UNIVERSIDADE DE LISBOA

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ANALYSIS OF DERMATOPHYTE PREVALENCE AND ASSOCIATED RISK FACTORS IN RABBITS

ELISA PAIS JACKSON

ORIENTADORA:
Doutora Ana Mafalda Lourenço
TUTOR:
Dr. Joel Tsou Ferraz

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ANÁLISE DA PREVALÊNCIA DE DERMATÓFITOS EM COELHOS E FACTORES DE RISCO ASSOCIADOS

Resumo

O número crescente de pacientes imunocomprometidos, a migração intercontinental, as mudanças no estilo de vida e as alterações climáticas mudaram a forma como encaramos as doenças fúngicas.

As micoses mais comuns são as que afectam a pele, cabelo/pêlo e unhas, sendo a Dermatofitose a mais prevalente. É uma micose superficial zoonótica causada por fungos dermatófitos, também conhecida como *ringworm*, devido às lesões comuns em forma de anel. No entanto, alopécia, eritema, crostas amarelas, pápulas, escamas, hiperpigmentação, hiperqueratose, impactação folicular, dermatite miliar e lesões nodulares são outras manifestações possíveis, tanto em animais como em humanos.

Suspeita-se que animais portadores assintomáticos sejam a principal fonte de infeção humana. Além disso, a dermatofitose há muito que tem sido associada a coelhos e roedores. Os coelhos, que são frequentemente portadores assintomáticos de dermatófitos, têm ganho popularidade enquanto "primeiro animal de estimação" de crianças. Pelo mencionado, este torna-se um tópico importante a considerar.

Neste contexto, o objetivo do presente estudo foi determinar a prevalência de dermatófitos em coelhos, acompanhados em consulta no Centro Veterinário de Exóticos, Porto, Portugal, entre Setembro 2021 e Fevereiro 2022, assim como os fatores de risco possivelmente associados (estação do ano, origem, sexo, esterilização, tipo de pelo, escovado pelos donos ou não, dieta, peso, alojamento, acesso ao exterior, desparasitação interna e externa, vacinação, estado de saúde e contacto com crianças).

Com base nos resultados obtidos, foi interessante notar que os coelhos são de facto frequentes portadores assintomáticos, com 21% apresentar esporos dermatófitos no seu pelo. Os resultados sugerem também que o estado de saúde, o sexo e o alojamento podem ser os factores que mais influenciam esta prevalência.

Esta informação poderá ser útil, não só para tutores de coelhos, como também para profissionais veterinários e trabalhadores de produção de coelhos. Ao entendermos o risco presente, conseguimos tomar passos para o prevenir; e com a dermatofitose a tornar-se cada vez mais comum, a prevenção é por certo o melhor caminho a tomar.

Palavras-Chave:

Trichophyton mentagrophytes; Dermatomicose; Portador são; *Tinea*; Animal de estimação

ANALYSIS OF DERMATOPHYTE PREVALENCE AND ASSOCIATED RISK FACTORS IN RABBITS

Abstract

The increase in immunocompromised patients, intercontinental migration, alterations in human lifestyle and climatic changes have transformed our perspective on fungal illnesses.

The most common mycoses affect the skin, hair, and nails, with dermatophytosis being the most prevalent. It is a superficial mycosis that is zoonotic and caused by Dermatophyte fungi. Due to its ring-shaped lesions, it is also known as ringworm. However, hair loss, erythema, dry yellow crusts, papules, scales, hyperpigmentation, hyperkeratosis, follicular clogging, miliary dermatitis, and nodular lesions are often other manifestations that occur in both animals and humans.

It is suspected that asymptomatic carrier pets are the primary source of human infection. In addition, dermatophytosis has been long linked to lagomorphs and rodents. Given that rabbits are typically asymptomatic dermatophyte carriers and are becoming increasingly popular as children's pets, this is an issue worthy of consideration.

In light of this, the purpose of the present study was to determine the prevalence of dermatophyte carriers in pet rabbits brought to Centro Veterinário de Exóticos, Porto, Portugal, between September 2021 and February 2022.

It was interesting to note that, according to the findings of this study, rabbits are indeed common asymptomatic carriers, with 21% having dermatophyte spores on their fur. The data also suggest that the health status of the rabbits, their sexes, and the type of housing they were provided may have the most influence on this occurrence.

This could potentially be useful information not only for rabbit owners, but also for veterinary professionals and rabbit farms’ employees. By understanding the risk, we may take measures to prevent it; and with dermatophytosis on the rise, prevention appears to be the best course of action.

Key words:

Trichophyton mentagrophytes; Dermatomycosis; Asymptomatic carrier state; Ringworm; Pet rabbits

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AST – Antibiotic Sensitivity Test

BID – *bis in di'e* (twice a day)

DTM – Dermatophyte Test Medium

GIT – Gastrointestinal tract

ISHAM – International Society of Human and Animals Mycology

MI – Molecular identification

pH – Potential hydrogen

PO – *per os* (administred orally)

POC – Point of Care

PPE – Personal protective equipment

RHVD2 – Rabbit hemorrhagic viral disease type 2

SDA – Sabouraud Dextrose Agar

SID – *sem'el in di'e* (once a day)

WHO – World Health Organization

1. Traineeship Activities

As part of the 6th year of the Integrated Master's in Veterinary Medicine, my traineeship took place in Centro Veterinário de Exóticos do Porto, in Porto, Portugal, under the guidance and orientation of Dr. Joel Ferraz. It began on the 1st of September, 2021, and ended on the 28th of February, 2022, totalizing 836 hours. During this time, several activities took place, allowing me to gain experience in different areas of Exotic Pet Medicine. I had the opportunity to participate in morning shifts (9am to 5pm), afternoon shifts (5pm to midnight) and night shifts (from midnight to 9am).

During the morning shift, I was involved in the appointments that took place during the day. Here, the main goal was to observe the doctor's way of working while they built anamneses, preformed physical and complementary exams, came to diagnoses and applied/prescribed treatments. Animal restraint, taking part in conducting complementary exams and discussing the process of reaching diagnoses were also present most days. In some of these morning shifts (usually twice a week) there would also be surgeries taking place, and I had the chance to have involvement in these as well. The most frequent ones were rabbit spayings and rabbit dental procedures. In surgery, I would mainly help with preparing the room for the specific procedure, monitoring the patient's anaesthesia, keeping their body temperature in a desired range as well as monitor them during post-operatory period. Last but not least, I also had the chance to work with the inpatients, mainly the mammals. In this context, I would be included in doctors' discussion of each case, clean the animal's cages, force-feed them, perform physical examinations and prepare and administer medications (oral or subcutaneous).

The afternoon shift would normally include the same activities from the previous one, with the addition of restocking materials throughout the clinic, as well as cleaning and tidying up the place.

At night, tending to the inpatients and taking part in any emergency cases that were presented to us was the main goal, although updating patients' clinical records would also occur.

During any of these shifts, reception work in a veterinary context was also a skill I got to practice.

2. Introduction

Fungi continue to be underestimated as major human and animal pathogens and have become a significant public health concern, with serious mortality and morbidity rates in people all over the world (Nucci and Marr 2005; Al-Janabi and Al-Khikani 2021). The fact that fungi illnesses do not spread at the same rate as other epidemic-causing pathogens does not make them less significant in terms of the number of affected individuals (Gnat et al. 2021).

The rise in immunocompromised people during the last 4-6 years, some of whom are especially susceptible to fungal infections, has drastically transformed our view on these diseases (Bongomin et al. 2017; Gnat et al. 2020). Moreover, it is known that climate change not only has a direct effect on the fungus' ability to cause damage to its host, but also enables it to spread geographically, thereby increasing its prevalence (Weitzman and Summerbell 1995; Clancy and Nguyen 2016; Shriber et al. 2017). Migration between continents, changes in human lifestyle, and the growing number of pets also play a significant contribution to this (Bontems et al. 2020; Colosi et al. 2020; Gnat et al. 2020; Łagowski, Gnat, Nowakiewicz, and Osińska 2020; Singh et al. 2020). These factors together make fungal infections that have been recognised for decades still an ongoing health risk (Gnat et al. 2021).

The most widespread mycoses are those concerning the skin, hair, and nails, affecting more than 25% of the world's population, and approximately €1.56 billion are spent annually on their treatment (Gupta et al. 2017; Gnat, Nowakiewicz, and Zięba 2019; Gnat, Nowakiewicz, Łagowski, et al. 2019; Gnat et al. 2020). According to Bongomin et al. (2017), the prevalence of these mycoses is comparable to that of migraines and dental caries, and despite the absence of comparable statistics for animals, this provides us a clear idea of the impact of these disorders (Gnat et al. 2021).

Dermatophytes can be found anywhere in the globe and are considered the principal causative agents of superficial mycoses in humans (Nweze and Eke 2018; Gnat, Nowakiewicz, and Zięba 2019; Bontems et al. 2020; Łagowski, Gnat, Nowakiewicz, and Osińska 2020). These fungi do not belong to the skin microbiota of neither animals nor humans, thus their presence cannot be deemed normal (Nweze and Eke 2018; Gnat, Nowakiewicz, and Zięba 2019). They can cause dermatophytosis, a skin illness which can clinically range from mild to severe depending on the host's immune state, the strain's virulence and other environmental factors (Łagowski, Gnat, Nowakiewicz, Osińska, and Zięba 2020).

Although the exact prevalence of dermatophytosis in humans is unknown because the condition is not reportable, this infection is quite common (Moriello 2003; d'Ovidio and

Santoro 2015). In fact, it is one of the most prevalent and significant zoonotic skin disorders globally and has been classified as a condition with epidemic proportion (Moriello 2003; Hoog et al. 2021). Additionally, in several countries over the past year, a significant increase in the prevalence of dermatophytosis was observed, as well as in the occurrences of chronic refractory disease, atypical presentations, recurrent relapses and treatment failures (Brouta et al. 2002; Mishra et al. 2022).

It is hypothesized that asymptomatic carrier pets play a crucial role in the epidemiology of this disease and are the primary route of fungal infection in humans, as well as in the rest of the household's pets (Łagowski, Gnat, Nowakiewicz, and Osińska 2020; Hoog et al. 2021). Furthermore, in economically developed countries, the shift in social and economical trends has led to an increase in pet ownership, particularly regarding exotic animals (Moriello 2003; Souza 2011). Dermatophytosis has also been linked to lagomorphs and rodents for quite a long time, and with rabbits growing increasingly popular as children's first pets, this becomes a topic worthy of attention (Donnelly et al. 2000; Vangeel et al. 2000).

3. Literature Review

3.1 Dermatophytes

Dermatophytes[1] are strictly aerobic fungi that produce keratinolytic proteases, allowing them to rely on keratin for nutrition (Donnelly et al. 2000; Robert and Pihet 2008; Surendran et al. 2014; Quinn et al. 2016; Ganguly et al. 2017). As a result, they have a significant affinity for both animal and human superficial keratinized skin and appendages, such as hair, feathers and horns, making them the only fungi that depend on animals for survival and dissemination (Donnelly et al. 2000; Dowd 2007; Quinn et al. 2011; Dey et al. 2016; Ganguly et al. 2017; Moriello 2020). They prefer moist body locations, and favour temperate and humid climates, having a maximum of keratinase activity between 25 and 35ºC (Moriello 2003; Gnat et al. 2021). This fact explains why tropical and subtropical countries, where temperature and humidity are high for the majority of the year, have a greater infection rate (Hoog et al. 2021).

In the Fungi kingdom, Dermatophytes belong to the mould group and are classified under the *Ascomycota* phylum, the *Onygenales* order and the *Arthrodemataceae* family (Quinn et al. 2011; Hoog et al. 2021).

[1] The term "dermatophyte" refers to pathogenic fungi capable of destroying keratin. Despite this, the name is routinely and widely used to refer to all the collective members of the seven genera, including those that are not pathogenic (Weitzman and Summerbell 1995; Gnat, Nowakiewicz, and Zięba 2019). This dissertation will employ the latter denomination.

Beginning as geophilic organisms, dermatophytes have existed for millions of years. Geophilic dermatophytes can be found in soil, around burrows, and in the vicinity of terrestrial mammals, including rodents and lagomorphs (Hoog et al. 2021). They breakdown keratin in fallen hair, feathers, horns, and skin (Weitzman and Summerbell 1995).

With the capacity to degrade keratine and subsequent intimate association with animals, it is theorised that numerous geophilic organisms evolved and began living asymptomatically in their fur and feathers (Weitzman and Summerbell 1995; Donnelly et al. 2000; Hoog et al. 2021). Not only was this crucial for the wider dissemination of these fungi, but it also contributed to the emergence of so-called zoophilic species (Hoog et al. 2021). These organisms form the majority of dermatophyte fungi and nearly exclusively parasitize animals, with mammals being their preferred hosts (Quinn et al. 2016; Moriello et al. 2017; Hoog et al. 2021).

Although the zoophilic process of host jumps has not been fully investigated, it is known that human-infecting dermatophytes originated from dermatophytes that infect animals (Hoog et al. 2021). It is hypothesised that, due to the prevalence of pets and domesticated animals, certain zoophilic species have developed to infect humans as well. This is of utmost importance since humans lack fur, which increases the likelihood of severely inflammatory infection rather than the asymptomatic colonisation observed before in animals. This allowed species that predominantly parasitize humans to emerge. These species belong to the most recently established ecological group: anthropophilic. Its evolution has been occurring for less than one million years and, as a result, there are only subtle and gradational changes across species, making them difficult to distinguish. Some are even doubtfully separate from their zoophilic forebears, with the mating type being the only differentiating trait (Hoog et al. 2021).

Keeping all of this in mind, it may be difficult to classify dermatophytes into genera and species; although geophilic species are molecularly far apart from each other and hence easily distinguished, zoophilic and anthropophilic species not only differ much less but are also still undergoing evolution. Consequently, taxonomic boundaries may not be as clear, and several intermediate genotypes may be encountered (Hoog et al. 2021). In order to taxonomically classify these species in the midst of their evolutionary process, a mixture of molecular, phenotypical, clinical, physiological, and ecological data must be considered (Gnat, Nowakiewicz, and Zięba 2019; Hoog et al. 2021).

Currently, they can be classified into 7 clades: *Trichophyton* (anthropophilic, or zoophilic when considering pets), *Epidermophyton fluccosum* (anthropophilic), *Microsporum canis* (zoophilic, maily pets), *Lophophyton* (zoophilic), *Paraphyton* (ecologically undefined), *Nannizzia* (ecologically undefined) and *Arthroderma* (geophilic), as can be seen in figure1 (Gnat, Nowakiewicz, and Zięba 2019; Hoog et al. 2021; Chabasse and Bouchara 2022).

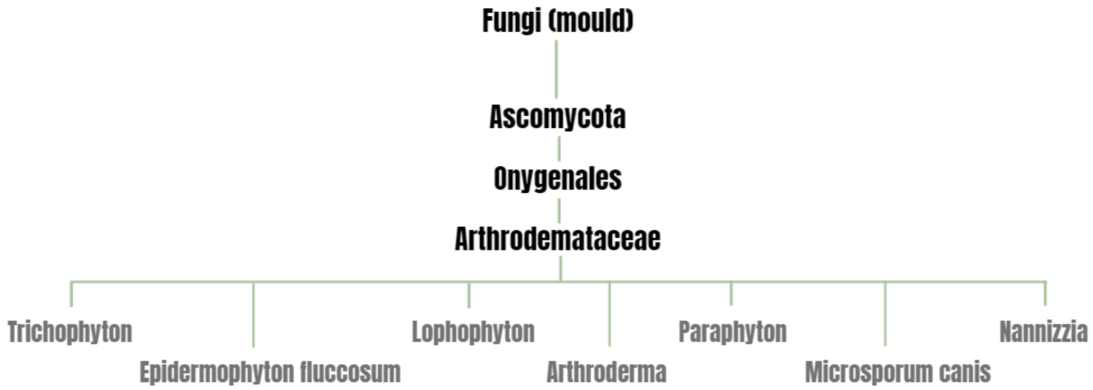

Figure 1 - Taxonomy of Dermatophytes. Source: original

Many organisms within these clades have both an anamorphic (asexual) and telemorphic (sexual) stage of their life cycle (Figure 2). This can be problematic since the morphology of dermatophytes between these two phases varies substantially. In the past, this resulted in the same fungus being classified as two separate fungi. With molecular testing however, many of those species have been revealed to be one and the same (Moriello 2020). Despite the reclassification brought about by this new knowledge, the anamorphic nomenclature is still more widely recognized and will be used in this dissertation. In addition, dermatophytosis is caused exclusively by fungus at the asexual point in their life cycle (Zhang et al. 2009; Gnat et al. 2021).

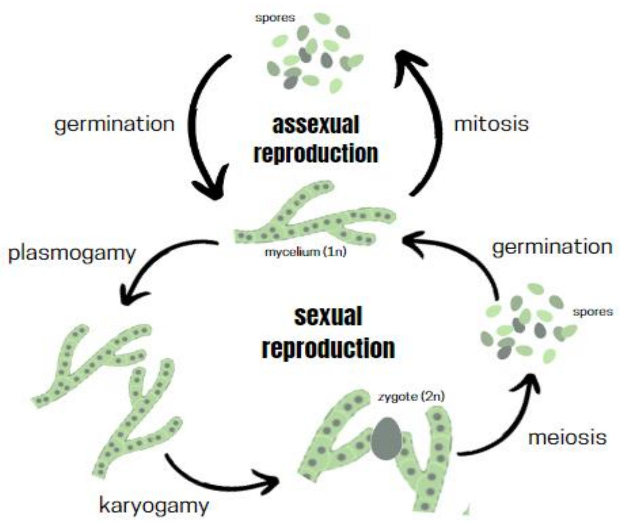

Figure 2 - Life cycle of fungi. Source: original

Dermatophytes are divided into well over fifty species. *Microsporum canis, Microsporum gypseum*, and *Trichophyton mentagrophytes* are the ones that are most pathogenic to pets and the ones that they most commonly carry (Moriello et al. 2017; Gnat et al. 2021).

