CHAPTER 2.5.13.

EPIZOOTIC LYMPHANGITIS

SUMMARY

Epizootic lymphangitis is a contagious, chronic disease of horses and other Equidae characterised clinically by a spreading, suppurative, ulcerating pyogranulomatous dermatitis and lymphangitis. This is seen particularly in the neck, legs and chest. It can also present as an ulcerating conjunctivitis, or rarely a multifocal pneumonia. Transmission is by contact of infected material with traumatised skin, by biting flies, ticks or inhalation. The causative agent, Histoplasma capsulatum *var.* farciminosum*, is a thermally dimorphic, fungal soil saprophyte. Differential diagnoses include glanders* (*farcy*), *caused by* Burkholderia mallei*, ulcerative lymphangitis due to* Corynebacterium pseudotuberculosis, *sporotrichosis caused by* Sporothrix schenckii, *and the skin lesions of histoplasmosis caused by* H. capsulatum *var.* capsulatum*. Local wound drainage and inorganic iodides are used to treat early cases.*

Identification of the agent: *Identification of the agent is made by its appearance in smears of the exudate or in histological sections of the lesion material. The yeast form of the organism is present in large numbers in well established lesions, and appears as pleomorphic ovoid to globose structures, approximately 2–5 µm in diameter, located both extracellularly and intracellularly in macrophages and giant cells. Organisms are usually surrounded by a 'halo' when stained with Gram stain, haematoxylin and eosin, Periodic acid–Schiff reaction or Gomori methenamine–silver stain. The mycelial form of the organism grows slowly under aerobic conditions at 25–30°C on a variety of media, including Mycobiotic agar, enriched Sabouraud's dextrose agar, brain–heart infusion agar, and pleuropneumonia-like organism nutrient agar. Conversion to the yeast phase at 37°C must be demonstrated.*

Serological and other tests: *Antibodies to* H. capsulatum *var*. farciminosum *develop at or before the onset of clinical signs. Assays reported for detection of antibody include fluorescent antibody, enzyme-linked immunosorbent assay, and passive haemagglutination tests. In addition, a skin hypersensitivity test has been described.*

Requirements for vaccines and diagnostic biologicals: *Killed and live vaccines have been used on a limited scale in endemic areas, but they are not readily available.*

A. INTRODUCTION

Epizootic lymphangitis is a contagious, chronic disease of horses, mules and donkeys. The disease is characterised clinically by a suppurative, 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. It has also been called pseudofarcy or pseudoglanders. Another synonym is equine histoplasmosis, which may be a more accurate name for the disease, as not all clinical cases present obvious lymphangitis. The form that the disease takes seems to depend primarily on the route of entry (18). The traumatised 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 (4). The conjunctival form of the disease is believed to be spread by flies of the *Musca* or *Stomoxys* genera (18). The pulmonary form of the disease is infrequent and is presumed to occur after inhalation of the organism. The incubation period is from about 3 weeks to 2 months (3). In all cases, the lesions are nodular and granulomatous in character, and the organism, once established, spreads locally by invasion and then via the lymphatics. There is often thickening, or 'cording', of lymphatics, with the formation of pyogranulomatous nodules. Regional lymph nodes may by enlarged and inflamed. Lesions usually heal spontaneously after 2–3 months, resulting in stellate scar formation. However, extensive lesions with high mortality rates can occur in areas where there is poor veterinary care and nutrition (3).

The causative agent, *Histoplasma capsulatum* var. *farciminosum*, is a thermally dimorphic fungus. The mycelial form is present in soil; the yeast form is usually found in lesions. *Histoplasma 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 mycelial and yeast forms (21). 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 (16). DNA sequences of four protein-coding genes have been analysed 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* (14).

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*, indolent ulcers acaused by *Rhodococcus equi*, sporotrichosis caused by *Sporothrix schenckii*, and histoplasmosis caused by *H. capsulatum* var. *capsulatum*, sarcoids and cutaneous lymphosarcomas (13, 15).

The disease is more common in the 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 metres above sea level (3, 4). 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 (21). 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 (12, 18). Infection in humans has also been reported (2, 6, 11).

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 (1). Localised 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.

As the clinical signs of epizootic lymphangitis can be confused with those of other diseases in the field, definitive diagnosis rests on laboratory confirmation.

B. DIAGNOSTIC TECHNIQUES

1. Identification of the agent

Material should be collected directly from unruptured nodules. For microbiological isolation, the material should be placed in a liquid nutrient medium with antibacterials and kept refrigerated until culturing, which should be attempted 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 dependent on the demonstration of *H. capsulatum* var. *farciminosum*.

a) Direct microscopic examination

- **Gram-stained smears**

Smears can be stained 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 (2). They may occur singly or in groups, and may be found either extracellularly or within macrophages. A halo around the organisms (unstained capsule) is frequently observed.

