

## EPIDEMIOLOGICAL SURVEY OF DERMATOPHYTOSIS IN MEAT RABBITS WITH ALOPECIA IN PORTUGAL

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**ABSTRACT:** An epidemiological dermatophytosis survey was carried out in farmed rabbits with alopecia in Northern and Central Portugal. Between August and October 2008, samples from suspected clinical cases of alopecia in meat rabbits on industrial farms were collected and cultured by conventional methods. Effects on the prevalence of several variables, such as breed, age, month of sample collection, configuration of the lesions and presence of concomitant infections in the rabbitries were evaluated using a logistic regression model. The overall prevalence of dermatophytes species was 82.7% (95% confidence interval, CI: 80.1-85.3%). Two dermatophytes species were isolated: *Trichophyton mentagrophytes* (91.9%) and *Microsporum canis* (8.1%). Five variables were associated with dermatophyte isolation in univariate analysis. The multivariate logistic regression model identified configuration of lesions (odds ratio, OR=3.15; 95% CI: 1.39-7.15%) and the presence of concomitant infections on the farms (OR=2.71; 95% CI: 1.03-7.12%) as risk factors. Considering the paucity of epidemiological reports in this country, these results could make a useful contribution towards the diagnosis and prevention of rabbit dermatophytosis.

**Key Words:** risk factors, prevalence, rabbits, dermatophytosis.

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## 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). These infections are a public health concern because of their transmissibility from human to human or from animal to human (Kim and Jang 1999; Van Rooij *et al.*, 2006; Chermette *et al.*, 2008). In rabbits, dermatophytosis most often occurs in kits. The most common fungal identified in rabbits with dermatophytosis is *T. mentagrophytes* (Cabañes *et al.*, 1997; Van Rooij *et al.*, 2006). Kits or immunocompromised rabbits are thought to be most susceptible. Clinically, dermatophytes infect the epidermis and adjacent structures, including hair follicles and shafts. This often results in localised lesions, most commonly on the face, usually on or around the head, and causes pruritus, patchy alopecia, erythema, and crusting. The disease is usually self-limiting (Kane *et al.*, 1997).

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 visualisation of tissue

invasion by organisms with compatible morphology. However, it is very difficult to culture these agents (Kane *et al.*, 1997; Uchida *et al.*, 2009).

In Portugal, few surveys have been organised to estimate dermatophytosis prevalence in animals (Bernardo *et al.*, 1989; Pinto, 1993; Bernardo *et al.*, 2005) and to the best of our knowledge the prevalence of dermatophytosis in meat rabbits has not been investigated in this country. The present study was initiated in response to concerns about the prevalence of the disease in meat rabbits in Northern and Central Portugal in order to discern its economic impact and prioritise the allocation of disease control resources.

## MATERIAL AND METHODS

### *Study design*

A cross-sectional study was carried out between August and October 2008. Sample size (208) was calculated using an expected prevalence of 10% and a confidence interval of 95%. Animals sampled were proportionally allocated according to the number of rabbit farms. Samples were taken from 208 meat rabbits with alopecia and suspected of having dermatophytosis, between August and October 2008, on 22 industrial rabbit farms raising animals for meat production in Northern and Central Portugal.

A carefully structured questionnaire was used to collect data for each animal. The effects on prevalence of several variables such as sex, breed, age, month of sample collection, configuration of the lesions and presence of concomitant infections in the farms were evaluated. During the sampling procedures, a clinical examination was performed with a detailed inspection of all skin surfaces (especially the ears), head and body hairs with a search for signs including erythema, vesicles or pustules, erosion, scaling and hyperkeratosis from ringworm infection. The sampling zone was disinfected beforehand with 70° alcohol. Samples (fur and scrapings) were collected with forceps or scalpel just behind the extending margin in the infected area. Hair with the root end was plucked and sent to laboratory of Medical Microbiology, Department of Veterinary Sciences at the University of Trás-os-Montes and Alto Douro, Portugal.

### *Culture and identification*

Inoculation was performed in Dermatophyte Test Medium (DTM, Merck), Mycobiotic agar medium, Sabouraud Dextrose agar medium (Oxoid) supplemented with cycloheximide (Sigma) to reduce the growth of non-dermatophytic fungi. The material was incubated at a temperature of 25 and 37°C and readings were taken daily for a period of 4 wk. Each mould was subcultured in Sabouraud dextrose agar medium for sample maintenance.

