



Original article

## Detection and clinical manifestation of Epizootic Lymphangitis in horses in Zaria and Kontagora Emirates, Nigeria

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### ABSTRACT

**Introduction:** A study was conducted to determine the clinical manifestation, isolation and identification of the aetiological agent of epizootic lymphangitis in horses in Zaria and Kontagora Emirates in Nigeria.

**Methods:** A total of seventeen (17) infected horses manifesting signs of epizootic lymphangitis were examined in Niger and Kaduna States of Nigeria. The pus samples were also examined using direct smears, Gram stained and examined under a light microscope. Infected lymphoid tissue samples were collected in 10% formal-saline from horses clinically manifesting infection for histopathological studies.

**Results:** Clinical signs observed were painless swellings (nodules) and suppurative ulcers along lymphatic channels with some areas of partial healing and reoccurrence. Culture and direct smear revealed Gram positive double contour and oval shaped yeast-like organisms with a violet cytoplasm. Histopathological examination revealed *Histoplasma farciminosum* cells as oval bodies in macrophages.

**Significance:** The study confirmed that Epizootic Lymphangitis is present in Nigeria.

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### Introduction

A number of diseases have limited the performance of equids in Nigeria, the most important of them and the most devastating being the viral disease- African horse sickness (Erasmus *et al.*, 1992). Epizootic lymphangitis (EL) and ulcerative lymphangitis are also serious health problems of horses in the Sub-Saharan Africa. Epizootic lymphangitis is caused by a dimorphic fungus, *Histoplasma farciminosum*. It is a chronic disease of equids characterized by spreading, suppurative, ulcerative pyogranomatous dermatitis and lymphangitis (Gabal and Khalifa, 1983; Ameni and Terefe, 2004).

Few reports indicated the presence of the disease worldwide, with foci in North Africa and Asia occurring in endemic form (Singh, 1965; Guerin *et al.*, 1992). However, reports on the prevalence of Epizootic lymphangitis among Nigerian horses are very scarce. Useh *et al.* (2005) in a study documented equine diseases in the northern guinea savannah zone of Nigeria categorically stated that documentation of equine diseases apart from helminthosis in the Ahmadu Bello University Teaching Hospital falls very far below expectation. The aim of this study was to determine the clinical manifestation of and isolate the causative agent of epizootic lymphangitis in horses in Zaria and Kontagora Emirates, Nigeria.

### Materials and methods

#### Study area

The study area comprised of Kontagora and Zazzau Emirates of Niger and Kaduna states, Kontagora Emirate is located between latitude 4° 30" and 6° 30" E and longitude 9° 20" and 11° 20" N and the climate of the state consists of distinct wet and dry seasons within the guinea savannah zone of Nigeria. Zazzau Emirate in Kaduna State is located between latitude 7° 46" and 7° 53" E and longitude 11° to 11° 30" N. The climate of the state consists of distinct wet and dry seasons within the guinea and part of Sahel (to North) savannah zone of Nigeria (Anonymous, 2000).

#### Clinical examination

A total of three hundred and four (304) horses in Kontagora and Zazzau Emirates were examined to identify epizootic lymphangitis. Horses were examined at a distance and at close range following adequate restraint by an assistant. Vital parameters (temperature, pulse and respiratory rates) were recorded. A complete clinical examination was done and was recorded on an examination data sheet.

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### Sample collection

All horses with clinical signs indicative of the disease sampled were identified. Sampling was between December 2008 and January 2009. Seventeen pus samples (4 from Kontagora and 13 from Zazzau Emirates) were collected from clinically infected horses for the isolation and identification of the causative organism. Horses with clinical lesions were sampled and nodules were cleaned, disinfected using 75% methanol and the skin was lanced using a scalpel blade to collect the exudates in a sterile swab. Samples collected were labelled in batches refrigerated before culturing. A portion of the sampled material was smeared on glass slides, fixed immediately over a burner and stained for observation under the light microscope (Ameni, 2006).

### Laboratory analysis of samples

#### Direct Smear Examination

Microscope slide smears were stained directly with Gram's reagents. The stained slides were examined under  $\times 100$  oil immersion objective with the aid of light microscope systematically for the typical yeast form of the organism (OIE, 2008).

### Culture

Sabouraud dextrose agar (SDA) slants enriched with 2.5% glycerol and Brain Heart Infusion (BHI) agar supplemented with 10% horse blood were prepared according to the method of Ameni (2007). Chloramphenicol (0.5 mg/L) was added to the media to reduce bacterial growth during incubation as described by OIE manual of standards (2008). Sample bottles containing the media were divided into two sets before inoculation and each of the two batches of bottles were inoculated with one sample and labelled. One sample was placed in the incubator at 37°C and the other one placed on the laboratory bench at room temperature (26°C) for 7 weeks after which all sample were examined for growths and the isolates were examined under a light microscope at  $\times 100$  magnification.

