LABORATORY LIMITS ON DERMATOPHYTE DIAGNOSIS IN RABBITS WITH CLINICAL LESIONS

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ABSTRACT

Dermatophyte infection or ringworm is a superficial cutaneous infection with one or more of the fungal species of the keratinophilic genera *Microsporum*, *Trichophyton*, or *Epidermophyton* and is a zoonosis with a great impact on Public Health. Dermatophytes were identified from rabbit sample cultures submitted to mycological examination in the Laboratory of Microbiology of the University of Trás-os-Montes e Alto Douro, Vila Real, Portugal. All samples were collected from suspected clinical cases. Dermatophytes were cultured from 4 of the 55 specimens (7.3%). The dermatophytes isolated were *Trichophyton mentagrophytes* var. *mentagrophytes* (1/4) and *Microsporum gypseum* (3/4). Microscopic examination was negative in all specimens. In this work, *Scopulariopsis* spp., a contaminant mould, was identified in 13 specimens (23.6%). The proportion of positive samples in relation to the number of samples examined from cases suspected was very low. As all samples were collected from rabbits with compatible signs, we presume that the low prevalence of isolation was due to laboratory constraints on dermatophytes diagnosis.

Key words: Dermatophytes, Rabbits, Diagnosis, Isolation.

INTRODUCTION

Dermatophytosis is a superficial cutaneous infection with one or more of the fungal species in the keratinophilic genera *Microsporum*, *Trichophyton*, or *Epidermophyton* (Kane *et al*., 1997; Hungerford *et al*., 1998). In rabbits, dermatophytosis most often occurs in young, newly weaned animals. The most common fungal identified in rabbits with dermatophytosis is *T. mentagrophytes* (Hagen and Gorham, 1972; Szili and Köhalmi, 1980; Van Cutsem *et al*., 1985; Torres-Rodriguez *et al*., 1992; Cabañes *et al*., 1997; Van Rooij *et al*., 2006). Young or immunocompromised rabbits are thought to be most susceptible. Clinically, dermatophytes infect the epidermis and annexe structures, including hair follicles and shafts. Often results in localized lesions most commonly on the face usually on or around the head, and cause pruritus, patchy alopecia, erythema, and crusting (Kane *et al*., 1997). Natural infection of laboratory rabbits may result in histopathologic changes which could confound studies involving the skin. Focal alopecia, with erythema, crusts and scabs, is seen around the eyes, nose and ears, with secondary lesions appearing on the feet. The disease is usually self-limiting. There are several constraints on laboratorial diagnosis of dermatophytes infection. The diagnosis must be made based on isolation of the organism from affected tissues and visualization of tissue invasion by organisms with compatible morphology. However, it is very difficult to culture these agents (Kane *et al*., 1997).

MATERIALS AND METHODS

Animals and samples

Samples were taken from 55 adult (1-2 years) female rabbits suspected of having dermatophytosis between a period from June and August 2007, in industrial rabbitry in the North east of Portugal. Previously, the sampling zone was disinfected with alcohol at 70º. Samples (hair and scrapings) were collected with forceps or scalpel just behind the extending margin in the infected area. Hair was plucked with the root end and sent to laboratory of Microbiology, department of Veterinary Sciences in the University of Trás-os-Montes e Alto Douro in up to 24 hours. Hair and scrapings were mounted for direct examination in 40% KOH and heated for 60 seconds and examined under x 400 magnification for fungal structures.

Culture

The inoculation was made in Dermatophyte Test Medium (DTM; Merck™), Mycobiotic agar medium, Sabouraud Dextrose agar medium (Oxoid™) supplement with cycloheximide (Sigma™) to reduce the growth of non-dermatophytic fungi. The material was incubated at a temperature of 25ºC and readings were taken daily, for a period of four weeks. Each mould was subcultured in Sabouraud dextrose agar medium for sample maintenance.

Identification

Colonies were subject to lactophenol (cotton-blue) staining and urease test. The fungi were identified by their macro and microscopic morphological characteristics based in the identification key of the Veterinary Mycology Laboratory Manual (Hungerford *et al*., 1998) and the Laboratory Handbook of dermatophytes (Kane *et al*., 1997).