3.1.1. *Trichophyton mentagrophytes*

Trichophyton mentagrophytes is the name of a species belonging to the *Trichophyton* genus (or clade). Until 2017, this group included 7 species (*T. tonsurans, T. mentagrophytes, T. interdigitale, T. equinum, T. quinckeanum, T. schoenleii* and *T. simii*) (de Hoog et al. 2016). Nowadays this has been reduced to 5 species - *T. mentagrophytes, T. interdigitale, T. erinacei, T. quinckeanum* and *T. benhamie* (Nenoff et al. 2019) (Figure 3). The main difference between all of these species is regarding their ecological preference. For instance, the species *T. mentagrophyte s* is considered to be a zoophilic fungus (Kim et al. 2018). It has worldwide distribution and a wide range of animal hosts, with rabbits being the most common one (Campbell, Colin et al. 2013; Kimura et al. 2015; de Hoog et al. 2016).

Figure 3 – Species of the *Tricophyton* group. Source: original

3.1.2. Dissemination of Dermatophytes

Dermatophytes are unable to reproduce sexually while growing on keratinized structures, only causing infection during their anamorphic life-cycle phase (Quinn et al. 2011). In this situation, the fungal hyphae break into very minute fragments known as arthrospores, allowing the transmission and dissemination of these fungi to take place (Quinn et al. 2011; Moriello et al. 2017). These arthrospores are predominantly found within or attached to the exterior of infected hairs and scales, making these structures the most contagious (Weitzman and Summerbell 1995; Chang et al. 2022).

Spores are spread not just through direct contact with these infected animals, but also by fomites (e.g. grooming appliances and collars). Furthermore, contaminated habitats might also be an effective transmission channel, as these spores can remain viable for more than a year (Quinn et al. 2016; Moriello et al. 2017). In this scenario, they would separate from skin cells in the environment and come into contact with other potential hosts (Weitzman and Summerbell 1995). However, some studies have concluded that this is not the case, and, as long as the pet is well-kept, it is not an effective method of Dermatophyte dissemination (Quinn et al. 2016; Moriello et al. 2017).

3.2 Dermatophytosis

Dermatophytes are one of the few fungi that can cause transmittable illness. Except for *Microsporum gallinae*, which is largely established in gallinaceous birds, they primarily affect mammals, both wild and domesticated (Weitzman and Summerbell 1995; Ganguly et al. 2017).

These fungi are not normally found on the skin and can cause a condition known as dermatophytosis, which can also be referred to as ringworm and *tinea* (Weitzman and Summerbell 1995; Brouta et al. 2002). It is an infection that is generally restricted to the dead cornified layers of the skin as dermatophytes are unable to penetrate deeper tissues of immunocompetent hosts (Weitzman and Summerbell 1995).

Although it is usually a self-limiting (from 4 to 12 weeks) and non-life threatening disorder, it is nevertheless significant since it is contagious and considered a zoonosis (Łagowski, Gnat, Nowakiewicz, Osińska, Trościańczyk, et al. 2020). Furthermore, despite the fact that significant complications are uncommon, this disease may require long-term treatment for complete eradication, potentially causing financial distress for the client. It can also raise emotional and social concerns, as it may cause disgust for the patient and those around them (Moriello et al. 2017; Turner et al. 2017; Moriello 2020; Hoog et al. 2021).

It is easy to see why dermatophytosis is such a serious issue in both Human and Veterinary Medicine when all of this information is considered, along with the fact that prevention of this disease may present itself to be difficult (Al-Janabi and Al-Khikani 2021).

3.2.1. Pathophysiology of Dermatophytosis

Dermatophytosis pathophysiological mechanisms are poorly known (Brouta et al. 2002). Dermatophyte infection does not occur on intact skin as the keratinized *stratum corneum* offers a physical barrier. However, only minor damage to this layer is required to make skin susceptible to these fungi (Donnelly et al. 2000; Tetsuya Koga 2009). Consequently, ordinary situations such as shampooing, *pruritus* and self-trauma, ectoparasites, poor-hygiene and pre-existing trauma can increase the risk for this type of infection to develop (Donnelly et al. 2000; Dowd 2007; Dey et al. 2016; Moriello et al. 2017; Moriello 2020). Warmth and moister are also indispensable for the infection to settle in. Furthermore, it has been found that lower levels of fatty acids and sphingosine in sebum also play a role in this process, being this of special importance in young individuals (Dey et al. 2016; Moriello 2020).

Given the fact that this infection is mainly restricted to the keratinized layer of the skin, dermatophytes rarely come into direct contact with immune effector cells. To counteract this and allow the organism to eliminate these invading fungi, the skin contains a unique subsystem, that includes both innate and acquired immunity. The former is primarily attained by epidermal keratinocytes, which not only serve as a physical barrier but also mediate cutaneous immune reactions by secreting proinflammatory cytokines and antimicrobial peptides. For the latter, delayed t-cell mediated response mechanisms are poorly understood, but they unquestionably play a role in both the host's long-term resistance to dermatophytes and the pathogenesis of the typical skin lesions (Tetsuya Koga 2009).

It is hypothesised that keratinolytic proteases are key virulence agents for dermatophytes, since they may play a role in the fungus' nourishment, tissue invasion and regulation of the host's defences, as well as in confining the fungi to the keratinized structures (Brouta et al. 2002). Dermatophytes have the ability to fight the host's local inflammatory response, by inhibiting lymphocytes, slowing keratinocyte turnover and modifying macrophage-lymphocyte interaction (Quesenberry et al. 2021). However, when the animal is healthy and has a top-functioning immunesystem, even in the presence of these fungi, it usually remains an asymptomatic carrier. Even so, any situation that lowers the effectiveness of immune response (such as stress or disease) increases the risk of active infection (Quesenberry et al. 2021).

3.2.2. Dermatophytosis in Rabbits

Dermatophytosis is listed as one of the most common causes of dermatological issues in animals, particularly domestic species (Neji et al. 2016; Quesenberry et al. 2021). It is a rather important infection in animals due to its zoonotic character as well as its consequences on the economic value of animal breeding and production (Al-Janabi and Al-Khikani 2021; Mishra et al. 2022). Additionally, it can affect wild animals, posing a potential threat to the ecosystem's equilibrium (Mishra et al. 2022).

Mycrosporum canis, Mycrosporum gypseum and *Trichophyton mentagrophytes* can cause Dermatophytosis in most mammal pets, with the latter being the most common in rabbits (Canny and Gamble 2003; Dey et al. 2016; Quinn et al. 2016; Moriello et al. 2017; Chang et al. 2022; Mishra et al. 2022). This is of extreme relevance since dermatophytoses are ubiquitous in rabbit farms, lab rabbits, and pet rabbits, and these species can also affect humans (Chang et al. 2022).

3.2.2.1. Clinical Findings

When dermatophytosis develops in our pets, they become a higher source of contamination for both people and other pets in the household, in addition to being uncomfortable and unhealthy.

There are no pathognomonic signs for dermatophytosis. The symptoms vary largely between individuals and mainly reflect the quality of their immune response, their level of susceptibility and resistance, and the virulence of the specific species of dermatophytes involved (Weitzman and Summerbell 1995; Donnelly et al. 2000; Quinn et al. 2011; Moriello et al. 2017; Moriello 2020). Because of this, clinical presentations can range from free-lesion animals all the way to complicated infections (Moriello 2020).

When clinical signs do develop, they mainly stem from the inflammatory response to the invasion of keratinized structures (Figure 4). This can include patchy areas of hair loss and breakage, diffused erythema, dry yellow crusts, papules, scales, hyperpigmentation, hyperkeratosis, follicular plugging, comedones, changes in nail growth and appearance, exsodative paronychia, *pruritus*, miliary dermatitis and even nodular lesions (kerions, pseudomycetoma and mycetoma) (Canny and Gamble 2003; Quinn et al. 2011; Moriello et al. 2017; Moriello 2020; Quesenberry et al. 2021). These lesions are typically situated on the face, head, auricles, and dorsal region of the animal's neck, as well as on its feet and legs with occasional spread to the nail bed (Canny and Gamble 2003; Chang et al. 2022; Mishra et al. 2022).

It is also important to note that secondary bacterial infection (especially in immunosuppressed animals) and gastrointestinal tract (GTI) obstruction (because of higher ingestion of hairs while self-grooming) may also be present (Dowd 2007; Quinn et al. 2016; Moriello 2020). Dermatophytosis can additionally lead to malnutrition, growth retardation and even death (Dey et al. 2016).

Deep or systemic mycoses in rabbits caused by dermatophytes are extremely uncommon, and are typically recorded only in very young kits (Canny and Gamble 2003).

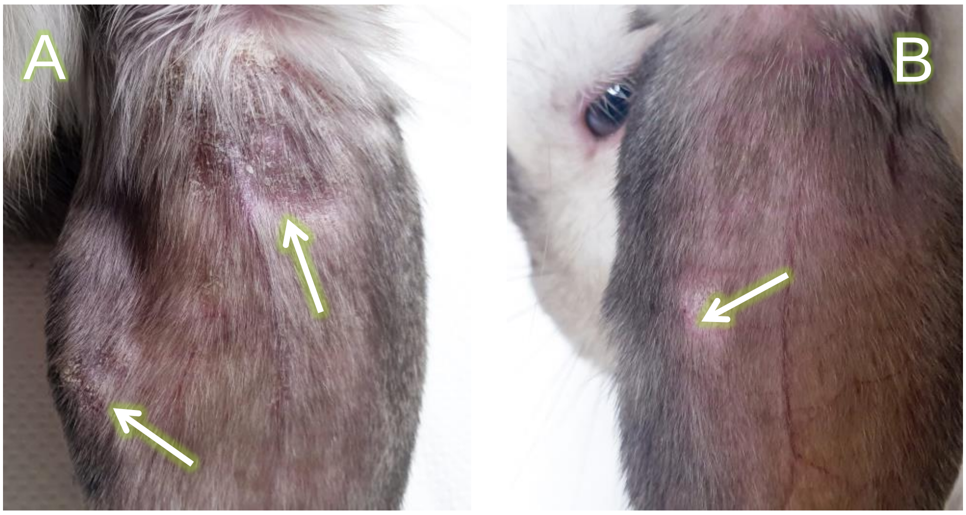

Figure 4 – Dermatophytosis skin lesions on a rabbit's ears. Source: original.

A: right ear, where patchy lesions (arrows) with hair loss and breakage, as well as crusts and scales can be seen. B: left ear, where circular lesion with hair loss can be seen (arrows)

3.2.2.2. Risk Factors

There are a number of factors that can contribute to an animal developing dermatophytosis. The most significant ones include exposure to contaminated surroundings, direct contact with diseased animals, and fomite transmission (e.g. grooming tools and bedding) (Moriello 2020). However, since the development of dermatophytosis requires certain environmental conditions and because the disease does not occur in individuals with intact skin and a healthy immune system, it is clear that additional factors must be involved.

Due to the fact that dermatophytes thrive in higher temperatures and humidity, an environment above 20ºC with a relative humidity rate of 62-65%, is ideal in enhancing the likelihood of this infection (Cafarchia et al. 2010). In Portugal, this could imply that there are seasonal changes in the prevalence of dermatophytes, with larger odds of pets developing dermatophytosis in spring and autumn.

Age has also been found to be a risk factor for dermatophytosis in rabbits, with young kits being the most vulnerable (Vangeel et al. 2000; Kraemer et al. 2012; Moreira et al. 2012; d'Ovidio and Santoro 2015). This is explained since, in addition to having lower quantities of fungistatic fatty acids in their sebum, juvenile rabbits also have underdeveloped immune systems (Chang et al. 2022; Mishra et al. 2022).

Furthermore, inadequate living conditions and poor sanitation have also been demonstrated to negatively impact skin health, hence increasing the chance of dermatophyte infection (Turner et al. 2017; Mishra et al. 2022). An unsatisfactory diet may also play a significant part in raising the risk of infection; not only is a proper diet vital for a rabbit's skin health, it is also crucial for its oral health and body weight, which are determining factors in the rabbits ability to groom itself. Unfortunately, rabbits are often victims of inferior living situations, mainly because of owners' lack of knowledge.

Dermatophytes are also isolated more frequently from free roaming and group-housed animals, as well as rabbits which have access to exterior environments (Moriello et al. 2017).

Mites, fleas, and ticks also contribute to a poor skin condition and, thus, the development of dermatophytosis (Mishra et al. 2022). These parasites alter the *stratum corneum* of the animal's skin, hence facilitating dermatophyte infection. In light of this, absence of parasite protection can be viewed in itself as a risk factor for this condition (Dey et al. 2016).

Additionally, rabbits have the habit of self-grooming. As it allows for the spores to be eliminated from the hair and skin, it is an effective defence mechanism against dermatophytes. However, when an animal has skin lesions, oral issues, is ill, in discomfort, or overweight, it often stops grooming. This can ultimately lead to skin infection (d'Ovidio and Santoro 2015; Moriello et al. 2017). In this sense and because the immune system is weakened, any other disease might cause asymptomatic carriers to develop lesions, and is therefore a risk factor for dermatophytosis (Mishra et al. 2022). Furthermore, when a rabbit stops grooming, it may seem prudent to brush their fur; particularly if they have long hair, as these animals seem to be more likely to exhibit more aggressive symptoms; however, grooming instruments can cause microtrauma to the skin and raise the risk of mycosis (Weitzman and Summerbell 1995; Quinn et al. 2011; Moriello 2020).

Moreover, a 2022 study revealed that pet rabbits acquired from pet stores were more likely to be infected with dermatophytes than rabbits obtained through adoption or home breeding (Chang et al. 2022). This can be explained by the fact that dermatophyte spores can remain viable for many years and infected cages or environments in pet stores can facilitate rabbits' mutual infection (Chang et al. 2022). In addition, contrasting with home breeding, animals in pet stores are typically not treated with parasite preventatives (Chang et al. 2022).

As can be seen, this disease is associated with a number of risk factors (Figure 5), all of which must be taken into account when evaluating the management of this zoonosis.

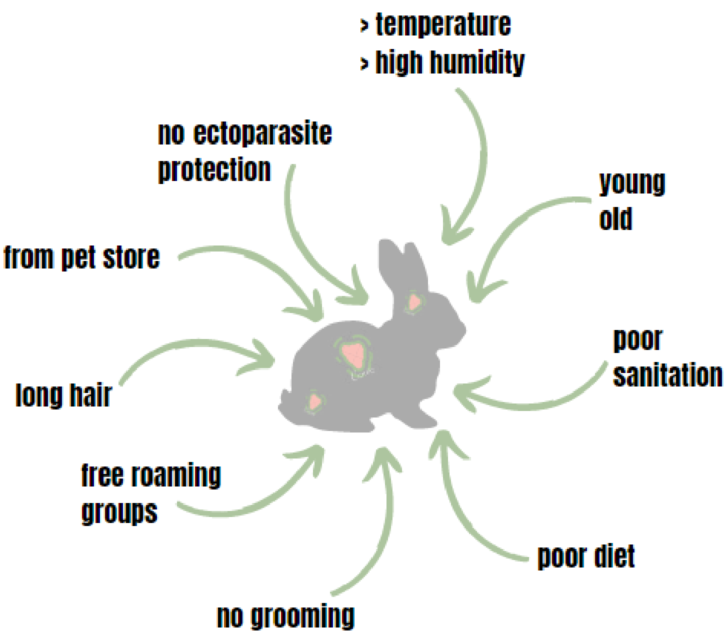

Figure 5 - Risk factors for dermatophytosis in rabbits.
Source: original

3.2.3. Dermatophytosis as a zoonosis

Globally, pets serve an essential role in human societies. They are valuable companions in many households, contributing to the physical, social, and emotional health of children and the well-being of their owners, particularly the elderly (Robertson et al. 2000). Although pets offer numerous advantages, there are risks involved with pet ownership, including increased exposure to various infectious agents (Robertson et al. 2000; Riley and Chomel 2005).

Dermatophytosis is highly zoonotic in nature (Mishra et al. 2022) and infected pets reflect a major role of these fungi's transmission to humans (Chang et al. 2022). People commonly receive dermatophytes from the infected/carrier animals' hair coat and skin flakes, or their nearby environment, rather than from human to human contact (Donnelly et al. 2000; Dey et al. 2016; Moriello et al. 2017).

3.2.3.1. Rabbits as Pets

Exotic mammalian animals have grown increasingly popular as pets (Quesenberry et al. 2021). Specifically, rabbits (*Oryctolagus cuniculus domesticus*) are becoming a common pet among children today (Vangeel et al. 2000). There are no data on the true frequency of zoonotic infections transmitted by exotic pets, but some studies claim rates as high as 25%, with youngsters being the most susceptible and frequently the only ones in the household to develop symptoms (Kraemer et al. 2013).

Dermatophytosis has been associated with lagomorphs and rodents for a very long time, as these mammals are frequently asymptomatic carriers for *T. mentagrophytes* and, consequently, one of the main hosts for these fungi (Vangeel et al. 2000; Kraemer et al. 2013; Dey et al. 2016; Chang et al. 2022). The quick growth and replacement of hair, as well as differences in skin secretions compared to other species, appear to have a role in facilitating dermatophyte presence in rabbits (Canny and Gamble 2003). Several studies have also indicated that rabbits can infect people through direct contact, even with very limited interaction (Donnelly et al. 2000; Skořepová et al. 2002; Riley and Chomel 2005; Zhang et al. 2009; Cafarchia et al. 2012). Consequently, rabbits can be considered a major source of dermatophytes for humans and other animals, hence the focus of this study.

Understanding the epidemiology of dermatophytes in rabbits is essential for lowering its prevalence in humans, rabbits, and other household pets. Furthermore, because juveniles are more likely to develop skin lesions as a result of dermatophyte infection, rabbit owners should be informed of the risks of keeping them as pets (Vangeel et al. 2000).

3.2.4. Dermatophytosis in Humans

Dermatophytosis can be considered the most frequent fungal illness in the world in humans, resulting in a large therapy cost each year (Achterman and White 2012). It is likely to impact every person at some point during their lives, and while the fatality rate is especially low, there is significant morbidity connected with these infections, as they cause severe skin reaction (Achterman and White 2012). Although uncommon, there are also case reports of systemic infections in humans, particularly in immunocompromised individuals (Al-Janabi and Al-Khikani 2021).