## Results

### Detection

A total of three hundred and four (304) horses were examined in the two emirates. Out of these one hundred and two (102) were from Kontagora Emirate, while two hundred and two (202) were from Zazzau Emirate. Out of the 304 horses examined only 17 horses were observed to have lesions suggestive of EL (Table 1). Of the 102 horses examined in Kontagora Emirate, only 4 (3.9%)

were observed to have lesions of EL. Similarly, only 13 (6.4%) of the 202 horses examined in Zazzau Emirate had lesions of the disease, giving an overall of 17 horses with the prevalence of 5.6 % for the two Emirates. Horses in Zaria City were mostly affected with 8 (2.63%) followed by Sabon Gari with 5 horses (1.64%), Kontagora 2 (0.66%) and then Wushishi 1 (0.33%) and Rijau 1 (0.33%). However, within each of the localities, the highest prevalence of 7.9% was found in Sabon Gari while the lowest was 2.8% found in Rijau.

Table 1. Distribution of horses examined at various areas of Kontagora and Zazzau Emirates

Emirate	Location	*Examined	<sup>β</sup> Lesions	p (%)
Kontagora	Kontagora	42	2	4.8
	Wushishi	24	1	4.2
	Rijau	36	1	2.8
Zazzau	Zaria City	139	8	5.8
	Sabon Gari	63	5	7.9
Total		304	17	5.6

p – prevalence, \* - Number of horses examined,  $\beta$  – Number of horses with clinical lesions

### Clinical manifestation

The clinical signs observed on the 17 horses includes painless swellings (nodules) and suppurative ulcers along the lymphatic vessels of the thoracic region of the chest 2 (11.8%), lesions on lymphatic of the forequarter and the thoracic region 4 (23.5%), areas of partial healing and reoccurrence around the axillary regions 2 (11.8%), lesions along the lymphatic of the hind quarters 1 (5.9%), lesions on the neck and facial (ocular) regions 1 (5.9%) and lesions on the chest, abdomen and both forelimb and hind limbs 7 (41.2%) as shown in Table 2.

Sixteen (94.1%) out of the seventeen infected horses had cutaneous lesions while the remaining one (5.9%) had ocular form of the disease. These cutaneous lesions were characterized by suppurative multifocal dermatitis and lymphangitis which appeared as nodules or ulcers. The chest, abdomen and the forelimbs were frequently involved, while the lesions at the neck and facial regions were less frequent (Table 2). The ocular form of the disease was observed in Zaria City.

Table 2. Nature, form, frequency and prevalence (%) of Epizootic Lymphangitis in horses

Form (%)	Nature	Frequency	%
Cutaneous (94.1%)	Painless swellings (nodules) and suppurative ulcers along the lymphatics of the thoracic region of the chest	2	11.8
	Lesions on lymphatic of the forequarter and the thoracic region	4	23.5
	Areas of partial healing and reoccurrence around the axillary regions	2	11.8
	Lesions along the lymphatic of the hind quarters	1	5.9
	Lesions on the chest, abdomen and both forelimbs and hind limbs	7	41.2
	Lesions on neck and facial (ocular) region	1	5.9
Ocular (5.9%)			
Total		17	100.0

### Gram staining and morphological appearance for direct smear

Organisms with double contour and oval shaped yeast like with a violet cytoplasm in gram stain, the cytoplasm of some of the cells did not take any stain exhibited a halo appearance. Some cells appear in singles while others appear to be budding (Figures 4 and 5).

### Cultural morphology

When cultured in SDA enriched with 2.5% glycerol at 25°C and full growth was observed after seven weeks as yellowish light

brown to grey, convoluted and cauliflower-like. The sample in the incubator at 37°C grew slowly at 8 weeks; the colonies appear as dry, grey-white, granular wrinkled mycelia and became brown with ageing. The samples growing on the bench developed aerial mycelial form producing a variety of conidia including chlamydospores. However, the large double walled macroconidia that are often observed in *H. faciminosum* were seen in stained smears (Figure 5). Isolates from SDA were subcultured on BHI agar with 5% horse blood at 37°C and complete conversion to yeast form was achieved after four repeated serial transfers on to fresh media every 7 days, with the growth observed as raised slightly wrinkled light grey and pasty appearance.