- **Histopathology**

In haematoxylin and eosin (H&E)-stained histological sections, the appearance of the lesion is quite characteristic and consists of pyogranulomatous inflammation with fibroplasia. Langhans giant cells are common. The presence of numerous organisms, both extracellularly and intracellularly within macrophages or giant cells in tissue sections stained with H&E, Periodic acid–Schiff reaction and Gomori methenamine–silver stain are observed (16). 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&E or Gram's stain (1).

- **Electron microscopy**

Electron microscopy 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 tetroxide. 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 (1).

b) Culture

The mycelial form of *H. capsulatum* var*. farciminosum* grows slowly on laboratory media (2–8 weeks at 26°C). Media that can be used include Mycobiotic agar (2), Sabouraud's dextrose agar agar enriched with 2.5% glycerol, brain–heart infusion agar supplemented with 10% horse blood, and pleuropneumonia-like organism (PPLO) nutrient agar enriched with 2% dextrose and 2.5% glycerol, pH 7.8 (11, 16). The addition of antibiotics to the media is recommended: cycloheximide (0.5 g/litre) and chloramphenicol (0.5 g/litre). Broad-spectrum antibacterial activity is obtained if gentamicin (50 mg/litre) and penicillin G (6×10^6 units/litre) are used instead of chloramphenicol. 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 mycelial 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 subculturing 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 (16). 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.

c) 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 (1).

2. Serological tests

There are published reports of various tests to detect antibodies as well as a skin hypersensitivity test for detection of cell-mediated immunity. Antibodies usually develop at or just after the onset of clinical signs.

a) Fluorescent antibody tests

- **Indirect fluorescent antibody test**

The following non-quantitative procedure is as described by Fawi (7).

i) Slides containing the organisms are made by smearing the lesion contents on to a glass slide or by emulsifying the cultured yeast phase of the organism in a saline solution and creating a thin film on a glass slide.

ii) The slides are heat-fixed by passing the slide through a flame.

iii) The slides are then washed in phosphate buffered saline (PBS) for 1 minute.

iv) Undiluted test sera are placed on the slides, which are then incubated for 30 minutes at 37°C.

v) The slides are washed in PBS three times for 10 minutes each.

vi) Fluorescein isothiocyanate (FITC)-conjugated anti-horse antibody at an appropriate dilution is flooded over the slides, which are then incubated for 30 minutes at 37°C.

vii) Washing in PBS is repeated three times for 10 minutes each.

viii) The slides are examined using fluorescence microscopy.

- **Direct fluorescent antibody test**

The following procedure is as described by Gabal *et al.* (8).

i) The globulin fraction of the test serum is precipitated, and then re-suspended to its original serum volume in saline. The serum is then conjugated to FITC.

ii) Small colony particles of the cultured mycelial form of the organism are suspended in 1–2 drops of saline on a glass slide. With a second slide, the colony particles are crushed and the solution is dragged across the slide to create a thin film.

iii) The smears are heat-fixed.

iv) The slides are washed in PBS for 1 minute.

v) The slides are incubated with dilutions of conjugated serum for 60 minutes at 37°C.

vi) The slides are washed in PBS three times for 5 minutes each.

vii) The slides are examined using fluorescence microscopy.

b) Indirect Enzyme-linked immunosorbent assay

The following procedure is as described by Gabal & Mohammed (10).

i) The mycelial form of the organism is produced on Sabouraud's dextrose agar in tubes, and incubated for 4 weeks at 26°C. Three colonies are ground in 50 ml of sterile PBS. The suspension is diluted 1/100 and the 96-well microtitre plates are coated with 100 µl/well.

ii) The plates are incubated at 4°C overnight.

iii) The plates are washed with PBS containing Tween 20 (0.5 ml/litre) (PBS-T) three times for 3 minutes each.

iv) The plates are incubated with 5% bovine serum albumin, 100 µl/well, at 23–25°C for 30 minutes, with shaking.

v) The plates are washed with PBS-T three times for 3 minutes each.

vi) The sera are serially diluted using twofold dilution in duplicate in PBS-T, starting with a 1/50 dilution and incubated for 30 minutes at 23–25°C.

vii) The plates are washed with PBS-T three times for 3 minutes each.

viii) Peroxidase-labelled goat anti-horse IgG is diluted 1/800 and used at 100 µl/well, with incubation for 30 minutes at 23–25°C, with shaking.

ix) The plates are washed with PBS-T three times for 3 minutes each.