3.2.4.1. Clinical Findings

Infections of the skin, hair, and nails by dermatophytes are extremely frequent in humans and quite diverse in appearance. In the case of zoophilic and geophilic dermatophyte infection, inflammatory symptoms in the skin are frequently evident (Weitzman and Summerbell 1995; Degreef 2008). With anthropophilic species, however, they are typically absent (Degreef 2008). This makes the anxiety linked with pet-associated dermatophyte infections even more realistic.

The infecting fungus, the site of infection and the degree of keratinisation at that site, as well as the immunological condition of the host, all play a significant role in the development of the various clinical manifestations (Degreef 2008).

The infection can range from mild to severe and the lesions are often ring shaped, hence the term “ringworm” (Ganguly et al. 2017). In contrast to rabbits, in humans the typical lesion is a well-defined, round, pink to slightly erythematous patch with a raised vesicular margin and a central clearing (Weitzman and Summerbell 1995; Canny and Gamble 2003; Degreef 2008). Inflammatory forms may also cause hyperpigmentation, scaling, fissuring, crusts, maceration, vesicles, papules or pustules (Degreef 2008). There may also be burning and itching, especially if a secondary bacterial infection is present (Weitzman and Summerbell 1995). When hairy areas are affected, patches of hair loss, folliculitis, and in the most severe cases, kerion may develop (Weitzman and Summerbell 1995; Degreef 2008). Additionally, the horny layer of the nail bed and nail plate becomes thicker when nails are afflicted. The nail may turn opaque, discoloured, and its free edge may become elevated. Moreover, white specks may be seen, and the texture of the entire nail can be altered. Furthermore, in the presence of secondary infection, the nail may potentially crumble and even disappear (Degreef 2008). These lesions can be observed in various parts of the human body and can be clinically classified based on their location: *tinea corporis* (body); *tinea cruris* (groin); *tinea faciei* (face); *tinea pedis* (feet); *tinea manus* (hands); *tinea capitis* (scalp, eyebrows and eyelashes); *tinea barbae* (beard and mustache); *tinea unguium* (nails) (Figure 6).

The National Health Insurance Research Database of Taiwan revealed in 2013 that *tinea pedis, tinea cruris*, and *tinea unguium* were the three most prevalent forms of dermatophytoses. Interes tingly, *Trichophyton mentagrophytes* is a relatively common cause for all of these various appearances (Chang et al. 2022).

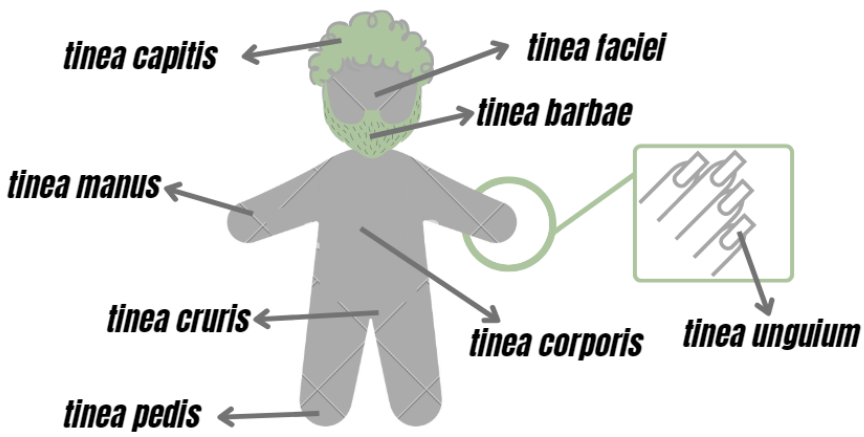

Figure 6 – Clinical findings of dermatophytosis in humans. Source: original

3.2.4.2. Risk Factors

Due to the temperature and humidity preferences of dermatophytes, the associated disease is more frequent in tropical and subtropical areas (Moriello 2003). This predominance will likely be exacerbated as a result of climate change. This is due to the fact that these shifts are not only expected to affect humans directly through more severe and extended weather events, but also through altering the global range of wildlife species and the periods when dermatophytosis can normally be transmitted to humans (Souza 2011).

Furthermore, places with a high population of feral animals have a higher incidence of dermatophytosis because the spores can be carried on their hair coats (Moriello 2003).

The expansion and globalisation of the human population and the concomitant encroachment on the habitats of reservoir species of zoonoses will certainly result in an increase in zoonotic disease prevalence (Souza 2011). In addition to wild animals, people with animals in their living and/or working environments are at a greater risk for developing dermatophytosis.

The primary mode of transmission of dermatophytes from pets to humans is direct contact with infected animals. This human-animal interaction includes casual contact, caressing, grooming and/or sleeping with the pet (Moriello 2003). However, a contaminated environment and ectoparasites that serve as vectors are also ways that people can become infected (Quesenberry et al. 2021).

The risk of human infection by pets, particularly by *T. mentagrophytes*, has been mostly connected with dermatophytosis in rabbits. Moreover, with the rising trading and ownership of exotic pets, this infection is predicted to reach even greater proportions (Van Rooij et al. 2006; Cafarchia et al. 2012). When owners play with their rabbits, they frequently hold them in their arms, rub them on their faces, and place them on their abdomens, raising the risk of dermatophyte infection (Chang et al. 2022).

Regarding the workplace, veterinary clinics and hospitals, laboratories, pet stores, necropsy establishments and rabbit farms have a greater incidence of dermatophytosis (Chang et al. 2022). The majority of occurrences involve *T. mentagrophytes,* and infected animals are a persistent source of this zoonotic infection for employees and their families (van Cutsem et al. 1985). During animal examination, treatment, and handling, direct transmission is possible. Indirect transmission however, includes used cages, equipment, surfaces, and laundry (Williams et al. 2015).

It is also important to note that animal-associated diseases can affect anyone, but children and people with compromised immune systems are the most vulnerable (Vangeel et al. 2000).

As is evident, there are factors (Figure 7) that put people at risk for dermatophyte infection which can be attributed to the presence of rabbits. Taking this into account is crucial if we wish to prevent the further spreading of this disease.

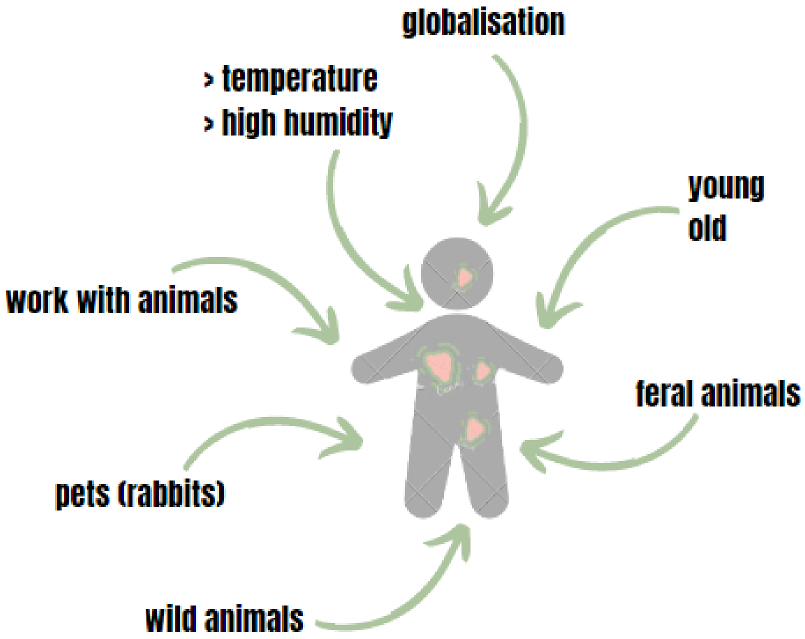

Figure 7 - Risk factors for dermatophytosis in humans. Source: original

3.3. Diagnosis

Because dermatophytosis is an infectious and contagious condition, establishing the diagnosis early is critical not only to begin treatment but also to limit the spread of infection (Moriello et al. 2017). Additionally, and despite the fact that therapy for dermatophytosis is the same for all species, recognising the one implicated is crucial for epidemiological considerations and to ensure that the necessary prophylactic measures are properly taken (Weitzman and Summerbell 1995; Canny and Gamble 2003).

Since the symptoms of dermatophytosis are not pathognomic and have a broad range, the majority of dermatological symptoms can indicate the presence of this disorder. This also means that the appearance of this condition may suggest a variety of other differential diagnoses, including ectoparasitism, trauma, overgrooming, barbering and wet dermatitis (Canny and Gamble 2003). Therefore, it is essential to determine whether dermatophytes are the cause of the problem and, if so, whether the animal presents a zoonotic risk to its household.

The first step in identifying dermatophyte presence should always involve gathering the clinical history of the pet and determining the reason for the veterinarian visit. As discussed previously, concurrent diseases are risk factors for this infection. In addition, any medications supplied beforehand can change the presence of these fungi in the animal's fur, and so altering the diagnostic results (Mishra et al. 2022).

Knowing the rabbit's age, sex, whether or not it is neutered, whether or not it has been dewormed, vaccinated and ectoparasite protected, how it is housed, and whether or not it has been in contact with other animals in the past year, are crucial for guiding our diagnosis, as they are considered risk factors for dermatophytosis (Dey et al. 2016; Moriello et al. 2017; Moriello 2020; Mishra et al. 2022). It is also important to inquire as to where the rabbit came from (pet store, home breeding), if the rabbit can groom itself and, if not, when was it last groomed, and what the rabbit's diet consists of. Taking into account the season when an animal’s symptoms started can also contribute to an accurate diagnosis (Weitzman and Summerbell 1995; Cafarchia et al. 2010; d’Ovidio and Santoro 2015; Moriello et al. 2017; Turner et al. 2017; Chang et al. 2022; Mishra et al. 2022).

Following this, a complete medical examination should be conducted on the rabbit. In addition to assessing their body score, we can check for the presence of other risk indicators, such as a poor overall health, a coat with long fur, or poor-quality hair (d’Ovidio and Santoro 2015; Moriello et al. 2017; Mishra et al. 2022). Detecting any skin lesions during this examination is essential, as samples taken from these locations are what will allow us to reach a definitive diagnosis of this condition.

3.3.1. Sample collection

3.3.1.1. Symptomatic animals

When sampling these animals, focus should be placed on the rabbits's skin lesions (Patel et al. 2008).

Typically, spores cling to the hair shafts of the rabbit's fur; hence, the goal is to gather enough fur for investigation without damaging these structures. The Mackenzie’s brush technique is ideal for this since it collects hair in a gentle and controlled manner (Patel et al. 2008; Moriello et al. 2017). A newly purchased toothbrush with soft bristles is combed through the rabbit’s hair, concentrating on the affected areas and avoiding those that had been recently medicated (Canny and Gamble 2003). Additionally, hair plucking and the collecting of scabs and crusts from the lesions should be performed. The hair plucking requires the gathering of 50-100 hairs from the periphery of the lesions, plucking them from the base, with sterile haemostatic forceps. In order to prevent damage to the hair and possible spores, pieces of IV fluid tubing can be used to shield the extremities of the forceps in. In the same manner, and also form the periphery of the lesions, scabs and crusts should be collected as well (Patel et al. 2008; Moriello et al. 2017).

Additionally, particularly in symptomatic patients, wearing gloves during the sampling procedure is of utmost importance for the clinician's safety.

3.3.1.2. Asymptomatic animals

In many instances, clinical symptoms in the family members rather than in the animal itself are what drive us to search for dermatophytes in asymptomatic pets (Kraemer et al. 2013). In this light, in rabbits without dermatological lesions, the primary objective is to determine whether or not they are mechanical carriers of dermatophytes.

In this scenario, Mackenzie's brush technique is also an excellent diagnostic tool (Weitzman and Summerbell 1995; Moriello 2003). This time, though, the toothbrush should be used to comb the animal's entire coat. The process should be done from front to back due to the fact that dermatophytes are typically more prevalent on the head and face area (Moriello et al. 2017). However, it is still important to remember to comb the paws, ears and belly (Canny and Gamble 2003). The technique is best done in the fur's direction of growth as this further prevents damage to happen to any targeted structures. A satisfactory sample can typically be obtained by brushing the animal for roughly 2 minutes (Patel et al. 2008).

Because rabbits normally enjoy this process, they remain calm and relaxed, making this procedure typically easy to perform (Patel et al. 2008).

3.3.2. Sample Analysis

3.3.2.1. Direct Microscopy (Trichogram)

Some of the hairs, scabs and crusts should be mounted on a microscope slide, immediately after collection, with liquid paraffin. They should be fixed with heat, using flame, a heater or a hair dryer. Once fixed, adding a drop of cotton blue dye 10 to 15 minutes before observation may be advantageous; however, it is not mandatory (Dey et al. 2016).

The slide is then examined under an optical microscope at a magnification of 4/10x. The goal is to look for damaged hairs, which are typically wider and paler than healthy ones (Figure 8). If any are discovered, they should be examined at higher magnifications for the presence of arthrospores and hyphae (Canny and Gamble 2003). With scabs and crusts, greater magnification should always be used (Dey et al. 2016).

Microscopically, all dermatophytes appear identical on infected skin and nails. Hyphae are septate, regular in thickness, branching, and frequently fractured into arthrospore chains. In hair however, these arthrospores may differ in size and disposition, depending on the species involved. *M. canis* arthrospores are 2-3 microns in diameter and form irregular clusters on the exterior of the hair shaft (ectothrix). *T. mentagrophytes* and *M. gypseum's* spores are bigger and can also grow within hair shafts (ecto-endothrix) (Campbell, Colin et al. 2013). However, fungal culture is required for species identification to be reliable.

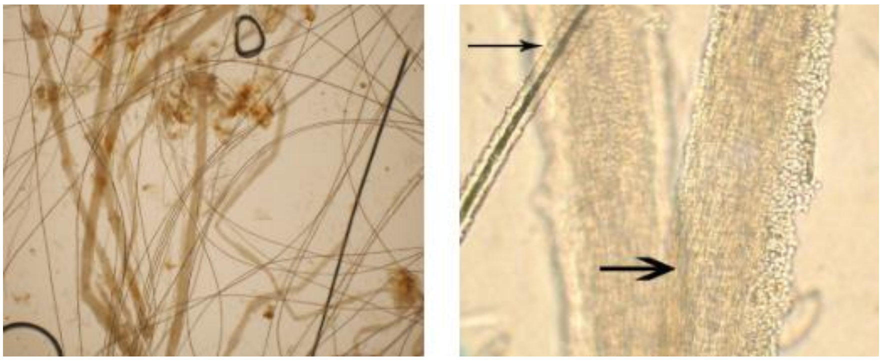

Figure 8 - Microscopic view of hairs damaged by dermatophyte infection Source: Courtesy of Dra. Karn A. Moriello (Moriello 2019)

3.3.2.2. Isolation and Identification of fungi

Direct microscopy is an extremely helpful technique, particularly in a Point of care (POC) context. It can, however, result in numerous false negatives and false positives. Furthermore, we cannot establish the species of dermatophyte involved (Moriello 2003).

For this, it is necessary to isolate the pathogen from the remaining hairs, scabs, and crusts (Canny and Gamble 2003; Anikar et al. 2022).

3.3.2.2.1. Growth medium

Colony growth for dermatophytes can be done in either one of two mediums: Sabouraud Dextrose Agar (SDA) and Dermatophyte Test Medium (DTM) (Moriello et al. 2017).

DTM is a selective medium for the growth of dermatophytes, composed of SDA, bacteria and saprophytic fungi inhibitors, and phenol red. The final component is a potential hydrogen (pH) indicator that turns the medium red when the pH level increases. As dermatophytes grow in this medium, they metabolise the available proteins and produce ammonium (an alkaline metabolite), causing the pH to rise above 8.2 and the colour of the medium to shift (Weitzman and Summerbell 1995; Canny and Gamble 2003). Within the first 10 days of colony growth, dermatophytes often produce sufficient alkaline metabolites for this change to occur (Canny and Gamble 2003). This medium however, has a number of drawbacks, including the need for daily examination of the plates to detect colour changes and assessment of the look of growing colonies. In addition, some dermatophyte species do not sporulate on DTM, but do so on SDA. Also possible with DTM is the occurrence of false negatives and false positives (up to 20%, according to Moriello 2020). The former occur when the colour change happens far later than what is typical for dermatophytes (this primarily occurs with *M. persicolor*, which only produces sufficient alkaline metabolites to alter the medium's colour after 10 days of development). False negatives can also occur if the sample was improperly collected and does not accurately represent the spores present on the animal's coat. When infecting saprophyte fungi cause the medium's colour to change, false positive results can occur. This often takes place later than with dermatophytes since these fungi begin by metabolising the sugars in the medium prior to degrading proteins; however, the two can still get confused (Patel et al. 2008; Moriello 2020). On another note, DTM is available in a variety of formats; however the plates are the optimal choice for a POC diagnosis. This is because toothbrushes are often larger than the opening of most medium filled tubes.. In addition, although the cost of DTM is very low, the plates and tubes have a short lifespan (months) and, if not used entirely, may be an unnecessary cost for the veterinarian.

SDA is an option for medium growth that would counteract DTM's limitations. Unlike DTM, however, it cannot be done in a POC setting and must be performed in a specialized laboratory. This implies the cost to be substantially greater and the waiting time to expand as well (Table 1).

Table 1 - Comparison of DTM and SDA as growth mediums for dermatophytes.

	DTM	SDA
Allows all dermatophyte species to sporulate	✗	✓
T. mentagrophytes	✓	✓
M. canis	✓	✓
M. gypseum	✓	✓
Bacterial and saprophytic inhibitors	✓	✓
pH indicator	✓	✗
Point of care friendly	✓	✗
Need for daily examination	✓	✓
False positives	✓	✗
False negatives	✓	✗
Cost	€	€
Time needed for results	≤2 weeks	>2 week

3.3.2.2.2. Inoculation

3.3.2.2.2.1. Mackenzie's Brush

After combing the animal's coat, when the toothbrush is full of hairs, the bristles should be pressed into the medium 6 to 8 times, so that individual impressions are evident (Moriello et al. 2017). This must be done so as to avoid over-inoculation, since it could impede sporulation and fungal growth from happening (Patel et al. 2008).

