Morrow and Sewell, 1990; Gilbert, 1998 and AL-Ani, 1999).

The disease was also being called pseudofarcy or pseudoglanders. Moreover, another synonym is Equine histoplasmosis, Histoplasmosis, Farciminosis, African farcy, Equine Blastomycosis and Equine Cryptococcosis. It is clinically characterized by a spreading suppurative inflammation of cutaneous lymphatic vessels, lymph nodes and adjacent skin (Negesse *et al.*, 2012).The form that the disease takes seems to depend primarily on the route of entry. The traumatized skin is either infected directly by infected pus, nasal or ocular excretions or indirectly by soil or contaminated harnesses, grooming equipment, feeding and watering utensils, wound dressings or flies. It is also believed that ticks may play a role in the transmission of this agent (Ameni and Tefere, 2004). The organism may also invade open lesions including ruptured strangles abscesses and castration wounds.

The disease is more common in tropics and subtropics and is endemic in north, east and north-east Africa and some parts of Asia including some countries bordering the Mediterranean Sea, India, Pakistan and Japan. 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 Tefere, 2004; Ameni, 2006). Reports from other parts of the world are sporadic and all cases must be verified by laboratory testing. The prevalence of the disease increases with assembling of animals; it was much more common, historically, when large numbers of horses were stabled together for cavalry and other transportation needs. Mainly, it is horses, mules and donkeys that are

affected by the disease although infection may occur in camels, cattle and dogs (Ueda *et al*., 2003). Experimentally, other animals are refractory to infection subsequent to inoculation, with the exception of certain laboratory animal species such as mice, guinea-pigs and rabbits (Herve *et al*., 1994). Infection in humans has also been reported (AL-Ani *et al*., 1998).

The incubation period is from about three weeks to two months (Ameni, 2006). In all cases the lesions are nodular and granulomatous character and the organism once established, spreads locally by invasion and then via the lymphatic system. There is often thickening, or 'cording' of lymphatic system with the formation of pyogranulomatous nodules. Regional lymph nodes may be enlarged and inflamed. Lesions usually heal spontaneously after two to three months, resulting in satellite scar formation. However, extensive lesions with high mortality rates can occur in areas where there is poor veterinary care and nutrition (Ameni, 2006). The form that the disease takes seems to depend primarily on the route of entry. Namely: cutaneous, respiratory, ocular and asymptomatic carriers. The conjunctival form of the disease is believed to be spread by flies of the *Musca* or *Stomoxys* genera. The pulmonary form of the disease is infrequent and is presumed to occur after inhalation of the organism. The cutaneous form of the disease may be confused with farcy (the skin form of glanders), ulcerative lymphangitis, indolent ulcers, sporotrichosis, cryptococcosis, strangles, sarcoid and cutaneous lymphosarcomas (Lehmann *et al*., 1996).

The disease is eradicated by the humane slaughter of infected horses, disinfection of infected premises and restricting the movement of equids from infected premises. In endemic areas where eradication is not possible inorganic iodides can be used for therapy in early cases. Localized nodules can also be lanced, the pus drained and the nodules packed with a 7% tincture of iodine. If affordable, Amphotericin B can be used (AL-Ani, 1999).

According to the study conducted by Edebu (1996) and SPANA (2003) in Ethiopia, epizootic lymphangitis is common mainly in cart horses which are still used as the major means of transport in many parts of the country and serves as a source of income for cart owners.

Therefore, the objectives of this paper are:

- To review epizootic lymphangitis
- To understand its diagnostic methods and the economic impact of the disease

2. LITERATURE REVIEW

2.1. Definition

Epizootic lymphangitis is a contagious relatively common infectious disease of horses and other equids caused by the dimorphic fungus, *Histoplasma capsulatumvariety farciminosum* (OIE, 2008; AL-Ani, 1999). It is a debilitating disease. Most cases of Epizootic lymphangitis are reported from horses (90%), and the remainder from mules and donkeys. Epizootic lymphangitis infection can occur in camels, cattle and dogs, (AL-Ani, 1999). Epizootic lymphangitis infection in humans has also been reported (AL-Ani *et al.*, 1988; Radostits *et al.*, 2006).

2.2. Etiology

Epizootic lymphangitis is caused by *Histoplasma farciminosum* (synonyms: *Cryptococcus farciminosum, Zymonema farciminosum, Histoplasma capsulatum* var. *farciminosum).* It waswhen the organism was known to produce tuberculated conidia that classification of the organism was known to be much related to *Histoplasma capsulatum* and henceconsidered to be one of the varieties of *Histoplasma* var. *farciminosum* (Weeks *et al*., 1985).