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

### *Statistical analysis*

The prevalence of dermatophytes species recovered from the sample was compared using chi-squared analysis with significance level defined at 95.0% confidence level ( $P < 0.05$ ). Analyses were carried out using SPSS version 15 software for Windows. Confidence limits for the proportions were established by exact binomial test with a 95% confidence interval (CI).

Univariate analysis was carried out using  $\chi^2$  test analysis. All variables from the questionnaire which showed  $P < 0.10$  at the 95% CI in the univariate analysis were subsequently introduced into a multivariate model (Hosmer and Lemeshow 1989). The logistic regression coefficients ( $\beta$ ) and their standard errors (S.E.  $\beta$ ) obtained from the chosen model were used to calculate the adjusted odds ratios (OR) and their corresponding 95% CI. A Wald test (Wald's  $P$ -value) was used to test the statistical significance of each coefficient ( $b$ ) in the model. For logistic regression purposes, the status of each animal (positive/negative) was used as a dependent variable in order to identify any risk factors associated with the prevalence of the disease.

## RESULTS

A total of 208 animals from 22 industrial rabbit farms were examined. All animals presented dermatological clinical signs such as scales, folliculitis, crusts and alopecic areas with various degrees of inflammation. Skin lesions were mainly observed on the head (100%) and ears. Lesions located in the tail and inguinal area were less frequent (1.3%).

Dermatophytes were cultured from 172 of the 208 specimens submitted (82.7%, 95% confidence interval [CI] 80.1 to 85.3%). Dermatophytosis positive animals (one or more) were detected in all industrial rabbit farms with suspected animals. Two dermatophytes species were isolated: urease positive *Trichophyton mentagrophytes* var. *mentagrophytes* was the most commonly isolated species (91.9%) and *Microsporum canis* (8.1%) was the second most isolated. Prevalence values among males and females were 86.2 and 79.8%, respectively. No gender-related differences in prevalence were found. Regarding age groups, the lowest prevalence value (77.8 %) was

**Table 1:** Factors associated with a positive result in culture. Univariate analysis ( $P < 0.10$ ).

Variables	No. of animals	% positive	$P$ -value	OR <sup>1</sup>	95% IC <sup>2</sup> (OR)
Breed				1	
HY (hyplus; hycat; hyla)	105	78.1			
Other breeds	103	87.4	0.080	1.94	0.92-4.1
Age					
More than 2 mo	117	77.8		1	
Less than 2 mo	91	89.0	0.037	2.31	1.1-5.1
Month of sample collection					
August	51	72.5		1	
September	101	81.2	0.029	4.09	1.16-14.51
October	56	94.6	0.005	6.69	1.79-24.92
Configuration of lesions					
Multifocal	137	89.1		1	
Single	71	70.4	0.001	0.29	0.14-0.61
Presence of other infections in the rabbit farms					
No	119	76.5		1	
Yes	89	91.0	0.008	3.12	1.34-7.22

<sup>1</sup>OR: odds ratio. <sup>2</sup> Confidence interval.

found in rabbits over 2 mo of age, and the highest (89.0%) in animals less than 2 mo of age –a difference which was statistically significant ( $P<0.05$ ).

Dermatophytes were more frequently isolated in animals with dark hair (85.1%), but no significant differences were detected in the hair colour distribution. Herds of less than 1000 females (83.7%) had higher positivity. There were no significant differences in prevalence among the number of females in the rabbit farms.

Five variables were associated ( $P<0.10$ ) with a positive culture for dermatophytes in the univariable analysis: breed, age, month of sample collection, configuration of lesions and the presence of concomitant infections in the industrial farms were associated with dermatophytosis in culture (Table 1).

These factors were analysed in a logistic regression model to determine their relative contribution to isolation, while adjusting for their effects (Table 2). A 3-fold increased risk of isolation was shown for rabbits from multifocal lesions compared with single lesions ( $P=0.006$ ). Rabbits on industrial farms with other infection problems had higher odds of being positive for culture, compared to rabbits living on farms without concomitant infections ( $P=0.043$ ).

## DISCUSSION

The risk of human contamination with *T. mentagrophytes* spread by rodents and lagomorphs has been proven and associated with the presence of infected animals in living and work areas (Van Rooij *et al.*, 2006). An understanding of ringworm epidemiology in rabbits is very important to reduce the spread of zoophilic fungal infections to humans. Around the world a considerable increase has been observed in human dermatophytosis transmitted by rabbits in recent years (Yukie *et al.*, 2004; Gallo *et al.*, 2005; Van Rooij *et al.*, 2006).

The clinical isolates in our study were identified as *T. mentagrophytes* var. *mentagrophytes* and *M. canis* on phenotypic examination. Specific studies of the prevalence rate of dermatophytosis in rabbits were scarce (Lopez-Martínez *et al.*, 1984; Torres-Rodríguez *et al.*, 1992; Vangeel *et al.*, 2000). However, in other studies the zoophilic dermatophyte *Trichophyton mentagrophytes* was also the most frequent species isolated from rodents, small mammals and lagomorphs (Cabañes *et al.*, 1997; Gallo *et al.*, 2005; Van Rooij *et al.*, 2006). *M. canis* is usually correlated with domestic environments and normally associated with humans, dogs and cats (Gallo *et al.*, 2005). In some studies, dogs and cats were implicated in *M. canis* contamination of

**Table 2:** Results of a multivariate analysis of studied factors in relation to laboratorial diagnosis of dermatophytosis<sup>1</sup>.

	$\beta$	Measures of variation	P-value	OR <sup>2</sup>	95% CI
Configuration of lesions					
Single	-	-	-	1	
Multifocal	1.147	0.419	0.006	3.15	1.39-7.15
Presence of other infections in the rabbit farms					
No	-	-	-	1	
Yes	0.997	0.493	0.043	2.71	1.03-7.12

<sup>1</sup>Overall data of the model. <sup>2</sup>OR: odds ratio.

lagomorph hair (Gallo *et al.*, 2005). Cats may be considered the prime reservoir of *M. canis*. Thus, the presence of stray cats near the farms, and whose role in the dissemination of *M. canis* has already been proven, cannot be ruled out (Moriello *et al.*, 1991; Moriello *et al.*, 1994; Gallo *et al.*, 2005). Incidence of dermatophytes in rabbit farms in Portugal is very similar to that of Spain, where dermatophytosis in rabbits are almost exclusively caused by *T. mentagrophytes* (Torres-Rodriguez *et al.*, 1992; Cabañes *et al.*, 1997). This zoophilic agent can be frequently transmitted to humans (Kim and Jang 1999; Gallo *et al.*, 2005; Van Rooij *et al.*, 2006).

Our results are in agreement with those of Cabañes *et al.* (1997) who found prevalence in rabbits of 83%.

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 a factor that influenced the results.

Previous research in rabbits has shown that dermatophytosis is more prevalent in males (Lopez-Martínez *et al.*, 1984), although in our study no significant difference was found.

Comparing our data with those of other studies, we confirm the higher frequency of dermatophytes in rabbits less than 2 mo-old (Kim and Jang 1999). Age was also considered a predisposing factor by several authors for different animals; young animals affected by dermatophytes appear more frequently (Lewis *et al.*, 1991, Cabañes *et al.*, 1997).

Given the nature of an industrial rabbit farm environment where the structure permits considerably more contact among animals, it was not surprising that the rate of fungal recovery in our population was reflective of a high-contact environment. However, no significant differences were found in the number of females on the farm. A 3-fold increased risk of isolation from multifocal lesions compared with single lesions was shown for rabbits. These findings are in agreement with previous reports where dermatophytosis was associated with multifocal lesions (Pinter and Stritof, 2004). Our results are in agreement with previous studies which report that dermatophytosis is more prevalent in the presence of other infections (Baran *et al.*, 2008; Rodwell *et al.*, 2008).

Since all samples were all from animals with clinical signs, veterinarians that work with rabbits should direct their clinical suspicion to initiate prompt investigation and treatment. Zoonotic potential of these isolates needs to be considered in the epidemiology of human dermatophytosis, in particular in handle-care workers and anyone coming into contact with these animals. These workers should be more aware of clinical and subclinical dermatophyte infections and there should be more proactive approaches with educational measures and management strategies to prevent further infections.

The data support the need for a re-evaluation of the current strategies and approaches used in the management of dermatophytosis infection in meat rabbits.

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