3.3.2.2.2.2. Plucked hairs, scabs and crusts

Using sterile haemostatic forceps, these materials must be forced into the growth medium until they are partially buried (Patel et al. 2008).

3.3.2.2.3. Incubation

The inoculated plates should be stored in individual plastic bags to prevent cross-contamination and desiccation. The bags are then placed within a box and stored in an area with a consistent temperature ranging between 25 and 30°C. If no such space is available, this can also be accomplished by placing the plates in a plastic container with a fish tank thermometer set to the proper temperature and sealing the container's lid during the incubation time (Dey et al. 2016; Moriello 2020) (Figure 9). Incubation should last for 7 to 14 days, and, except when samples are being gathered for examination, the plastic bags and boxes must always remain closed (Moriello 2020).

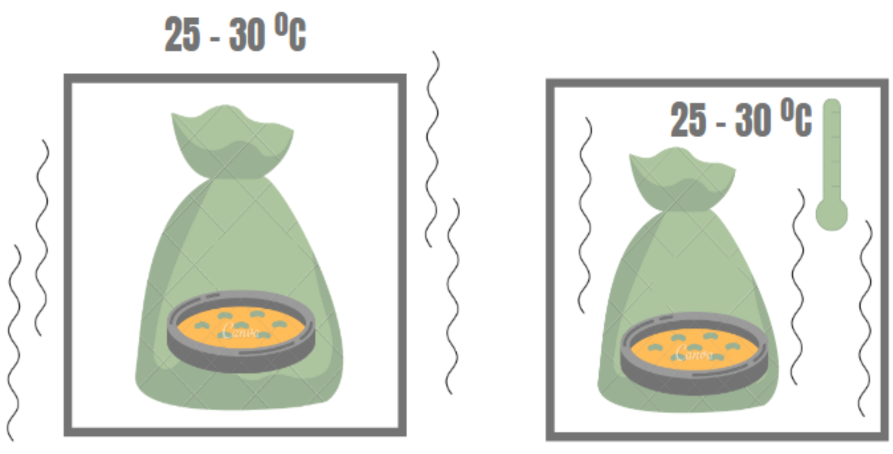

Figure 9 – Dermatophyte incubation POC set-up. Source: original

3.3.2.2.4. Macroscopic analysis

Dermatophytes differ widely in growth pace, colony topography, texture, and pigmentation. Before the microscopic analysis, it is helpful to assess these features (Campbell, Colin et al. 2013).

If no colony growth occurs within 14 days, the test is declared negative. In addition, the test is also considered negative if there is colony growth but no change in the colour of the medium within the given time range (Moriello 2020). Nonetheless, those colonies should also be observed under a microscope to make sure it is not a false-negative case.

The agar that the fungus is cultured on has a significant impact on its colonial characteristics (Campbell, Colin et al. 2013). The subsequent relates to dermatophytes growth on DTM and SDA.

The average growth rate of *Microsporum* species is moderate, with *M. gypseum* and *M. canis* increasing approximately 50 mm each week (Campbell, Colin et al. 2013; V. and Jr. 2017). *M. gypseum* produces flat, powdery colonies that range in colour from beige to brown and occasionally pink, whereas *M. canis* produces white colonies with a yellow margin. (Figure 10).

Trichosporum colonies often expand slowly. Each week, *T. mentagrophytes* grows *c.a.* 25 mm in a powdery and flat manner. In general, the colonies are white, cream, pink, grey, or yellow, and their undersides are often dark brown with radiating striations (Campbell, Colin et al. 2013; V. and Jr. 2017) (Figures 10 and 11).

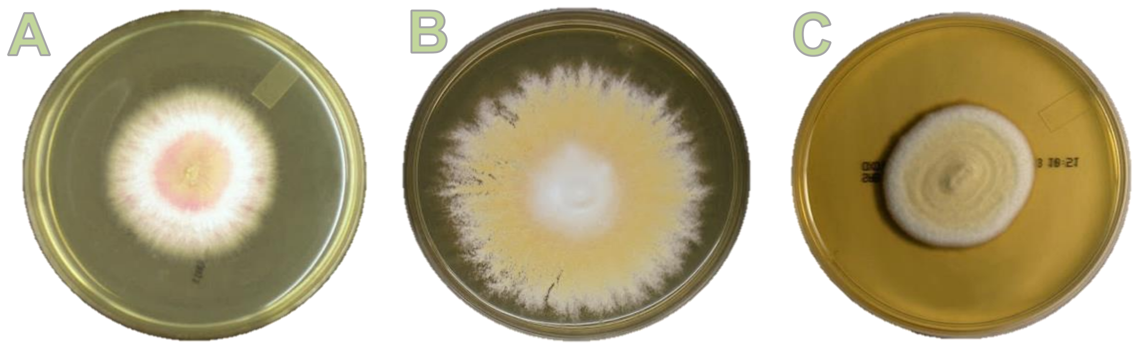

Figure 10 - Macroscopical view of *M. canis, M. gypseum* and *T. mentagrophytes* colonies.

Source: Courtesy of Yuri, from *Fun with Microbiology (what's buggin' you?).* Adapted from Yuri 2018.

A: Day 5 of *M. canis* growing in SDA, at 30ºC. B: Day 7 of *M. gypseum* growing in SDA, at 30ºC. C: Day 5 of *T. mentagrophytes* growing in SDA, at 30ºC

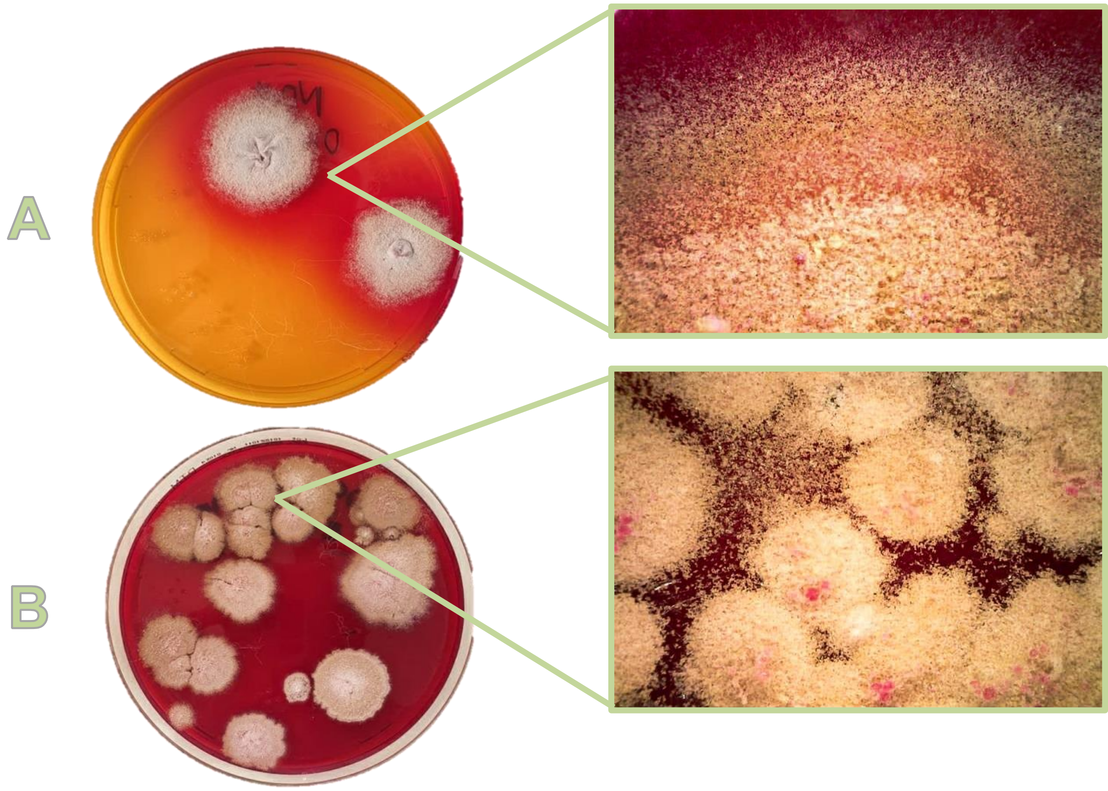

Figure 11 - Macroscopic appearance of *T mentagrophytes* growing in DTM with medium colour change.

Source: original.

A: 5 days of growth; B: 14 days of growth

3.3.2.2.5. Microscopic analysis

Although the differences in colonies' macroscopic appearances, DTM simply provides a "yes" or "no" result for dermatophyte presence; it does not reliably specify the species involved as this requires microscopic examination of the colonies (Quinn et al. 2011).

To identify a dermatophyte microscopically, it is necessary to determine hyphae morphology, whether macroconidia or microconidia predominate and whether spores are present (Frías-de-león et al. 2020). On the basis of a combination of microscopic and colony traits, this will eventually allow species-level distinction (Campbell, Colin et al. 2013).

When colony growth occurs within 14 days, especially if the medium changes colour, conducting a microscopic examination is essential. With clean forceps, a strip of tape should be applied to the surface of the colony with the adhesive side facing down so that biological material can adhere to it. The tape may then be dyed with Lactophenol Cotton blue to aid in the identification of fungal structures, or it may be left uncoloured (Patel et al. 2008).

The adhesive tape is then placed on a microscope slide and examined for macro- and microconidia using an optical microscope (Canny and Gamble 2003; Patel et al. 2008)

Microsporum species have both macro and microconidia, as well as septate hyphae. *M. gypseum* generates many macroconidia, and few microconidia. The macroconidia of this species are oval, have thin, rough walls, 4 to 6 septa, and occasionally a terminal filament. The microconidia are club-shaped and mostly form along the hyphae's sides (Campbell, Colin et al. 2013; V. and Jr. 2017). *M. canis* has mostly large macroconidia at the colony's centre. They are spindle-shaped and rough at the tip, which is frequently bent to one side. Most have 6 septa and sturdy walls. Narrow, club-shaped microconidia are also produced along the edges of the hyphae (Campbell, Colin et al. 2013) (Figure 12).

T. mentagrophytes has hyaline and septate hyphae that are typically smooth-walled and spiral-shaped (Bonagura and Twedt 2000). Numerous microconidia are produced individually or in huge clusters directly on the hyphae (Campbell, Colin et al. 2013). They are single-celled, have a smooth wall and are round, tear or irregularly shaped. Macroconidia are cylinder-shaped with thin, smooth walls and 3 to 4 septa, and originate laterally in the hyphae or in short pedicles (Symoens et al. 2011; Campbell, Colin et al. 2013; V. and Jr. 2017; Kim et al. 2018). The most consistent trait though, is the formation of aleuriospores organised in clusters like a grape bunch (Symoens et al. 2011; Kim et al. 2018) (Figures12 and 13).

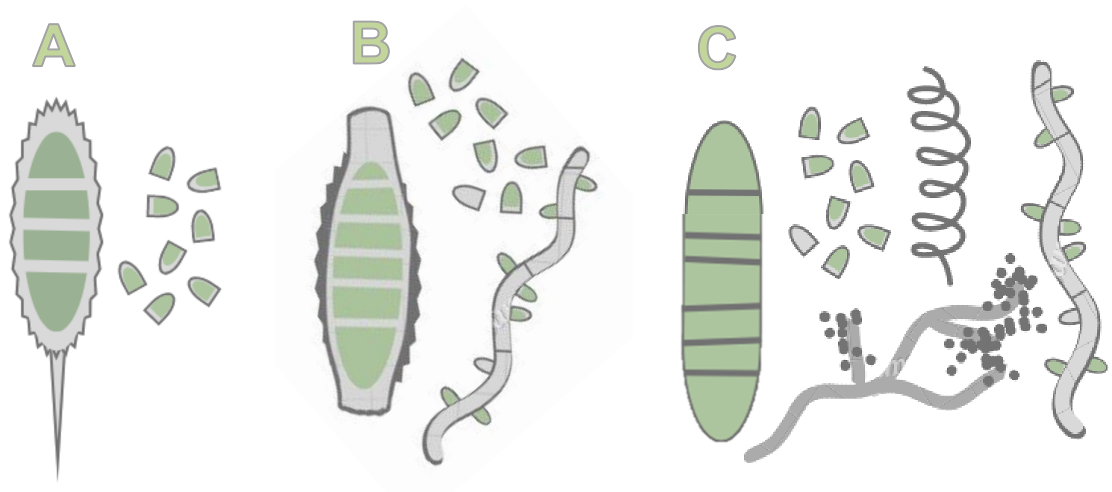

Figure 12 – Microscopic characteristics of dermatophytes. Source: original

A: *M. gypseum*; B: *M. canis*; C: *T. mentagrophytes*

Figure 13 - Spiral hyphae on a microscopic view of *T. mentagrophytes.*
Source: original

3.3.3. Other approaches to dermatophyte diagnosis

For the diagnosis of dermatophyte presence and dermatophytosis, there are further techniques to consider (Table 2).

Dermoscopy is a useful tool in POC circumstances. Although it only permits a more thorough skin and hair inspection, it may assist in the initial clinical screening as it facilitates the identification of possible fungi infected hairs for direct examination and culture (Pinheiro et al. 2012; Moriello et al. 2017). It utilizes a dermoscope, which is a hand-held non-invasive instrument that magnifies and illuminates the skin's surface. Infected hairs are often opaque, slightly curled or broken, with slanting and sharp ends and uniform in thickness (Scarampella et al. 2017). White to yellow scales may also be seen (Scarampella et al. 2017). This method does not aid in identifying the causative agent of dermatological lesions, nor does it contribute significantly to the diagnosis of dermatophytosis (Moriello et al. 2017).

Another tool that can and is often used in POC is the Wood’s lamp. However only 50% of *Microsporum* species will fluoresce under its light, with the other species and genera not being responsive to this diagnostic tool (Canny and Gamble 2003; Moriello et al. 2017). Because the most prevalent species in rabbits do not glow under this lamp, it is not an effective diagnostic tool for this animal.

Still in a POC scenario, cytology analysis may be beneficial, particularly in the case of exudative lesions. With this approach, arthrospores can be observed and infection can be confirmed. However, this technique produces several false negatives because samples are not necessarily indicative of the entire situation (Moriello 2019).

Working with a reference laboratory provides access to additional resources. PCR, for instance, has been investigated recently and is becoming more accessible for dermatophyte testing nowadays. The short turnaround time of days, as opposed to weeks, is its greatest advantage over fungal culturing. It is a highly sensitive test that can identify both viable and non-viable DNA. Because of this, similarly to fungal culture, it cannot differentiate between carriage and genuine disease; hence it is essential to collect samples only from the target lesion and not the entire coat. False negatives are uncommon unless inappropriate sampling procedures are done. This is also an effective tool for monitoring mycological recovery; for this however, it is necessary to bathe and dry the animal before sampling in order to eliminate any non-viable DNA material (V. and Jr. 2017; Moriello 2019). It is crucial to keep in mind, though, that PCR can be a more expensive alternative, and few clients opt for it.

The same holds true for a histological approach; it must be performed in a reference laboratory and may cost more than POC methods. Although it provides a more thorough and extensive investigation of the animal's skin and coat condition, it does not aid in identifying the aetiology for any potential lesions (Canny and Gamble 2003; Anikar et al. 2022). However, it may be quite useful in animals with uncommon dermatological manifestations and in circumstances where healing has proven challenging (Moriello et al. 2017). In addition, while sending the sample to the laboratory, it is essential to inform the pathologist that dermatophytosis is suspected, as ordinary stains are not as sensitive as periodic acid-Schiff or Gomori's methenamine silver for detecting fungal elements (Moriello 2019).

Also, due to recent dermatophyte antifungal drug resistance and increased virulence of these fungi, molecular identification (MI) and Antibiotic Sensitivity Tests (AST) are advised in cases of chronic, recurring, and atypical presentations (Cutler et al. 2007; Portuondo et al. 2014; Al-Janabi and Al-Khikani 2021; Hoog et al. 2021). However, currently there are no guidelines available for this to be done.

Table 2 - Comparison of various methods for dermatophyte diagnosis

	Comparison of diagnostic tests								
	POC	**fast**	**affordable**	**non-invasive**	**observ. lesions**	**caus. agent**	**Id. species**	**carriage / disease**	**false+ false -**
F. culture (DTM)	✓	✗	✓	✗	✗	✓	✗	✗	✓
Trichogram	✓	✓	✓	✗	✓	✗	✗	✗	✓
Dermoscopy	✓	✓	✓	✓	✓	✗	✗	✓	✓
Wood's Lamp	✓	✓	✓	✓	✗	✓✗	✗	✗	✓
Cytology	✓	✓	✓	✗	✓	✓	✗	✓	✓
F. culture (SDA)	✗	✓	✗	✗	✗	✓	✓	✗	✗
PCR	✗	✓	✗	✗	✗	✓	✓	✗	✓
Histology	✗	✗	✗	✗	✓	✗	✗	✓	✓
Molecular id.	✗	✗	✗	✗	✗	✓	✓	✗	✓

Note: ✓ - yes ✗ - no ✓✗ - semi (in wood's lamp case, it can identify *M. canis* but no other species).

3.4. Treatment and clinical management of dermatophytosis in rabbits

In immunocompetent individuals, dermatophytosis is frequently self-limiting (Weitzman and Summerbell 1995; Kraemer et al. 2013). However, because it might persist from weeks to months, treatment should still be provided in order to limit the disease's duration and thereby prevent transmission to other animals and certain public health concerns (Moriello et al. 2017; Melo et al. 2018). Additionally, not only do animals and humans respond well to therapy, but the available antimycotics have little systemic side effects, making treatment an overall win-win situation (Gupta et al. 2005).

3.4.1. Medical approach

Dermatophytosis is usually treated with both topical and systemic drugs (Figure 14), as combining them has been demonstrated to be more effective than individual therapy and can help reduce the duration of treatment (Anikar et al. 2022; Mishra et al. 2022). On the market, there is a sufficient selection of antifungal drugs for both presentations, and they are generally effective in treating the illness (Hoog et al. 2021; Anikar et al. 2022).