**Figure 1.** Swellings (nodules) on the chest suggestive of epizootic lymphangitis

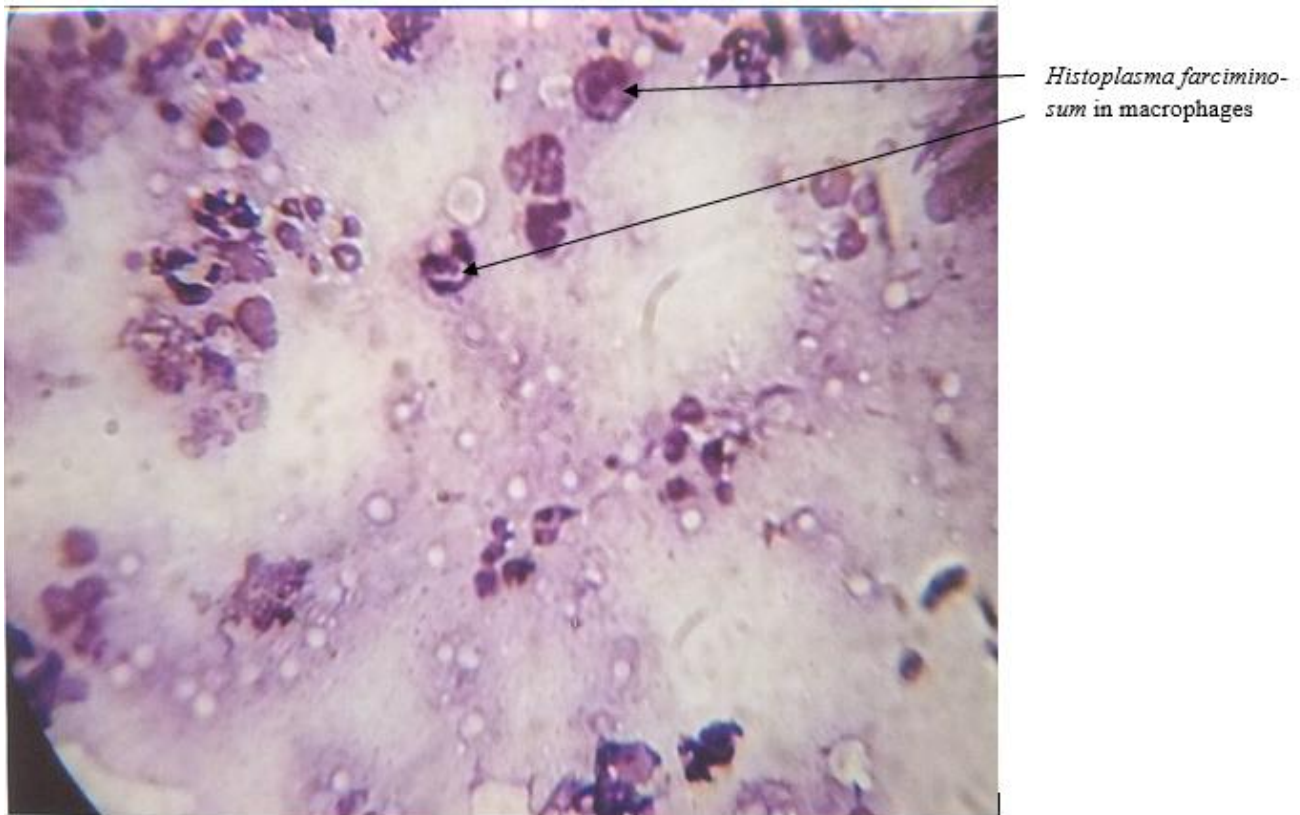


**Figure 2.** Lesion on shoulder showing a cord like appearance along the lymphatic vessels in Epizootic Lymphangitis infection

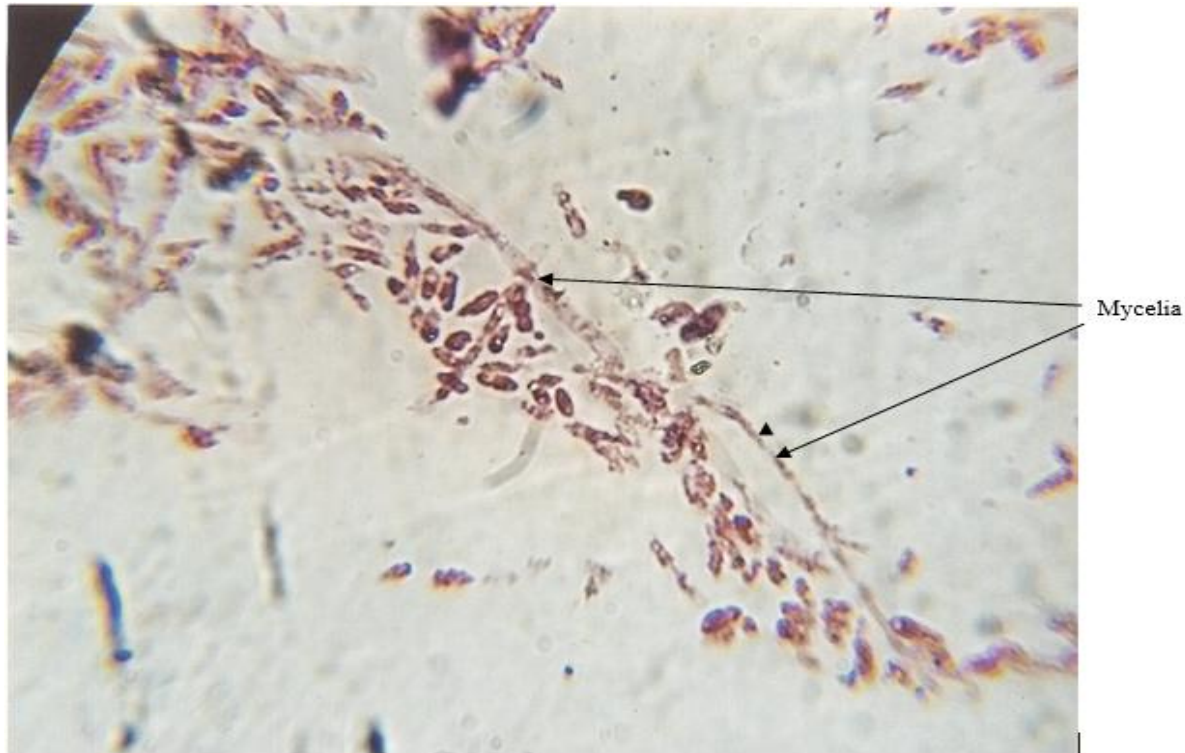




**Figure 3.** Suppurating ulcers and cord-like engorged lymphatic vessels in epizootic lymphangitis infection



**Figure 4.** Pus smear with yeasts of *H. farciminosum* in macrophages, ×100 mag. H&E stain.



**Figure 5.** Mycelial form of *H. farciminosum* from culture of Brain Heart Infusion agar at 37°C, 2 weeks and at  $\times 100$  mag.

## Discussion

The study carried out to detect epizootic lymphangitis on three hundred and four (304) horses in Kontagora and Zazzau Emirates has shown an overall prevalence of 5.6% (17/304) which is lower than 26.2% (97/307) reported in a similar study on cart horses in Ethiopia by Ameni and Siyoum (2002). The variation between the findings in this study and one reported from Ethiopia could be due to the fact that while cart horses are subjected to a lot of stress, while royal horses are taken out only on casual rides and during festivals. The lower prevalence of the disease in Kontagora 3.8% (4/102) compared to Zazzau Emirate could perhaps be mainly due to low horse population and less activity of the royal horses in the former. The higher prevalence of the disease in Zazzau 6.4% (13/17) could be explained by the high horse population with greater activities and therefore increase possibility of contact with infected horses and the common habit of sharing of harnessing equipment among owners. The finding in this study is consistent with the finding of Al-Ani (1999) that the disease is associated with congregation of horses in stud farms, experimental stations, military operations, durbar and polo fields during which infections are spread by the activities of biting flies, formites, tacs and direct contact with infected horses. The large number of infected animals obtained in Zaria could be due to the greater activities and more regular congregation of horses during weekend activities as youths use these horses for mini durbar during marriage ceremonies and other festivities as is common in most parts of Northern Nigeria.

The characteristic painless (nodular) swellings observed during

the study are indications of initial stages of the disease while cases of partial healing and reoccurrence are an indication of the chronic stage or there could be a relapse after treatment. This agreed with the observation of Bojia and Francois (2003). The higher prevalence of cutaneous form 94.1% (16/17) over the ocular form in these study has similarly been reported by Ameni (2007) in cart horses in Ethiopia.

The morphological appearance of the yeast form of the organism from direct smear and the cultural characteristics of both the yeast and the mycelial forms in SDA and BHI agar respectively were consistent with the description by Guerin *et al.* (1992), Robinson and Maxie (1993), Rodostits *et al.* (1995) and Bojia and Francois (2003).

## Conclusion

The study has shown that Epizootic Lymphangitis was found in Kontagora and Zazzau Emirates of Niger and Kaduna states of Nigeria with a prevalence of 5.6%. The study has shown that the clinical signs, cultural growth and morphological appearance as observed during the study were similar to those reported in Ethiopia. As this is the first report in these study areas, there is the need for further nationwide investigation of the disease to having a better understanding of the disease's status in Nigeria. Detailed study on the epidemiology, predisposing factors, level of susceptibility in other animal species and the zoonotic implications of the disease are needed in designing appropriate control strategies.

## Conflict of interest

None declared.

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