CHAPTER 2.4.9.

# DERMATOPHILOSIS


## SUMMARY

*Dermatophilosis* (*also known as streptothrichosis*) *is an exudative, pustular dermatitis that mainly affects cattle, sheep and horses, but also goats, dogs and cats, many wild mammals, reptiles and, occasionally, humans. The severe disease in ruminants is promoted by immunomodulatory effects induced by infestation with the tick,* Amblyomma variegatum*.*

*Laboratory diagnosis of dermatophilosis depends on the demonstration of the bacterium* Dermatophilus congolensis *in material from the skin or other organs. Sites other than the skin are rarely affected.*

***Identification of the agent:*** Dermatophilus congolensis *normally affects the epidermis, causing the formation of scabs. It may be demonstrated in smears made from scabs emulsified or softened in water or in impression smears from the base of freshly removed adherent scabs. The organism is Gram positive, but its morphology is more readily appreciated in smears stained with Giemsa. In stained smears, the organism is seen as branching filaments containing multiple rows of cocci. This characteristic appearance is diagnostic. In wet or secondarily infected scabs, only free cocci may be present, so that staining by immunofluorescence is necessary.* Dermatophilus congolensis *is demonstrated in histopathological sections by Giemsa staining or by immunofluorescence.* Dermatophilus cheloniae *may be found in crocodiles, chelonids and cobras.*

*Isolation of* D. congolensis *from freshly removed scabs is straightforward, but the organism is readily overgrown by other bacteria. When cultured from contaminated sites, special techniques involving filtration, chemotaxis, or selective media are necessary.*

*Demonstration and identification of* D. congolensis *by immunofluorescence is a reliable and very sensitive method of diagnosis, but requires that laboratories make their own diagnostic antisera as these are not available commercially. Although antigenic cross-reaction with* Nocardia *spp. has been reported, this is likely to give only weak fluorescence. Ideally, a monoclonal antibody specific to* D. congolensis *should be used. Polymerase chain reaction* (*PCR*)*-based characterisation has also been developed.*

***Serological tests:*** *A variety of serological tests has been used in studies of the epidemiology and pathogenesis of dermatophilosis. Antibody can be demonstrated in all but fetal blood in healthy ruminants, but the elevated levels associated with clinical infection can be used to identify animals that have been infected with the disease.*

***Requirements for vaccines and diagnostic biologicals:*** *Despite the identification of several virulence factors, no vaccines are available currently.*


## A. INTRODUCTION

Dermatophilosis (also known as streptothrichosis, or in sheep as 'lumpy wool disease') is an exudative, pustular dermatitis that affects mainly cattle, sheep and horses, but also goats, dogs and cats, many wild mammals, reptiles and, occasionally, humans. Dermatophilosis is caused by the bacterium *Dermatophilus congolensis,* the type species of the genus *Dermatophilus,* which is a member of the order Actinomycetales. Dermatophilosis is the commonest skin disease of crocodiles in Australia and has an impact on farming of this species (Buenviaje *et al*., 1998). It is provoked by *Dermatophilus cheloniae,* which has also been isolated from chelonids and cobra.

There is considerable variation in the clinical appearance of the disease and in the affected areas of the body. Typically, infection gives rise to the formation of dense scabs on the skin, but in certain areas, such as the

perineum in ruminants and the pastern in horses, moist lesions with thickened, folded skin may occur. In such lesions, relatively thin scabs are found. Where lesions are exposed to prolonged wetting, with or without secondary infection, exudative lesions may be present.

Scabs characteristically comprise alternating layers of parakeratotic keratinocytes invaded with branching bacterial filaments and infiltrates of neutrophils in serous exudate. This gives a palisaded appearence in stained sections. *D. congolensis* filaments remain confined to the epidermis and very rarely infect the dermis.

Extensive acute dermatophilosis cannot be reproduced easily in experimental conditions. *Dermatophilus congolensis* itself is not highly pathogenic, and a combination of factors is necessary for the development of clinical lesions. Malnutrition, intense rainfalls and mechanical traumas have been incriminated as favouring the disease. However, where dermatophilosis has an important economic impact in West and Central Africa as well as on some Caribbean islands, the major risk factor is the infestation by *Amblyomma variegatum* ticks. Severe disease may be promoted by immunomodulatory effects of saliva secreted during tick bite (Ambrose *et al*., 1999), but the fine underlying mechanisms are not understood. Susceptibility to dermatophilosis is also greatly influenced by the genetic background of ruminant breeds, animals from temperate regions and especially dairy cattle being extremely susceptible when introduced in regions at risk.