For systemic treatment, itraconazole 5 mg kg^{-1} or ketoconazole 10-15 mg kg^{-1}, both taken *per os* (PO), two times a day (BID) may be used. Griseofulvin can also be administered orally in this scenario at a dosage of 25 mg kg^{-1}, once a day (SID) or BID. There have been no reports of griseofulvin's negative effects on rabbits, however it is teratogenic in dogs and cats and can cause anorexia and suppression of bone marrow. Therefore, the previous alternatives should be given preference in this treatment. In addition, it is essential to highlight that all of these medications should be offered with a high-fat meal, as they are poorly water-soluble and fatty foods enhance gastrointestinal absorption (Zhi et al. 1995; Chang et al. 2022; Mishra et al. 2022).

Miconazole and clotrimazole are two excellent topical options for treating this problem. They are available as ointments, lotions, and creams and can be administered daily or weekly (depending on the product) on lesions on the skin. To reduce the likelihood of zoonotic transmission, owners should be encouraged to wear gloves. The use of lime sulphur baths, dips, and sprays should be avoided in rabbits, as they are extremely susceptible to stress and respiratory disease, and there have even been accounts of these animals passing away as a result (van Cutsem et al. 1985; Harvey 1995; Canny and Gamble 2003; Mishra et al. 2022). In order to apply topical treatment, local lesions should not be clipped so as not to abrade the skin and exacerbate the infection, as well as to prevent environmental contamination. However, if dealing with a long-haired breed, the hair surrounding the lesions may be trimmed and immediately disposed of in an appropriate manner (Canny and Gamble 2003).

Treatment should continue for at least two weeks, until clinical remission, or until two negative fungal cultures are obtained four weeks apart, whichever occurs first (Canny and Gamble 2003) (Moriello 2003).

Although antifungal drugs might have few adverse effects, it is many times protracted (Dowd 2007); thus, it is crucial to remember that this treatment may have a severe impact on hepatic and renal health. These functions should then be monitored throughout the extended duration of therapy and, additionally, hepatoprotective and renoprotective medicines may be recommended (Anikar et al. 2022). For instance, Silymarin and S-adenosyl-methionine are effective liver protectors in rabbit medicine. Vitamin A, mineral supplements, and topical iodine application are also claimed to increase the rate of healing, and are thus indicated (Ganguly et al. 2017).

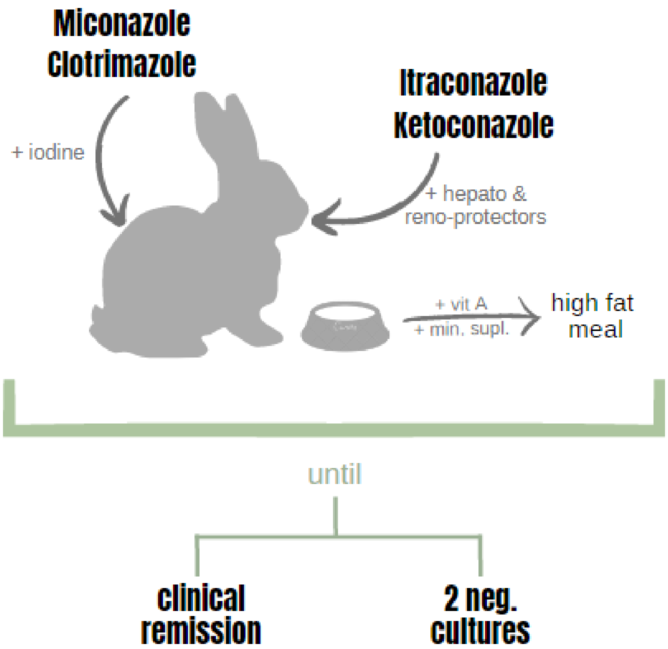

Figure 14 - Dermatophytosis treatment in rabbits. Source: original

3.4.2. Environmental approach

The health of humans, animals, and the environment are interconnected and must be managed as a whole in order to prevent continued or recurrent transmission and to deepen our awareness of the "One Health" idea (Souza 2011).

Affected animals must be separated from other animals, and all animals that have been in contact with them must be screened. Since it may take up to 3 weeks for the screening results to come, preventative topical therapy should be used in the meantime (Moriello 2003).

Continued meticulous and aggressive cleaning of the environment is required until the infection is cured (Canny and Gamble 2003; Moriello 2003). Recommendations include vacuuming (with safe disposal of the vacuum bag) and cleaning the area with 1:10 bleach dilution. Additionally, bedding, rugs, and brushes must be discarded (Canny and Gamble 2003).

3.4.3. Rabbit farms

Since rabbit breeding has become a large-scale industry, the prevalence of dermatophytes in these rabbits has increased and, as a result, has become a public health concern (Xiao et al. 2019). In addition, rabbit dermatophytosis poses a significant threat to the development of the rabbit breeding sector (Xiao et al. 2019); hence, it is essential that rabbit farms have access to efficient treatment options.

Individualized therapy is not a practical option in rabbit farms due to the frequent shortage and high cost of personnel (van Cutsem et al. 1985). Some authorities suggest exterminating the entire animal population. However, environmental disinfection may be a successful alternative. Spray or fogger formulations of 50 mg m^{-2} enilconazole emulsifiable concentrate have a fungistatic and fungicidal effect, destroying spores without requiring direct animal contact (van Cutsem et al. 1985). In addition, this treatment has no adverse effects and produces lasting results (van Cutsem et al. 1985). In this way, not only is the animal population preserved, but there is also no need for individual treatment, saving both time and money.

3.5. Dermatophyte Prevention

Because all of what was mentioned prior, dermatophyte infections can become extraordinarily expensive (Achterman and White 2012). In addition, widespread emergence of azole resistance has been recorded (as high as 19%), contributing to treatment failures and the persistence and chronicity of infections (Hoog et al. 2021). Remissions and relapses after therapy are typical with nodular lesions, and in that scenario euthanasia is frequently the conclusion in pet animals (Moriello et al. 2017). Taking all of this into account, it is evident why preventing this condition is so critical.

3.5.1. Preventing Dermatophytes in the Home

Education is essential to stopping the cycle of dermatophyte transmission, and veterinarians should be the primary provider of accurate information about this to owners (Robertson et al. 2000). These professionals have a responsibility to protect both animal and human health; therefore, they should recommend preventative measures to owners of animals with potential zoonotic diseases, advise clients on the dangers of exotic pets, provide educational materials, and work directly with physicians and other health care professionals to facilitate the "One Health" concept (Souza 2011).

3.5.1.1. Pet Rabbits

Due to the fact that pet rabbits are the primary source of dermatophytes for their owners, it is essential to take measures to prevent their presence in these animals.

A pet's healthy skin is largely dependent on its owners' and keepers' knowledge and commitment to various husbandry and management practices (Anikar et al. 2022). These techniques should be emphasised even more when working with young animals and/or when dealing with hot, humid environments and seasons.

Dermatophytes are transferred mostly through direct contact with sick or carrier animals. In this regard, and in order to reduce the prevalence of dermatophytosis in the home, it may be recommended to test newly acquired pets by fungal culture before introducing them to the already existing ones (Kraemer et al. 2013). This is relevant not only because the new pets may be asymptomatic carriers and transmission may occur, but also because, if the existing pets are carriers themselves, the stress of the new pets being introduced may promote the growth of dermatophytes and raise the likelihood of a home outbreak (Kraemer et al. 2013; d'Ovidio and Santoro 2015).

In addition to being transmitted via direct contact, dermatophytes can also be transferred to an animal's coat via a contaminated environment. Therefore, in order to prevent dermatophyte infections, it is crucial to maintain cleanliness and proper sanitation of the home. This is particularly necessary when dealing with rabbits that are allowed to roam free, as they are the most susceptible. This goes not only for the environment, but also for any potentially contaminated objects, such as grooming brushes and bowls (Moriello 2020).

The importance of grooming in preventing dermatophyte transmission cannot be overstated. Grooming tools, however, can cause micro-trauma to the skin while simultaneously acting as a fomite for dermatophyte spores. Given this, the best course of action is to ensure the animal can groom itself, and if not, to lightly groom them and disinfect tools prior and after usage (Weitzman and Summerbell 1995).

Like fomites, ectoparasites can also disrupt the *stratum corneum* and cause itching, microtrauma and scale production on the rabbit's skin, making it more susceptible to arthrospores. In this sense, parasite prevention could also reduce the risk of dermatophyte infection in these animals. Generally, 2 doses of Selamectin (18-20 mg kg^{-1}) with a 4 week interval, every 6 months, is the protocol used for this purpose (d'Ovidio and Santoro 2015; Chang et al. 2022).

A healthy diet also plays a part in maintaining the animal’s skin health, as well as maintaining good dental condition and body weight. It has been suggested that supplementing with minerals and vitamins can aid in reducing the occurrence of dermatophytosis in rabbits, as the animal's skin more easily keeps its integrity, and its immune system is maintained by the high nutritional value (Mishra et al. 2022).

As shown, there are numerous measures that may be taken to prevent dermatophytosis in pet rabbits and, ultimately, an outbreak in the household (Figure 15).

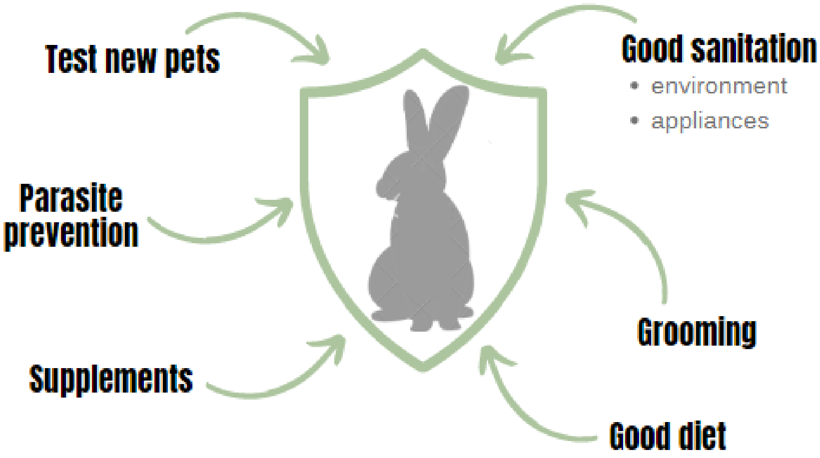

Figure 15 - Dermatophytosis prevention measures in rabbits.
Source: original

3.5.1.2. Humans

Regarding humans, the prevention of this disease rests primarily on proper hygiene and greater awareness, especially when young, elderly, or immunocompromised individuals are involved (Riley and Chomel 2005; Medici and Poeta 2015). As it is with rabbits, in a hot and humid environment, these precautions should be taken even more seriously (Moriello 2003). In addition, applying these preventative practices is ever more essential while residing in regions with a dense population of feral animals (in addition to avoiding contact with such animals), as they are mechanical vectors for dermatophyte spores (Moriello 2003).

Keeping a pet rabbit greatly increases the likelihood of coming into contact with dermatophytes; people should be informed and aware of this (Moriello 2003; Riley and Chomel 2005). Ensuring prophylactic measures are being taken to avoid dermatophytes on their pet rabbits, is a critical habit people must have to prevent these fungi in themselves. Additionally, rabbit owners should routinely wash their hands after handling their animal and ensure that everyone who contacted with their pet does the same. Similarly, these animals should not be kept in locations where food is prepared and house textiles and clothing should be regularly washed (Riley and Chomel 2005; Chang et al. 2022).

Although most worldwide scientific organisations have deemed antifungal vaccinations to be impracticable, The World Health Organization (WHO) and the International Society of Human and Animals Mycology (ISHAM) highly recommend dermatophytosis vaccination to be researched and developed in the foreseeable future (Deepe 1997; Brouta et al. 2002; Portuondo et al. 2014; Al-Janabi and Al-Khikani 2021). To date, no vaccine has been licensed for the use in humans (Portuondo et al. 2014). If this vaccine is successfully developed, it will play an important role in limiting the use of chemotherapy and antifungal agents for the control of fungal infections, as well as for the enhancement of the efficiency of the immune system (Cutler et al. 2007; Portuondo et al. 2014; Medici and Poeta 2015). It would also substantially reduce the treatment's time and cost, while also giving long-term protection (Al-Janabi and Al-Khikani 2021). Furthermore, and although animals would likely continue to be asymptomatic carriers, a vaccine would limit the transferring of dermatophytes between humans and animals while also reducing the toxicity and virulence effects of this disease in people (Criado et al. 2011; Al-Janabi and Al-Khikani 2021).

3.5.2. Preventing Dermatophytes in the workplace

People working with rabbits should be extra careful in avoiding dermatophyte transmission between the animals and themselves. They should take proper protection and hygiene measures when handling these animals, especially the diseased ones (Mishra et al. 2022) (Figure 16).

Firstly, regular cleaning and disinfection of equipment and surfaces between uses or whenever visually soiled, is essential for controlling infections in the workplace environment. Ideal practise is to use water and detergent prior to disinfection, using all materials according to the labels' instructions. Quaternary ammonium compounds and hypochlorites are the most commonly used. Examination tables, door knobs, cage latches, faucet handles, and sinks require special attention and should be made of nonporous and easily cleaned materials. Additionally, when cleaning the floors, vacuums with air filters and wet mopping should be utilised, as they reduce dust production and, consequently, spore dispersion. Integrated pest management (particularly for rodents) is also of the utmost importance, as these animals can spread dermatophyte spores (Williams et al. 2015).

The personnel should be supplied with a break room for eating and drinking, as well as dishwashing and storage areas where animal items are prohibited (i.e. food and water bowls, toys, litter boxes, etc). There should be a designated washing space for animal bowls, cages, toys, litter boxes, and other objects, for a proper cleaning between patients. Typically, routine dishwashing is sufficient for this task (Williams et al. 2015). In addition, these items should be routinely disinfected after washing, and their exchange between animals must be avoided (Chang et al. 2022). The same applies to laundry, where ordinary washing and drying by machine is sufficient. When handling any of the mentioned objects, Personal Protective Equipment (PPE) should be worn. This may include lab coats, smocks, aprons, coveralls, gowns, shoes, and head covers. Employers should be the ones supplying these materials and explaining how to use them to their employees (Williams et al. 2015).

Consistent and thorough hand hygiene is an additional step that must be taken to avoid the spread of fungi in the workplace. Hand cleansing with liquid soap and water, the use of alcohol-based hand rubs, and the proper usage of gloves constitute the single most essential precautions personnel can take in this regard. Moreover, disposable towels should be used for the drying process, and staff should not wear artificial nails or jewellery, as this affects the efficacy of hand hygiene. It is also recommended that employers offer employees individual hand moisturising lotions, not only to protect skin integrity but also to encourage staff adherence to this procedure. Likewise, strategic placement of the hand sanitizer stations may promote hand hygiene compliance (Williams et al. 2015).

As can be seen, there are a number of methods that can be used to minimise the likelihood of a dermatophyte outbreak in the workplace. Adding to this, and because pet shops have been identified as the primary source of pet rabbits in several countries, further preventative measures at these stores are vital. Each of the rabbits arriving at the store should be quarantined in a clean area and tested for fungi (Miller and Hurley 2009; Chang et al. 2022). In addition, despite the fact that rabbits are social creatures and this may end up being a source of stress, it is recommended to avoid keeping multiple rabbits in the same cage in order to prevent disease transmission (Williams et al. 2015).

At veterinary medical facilities, all of the abovementioned procedures must be performed consistently between examination of individual animals and groups of animals (*e.g*. litters). Furthermore, staff should pay great attention when treating any skin problem. In this case, workers must wear protective outerwear and gloves, as well as practise hand hygiene and remove their gear afterwards. Moreover, waiting rooms should be clean and safe for clients, animals, and medical personnel. This entails that reception staff asks incoming clients about the purpose of their visit and observes all pets for external indicators of skin illness. The sick animals should be taken promptly to an examination room, which should stay out of use until it has been properly cleaned and disinfected (Williams et al. 2015).

Likewise, professionals in necropsy environments and laboratories should wear gloves, face protection, and protective clothing. At the end, gloves must be discarded and handwashing is essential (Williams et al. 2015).

All of the aforementioned procedures are also applicable to workers in rabbitries. However, running water is often unavailable in these establishments, making hand hygiene somewhat more difficult. In this scenario, before applying an alcohol-based hand rub containing 60 to 95% ethanol, it is advisable to wipe the hands with a damp wipe to eliminate any organic material that may be present (Williams et al. 2015).

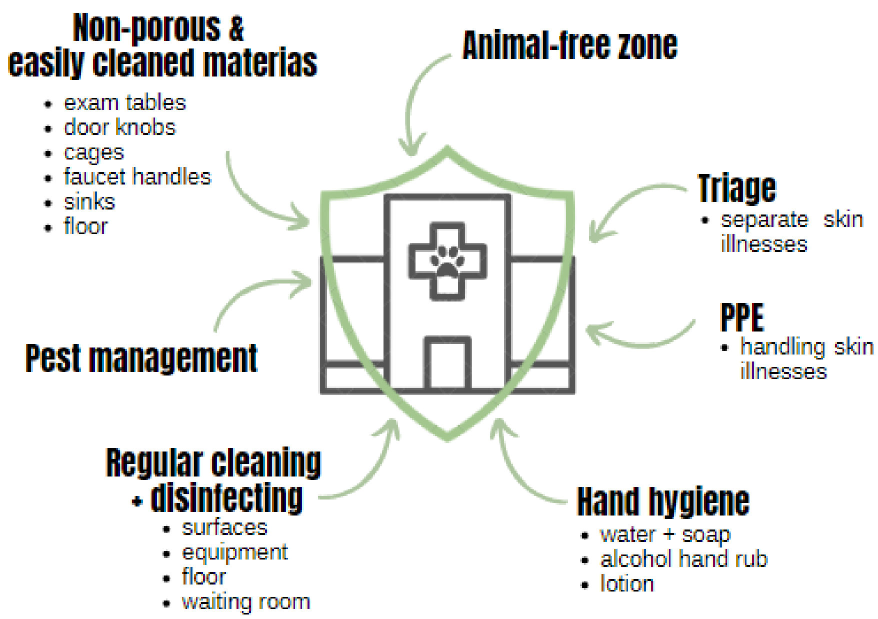

Figure 16 – Measures for dermatophyte presence in the workplace.
Source: original

4. Analysis of dermatophyte prevalence and associated risk factors in rabbits

In the view of rabbits being one of the primary sources of dermatophytes for humans and becoming increasingly popular as household pets, it is essential to comprehend the incidence of dermatophytes in these animals' fur. Furthermore, because asymptomatic animals are still important sources of these zoonotic fungi despite the absence of clinical symptoms, it is crucial to focus this investigation on asymptomatic animals in order to determine how many healthy animals still pose a significant risk to their families worldwide.