It is a dimorphic fungus which has a yeast form in the host and mycelia form in soil and on artificial laboratory media. In tissue, the organism is present in a yeast form; it forms mycelia in the environment (Carter*et al*., 1991). The yeast form of the organism appears in pus as a double-contoured oval or ovoid body, measuring 2.5-3.5 µm by 3-4 µm. The saprophytic stage is mycelia and both forms can be cultivated if suitable media, temperature of incubation and carbon dioxide (CO_2) tension are provided. The organism grows slowly when the yeast phase is grown on media rich in protein and in an atmosphere enriched with CO_2. Several culture media have been used, but the most satisfactory were Sabouraud dextrose agar enriched with 2.5% glycerol; brain heart infusion agar enriched with 10% horse blood; nutrient agar supplemented with 2% dextrose; mycobiotic agar and mycoplasma-like organism medium (Selim *et al*., 1985; Al-Ani *et al*., 1988).

Antigenically, *H. capsulatum* var. *farciminosum* and *H. capsulatum* var. *capsulatum* are indistinguishable, however, the latter is the cause of disseminated histoplasmosis is endemic in North America and has a wide host range (Robinson and Maxie, 1993). The DNA sequences of four protein-coding genes have been analyzed to elucidate the evolutionary relationships of *H. capsulatum* varieties. This

indicated that *H. capsulatum* var. *farciminosum* is deeply buried in the branch of SAm Hcc group A, (H60 to -64, -67, -71, -74 and -76), looking as if it were an isolate of South American *H. capsulatum* var. *capsulatum* (Kasuga *et al.*,1999).

2.3. Epidemiology

2.3.1. Host range

The disease mainly affects horses, mules and donkeys; however, infection may occur in camels, cattle and human beings (Radostits *et al.*, 1994). Mice and rabbits may also be infected experimentally (Knowles and Moulton, 1982). Horses under six years of age are most susceptible (Radostits *et al*., 1994).

2.3.2. Transmission

The mode of transmission of EPL includes transmission by direct or indirect contact with traumatized skin, by biting flies, by ticks or by inhalation of HCF (Ameni and Terefe, 2004). HCF is introduced via open wounds (Timoney, 2015). Spread of infection can also occur by indirect contact through contaminated objects such as grooming tools, feeding and watering utensils, and harnesses and through wound dressings (Jubb *et al.*, 2006). Little evidence is available to describe risk factors for EPL, such as factors favoring persistence of the organism within the environment, the routes of transmission and potential vectors (Scantlebury *et al*., 2015). The wounds caused by harness are reported as major predisposing factors of EPL in carthorses in Ethiopia (Asfaw *et al*., 2012). Experimentally, the disease can be transmitted by biting flies, e.g. *Musca* and *Stomoxys* spp that feed on open, discharging lesions. Flies may also transmit the skin form mechanically when they feed on lesions and exudates (Scantlebury *et al*., 2015).Fungal spores can be trans-mitted to healthy animals by direct contact with infected animals or with inanimate objects or fomites, suchas grooming equipment, bedding, saddler, etc., and enter the skin through cutaneous abrasions (Ameni, 2006)

Saprophytic stage in the soil *Histoplasma capsulatum* var. *farciminosum* is relatively resistant to environmental conditions. It can survive for many months in warm and moist environment (OIE, 2005). It is also believed that ticks may play a role in the transmission of this agent. In endemic areas in certain regions of the world, the seasonal dusty winds expose horses to the inhalation of dust and spores, leading to pneumonia. The organism has been isolated from the alimentary tract of biting flies that had alighted on open lesions, and the disease has developed in horses 4.8 km from the nearest case (Ameni and Tefere, 2004).

2.3.3. Occurrence

The disease is endemic in countries bordering the Mediterranean, particularly in Italy and North Africa and is also found in Central and Southern Africa, and in regions of Asia and Russia (Addo, 1980; Al-Ani, 1989; Herve *et al.*, 1994 and Jerabek, 1994). Some doubt exists concerning the validity of the reported cases of epizootic lymphangitis in the USA. The three major outbreaks of epizootic lymphangitis during the 20th century have been associated with the massing together of large numbers of horses due to military operations (Gillespie and Timoney, 1981). 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. Reports from other parts of the world are sporadic and all cases must be verified by laboratory testing (Ameni and Tefere, 2004; Ameni, 2006).

2.4. Pathogenesis

Histoplasmafarciminosum invades subcutaneous tissue via local granuloma or ulcer and spreads along the lymphatic vessels (Radostits *et al*., 1994). Following the initial invasion of the skin, the organism spreads through the lymphatic vessels to the regional lymph nodes, and in more advanced cases involves the internal organs. Nodular and chronic suppurating lesions are evident in the skin overlying lymph vessels and nodes. When mucosal lesions occur, most are confined to the upper respiratory tract and eyes (Al-Ani and Al-Delaimi, 1986). The nasal infection is usually accompanied by mucopurulent discharge containing large numbers of the fungus. In the Sudan, *H. farciminosum* has been isolated from granulomatous lung lesions of two horses suffering from pneumonia. A fatal pneumonia due to *H.farciminosum* has been reported in an immune suppressed foal (Ramachandran, 1995).