RESULTS AND DISCUSSION

Thirteen cultures from the 55 rabbits does suspected of having dermatophytosis were macroscopically compatible with this kind of affection. However, dermatophytes were cultured and identified only from 4 of the 55 specimens submitted (7.3%). Two dermatophytes species were isolated: *Trichophyton mentagrophytes* var. *mentagrophytes*, urease positive, in one female and *Microsporum gypseum* in the 3 other females. In other studies *Trichophyton mentagrophytes* was the most frequent species isolated from rabbits (Hagen and Gorham, 1972; Szili and Köhalmi, 1980; Van Cutsem *et al*., 1985; Torres-Rodriguez *et al*., 1992; Cabañes *et al*., 1997; Van Rooij *et al*., 2006). Microscopic examination was negative in all samples. In this work, *Scopulariopsis* spp., a contaminant mould, was identified in 13 cultured samples (23.6%). The proportion of positive samples to dermatophytes, in relation to the number of samples examined from cases suspected was very low (4/55; 7.3%). However the value is in agreement with previous studies in other species such as in dogs (Pepin and Austwick, 1968; Cabañes *et al*., 1997). Laboratory constraints in dermatophytosis diagnosis in different species are well document in the literature (Cabañes *et al*., 1997); however there are few studies about the relative low prevalence of dermatophytes in rabbits with suspected lesions. This low rate of isolation is probably due to the laboratory limits in general more than associated with clinical false positives. In the diagnosis of dermatophytosis there is a lack of correlation between etiologic agents and clinical disease manifestations (Sibbald, 1997). This is due to problems with growth of dermatophytes in culture media, contamination of culture media, limits in identification of cultured fungi; sampling collection, etc. It has been reported that the number of positive cultures is related with the kind of selection of samples made by the practitioners (Cabañes *et al*., 1997). In this study all samples were collected and processed by the authors so, we believe that this was not a factor that can influence the results. The high number of negative cultures may be explained by the culture media used that permit the growth of contaminant moulds. We used cycloheximide in the culture media, but the rate of non-dermatophytic fungi that growth was high. DTM™ has a good sensitivity, but has the disadvantage of

not allowing visualization of colony reverse pigmentation, a character often important in identification. As all samples were collected from rabbits with compatible signs we presume that the low prevalence of isolation was due to laboratory limits on dermatophytes diagnosis. Zoonotic potential of these isolates needs to be considered in the epidemiology of human dermatophytosis in the North east of Portugal.

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REFERENCES

Cabañes F.J., Abarca M.L., Bragulat M.R. 1997. Dermatophytes isolated from domestic animals in Barcelona, Spain. *Mycopathologia, 137, 107-113*.

Hagen K.W., Gorham J.R. 1972. Dermatomycoses in four animals: chinchilla, ferret, mink and rabbit. *Vet. Med. Small Anim. Clin., 67, 43–48.*

Hungerford L.L., Campbell C.L., Smith A.R. 1998. Veterinary mycology laboratory manual. *Iowa State University Press, Ames.*

Kane J., Summerbell R., Sigler L., Krajden S., Land G. 1997. Laboratory handbook of dermatophytes. *Star Publishing Company, Korea.*

Pepin G.A., Austwick P.K. 1968. Skin disease, mycological origin. *Vet. Rec., 82, 208-221*.

Sibbald R.G. 1997. Foreword. *In: Kane J., Summerbell R., Sigler L., Krajden S., Land G. (Eds). Laboratory handbook of dermatophytes. Star Publishing Company, Korea, VII-IX.*

Szili M., Köhalmi I. 1980. Endemic *Trichophyton mentagrophytes* infection of rabbit origin. *Mykosen, 24, 412–20*.

Torres-Rodriguez J.M., Dronda M.A., Rossell J., Madrenys N. 1992. Incidence of dermatophytoses in rabbit farms in Catalonia, Spain, and its repercussion on human health. *Eur. J. Epidemiol., 8, 326-329*.

Van Rooij P., Detandt M., Nolard N. 2006. *Trichophyton mentagrophytes* of rabbit origin causing family incidence of kerion: an environmental study. *Mycoses, 49, 426-430*.

Van Cutsem J., Van Gerven F., Geerts H., Rochette F. 1985. Treatment with enilconazole spray of dermatophytosis in rabbit farms. *Mykosen, 28, 400-407*.