4.1. Aims of this Study

This study mainly intends to contribute to the current understanding of the prevalence of dermatophytes in the fur of pet rabbits in Porto, Portugal, as well as the fraction of asymptomatic carriers and the associated risk factors. To achieve this, the research was guided by a number of minor goals, which can be summarised as follows:

- Gathering the medical and risk factor histories of the maximum number of rabbits brought to the clinic.
- Collecting hair samples from those rabbits.
- Assessing every sample for the presence of dermatophytes.
- Determining the incidence of dermatophyte carriers as well as the proportion of asymptomatic individuals.
- Predicting the most effective prevention measures against dermatophytes in the home and workplace.

4.2. Materials and Methods

This study took place in Centro Veterinário de Exóticos do Porto, in Porto, Portugal. The study was conducted on rabbits brought to the clinic between September 2021 and February 2022.

4.2.1. Consultation

In order to determine the existence of dermatophytes, several diagnostic methods had to be implemented; however, some information regarding the health of these animals was required beforehand (Figure 17). All of the participants' owners in this study were willing to provide their pet's information anonymously and signed an informed consent form, which is presented in Annexe 1.

Firstly, a medical examination was conducted on each rabbit, not only to establish their overall well-being, but also to note body weight, sex, fur type and condition, skin lesions and possible ectoparasite presence. Secondly, the owners were questioned for more valuable information; this included where the rabbit had come from (pet store, home breeding), how old it was, if it had been neutered before, what was the housing situation (cage, park, free roaming) and what did the rabbit's diet consist of, if it had contact with any other animals or exterior environments, if parasite prevention was routinely done, as well as vaccination, if the animal could groom itself or was otherwise groomed by the owners and lastly, whether there were any children living in the household.

Clinical history and the cause for the veterinarian visit were also taken into account, as concomitant illnesses and any applied drugs can affect the presence of dermatophytes in the animal's fur.

All of this information was systematically collected on all rabbits, as detailed in Annexe 2.

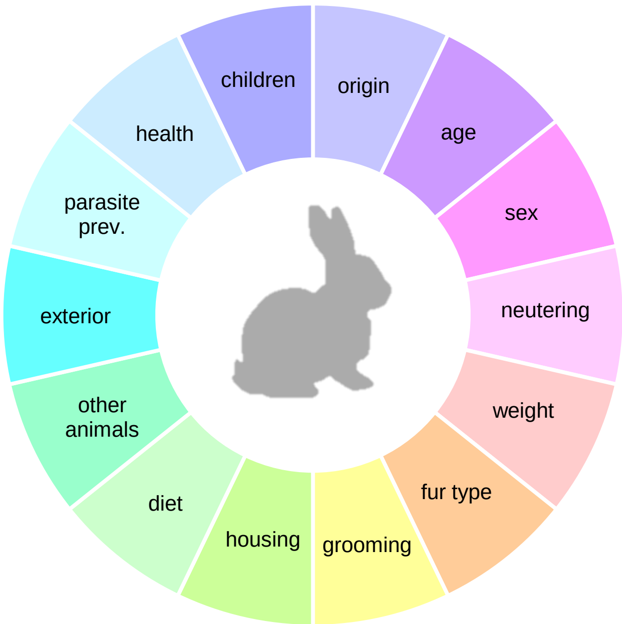

Figure 17 - Data collected during consultation. Source: original

Because information such as age, nutrition, weight, etc. might be relatively general and vary much between rabbits, it was necessary to build groups to accommodate the results. This allowed for the creation of a database that was much more cohesive and workable.

4.2.1.1. Age

For statistical convenience, the animals were separated into groups according to their ages. 5 groups were established, as depicted in figure 18.

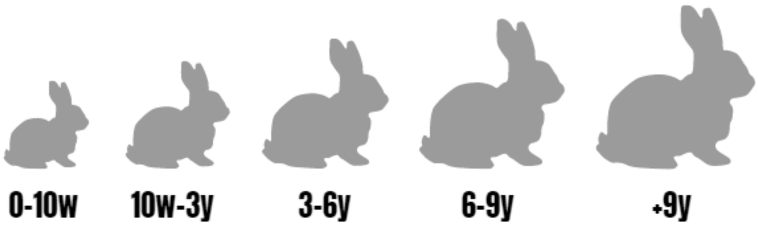

Figure 18 - 5 age groups created for this study. Source: Original
w = weeks ; y = years

4.2.1.2. Weight

The weight condition of these rabbits was evaluated by dividing them into three groups: underweight, ideal weight, and overweight. The first group consisted of animals with sharp hip bones, ribs, and spine, loss of muscle and zero to minimal fat cover, and an inwardly curved rump area. Ideal weighted rabbits had easily palpable hip bones, ribs, and a spine that were not sharp, no abdominal bulge, and a flat rump. Finally, overweight rabbits featured difficult-to-feel hip bones, spine, and ribs, fat layers with a sagging abdomen, and a rounded, protruding rump (Figure 19).

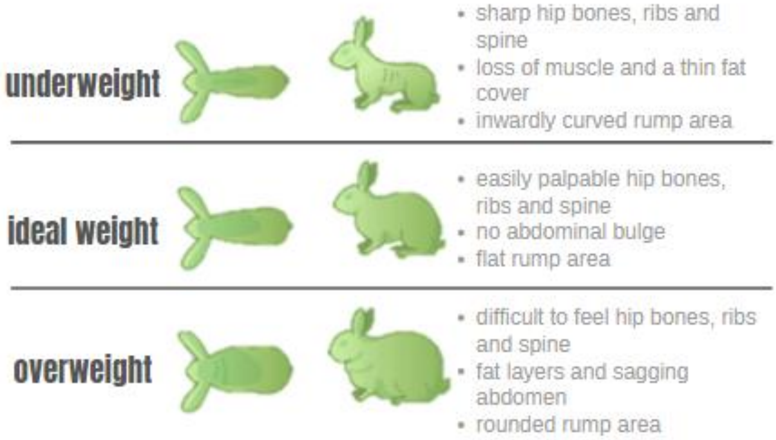

Figure 19 - Simplified weight categorization. Source: adapted from (PFMA 2015)

4.2.1.3. Fur Type

The sampled animals were also divided into three fur length groups: short-haired (<2cm), medium-length haired (2-5cm), and long-haired (>5cm).

4.2.1.4. Housing

Based on their housing conditions, the rabbits were categorised into 3 groups: cage, park and free roaming (Figure 20).

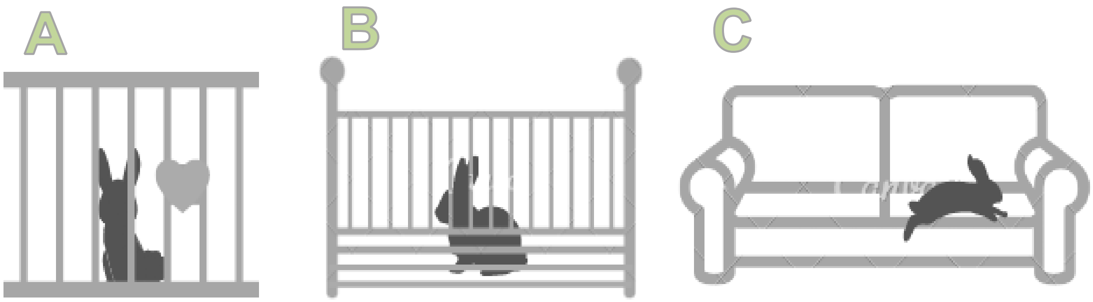

Figure 20 - Types of rabbit housing. Source: original
A – Cage; B – Park ; C – Free roaming

4.2.1.5. Diet

For diet assessment in the context of this study, 3 primary factors were considered: if the diet was nutritionally balanced, whether it included fern and if it was provided in an adequate amount. Based on that, 3 groups were formed: bad diet (fulfilled 0-1 of the requirements), medium diet (achieved 2 of the requirements), and good diet (all criteria were met).

4.2.1.6. Health

The majority of the rabbits involved in this experiment were brought to the veterinarian's office because they were experiencing some kind of health problem. For the sake of analysis and statistics, these problems had to be grouped. In the end, 4 "health-problem categories" were identified: oral issues (O), mobility difficulties (M), dermatological symptoms (D), and systemic conditions (S).

4.2.2. Diagnostic methods used

In this research, trichogram and DTM fungal culture were used to detect the presence of dermatopytes on rabbit fur.

In addition to identifying damaged hairs for trichogram, dermoscopy could facilitate a closer examination of skin lesions. However, as the majority of the participants in this study were asymptomatic carriers, its value was limited and it was therefore not utilised. The same holds true for cytology and histology, since in most cases, there were no lesions from which to get samples.

The Wood's lamp test, despite being a POC and inexpensive procedure, would only be beneficial for a few *Microsporum* species, and as the majority of dermatophytes affecting rabbits are *Trichophyton* species, it was of little use in this study.

Although trichogram is an unreliable approach for finding dermatophytes in asymptomatic carriers, it is a simple, accessible, and inexpensive diagnostic tool. In addition, when paired with fungal culture, it increases the diagnostic value.

Fungal culture was crucial to the success of this project. SDA growth medium provides a more accurate diagnosis, but it is not POC-friendly, as opposed to DTM. Since this project was self-funded, PCR and molecular species identification were excluded from this project as they are significantly more expensive tools.

4.2.3. Sample collection

4.2.3.1. Dermatologically healthy rabbits

In rabbits without dermatophyte-compatible lesions, the primary goal was to determine whether or not they were asymptomatic carriers and if they posed a threat to the welfare of their family.

As mentioned before, spores typically adhere to the hair shafts of the rabbit’s hair. In this regard, the objective was to collect sufficient fur for study without harming these structures. To achieve this, the Mackenzie's brush technique was utilised, combing the animal's entire coat from front to back.

4.2.3.2. Rabbits with Dermatological signs compatible with dermatophytosis

For ill animals, the previously described Mackenzie's brush technique was used, concentrating on the affected areas and avoiding those that had been recently medicated. Additionally, more samples were obtained from these animals utilising various techniques. These included hair plucking and the collecting of scabs and crusts, all of which from the animal's lesions' periphery.

4.2.4. Sample analysis

4.2.4.1. Direct Microscopy

Some of the hairs and scabs obtained from each animal were immediately mounted on a microscope slide with liquid paraffin. They were then fixed with heat (in this project, a hair dryer was chosen), and covered with a glass lamella.

Using an optical microscope (*Leica Microsystems* ®) at a magnification of 4/10x, the slide was examined for damaged hairs. When any were discovered, those hairs were examined at higher magnifications for the presence of dermatophytes. When observing scabs and crusts however, a greater magnification was always used.

4.2.4.2. Isolation and Identification of fungi

The remaining hairs and scabs that were not used for direct microscopy were used for inoculation and fungus growth.

For this purpose, after combing the animal's coat, when the toothbrush was full of hairs, the bristles were pressed into the medium 6 to 8 times, so that individual impressions were evident. This was done avoiding over-inoculation, which could subsequently impede sporulation and fungal growth. Plucked hairs, crusts and scabs were also pressed into the growth medium using sterile haemostatic forceps to ensure its stay in the plate.

The plates were then stored in individual plastic bags, preventing cross contamination. The bags were then placed inside a box that was kept in a room with a constant temperature of 30ºC, for a 21 day period. Except for when samples were being collected for analysis, the box was kept closed at all times.

4.2.4.2.1. Macroscopic Analysis

The DTM plates were examined daily for changes in colour and colony growth. Within 21 days, if no colony development or colour change happened, the test was ruled negative. However, where there was no colour change yet colony formation was visible, these colonies were still inspected under a microscope to ensure it was not a case of false-negativity (Figure 21). Furthermore, as noted previously, dermatophyte colonies developing on this medium have a distinct appearance, thus a comprehensive macroscopic examination of these colonies was also conducted.

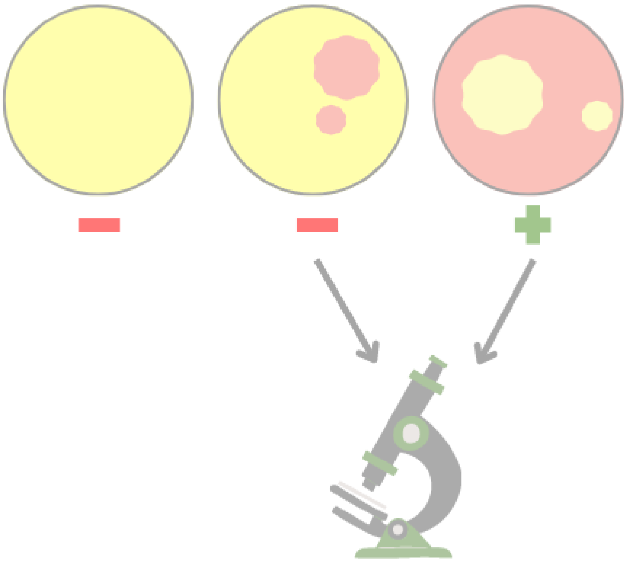

Figure 21 – Samples' colonies which go through microscopic analysis.
Source: original

4.2.4.2.2. Microscopic Analysis

If colony growth occurred within 21 days, regardless of whether the medium changed colour, a microscopic examination was conducted. Using clean forceps, the adhesive side of a strip of tape was applied to the surface of the colony so that biological material adhered to it. The sticky tape was then placed on a microscope slide and inspected under the optical microscope for macro- and microconidia.

4.2.5. Material Disposal

Following the required guidelines, all biological waste was transferred for proper disposal. This included DTM dishes, microscope slides, and toothbrush heads that had been previously detached from their handles.

The haemostatic forceps and toothbrush handles were carefully disinfected, and the brushes' handles were then donated to a solidarity initiative.

4.2.6. Data management

The acquired data for this investigation were organised, summarised, and analysed with Microsoft Excel 2010.

4.3. Results

4.3.1. Sample characterization

The sample included 63 healthy and sick animals, with 62 dwarf rabbits and 1 non-dwarf. To maintain the sample as homogeneous as possible, the latter participant was excluded from the study.

4.3.1.1. Season

The majority of this study's samples (90%) were collected during winter, while the remaining 10% were collected in autumn (Table 3).

Table 3 – Distribution of samples by season of sampling

	Rabbits	
	nº	%
Winter	56	90
Autumn	6	10
Total	62	100

4.3.1.2. Origin

Pet stores were the source of 84% of the rabbits in this sample, while the remaining 16% were the result of home breeding of different sorts (Table 4).

Table 4 – Distribution of animals by place of origin

	Rabbits	
	nº	%
Pet Shop	52	84
Home Breeding	10	16
Total	62	100

4.3.1.3. Age

The average age of the rabbits that took part in this research was 4 years old ± 0,5 (Figure 22), with the smallest participant being only 4 weeks old and the oldest participant being 12.5 years old.

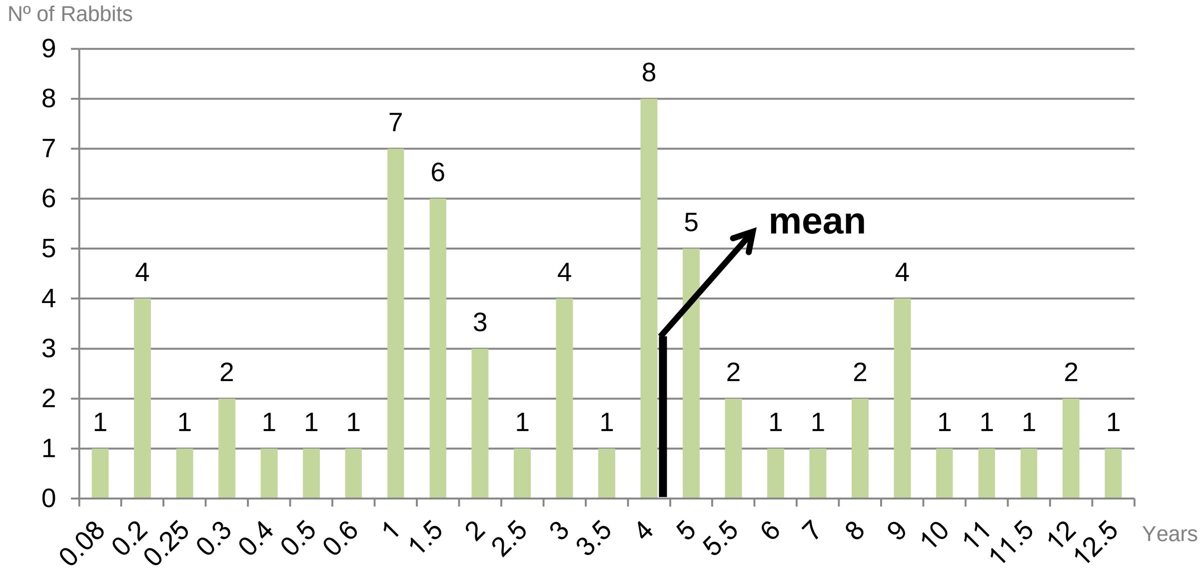

Figure 22 - Animals' age distribution

The largest proportion of this study's rabbits (44% of the total) was between the ages of 10 weeks and 3 years. After this category, the rabbits that were between 3 and 6 years old were the most prevalent, making about 27% of the overall sample. 11% were between the ages of 6 and 9 years old, 10% were between the ages of 9 and 13 years old, and 8% were between 0 and 10 weeks old (Figure 23).