x) Finally, 100 µl/well of hydrogen peroxide and ABTS (2,2'-Azino-di-[3-ethyl-benzthiazoline]-6-sulphonic acid) in a citric acid buffer, pH 4, is added.

xi) The plates are read at 60 minutes in a spectrophotometer at wavelength 405 nm.

xii) The absorbance values are obtained twice from each serum dilution and the standard deviation and average percentage of the absorbance values of the different serum samples are considered in the interpretation of the results.

c) Passive haemagglutination test

The following procedure is as described by Gabal & Khalifa (9).

i) The organism is propagated for 8 weeks on Sabouraud's dextrose agar. Five colonies are scraped, ground, suspended in 200 ml of saline, and sonicated for 20 minutes. The remaining mycelial elements are filtered out, and the filtrate is diluted 1/160.

ii) Normal sheep red blood cells (RBCs) are washed, treated with tannic acid, washed, and re-suspended as a 1% cell suspension.

iii) Different dilutions of the antigen preparation are mixed with the tanned RBCs and incubated in a water bath at 37°C for 1 hour. The RBCs are collected by centrifugation, washed three times in buffered saline and re-suspended to make a 1% cell suspension.

iv) Test sera are inactivated by heating at 56°C for 30 minutes and then absorbed with an equal volume of washed RBCs.

v) Dilutions of serum (0.5 ml) are placed in test tubes with 0.05 ml of antigen-coated tanned RBCs.

vi) Agglutination is recorded at 2 and 12 hours.

vii) Agglutination is detected when the RBCs form a uniform mat on the bottom of the tube. A negative test is indicated by the formation of a 'button' of RBCs at the bottom of the tube.

d) Skin hypersensitivity tests

Two skin hypersensitivity tests for the diagnosis of epizootic lymphangitis have been described. The first test was described by Gabal & Khalifa and adapted by Armeni *et al.* (5, 9).

i) A pure culture of *H. farcimonsum* is propagated for 8 weeks on Sabouraud's dextrose agar containing 2.5% glycerol. Five colonies are scraped, ground, suspended in 200 ml of saline, undergo five freeze–thaw cycles and are sonicated at an amplitude of 40° for 20 minutes. The remaining mycelial elements are removed by centrifugation at 1006 ***g*** at 4°C for 11 minutes. Sterility of the preparation is verified by incubating an aliquot on Sabouraud's dextrose agar at 26°C for 4 weeks.

ii) Animals are inoculated intradermally with 0.1 ml containing 0.2 mg/ml protein in the neck.

iii) The inoculation site is examined for the presence of a local indurated and elevated area at 24–48 hours post-injection. An increase in skin thickness of > 4 mm is considered to be positive.

Alternatively, a 'histofarcin' test has been described by Soliman *et al*. (19).

i) The mycelial form of the organism is grown on polystyrene discs floating on 250 ml of PPLO media containing 2% glucose and 2.5% glycerine at 23–25°C for 4 months.

ii) The fungus-free culture filtrate is mixed with acetone (2/1) and held at 4°C for 48 hours.

iii) The supernatant is decanted and the acetone is allowed to evaporate.

iv) Precipitate is suspended to 1/10 original volume in distilled water.

v) Animals are inoculated intradermally with 0.1 ml of antigen in the neck.

vi) The inoculation site is examined for the presence of a local indurated and elevated area at 24, 48 and 72 hours post-injection.

C. REQUIREMENTS FOR VACCINES AND DIAGNOSTIC BIOLOGICALS

Control of the disease is usually through elimination of the infection. This is achieved by culling infected horses and application of strict hygiene practices to prevent spread of the organism. There are published reports on the use of killed (2) and live attenuated vaccines (23) in areas where epizootic lymphangitis is endemic, apparently with relatively good results.

The antigens used for skin hypersensitivity testing are described in the previous section.

REFERENCES

1. AL-ANI F.K. (1999). Epizootic lymphangitis in horses: a review of the literature. *Rev. sci. tech. Off. int. Epiz.*, **18**, 691–699.

2. AL-ANI F.K., ALI A.H. & BANNA H.B. (1998). *Histoplasma farciminosum* infection of horses in Iraq. *Veterinarski Arhiv*., 68, 101–107.

3. AMENI G. (2005). Preliminary trial on the reproducibility of epizootic lymphangitis through experimental infection of two horses. Short Communication. *Veterinary J.,* (In Press).

4. AMENI G. & TEREFE W. (2004). A cross-sectional study of epizootic lymphangitis in cart-mules in western Ethiopia. *Preventive Vet. Med*., **66**, 93–99.

5. AMENI G. TEREFE W. & HAILU A. (2006). Histofarcin test for the diagnosis of epizootic lymphanigitis in Ethiopia: development, optimisation and validation in the field. *Veterinary J.*, **171**, 358–362.

6. CHANDLER F.W., KAPLAN W. & AJELLO L. (1980). Histopathology of Mycotic Diseases. Year Book Medical Publishers, Chicago, USA, 70–72 and 216–217.

7. FAWI M.T. (1969). Fluorescent antibody test for the serodiagnosis of *Histoplasma farciminosum* infections in Equidae. *Br. Vet. J.*, **125,** 231–234.

8. GABAL M.A., BANA A.A. & GENDI M.E. (1983). The fluorescent antibody technique for diagnosis of equine histoplasmosis (epizootic lymphangitis). *Zentralbl. Veterinarmed. [B]*, **30,** 283–287.

9. GABAL M.A. & KHALIFA K. (1983). Study on the immune response and serological diagnosis of equine histoplasmosis (epizootic lymphangitis). *Zentralbl. Veterinarmed. [B]*, **30,** 317–321.

10. GABAL M.A. & MOHAMMED K.A. (1985). Use of enzyme-linked immunosorbent assay for the diagnosis of equine *Histoplasma farciminosi* (epizootic lymphangitis). *Mycopathologia*, **91,** 35–37.

11. GUERIN C., ABEBE S. & TOUATI F. (1992). Epizootic lymphangitis in horses in Ethiopia. *J. Mycol. Med.*, **2**, 1–5.

12. HERVE V., LE GALL-CAMPODONICO P., BLANC F., IMPROVISI, L., DUPONT, B, MATHIOT C. & LE GALL F. (1994). Histoplasmose a *Histoplasma farciminosum* chez un cheval africain. *J. Mycologie Med.*, **4**, 54.

13. JUNGERMAN P.F. & SCHWARTZMAN R.M. (1972). Veterinary Medical Mycology. Lea & Febiger. Philadelphia, USA.

14. KASUGA T., TAYLOR T.W. & WHITE T.J. (1999). Phylogenetic relationships of varieties and geographical groups of the human pathogenic fungus *Histoplasma capsulatum* darling. *J. Clin. Microbiol*., **37**, 653–663.

15. LEHMANN P.F. HOWARD D.H. & MILLER J.D. (1996). Veterinary Mycology. Springer-Verlag, Berlin, Germany, **96**, 251–263.

16. ROBINSON W.F. & MAXIE M.G. (1993). The cardiovascular system. *In:* Pathology of Domestic Animals*,* Vol. 3. Academic Press, New York, USA, 82–84.

17. SELIM S.A., SOLIMAN R., OSMAN K., PADHYE A.A. & AJELLO L. (1985). Studies on histoplasmosis farciminosi (epizootic lymphangitis) in Egypt. Isolation of *Histoplasma farciminosum* from cases of histoplasmosis farciminosi in horses and its morphological characteristics. *Eur. J. Epidemiol*., **1,** 84–89.

18. SINGH T. (1965). Studies on epizootic lymphangitis. I. Modes of infection and transmission of equine histoplasmosis (epizootic lymphangitis). *Indian J. Vet. Sci.*, **35,** 102–110.

19. SOLIMAN R., SAAD M.A. & REFAI M. (1985). Studies on histoplasmosis farciminosii (epizootic lymphangitis) in Egypt. III. Application of a skin test ('histofarcin') in the diagnosis of epizootic lymphangitis in horses. *Mykosen,* **28,** 457–461.

20. STANDARD P.G. & KAUFMAN L. (1976). Specific immunological test for the rapid identification of members of the genus *Histoplasma*. *J. Clin. Microbiol*., **3**, 191–199.

21. UEDA Y., SANO A. TAMURA M., INOMATA T., KAMEI K., YOKOYAMA K., KISHI F., ITO J., Y., MIYAJI M. & NISHIMURA K. (2003). Diagnosis of histoplasmosis by detection of the internal transcribed spacer region of fungal rRNA gene from a paraffin-embedded skin sample from a dog in Japan. *Vet. Microbiol.,* **94**, 219–224.

22. WEEKS R.J., PADHYE A.A. & AJELLO L. (1985). *Histoplasma capsulatum* variety *farciminosum:* a new combination for *Histoplasma farciminosum*. *Mycologia*, **77**, 964–970.

23. ZHANG W.T., WANG Z.R., LIU Y.P., ZHANG D.L., LIANG P.Q., FANG Y.Z., HUANG Y.J. & GAO S.D. (1986). Attenuated vaccine against epizootic lymphangitis in horses. *Chinese J. Vet. Sci. Tech*., **7**, 3–5.

*

* *