# B. DIAGNOSTIC TECHNIQUES

## 1. Identification of the agent

### 1.1. Microscopic observation

Diagnosis can usually be made by demonstrating the causal organism in scabs from the lesions or in exudate beneath the scabs. The organism has a characteristic microscopic appearance – its septate, branching filaments become longitudinally, as well as transversely, divided to form ribbons of spherical or ovoid cocci, each about 0.5 µm in diameter, in multiple rows. This appearance is diagnostic, provided that cocci are found in transverse rows of four or more, and is readily seen in stained preparations. However, the distinctive formation can be disrupted during the preparation of smears for examination if the material is spread too vigorously over the slide.

Impression smears may be made from the moist, concave under surfaces of freshly removed scabs. Otherwise, thick smears are best prepared from scabs emulsified in sterile distilled water. Alternatively, scabs can be soaked overnight in sterile water or saline to sufficiently moisten them so that the under surface of the scab can be used to make effective impression smears by firmly pressing this surface on to a microscope slide. Smears are then air-dried, fixed by heating or immersion in methanol for 5 minutes, and stained. The organism stains well in dilute carbol fuchsin or methylene blue stain, but Gram's stain or, preferably, a 1 in 10 dilution of Giemsa stain for 30 minutes, gives better differentiation in thick smears, the darkly stained *D. congolensis* contrasting with the paler or pink counterstained background of keratinocytes and neutrophils. Gram staining does not give as good results as Giemsa because it may overstain the background and does not clearly show the characteristic laddering of the coccoid forms.

Wet or secondarily infected scabs often contain few, if any, intact filaments, and the organism may not stain Gram positive. In such material, the cocci cannot be differentiated morphologically from other coccoid bacteria, so that staining by immunofluorescence is required. However, specific antisera for immunofluorescence are not commercially available. Thin, heat-fixed smears are used. In difficult cases and when infection of organs other than the skin is suspected, histopathological examination of biopsy or necropsy material is advisable. Giemsa stain or immunofluorescence is used.

The characteristic appearance of the lesions and of the organism in smears from typical bovine dermatophilosis makes culture unnecessary in most cases. However, in the rare cases in which a Giemsa-stained smear does not give a definitive result, confirmation of the diagnosis may be made by isolating the bacterium. Cultures are made on blood agar and incubated at 37°C. Growth is accelerated under microaerophilic conditions; rough, usually haemolytic, greyish-yellow colonies, about 1 mm in diameter, are seen pitting the medium after 24 hours. Incubation in air produces similar pinpoint colonies at 24 hours that grow to about 1 mm at 48 hours. The rough colonies are formed by the branching filaments, but continued growth in air stimulates the production of the cocci, which are commonly yellow in colour. Colonies take on a smooth, often yellowish, appearance. The cocci are normally vigorously motile when taken from young cultures. The colonies must be differentiated from *Nocardia* spp. and *Streptomyces* spp., neither of which produces filaments that break up into multiple rows of motile cocci.

### 1.2. Culture

For isolation, material can be streaked out directly from the moist under surfaces of freshly removed, uncontaminated scabs or from scab emulsions, but the relatively slow-growing *D. congolensis* is readily overgrown by other bacteria. Special isolation techniques are thus required for contaminated specimens. In most specimens, free cocci, whether motile or not, will be present in emulsions of the material. Filtration of the emulsion through a 0.45 µm membrane filter is usually sufficient to reduce or eliminate contaminants and permits isolation from the filtrate, as described above. Alternatively, Haalstra's method (Haalstra, 1965) may be used. Small pieces of scab are placed in a bijou bottle containing 1 ml of sterile distilled water and allowed to stand at room temperature for 3–4 hours. The open bottle is then placed for 15 minutes in a candle jar. Samples of the surface liquid are removed with a bacteriological loop and cultured. The method depends on the release from the scab of the motile cocci of *D. congolensis* and their chemotropic attraction towards the carbon-dioxide-rich atmosphere of the candle jar. A selective medium consisting of 1000 units/ml of polymyxin B in blood agar can also be used, and is effective when the contaminants are sensitive to this antibiotic.

### 1.3. Immunological methods

Immunofluorescence staining of smears or tissues is the most reliable and sensitive immunological technique for the identification of *D. congolensis* antigens and for the diagnosis of dermatophilosis. Polyclonal antibody obtained from animals inoculated with *D. congolensis* can be easily prepared using standard methods, but there is a risk of possible cross-reaction with some strains of *Nocardia* spp. Monoclonal antibody to species-specific antigen (How *et al*., 1988) is preferable. However, monoclonal antibodies have not been widely distributed and validated by interlaboratory tests. Thin, heat-fixed smears of scab emulsions, or impression smears, are stained. Known positive and negative control specimens should always be included.

### 1.4. Nucleic acid recognition methods

In absence of extensive genome sequence information, randomly amplified polymorphic DNA methods (RAPD) as well as pulsed-field gel electrophoresis (PGFE) have been used and proved to be useful for the molecular typing of *D. congolensis* (Larrasa *et al*., 2004). An alkaline ceramidase gene was cloned from RADP fragments, and a polymerase chain reaction (PCR) using primers designed from the nucleotide sequence from this gene gave an amplification product with *D. congolensis* DNA. No amplification product was observed with *M bovis*, *C. propinquum* and *D. cheloniae*, suggesting a possible use in diagnosis or detection of *D. congolensis* (Garcia-Sanchez *et al*., 2004). Alternatively, 16S rDNA sequence obtained after amplification can be used to confirm the presence of *D. congolensis*.

## 2. Serological tests

Clinical diagnosis is best performed using the methods described above rather than serological methods. Antibody can be demonstrated in all but fetal blood in healthy ruminants, but levels are raised following clinical infection. The enzyme-linked immunosorbent assay (ELISA) has proved to be a sensitive and convenient assay technique, and elevation of titres above baseline values can be used in epidemiological studies to identify animals that have had the disease (Martinez *et al*., 1993). The test being based on a crude antigen, cross-reactivity with other bacteria can occur as in immunofluorescence. At present, the ELISA remains as a research and investigation method. Serology, either using ELISA or older methods such as haemagglutination and counter-immunelectro-phoresis, is not used for routine diagnosis of dermatophilosis where direct detection of the bacterium is easy.

# C. REQUIREMENTS FOR VACCINES AND DIAGNOSTIC BIOLOGICALS

*Dermatophilus congolensis* produces virulence factors such as haemolysin, phospholipases, ceramidases and proteolytic enzymes, which may be used to penetrate the epidermis barrier and interact with the inflammatory response of the host. These virulence factors are considered candidate antigens for vaccines. Research on vaccines for prevention of dermatophilosis has been conducted (How *et al*., 1990; Sutherland & Robertson, 1988); however, no vaccine is currently available. Research in this domain is hampered by the inability to reproduce the disease experimentally and the poor understanding of skin immunity. Much emphasis has therefore been put on tick control and identification of genetic markers of resistance or susceptibility with promising results in cattle (Maillard *et al*., 2003).

## REFERENCES

AMBROSE N., LLOYD D.H. & MAILLARD J.C. (1999). Immune responses to *Dermatophilus congolensis* infections. *Parasitol. Today*, **15**, 295–300.

BUENVIAJE G.N., LADDS P.W. & MARTIN Y. (1998). Pathology of skin disease in crocodiles. *Aust. Vet. J*., **76**, 357–363.

GARCIA-SANCHEZ A., CERRATO C., LARRASA J., AMBROSE C.N., PARRA A., ALONSO J.M., HERMOSO-DE-MENDOZA M., REY J.M. & HERMOSO-DE-MENDOZA J. (2004). Identification of an alkaline ceramidase gene from *Dermatophilus congolensis*. *Vet. Microbiol*., **99**, 67–74.

HAALSTRA R.T. (1965). Isolation of *Dermatophilus congolensis* from skin lesions in the diagnosis of streptothricosis. *Vet. Rec.*, **77**, 824–825.

HOW S.J., LLOYD D.H. & LIDA J. (1988). Use of a monoclonal antibody in the diagnosis of infection by *Dermatophilus congolensis*. *Res. Vet. Sci.*, **45**, 416–417.

HOW S.J., LLOYD D.H. & SANDERS A.B. (1990). Vaccination against *Dermatophilus congolensis* infection in ruminants: prospects for control. *In:* Advances in Veterinary Dermatology, Volume 1, Von Tscharner C. & R.E.W. Halliwell, eds. Bailliere Tindall, London UK.

LARRASA J., GARCIA-SANCHEZ A., AMBROSE C.N., PARRA A., ALONSO J.M., REY J.M., HERMOSO-DE-MENDOZA M. & HERMOSO-DE-MENDOZA J. (2004). Evaluation of randomly amplified polymorphic DNA and pulsed field gel electrophoresis techniques for molecular typing of *Dermatophilus congolensis*. *FEMS Microbiol. Lett*., **240**, 87–97.

MAILLARD J.C., BERTHIER D., CHANTAL I., THEVENON, S., SIDIBE I., STACHURSKI F., BELEMSAGA D., RAZAFINDRAIBE H & ELSEN J.M. (2003). Selection assisted by a BoLA-DR/DQ haplotype against susceptibility to bovine dermatophilosis. *Genet. Sel. Evol*., **35**, 193–200.

MARTINEZ D., AUMONT G., MOUTOUSSAMY M., GABRIEL D., TATAREAU J.C., BARRE N., VALLEE F. & MARI B. (1993). Epidemiological studies on dermatophilosis in the Caribbean. *Rev. Elev. Med. Vet. Pays Trop.*, **46**, 323–327.

SUTHERLAND S.S. & ROBERTSON G.M. (1988). Vaccination against ovine dermatophilosis. *Vet. Microbiol.,* **18**, 285–288.

*
* *