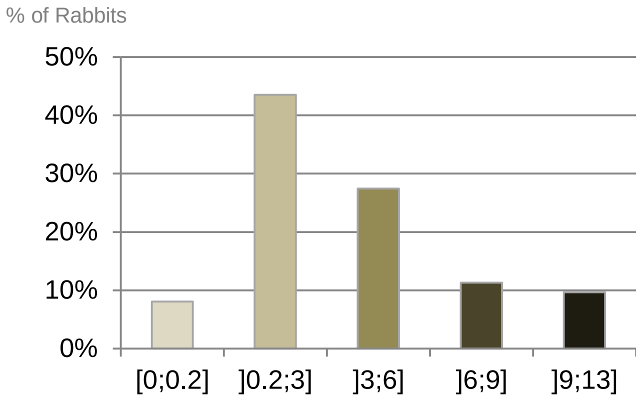

Figure 203 - Percentage of animals per age group

4.3.1.4. Sex and Neutering

Five of the 62 rabbits in the study were too immature to determine their sexes. For this reason, they have been excluded from sex and neutering related statistics.

The sample for this investigation was made up of 74% male rabbits, while the female rabbits made up roughly 26% of the total number of animals (Table 5). 44% of the rabbits were neutered, while the remaining 56% were not. 38% of the males had undergone the castration procedure and 62% had not. However, this is not the case with female animals, since more than half of them (60%) had been spayed while the others had not (40%).

Table 5 - Distribution of animals by sex and neutering status

	Rabbits	
	nº	%
Males	42	74
Neutered	16	28
Unneutered	26	46
Females	15	26
Neutered	9	16
Unneutered	6	10
Total	57	100

4.3.1.5. Fur type

The bulk of the rabbits who took part in this investigation had short fur; in fact, 50% of the total sample was comprised of animals that fell into this group. The remaining 50% either had medium length hair or longer (Table 6).

Table 6 - Distribution of animals by fur type

	Rabbits	
	nº	%
Short	31	50
Medium	17	27
Long	14	23
Total	62	100

4.3.1.6. Grooming

The animals' caretakers reported that 21% of the rabbits had their fur groomed on a regular basis, while the remaining 79% did not receive this type of maintenance (Table 7). It is interesting to note that the majority of animals who had their hair routinely brushed (87%) also had lengthy hair.

Table 7 - Distribution of animals by grooming situation

	Rabbits	
	nº	%
Groomed	13	21
Not Groomed	49	79
Total	62	100

4.3.1.7. Diet

It was determined that approximately half of the animals used in this investigation were provided with appropriate dietary management (47%). 22% of them received average care in this regard, while the remaining 33% did not have a diet that was beneficial to their skin, teeth, body weight or overall health (Table 8).

Table 8 – Distribution of animals by diet quality

	Rabbits	
	nº	%
Good diet	29	47
Medium diet	14	22
Bad diet	19	31
Total	62	100

4.3.1.8. Body Weight

The majority of the rabbits that were sampled, which made up 76% of the total, were classified as having a "desired weight." Only 3% of the remaining 24 were assessed to have an unhealthy high weight, while the other

Twenty-one percent were considered to be underweight (Table 9).

Table 9 - Distribution of animalss by weight

	Rabbits	
	nº	%
Underweight	14	23
Average	44	71
Overweight	4	6
Total	62	100

4.3.1.9. Housing

Thirty-two percent of the rabbits in this study were kept in cages, 39% were allowed to run free, and 29% were kept in a park (Table 10).

Table 10 - Distribution of animals by housing situation

	Rabbits	
	nº	%
Cage	21	34
Park	22	35
Free Roaming	19	31
Total	62	100

4.2.1.10. Contact with other animals

The vast majority of the animals in this sample, which accounts for 63% of the total, had not interacted with any other animals previous to their appointment at the clinic. The remaining 37% either had frequent contact with another animal or had come into contact with one in the year leading up to the consultation (Table 11).

Table 11 - Distribution of animals by existence of contact with other animals

	Rabbits	
	nº	%
Contact with other animals	23	37
No contact with other animals	39	63
Total	62	100

4.3.1.11. Access to exterior environments

The vast majority of these study's animals, which accounted for 94%, did not have any access to exterior environments; only 6% did (Table 12).

Table 12 - Distribution of animals by access to exterior environments

	Rabbits	
	nº	%
Access to exterior	4	6
No access to exterior	58	94
Total	62	100

4.3.1.12. Deworming and external parasite prevention

Sixty-seven percent of the rabbits included in this study had been previously dewormed, while 33% had not. In relation to external parasite treatment and prevention, these proportions are flipped, with only 16% of clients having taken preventative measures. In addition, 53% of the rabbits received only an internal deworming and no exterior treatment. The bulk of the remaining 47% did not receive any treatment (31%), while 14% received both and 2% received only the external one (Table 13).

Table 13 - Distribution of animals by parasite preventative care

	Rabbits	
	nº	%
No prevention	19	31
External prevention only	1	2
Internal prevention only	33	53
Both preventions	9	14
Total	62	100

4.3.1.13. Vaccination

The primary vaccinations considered for this investigation were those for Myxomatosis and Rabbit Haemorrhagic Disease types I and II.

The majority of the animals (68%) had their vaccines up to date, while 32% did not (Table 14). The former matched up with those that also had their treatment for internal parasites up to date.

Table 14 - Distribution of animals by vaccination status

	Rabbits	
	nº	%
Vaccinated	42	68
Not vaccinated	20	32
Total	62	100

4.3.1.14. Health situation

In total, 81% of the animals were not in an ideal health condition. However, 19% were in fine shape and were just seeking medical care for preventative measures such as checkups, vaccinations, and deworming.

Among the sick animals, 63% had some type of systemic disease (GIT obstruction, RHVD2, floppy rabbit syndrome and urinary obstruction were the most commonly diagnosed), 77% had dental problems, 32% had diseases that hindered normal movement, and 12% had dermatological symptoms. Combining this information, and as shown in figure

24, we come to understand that the majority of animals could be grouped into 3 categories: healthy, with systemic disease or with systemic and movement-impairing disorders.

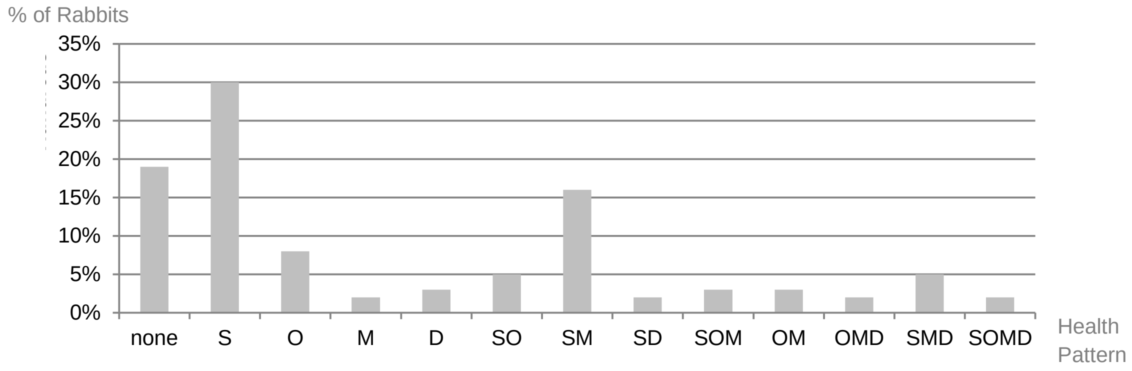

Figure 24 - Distribution of animals per health pattern observed

none – healthy; S – Systemic Disease; O – Oral disease; M – movement impairment; D – dermatological disease; SO – S + O; SM – S + M ; SD – S + D; SOM – S + O + M; OM – O + M; OMD – O + M + D; SMD – S + M + D; SOMD – S + O + M + D

4.3.1.15. Children in the household

Sixty percent of the rabbits in this study came from houses with children (Table 15). Moreover, these families reported greater direct animal contact than the 40% of households without children: 87% of them reported that members of the household frequently cuddled and petted the rabbit.

Table 15 - Distribution of animals by households with and without children

	Rabbits	
	nº	**%**
Children	37	60
No children	25	40
Total	62	100

4.3.2. Dermatophyte presence in the rabbits' fur

Twenty-one percent of the participant rabbits in this study had dermatophytes on their fur (Table 16) Even though the results of 79% of the samples were negative, the fact that 21% were positive implies that there are still a significant number of dermatophyte carriers in people's homes.

The dermatophyte *Trichophyton mentagrophytes* was the one identified in the fur of all rabbits that tested positive during the course of this study.

Table 16 – Samples' dermatophyte prevalence

	Rabbits	
	nº	%
Positive	13	21
Negative	49	79
Total	62	100

4.3.2.1. Season

All of the positive dermatophyte samples were collected during the winter season. Among the negative ones, however, 12% were gathered in autumn, while the remaining 88% were also collected in winter (Table 17).

From a different angle, 23% of winter samples resulted in positive tests while the remaining 77% and all of autumn samples were negative for dermatophytes.

Table 17 - Distribution of positive and negative samples by season

	Rabbits	
	nº	%
Positive	**13**	**21**
Autumn	0	0
Winter	13	21
Negative	**49**	**79**
Autumn	6	10
Winter	43	70
Total	62	100

4.3.2.2. Origin

There were no significant variations in origin between the positive and negative animals. In both groups, the majority of animals (85% of total positive animals and 84% of negative ones) originated from a pet store, while the remainder were born in a home breeding scenario (Table 18).

Even when analysing the data with focus on the different origins themselves, this lack of distinctiveness between groups remains. In both groups, around 20% of rabbits tested positive for the presence of dermatophytes on their fur: 20% of the home-bred rabbits and 21% of the pet store rabbits.

Table 18 - Distribution of positive and negative samples by place of origin

	Rabbits	
	nº	**%**
Positive	**13**	**21**
Home Breeding	2	3
Pet shop	11	18
Negative	**49**	**79**
Home Breeding	8	13
Pet shop	41	66
Total	62	100

4.3.2.3. Age

The majority (84%) of rabbits whose fur tested positive for dermatophytes were aged between 10 weeks and 6 years. The remaining 16% were evenly split between the 0 to 10-week-old and 6 to 9-year-old age categories; none were older than 9 years. The bulk (68%) of negative testing animals were also aged between 10 weeks and 6 years, whilst the remaining 32% were dispersed between the 0 to 10 week, 6 to 9 year, and 9+ age groups (Figure 25)

When examining the data from within each group, it is clear that the group of rabbits between 10 weeks and 3 years had the largest proportion of positive tests. The coats of 30% of these animals tested positive for dermatophytes. Following this group, the rabbits aged 0 to 10 weeks had a 20% positive test rate. 18% of rabbits aged 3 to 6 years tested positive, compared to 14% of rabbits aged 6 to 9 years. At the bottom of the list, none of the rabbits older than 9 years got positive results for the presence of dermatophytes on their fur (Figure 26).

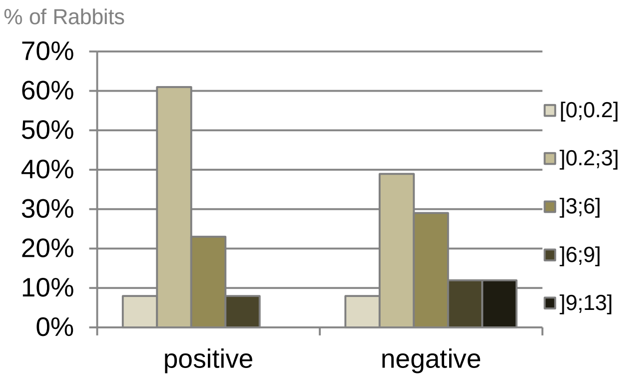

Figure 25 – percentage of each age group in positive and negative samples

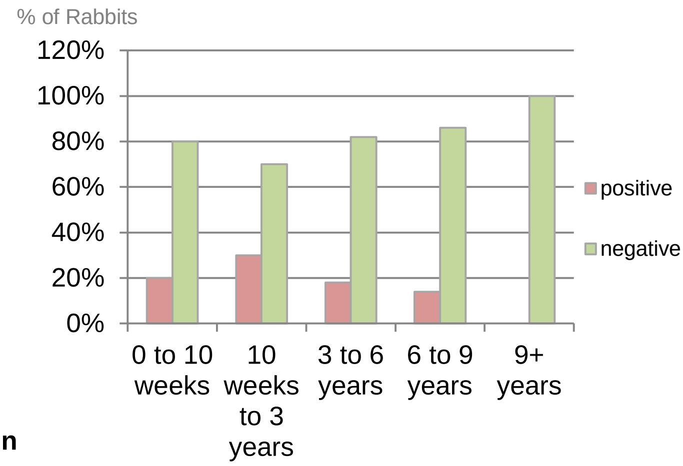

Figure 26 - percentage of positive and negative samples per age group

4.3.2.4. Sex and Neutering

Sixteen percent of the test-positive animals were female, while 84% were male. In addition, 33% were neutered (of which 25% were females and 75% were males), whereas 67% were not neutered (among those, 13% were females and 87% were males). 71% of the negative animals were males and 29% were females. Regarding neutering, 47% had been neutered (with 38% being female and 62% being male), whilst 53% had not been neutered (21% being females and 79% being males) (Figure 27).

Focusing on females, the presence of dermatophytes on the coats of 14% rabbits was confirmed. Half of these animals were neutered, while the other half were not. In males however, 24% of animals tested positive for dermatophyte presence, with 29% being neutered and 71% not neutered (Figure 28).

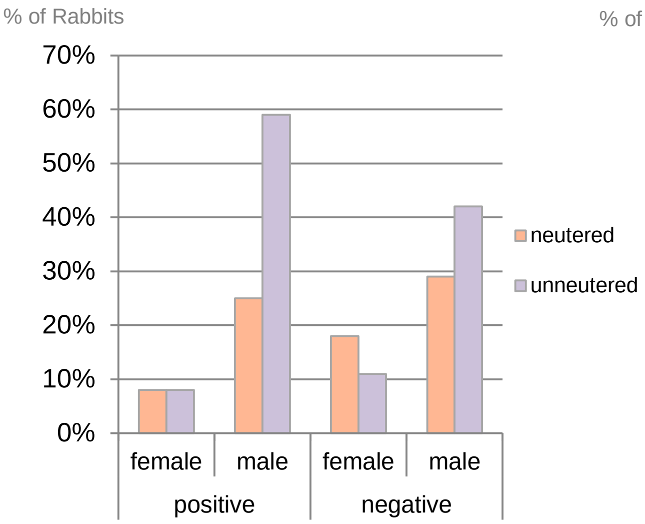

Figure 27 - percentage of each sex and neutering status in positive and negative samples

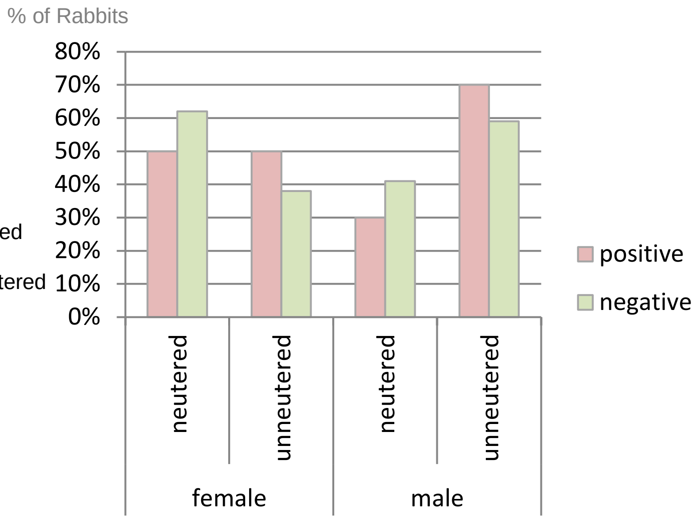

Figure 28 - Percentage of positive and negative samples per sex and neutering status

4.3.2.5. Fur Type

Nearly half (46%) of all positive test animals had short hair. The remaining 54% had either long (23%) or medium-length (31%) hair. Similarly, 51% of negative animals had short hair, 22% had long hair, and 27% had hair of medium length (Table 19).

If we take this data and focus on the various categories of fur type, we can find that the proportion of positive and negative tests is almost identical. 19% of short haired rabbits, 24% of medium haired rabbits, and 21% of long haired rabbits tested positive for dermatophytes.

Table 19 - Distribution of positive and negative samples by fur type

	Rabbits	
	nº	**%**
Positive	**13**	**21**
Short	6	10
Medium	4	6
Long	3	5
Negative	**49**	**79**
Short	25	40
Medium	13	21
Long	11	18
Total	62	100

4.3.2.6. Grooming

Fifteen percent of the positive testing animals were groomed by their owners on a regular basis, while 85% were not. Amongst the negative ones, 22% were groomed and 78% were not (Table 20).

Fifteen percent of the groomed and 22% of the ungroomed rabbits tested positive for dermatophyte presence on their fur, indicating there is a modest distinction between the two groups (Table 20).

Table 20 - Distribution of positive and negative samples by grooming situation

	Rabbits	
	nº	**%**
Positive	**13**	**21**
Groomed	2	3
Not groomed	11	18
Negative	**49**	**79**
Groomed	11	18
Not groomed	38	61
Total	62	100

4.3.2.7. Diet

Examining the diets given to the animals in this study reveals that, among those having dermatophytes on their fur, 46% were provided with a good quality diet, 38% had a medium diet, and 15% had poor diet management. In the negative animals, 49% had a medium quality diet, 26% had a good diet, and 24% had a poor diet (Table 21).

By separating the animals according to the sort of diet they were given, it was found that 32% of those with a healthy diet tested positive for dermatophytes. With a moderate diet, this number decreased to 17%, and with a poor diet, it dropped to roughly 14%.

Table 21 - Distribution of positive and negative samples per diet quality

	Rabbits	
	nº	**%**
Positive	**13**	**21**
Good diet	6	10
Medium diet	5	8
Bad diet	2	3
Negative	**49**	**79**
Good diet	13	21
Medium diet	24	39
Bad diet	12	19
Total	62	100

4.3.2.8. Body Weight

At the time of sampling, 23% of animals who tested positive for the presence of dermatophytes were considered underweight. All of the overweight rabbits tested negative, making up 8% of all negative animals. Moreover, 69% of these negative animals were of average weight, while 23% are thought to be underweight (Table 22).