2.5. Clinical Signs

The incubation period ranges from several weeks to six months (Ramachandran, 1995). The clinical signs of epizootic lymphangitis can be grouped into four different forms; the form that the disease takes seems to depend primarily on the route of entry; namely: cutaneous, respiratory, ocular and asymptomatic carriers (Al-Ani and Al-Delaimi, 1986). The cutaneous form of the disease, after which the disease was named, is the most common (Guerin *et al*., 1992). The initial lesion is an open granulomatous wound along the course of a lymphatic vessel which has a

tendency to ulcerate or to undergo alternating periods of discharge and closure for some weeks before healing with residual scar formation. Lesions are most common in the forelimbs, the chest wall and the neck (Figure 1). In severe cases, skin over the entire body may be affected. The lesions begin as indolent, chancre-like papules, becoming larger over the course of weeks and eventually form irregular pyogranulomatous nodules which frequently ulcerate (Al-Ani and Al-Delaimi, 1986).

Figure.1. Cutaneous form of epizootic lymphangitis in horse
Source: (Stephanie, 2017)

The ophthalmic form of the disease is less frequent. Infection may occur as conjunctivitis or a naso-lachrymal infection. The infection rarely becomes generalized. Initial infection is characterized by a watery discharge from one or both eyes and some swelling of the eyelids, followed by the development of papules and ulcerating button-like growths on the conjunctiva and/or on the nictitating membrane (Al-Ani and Al-Delaimi, 1986).

The respiratory form of the disease is characterized by lesions which are mostly confined to the upper respiratory tract. This form usually occurs as a late development in the cutaneous form of the disease. On the nasal mucosa, the lesions begin as yellowish papules or nodules and these soon form crater like granulating ulcers that bleed easily. The lesions are usually found near the external nares (Figure 2). These lesions may also occur in the lungs (Al-Ani and Al-Delaimi, 1986).

3. Figure. Respiratory form of epizootic lymphangitis in horse
Source: (Scantlebury and Reed, 2009)

Asymptomatic carriers can be identified clinically by the identification of fibrocalcific skin lesions at previous sites of infection (Al-Ani and Al-Delaimi, 1986). Such horses will give a positive result to an intradermal sensitivity test and positive reactions to serological tests (Soliman *et al*., 1985).

2.6 Diagnosis

Diagnosis of epizootic lymphangitis can be done based on the result of the tentative and definitive diagnoses of the disease**.**

2.6.1. Tentative Diagnosis

Although the clinical manifestations of epizootic lymphangitis are well described, the diagnosis cannot be achieved on the basis of clinical information alone, since there is significant overlap of with other diseases. For instance, mild acute epizootic lymphangitis is similar to diverse viral respiratory tract infections. Pulmonary manifestations of more aggressive disease with pulmonary infiltrates and hilar lymphadenopathy overlap with infections by other dimorphic fungi or *Mycobacterium* species (Leimann*et al*., 2005).

Necropsy findings together with histopathological findings can also help us to reach on the tentative diagnosis of the disease. Gross lesions are manifested by pyogranulomas, purulent discharge of thickened superficial lymphatic vessels and enlargement of regional lymph nodes (Leimann *et al*., 2005).
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Hematological picture showed leucocytosis, neutrophilia and an increase in the erythrocyte sedimentation rates. The tests described below are used in the diagnosis of epizootic lymphangitis (Al - Ani and Al - Delaimi, 1986).

2.6.2. Definitive Diagnosis

The definitive diagnosis requires the isolation and identification of *H. farciminosum* var. *farciminosum* on specific culture medium or visualization of the yeast form in direct examination of clinical specimens using specific fungal staining techniques. However, these procedures are time consuming, usually taking a minimum of 15 days and lack sensitivity. Furthermore *H. farciminosum* structures visualized microscopically can be confused with structures from other fungal pathogens. The definitive diagnosis is also depending on the material processed for these applications, since some contaminants can out grow *H. farciminosum* complicating the diagnosis. Given these difficulties, other techniques have been developed to supplement culture and microscopic examination that incorporate clinical information and serology. Laboratory tests have the benefit of a rapid turnaround time and reasonable specificity and sensitivity. Detection of antibodies and antigens provides information

indicative of current disease that helps in the management of the infection. Detection of precipitins by immunodiffusion to the two most important diagnostic antigens from *H. farciminosum* with the H and M antigen is one of the most widely available techniques for diagnosis, but the assay has a specificity of 70 to 100%. Complement fixation tests have a sensitivity range of 70-90% but are less specific than immunodiffusion (70-80%). Antigen detection methods are also used, especially when the antibody detection is unlikely. This most often occurs in immunocompromised patients with disseminated infection who often fail to manifest an immune response. However, antigen detection assays are not universally available (Leimann *et al.*, 2005).

Sensitivity of laboratory tests for diagnosis of epizootic lymphangitis is different. The choice of each test to be applied for diagnosis depends mainly on the clinical manifestation and host factors (Leimann *et al.*, 2005). Although attempt to culture the organism should always be pursued, culture based methods are most effective when the fungal burden is high, for instance, in some patients with chronic or disseminated forms of epizootic lymphangitis. However, culturing is insensitive in sub-acute and acute form epizootic lymphangitis. Non-culture based methods are used in conjunction with culture to improve our ability to diagnosis *H. farciminosum* infection and can also guide therapy for epizootic lymphangitis. Recently, some molecular biology techniques have been developed that may require further improvement in the diagnosis of epizootic lymphangitis, particularly for the detection of disease in an early stage and to improve the specificity of the diagnosis (Gabal and Khalifa, 1983).

2.6.2.1. Isolation of the Agent

This includes the isolation of the causative agent by culturing on appropriate medium in the laboratory (Gabal and Khalifa, 1983**;** Al-Delaimi and Khairallah, 1984**;** Al-Ani, 1989).

Samples for laboratory diagnosis should be collected directly from enraptured nodules. For microbiological isolation, the material should be placed in a liquid nutrient medium with antimicrobials and kept in refrigerator at + 4 ^{0}C until culturing but it should be attempted to culture as soon as possible. For direct examination, swabs of lesion material can be smeared on glass slides and fixed immediately. For histopathology, sections of lesion material, including both viable and nonviable tissue should be placed in 10% neutral buffered formalin. Confirmation of the disease is depending on the demonstration of *H. farciminosum* var. *farciminosum* (AL-Ani, 1999).

Isolation of the fungus can be achieved on special medium such as Sabouraud Dextrose Agar (SDA). The mycelia form of *H. farciminosum* var. *farciminosum* grows slowly on laboratory media (2-8 weeks at 26°C). Media that can be used include Mycobiotic agar (AL-Ani *et al*., 1998), Sabouraud Dextrose Agar enriched with 2.5% glycerol, brain–heart infusion agar supplemented with 10% horse blood, and plueropneumonia-like organism (PPLO) nutrient agar enriched with 2% dextrose and 2.5% glycerol, PH 7.8 (Guerin, 1992; Robinson and Maxie, 1993). The addition of antibiotics to the media is recommended: cycloheximide (0.5g/ litre) and chloramphenicol (0.5g/litre). Broad-spectrum antibacterial activity is obtained if gentamycin (50 mg/liter) and penicillin G (6×10^6 units/litre) are used instead of chloramphenicol.

Colonies appear in 2-8 weeks as dry, grey-white, granular and wrinkled mycelia. The colonies become brown with aging but it may have aerial form occur rarely. The mycelia form produces a variety of conidia, including chlamydospores, arthroconidia and some blastoconidia. However, the large round double-walled macroconidia that are often observed in *H. capsulatum* var. *capsulatum* are lacking. As a confirmatory test the yeast form of *H. capsulatum* var. *farciminosum* can be induced by sub-culturing some of the mycelium into brain–heart infusion agar containing 5% horse blood or by using Pine's medium alone at 35 – 37°C in 5 % CO_2. Yeast colonies are flat, raised, wrinkled, white to greyish brown and pasty in consistency. However, complete conversion to the yeast phase may only be achieved after four to five repeated serial transfers on to fresh media every 8 days (Robinson and Maxie, 1993).

Allergic test can also use as screening test for epizootic lymphangitis. This method evaluates the reactivity of the patients with histoplasmosis when challenged intradermally with fungal proteins (HMIN). Some studies have suggested that this methodology has an application on diagnosis of primary, symptomatic or asymptomatic infections caused by *H. capsulatum* in immunocompetent individuals (Torres, 1999). However, HMIN skin testing is currently largely used for research rather than for clinical purposes. Testing with HMIN is particularly useful in epidemiological studies in non-endemic geographic regions in individuals who occasionally visit endemic areas or during epidemics. An accurate and reliable method of skin testing is the intradermal test (Soliman *et al.*, 1985; Soliman *et al*., 1986). This consists of intradermal injection of 0.1 ml of soluble antigen prepared from

H.farciminosum. An increase in the thickness of the skin of 8 mm to 20 mm, 24 hr after injection of the antigen can be regarded as a positive result (Soliman *et al*., 1986).

2.6.2.2. Identification of the agent

The isolated organism can be identified by using the following methods:

i. Direct microscopic examination

The organism can be identified by direct microscopic examination of the organism from specimens and from isolated colonies in the laboratory. The organism can be identified from specimens directly with Gram's stain and examined for the typical yeast form of the organism, which will appear as Gram-positive, pleomorphic, ovoid to globose structures, approximately 2-5 µm in diameter. They may occur singly or in groups and may be found either extracellular or within macrophages and a halo around the organisms (unstained capsule) is frequently observed (AL-Ani *et al*., 1998).

Histopathological examination: The disease can be diagnosed by hematoxylin and eosin stained histological sections, the appearance of the lesion is quite characteristic and consists of pyogranulomatous inflammation with fibroplasia and Langhans giant cells. The presence of numerous organisms, both extracellularly and interacellularly within macrophages or multinucleated giant cells in tissue sections stained with hematoxylin and eosin, Periodic Acid–Schiff reaction and Gomori methylamine–silver stain are observed (Robinson and Maxie, 1993). There is some indication that the number of organisms increases with chronicity. The organisms are pleomorphic, often described as slightly lemon-shaped basophilic masses, varying from 2 to 5 µm in diameter, that are surrounded by a 'halo' when stained with H and E or Gram's stain (AL-Ani, 1999).

Electron microscopy: it has been applied to skin biopsy samples of 1.5–2.0 mm immediately prefixed in phosphate buffered 2% glutaraldehyde solution at 4°C and post-fixed in 1% osmium tetra oxide. Ultra-thin sections were cut and stained with uranyl acetate and lead citrate. Examination demonstrated the fine internal structure of the organism, *H. capsulatum* var. *farciminosum*, including the cell envelope, plasma membrane, cell wall, capsule and inner cell structures (AL-Ani, 1999).

Laboratory tests: used in the diagnosis of epizootic lymphangitis include isolation of the causative agent by culture and tests for the presence of antibodies in the blood (Gabal and Khalifa, 1983; Al-Delaimi and Khairallah, 1984; Al-Ani, 1989). Hematological picture showed leucocytosis, neutrophilia, and an increase in the erythrocyte sedimentation rates (Al-Ani and Al-Delaimi, 1986). The tests described below are used in the diagnosis of epizootic lymphangitis.

ii. Colony morphology

The causative agent, *H. capsulatum* var. *farciminosum (HCF)*, is a thermally dimorphic fungus. The mycelia form is present in soil; the yeast form is usually found in lesions. The fungus can be isolated on Sabroud Dextrose Agar (SDA) in laboratory. Colonies appear in 2-8 weeks as dry, grey-white, granular, wrinkled mycelia. The colonies become brown with aging. Aerial forms occur, but are rare. The mycelia form produces a variety of conidia, including chlamydospores, arthroconidia and some blastoconidia. *H. farciminosum* was formerly described as an independent species but this assessment has been changed and it is now considered to be a variety of *H. capsulatum* due to the close morphological similarities of both the mycelia and yeast forms (Ueda *et al*., 2003).

iii. Animal inoculation

*Histoplasma capsulatum*var. *farciminosum* can be identified by animal inoculation. Experimental transmission of *H. capsulatum*var. *farciminosum*has been attempted in mice, guinea-pigs and rabbits. Immunosuppressed mice are highly susceptible to experimental infection and can be used for diagnostic purposes (AL-Ani, 1999).

iv. Serological methods

The isolated colonies of *H. capsulatum* var. *farciminosum* can be identified by using many different serological techniques. They are also used to diagnose the disease in the absence of positive culture of *H. capsulatum* var. *farciminosum.* The methods are also used as a presumptive diagnosis and is usually made, based on the presence of antibodies in the serum. Although several serological tests have been used for the diagnosis of epizootic lymphangitis, none of the tests are sufficiently sensitive or specific to confirm the diagnosis. Nowadays, four serological tests described below are relevant. Fluorescent antibody technique is one of the most common serological tests to diagnose epizootic lymphangitis. The usefulness of the fluorescent antibody (FA) technique as a diagnostic tool for many infectious diseases has been firmly established. A number of investigators have explored the possibility of using the FA procedure for diagnosis of *H.capsulatum* var. *farciminosum* infection. The test is rapid and reliable, especially in cases where detection and isolation of the organism is

unsuccessful (Gabal *et al*., 1983; Al-Delaimi and Khairallah, 1984).

The second serological test to diagnose epizootic lymphangitis is agar gel immunodiffusion test has been developed (Soliman *et al*., 1984). The antigen used has been prepared from the mycelial (Soliman *et al*., 1985) and the yeast forms of the organism. This method is based on the fact that all *Histoplasma* spp. produce H and M histoplasmin antigens (Al-Ani, 1989).

An enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies in horses infected with epizootic lymphangitis was evaluated (Gabal and Mohammed, 1985). Mincing preparation of four weeks growth of the fungus in a phosphate buffer saline can be used as antigen. A peroxidase labeled goat anti-equine IgG (immunoglobulin G) was used as a conjugate.
The ELISA is simple and reliable for the diagnosis of the disease (Gabal and Mohammed, 1985).

The serum agglutination test (haemagglutination) is one of the traditional standard tests which are used widely. It is highly suitable for thelarge-scale screening of sera. A titer of 1:80 or more can be considered positive (Al-Ani *et al*., 1989)

v. Molecular based methods

DNA sequences of four protein-coding genes have been analyzed to elucidate the evolutionary relationships of *H. capsulatum* varieties. This indicated that *H. capsulatum* var. *farciminosum* is deeply buried in the branch of SAmHcc group A, (H60 to -64, -67, -71, -74 and -76), looking as if it were an isolate of South American *H. capsulatum var. capsulatum* (Kasugat *et al*.,1999).
Polymerase Chain Reaction (PCR) diagnosis based on the amplification of fungal gene sequences is a powerful tool for identifying invasive mycoses. A new nested PCR has been tested in murine models of histoplasmosis and compared to quantitative cultures (Bialek*et al., 2001*). The primers sequence used in this method were based on the small-subunit (18S) rRNA gene of *H. capsulatum*. The nested PCR could detect *H.capsulatum* DNA in tissue and blood samples from infected animals. But not specifically because preliminary data showed that the assay could also detect DNA of *Blastomyces dermatitidis* and *Paracoccidioides brasiliensis*. This method, when compared with other staining methods for *H. capsulatum* detection, was the most sensitive for detecting the fungus in the spleens of experimentally infected mice and all the samples used were paraffin-embedded to reflect routine procedures in histopathology and also provides the possibility of repetitive examinations (Bialek*et al*., 2002**)**.

2.7. Differential Diagnosis

The differential diagnosis includes the skin form of glanders (farcy), strangles, ulcerative lymphangitis, sporotrichosis, cryptococcosis, sarcoids and cutaneous lymphosarcomas. Epizootic lymphangitis also resembles histoplasmosis which is caused by *Histoplasma capsulatum* var. *capsulatum*. The cutaneous form of the disease may be confused with farcy (the skin form of glanders) which is caused by *Burkholderia mallei*, ulcerative lymphangitis which is caused by *Corynebacterium pseudotuberculosis*, and indolent ulcers which are caused by *Rhodococcus equi* and from sporotrichosis caused by *Sporothrix schenckii*. Epizootic lymphangitis must be differentiated from diseases like cryptococcosis, strangles, sarcoids and cutaneous lymphosarcomas (Lehmann *et al*., 1996)

2.8. Treatment

Epizootic lymphangitis is a chronic disease although some cases may heal spontaneously a few weeks after the development of clinical signs (Radostits *et al*., 1994). There are many chemicals those are used to treat epizootic lymphangitis but 20%sodium iodide solution given intravenously at the dose of 125 ml/ 250 kg once a day, for 3 consecutive days gives a good treatment effect. Intravenous administration must be followed by its oral administration of 30 g dissolved in one liter of clean tap water for 30 days. Administered orally the solution immediately after working hours, when it got thirsty. Unwilling horses were given with a handful of crop residue (Hadril, 2002).

Another chemical that is used to treat epizootic lymphangitis is potassium iodide (KI). For the administration of KI, the procedure adopted by Endebu in 1996 was followed. Briefly, 15 g of KI was dissolved in one liter of water first and this followed by additional volume of water until all the iodide was dissolved. The treatment using KI was given for 30 days as recommended by Aiello and Mays in 1998.

“Endod” (*Phytolacca dodecandra*) also can be used to treat epizootic lymphangitis. A crude dried grounded berry of “Endod” (*L. herit* type 44) was used for this experiment. One sachet (20 g) of the powder was suspended in a liter of clean tap water and left to stay for 14 hours allowing the extraction of water-soluble

chemicals from the crude powder. The area of the lesion was washed with tap water to remove dead tissue and cell debris. Then, the lesion was washed with 2% suspension of "Endod" in the late afternoon, to avoid evaporation. The procedure was repeated daily for a maximum of 6 weeks (Lemma, 1984 and Ameni and Tilahun, 2003).

Broad spectrum antibiotics like pen strip can be used to treat epizootic lymphangitis in equines. The recommended daily dose for horse (8 mg procaine penicillin and 10 mg dihydrostreptomycin sulphate per kilogram body weight) was achieved by the administration of 1 ml penstrip solution per25 kg body weight (Hadril, 2002). This dose was given once a day intramuscularly for five consecutive days.

Different antifungal drugs have also been used and successful treatment with amphotericin B has been reported. The infected horses were treated with an intravenous injection of amphotericin B at a dose of 0.2 mg/kg body weight three times on alternate days. The scabs were removed and the areas cleaned daily with an iodine solution for seven days. The lesions should heal fully within four weeks (Al-Ani, 1989). Testing "*in vitro*" at a concentration of 50 mol. /ml to 100 mol/ml of amphotericin B inhibited strongly the growth of the yeast phase of *H.farciminosum* (Gabal, 1984). Repeated administration of griseofulvin has given good result when combined with iodides and local surgical treatment. The surgical treatment usually consists of opening the nodules and packing with gauze soaked in 7% tincture of iodine (Richer, 1977).

2.9. Control and Prevention

Outbreaks in non-endemic areas are probably best controlled by the slaughter of affected animals. The long incubation period of the disease, the high resistance of the causative agent and the presence of clinically healthy carriers make control of the disease difficult in endemic areas. The method used to control epizootic lymphangitis in large endemic areas will depend on the incidence of the disease, methods of husbandry, attitude and economic capacity of the farming community and the acceptance of the latter of a test and culling programme. Control of the disease depends upon elimination of the infection by culling infected horses and preventing the spread of the disease by hygiene precautions. Cleaning and disinfection will help to prevent the disease from spreading. This method of control is the most satisfactory and proven to be mandatory for large breeding companies in endemic areas (Al-Ani, 1989).

In many countries where epizootic lymphangitis has been introduced, the disease has subsequently been eradicated and thereby has been prevented from becoming endemic. The disease was eradicated from Great Britain in 1906 and no case has occurred since then. In recent years, immunization against epizootic lymphangitis has become an option. A killed formalized vaccine prepared from the yeast form of the fungus, administered subcutaneously in a dose of 5 ml once a year has given good results (Al-Ani, 1989).

An attenuated vaccine was developed by exposure of the causative agent to high temperature. Horses inoculated subcutaneously with 3 ml in a single dose had a protection rate of 75.5% and the duration of immunity exceeded 31 months following vaccination (Zhang *et al.,* 1986*).* Vaccinated animals may be serologically positive which may interfere with control and eradication programmes (Al-Ani, 1989).

2.10 Economic Importance of Epizootic Lymphangitis

Although the disease is common in Ethiopia, its economic importance of the disease is not well studied. The disease is common in cart horses and affecting an average of 18.8% of horses in warm, humid areas between 1500 and 2300 meters above sea level (Ameni and Tefere, 2004 and Ameni, 2006). Reports from other parts of the world are sporadic and all cases must be verified by laboratory testing. The prevalence of the disease increases with assembling of animals; it was much more common, historically, when large numbers of horses were stabled together for cavalry and other transportation needs. The economic impact of epizootic lymphangitis comprises from mortality and inability of the animals to work although mortality does not usually exceed 10% to 15%. The main loss results from the inability of animals to work for several weeks because of extremely painful lesions. Extensive lesions with high mortality rates can occur in areas where there is poor veterinary care and nutrition (Ameni, 2006).

2.11 Status of Epizootic Lymphangitis in Ethiopia

Although Epizootic lymphangitis has been eradicated from Europe, it is currently prevalent in Ethiopia, where between 0% and 39% of equids may be infected, with the rate being dependent upon the region (Asfaw *et al.,* 2012). Epizootic lymphangitis is a common infectious disease of horses in Ethiopia. It is a significant concern in the country, where the prevalence in carthorses is nearly 19%, and economic losses from this disease are high (Ameni, 2006a). It is

particularly prevalent in carthorses in most parts of Ethiopia studied. For instance, it occurs in 24.9% horses in Woliso (Asfaw *et al.*, 2012). Similarly, studies conducted in Ethiopia in horses showed that EPL is endemic to Ethiopia and its distribution covers humid and hot areas (Ameni *et al.*, 2004). Epizootic lymphangitis is second only to African Horse Sickness as a most important disease of horses in Ethiopia (Ameni, 2005). The disease primarily occurs in areas with altitudes ranging from 1600 to 2400mm above sea level, which allows the environmental form of HCF to persist for several months (Ameni *et al.*, 2004). It is common in Ethiopia, especially in cart horses, affecting an average of 18.8% of horses in warm and humid areas (Ameni and Siyoum, 2002). The prevalence of disease accounts for 26.2% in cart horses and 21% in cart mules (Ameni and Terefe 2004). Recently, an overall prevalence of 18.8% was recorded for EPL in carthorses in 28 Ethiopian towns (Ameni, 2005).

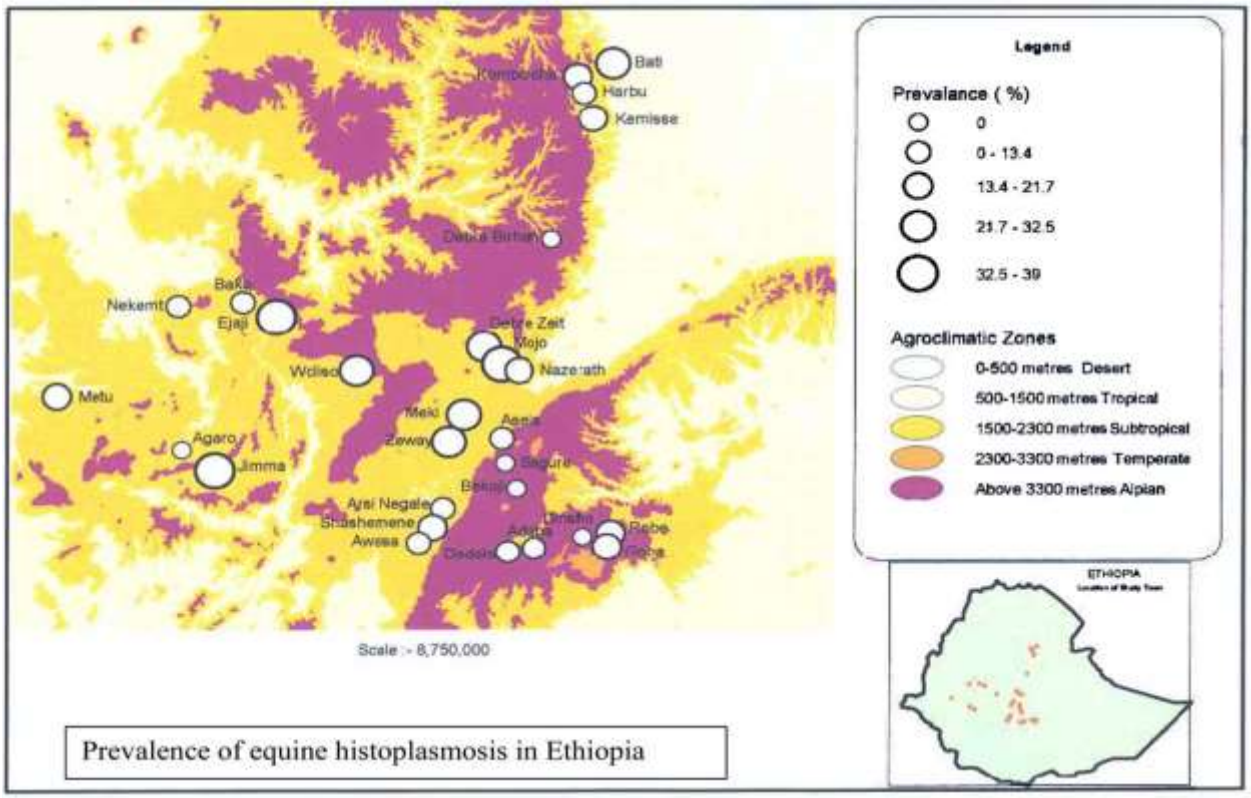

Figure.1.map representing equine histoplasmosis in 28 towns located in different agroecological zones of Ethiopian. **Source**: (Ameni, 2006)

3. CONCLUSIONS AND RECOMMENDATIONS

Epizootic lymphangitis is a debilitating fungal disease mainly occurs in equids. The disease results from infection by dimorphic fungus, *Histoplasma capsulatum var. farsiminosum.* The disease is more common in tropical and subtropical regions than in temperate zones. There are three forms of the disease: cutaneous (skin), ocular, and respiratory forms. The diagnosis of histoplasmosis cannot be achieved on the basis of clinical information alone, since there is significant overlap of histoplasmosis with other diseases. Laboratory diagnosis is mandatory to detect the disease than the clinical findings. Early diagnosis of the disease is preferable way to treat the animal on time and to reduce the loss because of the disease. Although slaughtering or elimination of severely sick animals controls the spread of epizootic lymphangitis in many developed countries, it is impractical to implement in many developing countries like Ethiopia.Intravenous administration of sodium iodide, oral administration of potassium iodide and surgical excision of lesions can be used as a treatment. Strict hygienic precautions are essential to prevent spread of epizootic lymphangitis moreover great care should be taken to prevent spread by grooming or harness equipment. The disease is eradicated by the humane slaughter of infected horses, disinfection of infected premises and restricting the movement of equids from

infected premises. Thus, based on this conclusion the following recommendations are forwarded:

- Large scale training on the knowhow of epizootic lymphangitis for carthorse owners has to be programmed and implemented in a sustainable way.
- More researches have to be done on pathology, pathogenesis, immunology and treatment of epizootic lymphangitis to fill the gap.
- The medicinal plants that the local healers use has to be investigated in depth
- Attention should be given to the management of the abandoned carthorse and there should be rule and regulation on the welfare of them and responsibility of abandoned carthorse owner

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