From another point of view, 21% of animals in the underweight group and 22% of animals in the average group tested positive for the presence of the fungus on their fur (Table 22).

Table 22 - Distribution of positive and negative samples by weight

	Rabbits	
	nº	**%**
Positive	**13**	**21**
Low	3	5
Average	10	16
High	0	0
Negative	**49**	**79**
Low	11	18
Average	34	55
High	4	6
Total	62	100

4.3.2.9. Housing

Fifty-four percent of positive animals were allowed to roam freely, 23% were housed in parks, and the remaining 23% were kept in cages. In the case of the negative test animals, the proportion of rabbits with each form of housing was similar: 36% in cages, 31% in parks, and 33% free to roam (Table 23).

Looking at the housing categories themselves, 14% of caged animals, 16% of parked ones and 32% of the free roaming rabbits tested positive for the presence of dermatophytes.

Table 23 - Distribution of positive and negative samples by housing situation

	Rabbits	
	nº	**%**
Positive	**13**	**21**
Cage	3	5
Park	3	5
Free	7	11
Negative	**49**	**79**
Cage	18	29
Park	16	26
Free	15	24
Total	62	100

4.3.2.10. Contact with other animals

Thirty-one percent of the dermatophyte-positive rabbits had interaction with other animals, while 69% had no such contact. Similar to the positive ones, 39% of the negative rabbits had regular contact with other animals, whereas 61% did not (Table 24).

Seventeen percent of rabbits who had contact with other animals tested positive for dermatophytes. Although rather slightly, this number rises in the rabbits that did not have this contact, with 23% having these fungi on their coats (Table 24).

Table 24 - Distribution of positive and negative samples by contact with other animals

	Rabbits	
	nº	%
Positive	**13**	**21**
Contact	4	6
No contact	9	15
Negative	**49**	**79**
Contact	19	31
No contact	30	48
Total	62	100

4.3.2.11. Access to exterior environments

Among the individuals whose tests came out positive, 8% had access to an exterior environment, while 92% did not. This is also what very similarly happens with the negative animals, where 6% had access to the exterior and 94% did not (Table 25).

Twenty-five percent of the rabbits that did have access to the outdoors tested positive, which is slightly higher than the 21% of rabbits that lived exclusively indoors.

Table 25 - Distribution of positive and negative samples by exterior access

	Rabbits	
	nº	%
Positive	**13**	**21**
Access to exterior	1	2
No access to exterior	12	19
Negative	**49**	**79**
Access to exterior	3	5
No access to exterior	46	74
Total	62	100

4.3.2.12. Deworming and external parasite prevention

When examining the animals that were determined to have dermatophytes on their coat, we find that 31% had not had any parasite prevention prior to the visit, 15% had received both internal and external preventions, and 54% had only received internal preventive. Curiously, these percentages are quite similar in the group that had negative testing (Table 26).

In order to look further into this topic, the various parasite prevention groups were compared amongst themselves for positive and negative testing. Due to only one animal having performed only exterior prevention and not interior prevention, it was eliminated from this particular analysis. Interestingly, the proportions of positives (21%) and negatives (79%) in the remaining three groups were virtually identical.

Table 26 - Distribution of positive and negative samples by parasite preventative care

	Rabbits	
	nº	**%**
Positive	**13**	**21**
None	4	7
Only external	0	0
Only internal	7	11
Both	2	3
Negative	**48**	**79**
None	15	25
Only external	0	0
Only internal	26	43
Both	7	11
Total	61	100

4.3.2.13. Vaccination

Sixty-two percent of positive-testing-animals had been previously vaccinated, whilst 38% had not. Similar proportions of vaccinated and unvaccinated animals may be observed in the negative animal population, where 69% had their vaccines and 31% did not (Table 27).

Furthermore, by comparing the vaccinated and unvaccinated groups, it is revealed that 19% of the former and 25% of the latter tested positive for dermatophytes.

Table 27 - Distribution of positive and negative samples by vaccination status

	Rabbits	
	nº	%
Positive	**13**	**21**
Vaccinated	8	13
Not vaccinated	5	8
Negative	**49**	**79**
Vaccinated	34	55
Not vaccinated	15	24
Total	62	100

4.3.2.14. Health Situation

Thirty-eight percent of animals with dermatophytes on their coats were healthy, whereas 62% were experiencing some sort of health issue. These 62% were comprised of 38% animals with systemic disorders, 15% animals with systemic and mobility disorders, and 8% animals with systemic, mobility, and dermatological disorders. Other health patterns were observed in animals that tested negative for these fungi. 86% of them had some issue that prompted them to the veterinarian, while 14% were deemed healthy. 29% of those 86% only had systemic problems, 10% just experienced oral ones, 2% suffered only from mobility restraints, and 4% only experienced dermatological symptoms. Additionally, 6% had both systemic and dental concerns, 17% had both systemic and mobility issues, 2% had both systemic and dermatological issues, and 4% had both dental and mobility issues. Another 4% had systemic, dental, and mobility problems, 4% had systemic, mobility, and dermatological problems, 2% had dental, mobility, and dermatological symptoms, and the remaining 2% had all four types of the clinical symptoms mentioned (Figure 29).

Examining each clinical pattern present in this study reveals that 42% of animals with no symptoms whatsoever showed positive test results for dermatophyte presence on their fur. In addition, 26% of systemically ill rabbits tested positive, as did 20% of those with systemic and mobility problems and 33% of those with systemic, mobility, and dermatological problems. The remaining health patterns contained no positive results (Figure 29).

Figure 29 - Percentage of positive and negative samples per health pattern

none – healthy; S – Systemic Disease; O – Oral disease; M – movement impairment; D – dermatological disease; SO – S + O; SM – S + M ; SD – S + D; SOM – S + O + M; OM – O + M; OMD – O + M + D; SMD – S + M + D; SOMD – S + O + M + D

4.3.2.15. Children in the household

The majority (62%) of positive cases in this study occurred in animals that shared living areas with children on a daily basis. The remaining 38% either did not live in a household with children or did, but had no interaction with them (Table 28).

Table 28 - Distribution of positive and negative samples by households with and without children

	Rabbits	
	nº	%
Positive	**13**	**21**
Children	8	13
No children	5	8
Negative	**49**	**79**
Children	29	47
No children	20	32
Total	62	100

4.4. Discussion

Due to the frequency with which rabbits are asymptomatic dermatophyte carriers, it is essential to take steps to prevent transmission and infection. In addition, it is essential to determine which risk factors are the most significant, not only to know which areas require the most attention, but also because owners are far more likely to comply to changes if fewer are required.

In this study, 21% of rabbits tested positive for *T. mentagrophytes*, indicating that 1 in 5 rabbits are carriers of this fungus on their fur, which was interesting since the prevalence from the study by (SILVA 2019) was way lower than this. This is potentially cause for alarm. Since no other dermatophyte species were discovered, we may be dealing with a case of a single-source epidemic. However, additional research would be required to study this further. Moreover, only one of the positive animals exhibited dermatological symptoms, indicating that 92% of the positive-testing rabbits (and 19% of the whole sample) were asymptomatic carriers. Drouot et al. (2009) also found that *T. mentagrophytes* was the species most isolated from pet rabbits and rodents however, other authors believe *M. canis* and *M. gypseum* to be the most prevalent (Canny and Gamble 2003; Quesenberry et al. 2021). Additionally, rabbits being asymptomatic carriers of dermatophytes is not a new issue; it has been previously documented that as many as 60% of rabbits are asymptomatic carriers (Franklin et al. 1991; Quesenberry et al. 2021).

Regarding seasonality, whereas 23% of winter samples were positive for dermatophytes, there were not enough data from other seasons to determine if there is an association between seasonality and dermatophyte presence. Theoretically, because these fungi flourish in heat and humidity, spring and autumn would be the peak seasons for their occurrence. According to a 2016 study conducted in Italy, the season with the highest risk of dermatophyte infection in all pets is autumn (Allizond et al. 2016). However, other studies found no association between the two events and determined that season should not be regarded a risk factor for this issue (Cafarchia et al. 2004; Ilhan et al. 2016; Chang et al. 2022). Nonetheless, taking into account false positives and negatives, it would also be interesting to do a more recent study that examines an equal number of homogeneous samples from each season, along with an adequate statistical analysis, to corroborate these findings.

According to Chang et al. 2022, origin is a risk factor for dermatophytes, with a higher prevalence of these fungi on the fur of rabbits from pet stores. However, this study revealed no difference between animals from pet stores and those from home breeding. This may indicate that there is no risk factor linked with pet stores in Portugal (as the study mentioned before was conducted in the Netherlands). However, the fact that the majority of the animals in this study's sample came from pet stores (84%) could potentially have a role in sabotaging the results. Another theory is that pet businesses today take security and preventative measures more seriously, hence decreasing the danger of transmission. Overall, it would be advantageous to carry out a study in Portugal using equivalent samples from each origin to determine whether or not there is a higher chance of animals from pet stores carrying dermatophytes on their fur.

Age has been mentioned as a factor influencing dermatophytosis in rabbits (Chang et al. 2022). Allegedly, young kits and elderly rabbits have the weakest immune systems and skin defences, making them the most susceptible to take on this carriage (Nimni et al. 1965; Vangeel et al. 2000). In this study, although juveniles had a 20% frequency and elderly animals had 14% prevalence; 30% of medium aged rabbits were positive. This could be because there were significantly more middle-aged rabbits in this sample (82%) than in the other two age-groups (8% babies and 10% seniors), but it could also indicate that a weakened immune system and a damaged skin condition do not result in a greater presence of dermatophyte spores on the animal's coat, but rather in the development of the disease if the spores are present. Concluding, age may increase the chances of contracting dermatophytosis, but not necessarily of carrying the fungi on their fur.

To the author's knowledge, no study has identified sex as a risk factor for dermatophytes in rabbits. However, due to the fact that males and females have distinct skin thickness and density (Shuster et al. 1975), this factor was nonetheless taken into account. 24% of male rabbits and 14% of female rabbits were infected with dermatophytes. Although the difference between the two groups is modest, the fact that it exists is nevertheless intriguing. However, this sample had far more male rabbits (74%) than female rabbits (26%), which may have affected the results. It would be interesting to examine the relationship between sex and skin condition to determine whether sex might be considered a risk factor for skin infections and asymptomatic carriage. In addition, the status of sterilisation did not appear to have any effect on the presence of fungi.

Since long-haired animals tend to exhibit more severe dermatophytosis symptoms (Moriello 2020), it was essential to analyse this aspect. It was intriguing to discover that these fungus were practically equally prevalent in all 3 hair types (short, medium and long). In addition, there was a representative sample of each type, thus it was safe to compare the 3. This is still consistent with what Moriello (2020) stated, because, as mentioned previously, sickness only occurs when other conditions are present. Thus, long-haired rabbits may not always carry more dermatophytes on their fur, but rather only develop more severe disease under certain conditions. In addition, the majority of the long-haired animals in this study were groomed by their owners, which may have reduced spore presence. It would be interesting to obtain homogeneous samples of each type of hair from both groomed and ungroomed animals in order to determine whether grooming and hair length affects the occurrence of dermatophyte carriage.

Diet has been shown to affect rabbits' teeth and skin barrier condition (Meredith et al. 2015; Parke et al. 2021). In addition, it can also affect its weight, and because these factors may increase the risk of dermatophyte infection in animals (by preventing the animal from grooming itself and reducing the skin's ability to fight fungi) (Abreu et al. 2021), they were evaluated as risk factors for the presence of dermatophytes. The acquired data, however, did not support this. Intriguingly, the rabbits with the best diets (including appropriate nutrition and dental wear agents) had the highest percentage of positive tests (32%). Furthermore, all positive tests were housed by underweight and normal-weight rabbits. This could be attributed to the usual rabbit behaviour of mutual grooming, the owners grooming the animal themselves, or the sample not being representative enough.

According to Moriello et al. (2017), the prevalence of dermatophytes on the fur of free-roaming animals appears to be higher. This is also shown by the results of this study, where 32% of free-roaming animals tested positive for these fungi, while only 15% of caged and parked animals did.

Another finding by Moriello et al. (2017) showed that animals in contact with other animals reported dermatophyte presence more frequently than those without contact. This was not the case in this study, as both isolated and grouped animals possessed the same proportion of these fungi on their coats. This could be explained by the fact that the animals they interacted with (mainly cats and dogs) were frequently groomed, bathed, and treated for external parasites.

It would also be reasonable to assume that animals with access to outdoor spaces would be at a higher risk of carrying dermatophytes (Moriello et al. 2017); however, this was not the case in the present study, as both indoor and outdoor animals had nearly the same percentage of dermatophytes on their fur (21% and 25%, respectively). Not only is this a surprise discovery, but it also begs the question of where indoor-only rabbits acquire dermatophytes, if not through pet stores. One interesting hypothesis includes looking at store-bought hay as a possible fomite for dermatophytes. It would be interesting to conduct more detailed research on this topic and possibly understand the source of fungi for indoor animals.

Although external parasite prevention makes sense when also preventing dermatophytosis because it helps maintain skin integrity (Mishra et al. 2022), it does not likely prevent the simple presence of dermatophyte spores on the coat, as it is not fungal-targeted. This was supported by the fact that the prevalence of these fungi in both groups (with preventative treatment and without) was almost identical. In addition, the majority of the animals in this study were kept exclusively indoors and so had less contact with external parasites. The same holds true for vaccination, as preventing other diseases helps preserve the proper functioning of the Immune system, hence preventing the onset of dermatophytosis. However, this does not prevent the occurrence of asymptomatic carriers, which was supported by the findings of this investigation, as their presence was again, almost identical in both groups (vaccinated and non-vaccinated). In addition, immunizations only protect against a subset of diseases, leaving potential for others to still impair the immune system's health and hence the likelihood of skin disease.

In this study's linkage between health status and dermatophyte carriage, it is noteworthy that the majority of healthy animals were asymptomatic carriers. Although statistical significance was not determined, it is also important to note that this group had a significantly higher dermatophyte prevalence than the other groups. This was unexpected, as other studies have reached the opposite conclusion (d'Ovidio and Santoro 2015; Mishra et al. 2022). However, upon further consideration, as unhealthy animals are typically on medication (which was the case in this study), it could have affected the results by reducing the amount of dermatophytes on their fur. The only animals that were actually sick and had dermatophyte presence were those with a systemic disease; rabbits with merely mobility, dental, and dermatological disease did not accuse dermatophyte presence. It would be interesting to investigate if this factor has statistical and clinical significance, and if so, why.

Concerningly, 62% of the positive tests in this study were conducted on rabbits that experienced daily interaction with children. This did not come as a surprise, as it has been stated in past research (Vangeel et al. 2000). Children are the most susceptible to dermatophyte-caused skin illness, thus it is crucial to take this information into consideration and determine which preventative measures should be more urgently implemented.

Based on the results of this study, we conclude that keeping a rabbit as a pet in itself represents a substantial risk of introducing dermatophytes into the household. In light of this it is essential to take all precautionary measures mentioned to keep the household dermatophyte-free. However, and even though every protocol was strictly followed, human error must still be considered. In addition, and although false positives were avoided through microscopic examination, DTM can be less accurate than SDA, so this also might have resulted in inaccurate data. It would be of great worth to have an expert conduct the same research in a proper laboratory using SDA to determine whether the same results would be obtained. Moreover, adding PCR and Molecular identification would further strengthen the reliability of this investigation.

Additionally, based on the data presented in this study, we may also be inclined to conclude that sex, housing, and health status are the most influential risk factors for the occurrence of dermatophytes on rabbit fur; however, a proper statistical analysis was not conducted in order for us to assume such conclusion. Nevertheless, utilizing the data acquired in this dissertation, there is a second study currently taking place, targeting exactly that.

4.5. Conclusions

Trichophyton mentagrophytes, a dermatophyte that can cause severe inflammatory skin reactions in people, particularly children, appears to be commonly carried asymptomatically by pet rabbits, in Porto, Portugal. As the popularity of these animals as children's pets continues to rise, it is crucial to take precautions against transmission by practicing good animal husbandry and provide effective medical care for both the pet and the family.

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Annexes

Annexe 1. Informed Agreement

Consentimento Informado para Participação em Projeto Científico

Eu,____ , portador/a do CC nº ______________________ , titular do animal _________________________ , declaro que autorizo a sua participação no projeto "Prevalência de Dermatófitos em Coelhos", autorizando:

1. A colheita de pêlos através de escovagem e extração com pinça.
2. A observação direta da amostra colhida ao microscópio
3. A cultura fúngica da amostra colhida

Fui informado/a que o projeto se destina à determinação da prevalência de fungos dermatófitos em coelhos domésticos, e de que os dados resultantes da análise serão usados exclusivamente na elaboração da tese de mestrado em Medicina Veterinária da aluna estagiária Elisa Pais Jackson.

Declaro também que a minha participação neste projeto é totalmente voluntária e estou ciente de que a posso interromper a qualquer momento.

____/____/________

____

(o/a tutor/a)

Annexe 2. Guidelines for Animal Information Collection

Consultation guide

1. Health Situation

Motive for the vet visit:____

____

Previous Medications:____

____

2. Animal's History

Origin: ☐ Pet Store ☐ Home Breeding

Age: ________ years

Housing: ☐ Cage ☐ Park ☐ Free Roaming

Diet: ☐ Good ☐ Medium ☐ Bad

Grooming: ☐ Able to groom itself ☐ Groomed by owners ☐ Not groomed

Parasite prevention: ☐ Yes ☐ No

Vaccination: ☐ Yes ☐ No

Neutering status: ☐ Neutered ☐ Non-neutered

Contact with other animals in past year: ☐ Yes ☐ No

Access to exterior environments in past year: ☐ Yes ☐ No

Children in the Household: ☐ Yes ☐ No

3. Physical examination

Overall Well-being:____

____

Weight: ________ Kg

Sex: ☐ Female ☐ Male

Fur type: ☐ Short ☐ Medium ☐ Long

Skin lesions: ☐ Yes ☐ No

Ectoparasites present: ☐ Yes ☐ No

Notes: