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Parasitic otitis and dermatitis in dogs in Tuscany

Otiti e dermatiti parassitarie in cani della Toscana

Candidato: Yaacov Irit

I° Relatore: Prof. Perrucci Stefania

II° Relatore: Prof. Corazza Michele

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To my family,

Without their love and support I wouldn’t be here

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Abstract

Otitis externa (OE) and dermatitis are very common diseases in dogs. In a population of affected dogs we focused on parasitic dermatitis and OE with the aim to evaluate their frequency and the parasitic species more frequently involved. The main symptoms associated with each isolated parasite and the frequency of some predisposing factors (sex, age, breed and living situation), were also evaluated. In the period between March 2011 and September 2012, skin and wax samples were collected from 87 dogs including owned animals living in Pisa municipality and animals hosted in kennels located in Pisa and Florence districts. For sampling collection, skin scraping, scotch test, trichogram and ear swab methods were used. In order to find parasitic arthropods, skin scrapings were digested by NaOH 10% or just mixed with few drops of paraffin oil and then checked under the microscope (100 X- 400 X), ear wax samples were mixed with paraffin oil or directly observed, while the scotch test and trichogram samples were directly observed under the microscope. To find *Malassezia*, smears of ear wax samples were stained with Diff-Quick and microscopically observed at 400 X and 1000X magnifications. All data was statistically analyzed. Overall, 54% of the dogs had dermatitis, 43% had otitis and 3% had both. Out of the cases of dermatitis 44% were diagnosed with **Demodicosis** and 38% with **Sarcoptic mange**. Out of the cases of OE 67.5% were diagnosed with *Malassezia*, 7.5% with *Malassezia* and *Otodectes cynotis*, 2.5% with *Malassezia* and *Trombicula autumnalis*, 2.5% with *O. cynotis* and 20% were negative for parasites. From statistical analysis, parasitic OE was more frequent in kenneled and crossbreed dogs ($P<0.01$), while purebred dogs were more likely to have a parasitic dermatitis ($P<0.01$). Age resulted an important predisposing factor in parasitic dermatitis (mainly demodicosis and sarcoptic mange), with a significant higher prevalence in the 0-2 years age group ($P<0.05$). However, age was not found to be a predisposing factor in parasitic otitis ($P>0.05$). Sex was not correlated with parasitic dermatitis or with parasitic OE. Pruritus (100%) and erythema (89%) were the main clinical signs of sarcoptic mange, while pruritus (82%), alopecia (68%) and erythema (68%) were the main clinical signs of demodicosis. The main symptoms of parasitic OE (*Malassezia* and *O. cynotis*) were abundant (77%, 100%) and dark (68%, 100%) wax.

Key words: Dermatitis, *Otitis externa* (OE), parasites, dogs.

Riassunto

Le otiti esterne (OE) e le dermatiti sono patologie molto comuni nel cane. Nel presente studio è stata esaminata una popolazione di cani affetti da queste patologie al fine di valutare la frequenza delle OE e delle dermatiti parassitarie ed i parassiti coinvolti. Sono stati inoltre valutati i principali sintomi associati a ciascun parassita isolato e la frequenza di alcuni fattori predisponenti, quali sesso, età, razza e condizioni di vita. Nel periodo compreso tra marzo 2011 e settembre 2012, campioni di cute e di cerume sono stati raccolti da 87 cani comprendenti animali di proprietà che vivono nel comune di Pisa e animali ospitati in canili delle provincie di Pisa e di Firenze. Per la raccolta dei campioni sono stati eseguiti raschiati cutanei, scotch test, tricogramma e tamponi auricolari. Per la ricerca di artropodi, i raschiati cutanei sono stati digeriti con NaOH 10% o semplicemente miscelati con alcune gocce di olio di paraffina e poi esaminati al microscopio (100 X-400 X), i campioni di cerume sono stati miscelati con olio di paraffina o direttamente osservati, mentre per l'esecuzione dello scotch test e del tricogramma i campioni erano direttamente osservati al microscopio. Per la ricerca di *Malassezia*, strisci di cerume auricolare sono state colorati con Diff-Quick e osservati al microscopio a 400 X e1000X. Tutti i dati sono stati analizzati statisticamente. Il 54% dei cani esaminati era affetto da dermatite, il 43% da otite, il 3% da entrambe le patologie. Tra i casi di dermatite, il 44% erano rappresentati da demodicosi ed il 38% da rogna sarcoptica. Tra i casi di OE, nel 67,5% è stata isolata *Malassezia*, nel 7,5% dei casi *Malassezia* e *Otodectes cynotis*, nel 2,5% dei casi *Malassezia* e *Trombicula autumnalis*, nel 2,5% *O. cynotis*. Il 20% dei cani sono risultati negativi per i parassiti. Dall'analisi statistica, l'otite parassitaria è risultata più frequente nei cani dei canili e negli incroci ($P <0,01$), mentre nei cani di razza è risultata un maggiore probabilità di dermatiti parassitarie ($P <0,01$).L'età è risultata un importante fattore predisponente per le dermatiti parassitarie (principalmente demodicosi e rogna sarcoptica), con una prevalenza significativamente maggiore nel gruppoda 0 a 2 anni ($P <0,05$), ma non per le otiti parassitarie ($P> 0.05$). Il sesso non è risultato un fattore significativo né per le dermatiti nè per le otiti parassitarie. Il prurito (100%) e l'eritema (89%) sono risultati i principali sintomi associati alla rogna sarcoptica, mentre il prurito (82%), l'alopecia (68%) e l'eritema (68%) sono stati i principali segni clinici della demodicosi. I sintomi principali delle otiti parassitarie OE (da *Malassezia* e *O. cynotis*) sono stati cerume abbondante (77%, 100%) e scuro (68%, 100%).

Parole chiave: dermatite, otite esterna (OE), parassiti, cane.

INTRODUCTION

Dermatitis, including otitis externa (OE), is a very common and important disease of the dog. For example, OE is estimated to occur in 10 to 20% of canine patients seen by veterinarians (Scott et al., 2001). The diagnosis of these diseases is not always simple since they may be caused simultaneously by one or more factors, including different pathogens.

Parasites are common causes of dermatitis (Chee et al. 2008; Taylor et al. 2010). Several arthropods and fungi live as ectoparasites and/or commensals on the domestic dog. These parasites, generally associated with dermatitis, affect animals to different degrees depending on their nutrition, immunological condition and parasite intensity, and in extreme cases can lead to death. Among these parasites, in this study we focused on mites and yeasts of the genus *Malassezia*. Mites are primary causes of dermatitis and otitis, while *Malassezia* is more often secondary.

Considering the scarce number of studies conducted in Italy, the main aim of this study was to evaluate, in a canine population affected with dermatitis and/or otitis, the prevalence of parasitic otitis and dermatitis and that of each identified parasitic species. The evaluation of symptoms and lesions associated with each isolated parasite and of differences in the prevalence of parasitic otitis and dermatitis according to breed (pure or crossbred), age, sex and the origin (owned or kenneled) of examined dogs were other important aims of the present study.

MITES, DERMATITIS AND OTITIS

Mites are included among the main causes of dermatitis and otitis in dogs (Chee et al. 2008).

The species of mites that can cause dermatitis and/or otitis are:

- *Sarcoptes scabiei* var. *canis*
- *Cheyletiella yasguri*
- *Demodex* spp.
- *Otodectes cynotis*
- *Neotrombicula autumnalis*

Sarcoptes scabiei and sarcoptic mange

Biology

Sarcoptic mange is caused by *Sarcoptes scabiei*, a mite species reported in 10 orders, 27 families and 104 species of domestic, free-ranging and wild mammals (Currier et al., 2004). *Sarcoptes scabiei var. canis* has been isolated from species other than domestic and wild canids and has been experimentally established on rabbits, guinea pigs, sheep, goats, calves, cats and humans (Curtis, 2004).

It is a roundish mite, dorsally convex and flat ventrally. The adult female is about 300-450 µm in length and 250-350µm wide; the male is smaller, no more than 300µm long and 100-200µm in wide. These mites have 4 pairs of limbs – 2 pairs in the front and 2 in the rear end of the mite. The two posterior pairs of limbs do not extend beyond the margin of the body. The pairs in the front have claws and pulvilli in a form of a suction pad which are used to anchor themselves to the substrate during their movement. In the female both of the posterior pairs of limbs, and in the male only the 3rd pair finish with long setae. Both sexes have a round shaped mouth. The dorsal surface of the body is covered with transverse ridges and triangular flakes. The anus is located in a terminal, lightly dorsal position. Stigmas and eyes are absent (Taylor et al., 2010).

This mite completes its whole life cycle in the epidermis.

The life cycle, from eggs to adults, going through one larval and two nymphal stages, and may be completed in only 10 days under favorable conditions. However, on average, the normal development time is 2– 3 weeks. The larval stage hatches from the egg and moults to the nymphal stage within the burrows in the skin. However, both larvae and nymphae frequently wander about on the skin surface (Arlian and Vyszenski-Moher 1988).

Copulation takes place in burrows created by the females, where they lay up to three eggs per day over a period of 2-3 weeks.

The mites can live off of a host for days to weeks depending on their life stage, but they are only infective for 36 hours (Abdel-Ghaffar et al. 2008).

Canine scabies, in addition to being a severe and distressing condition, can also cause a transient dermatitis in human beings, although these infestations usually resolve after treatment of the canine source. Canine mites are capable of burrowing, feeding, and producing eggs in human skin for a limited period. However, skin scrapings obtained from human beings affected by canine scabies are usually negative, and diagnosis is based on clinical signs and demonstration of the mite on the animal host (Shanks et al., 2000).

Epidemiology

Sarcoptic mange in animals remains a problem in most areas of the world (Currier et al., 2012). Canine scabies is a non-seasonal, intensely pruritic, transmissible skin condition (Terada et al., 2010). It spreads through close contact between infested dogs or by contaminated fomites. Strains of this species appear to be physiologically adapted to a particular host to a greater or lesser extent, and cross-infestation between mammalian host species may be successful, unsuccessful, or transient. This has led to the conclusion that the mite strains are not host-specific but possess a degree of host preference (Arlian et al., 1984).

There have been no reports that sex is a predisposing risk factor to dogs contracting sarcoptic mange as there was around a 50% split between each sex. Also, whether the animal was neutered or not did not seem to affect the chance of the animal becoming infested (Feather et al., 2010).

The age range of dogs is between 4 months to 14 years (Wagner and Wendlberger, 2000), however dogs under the age of 2 years were found to be significantly more likely to contract the disease than dogs over this age (Feather et al., 2010).

It seems that there are few breeds who are more predisposed, such as Labrador, Border Collie and Jack Russell Terrier (Feather et al., 2010).

Foxes appear to be an important vector of the disease in some geographical areas having a large population of infested foxes, such as Sweden (Bornstein 1991). In southern England there are many anecdotal reports of infestation of dogs following direct or indirect exposure to foxes (Bond, 1998).

Canine scabies can also cause a transient dermatitis in human beings (Aydıngöz and Mansur 2011; Jofré et al., 2009), although these infestations usually resolve after treatment of the canine source (Shanks et al., 2000; Terada et al., 2010).

The use of molecular markers has enabled significant progress to be made in understanding the genetic relatedness of Sarcoptic host-associated populations, with molecular data providing insight into both host selection and host-mediated influences on *S. scabiei* population structures (Alasaad et al. 2011; Walton 2004). Current indications suggest that the adaptive evolution of the *Sarcoptes* varieties appear to be strongly related to the phylogenetic similarity of the host species. Convincing evidence of limited gene flow was shown between sympatric populations of human and dog mites in scabies-endemic indigenous communities in Australia by Walton and colleagues (2004). These workers were the first to demonstrate, using both nuclear and mitochondrial markers, genetically distinct host-associated populations (Soglia et al., 2007).

Clinical signs

The lesions might be on the head and the pinnae, on the elbow and the hock of the legs and on the ventral part of the thorax and abdomen. It might involve one (localized form) or few of these areas, or even being widespread all over the body (Albanese and Leone, 2007). Sarcoptic mange is probably underdiagnosed in dogs due to mild signs in some cases (Pin et al., 2006). After contact with *S. scabiei*, clinical signs may develop anywhere within 10 days to 8 weeks (Abdel-Ghaffar et al., 2008). The disease typically presents as an intensely pruritic, papulocrustous dermatosis affecting the periocular skin, pinnal margins, elbows and hocks which may generalize (Curtis, 2004; Pin et al., 2006). Dogs with a chronic generalized disease tend to have seborrhea, severe thickening of the skin, crusting, lymphadenopathy, and emaciation (Taylor, et al. 2010). They might also show pruritus, even intensive, alopecia, erythema, excoriations and hyperpigmentation. It is quite common to have bacterial and fungal (*Malassezia*) complications (Salkin et al. 1980; Albanese and Leone, 2007). In 80% of the dogs the otopodal reflex is positive (Albanese and Leone, 2007). Most

dogs show a combination of the symptoms reported above, but not all of them together (Feather et al., 2010). Clinical lesions may be atypical and localized, particularly in dogs that receive flea-control products regularly (containing fipronil or permethrin). These preparations may prevent extension of the disease because of their acaricidal properties. The local form of this disease is usually described as having a tendency for lesions to spread over the body (Pin et al., 2006).

The characteristic features of the condition in humans include the sudden onset of intensely pruritic papules and vesicles on areas of contact with the pet, often occurring simultaneously in several members of the family (Smith and Claypoole, 1967).

At hystological examination, generally widespread and uniform severe hyperplasia of the epidermis and light perivascular eosinophilic dermatitis, are observed.

Diagnosis

Sarcoptic mange should always be included in the differential diagnosis of pruritic, hyperplastic dermatitis (Taylor et al., 2010).

When faced a dog with the symptoms described earlier it is important to do differential diagnosis of the sarcoptic mange with other diseases who have similar clinical manifestations in dogs, as allergic dermatitis, cheiletiellosis, otoacariasis, dermatophytosis and demodicosis (Albanese and Leone, 2007).

Diagnosis is performed using the superficial skin scraping technique in which adults, larvae, nymphs, eggs and faeces could be found. However, experience has shown that the sensitivity of this traditional test is less than 50%. (Walton and Currie 2007; Albanese and Leone, 2007; Arlian and Morgan 2000). Positive skin scrapings are usually observed only in dogs with massive infestation (Bornstein, 1991).

Another way to diagnose is performing a serodiagnostic test with an antigen of *S. scabiei* -based ELISA assay. The ELISA applied to sera of dogs suspected with scabies showed a sensitivity and a specificity of 92 and 96%, respectively, in the study of Bornstein et al. (1996), of 83% and 92%, respectively, in the study of Curtis (2001) and of 84.2% and 89.5% ,respectively, in the study of Lower et al. (2001).

Another possible way to diagnose is called “diagnosis by treatment” or “trial therapy”. In this case the diagnosis is done by treating the dog with a topical medication that specifically targets and kills these mites (Albanese and Leone, 2007; Mueller and Bettenay 1999).

It is also possible to diagnose this disease with a skin biopsy. Although the mites are rarely seen on a skin biopsy sample, the mite burrows are surrounded by inflammatory cell infiltrates comprising eosinophils, lymphocytes, and histiocytes (Walton and Currie 2007).

When mites evade detection, a Sarcoptes IgG enzyme-linked immunosorbent assay can be used as a diagnostic test for canine scabies in Europe and North America, as well (Curtis, 2004; Terada et al., 2010).

According to Arlian and Moragan (2000) many random-source dogs without scabies or signs of atopic dermatitis or other visible skin lesions may already have low levels of circulating antibody to house dust mites. Consequently, because of the cross-reactivity of dust mite and scabies antigens, these dogs also have low levels of circulating antibodies that recognize a few antigens in an extract of SS. However, in spite of pre-existing low levels of circulating antibodies to *S. scabiei* and dust mites, all the dogs infested with scabies developed new or increased levels of antibody to many proteins in extracts of both *S. scabiei* and of the house dust mites (Arlian and Morgan 2000).

Treatment and prevention

Current control for canine scabies includes several types of treatments: dips, oral/injectable medications, or spot-on products (Abdel-Ghaffar et al., 2008). In any case it is based upon the use of acaricides (Curtis, 2004). Based on the localization of the mite on the body and on the duration of its life cycle, most acaricides must be used once a week for 4 weeks, or more if necessary, until the disappearance of the lesions. Since sarcoptic mange is a highly contagious disease, the infected animals should be isolated from other animals, and all the dogs came in contact with the infected animals should be treated as well, even if they do not manifest any symptoms. In dogs with poor general conditions, it is recommended to add cortisone by parenteral administration to the acaricide treatment (Taylor et al., 2010).

In the past 50 years lindane has been the preferred therapy for scabies. During recent years, resistance to lindane seems to be rising worldwide. Permethrin cream (5%) was introduced in 1989 for the treatment of scabies and seems to be a good substitute for lindane. It is considered to be the drug of choice in many countries. The 5% permethrin preparation kills the organisms and eggs and

has an extremely low rated of absorption, making the toxicity potential nonexistent. However, resistance to permethrin has been reported in developed countries (Bigby, 2000).

Ivermectin is an antiparasitic agent effective against a variety of ectoparasites. Although ivermectin is as effective as permethrin, it has few out weighing advantages over topical permethrin. It is cost effective and can be given to masses with better compliance with or without supervision. It can also be given safely to patients with scabies with secondary eczema, erosions or ulcers where topical therapies such as permethrin and benzyl benzoate can cause serious cutaneous and systemic side-effects in addition to the problem of compliance (Goldust et al., 2012). However, mite strains refractory to ivermectin have been reported (Yuri Terada et al., 2010).

Ivermectin can be administered by subcutaneous (sc) injection, orally or topically as a pour-on, but owing to the possibility of an idiosyncratic reaction in collies and sheepdogs, it should not be used in these breeds or their crosses as it can cause ataxia, tremors, mydriasis, salivation, depression and even coma and death (Curtis, 2004). Although more expensive than ivermectin, milbemycin is fairly well tolerated in collies and related breeds and therefore it is a safer alternative in high-risk breeds (Curtis, 2004). Selamectin, a novel avermectin, has an easy application and it is apparent safety in collies and related breeds. However, dermatologists have anecdotally reported delayed responses to selamectin and a small number of treatment failures when using the drug according to the manufacturer's recommendations (Shanks et al., 2000; Curtis, 2004). Its spot-on formulation is the only systemic treatment licensed for the control of sarcoptic mites (Curtis, 2004) and is effective in more than 95% by day 30, and 100% by day 60 (Six et al., 2000).

Fipronil applied by pump spray on three occasions at 3-weekly intervals, has been used to control an outbreak of scabies in puppies. It has also been used successfully as a sponge-on in adult dogs when applied once a week for two weeks (Curtis, 2004).

Amitraz is an alternative topical scabicide and it is applied as a 0.025% sponge-on solution. In the UK, the product is licensed for weekly use (Curtis, 2004). It should not be used in Chihuahuas, in pregnant or nursing bitches or puppies less than three months of age. Care should be exercised if the product is being handled by, or applied to, a diabetic owner or patient, respectively, as individuals exposed to the active component and its vapors can develop transient hyperglycemia (Curtis, 2004). It might induce depression, bradycardia and sedation and these effects can last for 24 hours following application (Curtis, 2004).

It was found that a treatment with water-free neem seed extract shampoo of infested sites resulted in a rapid reduction in mite counts and improvement of clinical signs of sarcoptic mange (Abdel-

Ghaffar et al., 2008). By the end of the treatment (after 14 days), only a small number of mites were found in two dogs, while eight dogs were completely cured as was proven by mite counts and disappearance of clinical signs. No remarkable signs of side effects or adverse reactions were observed throughout the study. This product could be an alternative early biodegradable natural product (Abdel-Ghaffar, et al. 2008).

Another study found that treatment of dogs with pyriprole, applied twice with a monthly interval between treatments and at the dosage rate recommended for the control of ticks and fleas, rapidly reduced the level of *Sarcoptes* infestation in treated dogs and resulted in a slow but marked improvement in the clinical signs associated with infestation (Fourie et al., 2010).

Cheyletiella yasguri

Biology

Cheyletiella yasguri was first found in 1965. The male is 270-360μm long and 170-250μm wide, while the female is 350-540μm long and 230-340μm wide.

The adults have a roundish form with a striated cuticle. The rostrum is developed and has terminal pulps, with 2 opposing prominent curved hooks. The limbs are developed and divided in 2 groups by a transverse groove, and ending with appendix (empodium) that looks like comb. On the limbs there are plumose bristles.

Species of this genus are differentiated on the basis of the morphology of the sensorial bristle (also known as "Soledinion"), located on the 3rd segment of the 1st pair of limbs: in *C. yasguri* it looks like a heart (Albanese and Leone, 2007). However, the morphology of this structure may vary among different individual mites and stages of the life cycle and, therefore, species identification is not simple (Taylor, et al., 2010).

Cheyletiella mites, also known as the "walking dandruff", live in the keratin layer of the epidermis and feed on surface debris and tissue fluids. Their ova are smaller than louse nits (120-230μm) and are attached to hairs by fine fibrillar strands. The egg-larval-nymphal-adult stages are completed within 21 days on one host. Adult females may live free of their host for up to 10 days. Eggs are shed that can be dispersed into the environment with the hair playing an important role as source of re-infestation. Young animals are at higher risk and humans may serve as an accidental or transitory host (Wagner and Stallmeister, 2008). It is rare to diagnose this mite on animals that are on regular flea preventative treatments (Ghubash, 2006).

Inside the eggs are developing the pre-larvae that become larvae, followed by the octopod nymph that hatches from the egg. There are two more stages of nymphs, before becoming adults.

The mites live between the hair and the dandruff moving (fast) on the skin only to feed. The nutrition takes place via puncture of the epidermis with the chelicerae (Taylor, et al., 2010).

Epidemiology

Cheyletiellosis is typically a mild, albeit very contagious dermatitis caused by mites living on the skin surface. The family Cheyletiellidae is not believed to be host specific and may readily transfer between dogs, cats and rabbits (Curits 2004).

Cheyletiellosis affects animals of both sexes, young animals are particularly susceptible. According to Curtis (2004), boxer and cocker spaniel breeds are more commonly infected by the mites.

Humans in contact with pets carrying *Cheyletiella* spp. may also become transiently infested and develop pruritic papular lesions on the dorsal areas of the body and on arms (Curtis 2004).

Clinical signs

In dogs, mites live mostly on the skin of the dorsal-lumbar region, but can spread all over the body (Albanese F, Leone F., 2007).

Skin debris appears on the hair, which gives a dusty appearance to the infected dogs. The mites are white and move fast between the skin debris and for this reason they are called "dandruff that walk" (M.A Taylor, et al., 2010).

Papules and lesions, such as alopecia crusts and excoriation, could be caused by the self-trauma induced by pruritus. The disease is often complicated by bacteria and fungi (Albanese F, Leone F., 2007).

Some affected animals show no symptoms and can act as asymptomatic carriers for other pets and a source for environmental contamination (Ghubash, 2006).

Diagnosis

The differential diagnosis must include (Albanese F, Leone F., 2007):

- Lice
- Sarcoptic mange
- Allergic dermatitis
- Dermatophytosis
- Leishmaniasis
- Malnutrition
- Defects of skin keratinization.

These mites live on the skin surface and can be diagnosed microscopically on the 100X to 400X magnification by visualization of superficial skin scrapings, tape impressions, or collections of scale. A preferred method is the collection of dander and scales by using a comb to brush the coat and examining microscopically the collected material. When the mite is collected this way the scale and hair is transferred to a slide, immersed in mineral oil, covered with a cover slip, and then evaluated microscopically (Ghubash, 2006).

It is also possible to diagnose the mites at hairs examination (the eggs are adherent to the hair shafts) and at coprological examination (Albanese and Leone, 2007).

Treatment and prevention

Treatment is based upon the use of acaricides. The main drugs used against the Cheyletiella are:

- Fipronil – it's a safe and effective treatment and both the 0.25% spray and the 10% spot-on products have been found to be effective
- Selamectin
- Lime sulfur - can be an effective treatment for canine and feline cheyletiellosis, and has the benefit of being antipruritic (Ghubash, 2006)
- Ivermectin - In collies and Australian shepherds it may have a possible lethal effect. (Wagner and Stallmeister, 2008)
- Milbemycin - Because of the expense of this medication, it should be reserved for cases refractory to other treatments
- Amitraz (Ghubash, 2006).

Infected dogs should be re-examined and monitored throughout the treatment period to screen for residual mites and eggs. Treatment should continue for a few weeks beyond clinical resolution and when multiple tape strippings and superficial skin scrapings fail to reveal any microscopic evidence of mites (Curtis, 2003).

Environmental decontamination and treatment of all animals came in contact with infected dogs are important to avoid re-infestation. If pruritus is a significant component of the disease and glucocorticoids are not contraindicated the use anti-inflammatory of prednisone is recommended at the beginning of treatment (Curtis 2004).

***Demodex* spp.**

Biology

Demodectic mange in dogs is a widespread parasitic disease all over the world, including Italy, caused mainly by *Demodex canis*. In recent years there have been found 2 new morphotype – *Demodex injai* and a short form of *Demodex* also known as *Demodex cornei* (Izdebska, 2010)

Demodex canis:

This mite is known in the world since the 19th century, and has been studied extensively (Joanna N. Izdebska, 2010).

It's a wormlike parasite. The male is 220-250μm long and 45μm wide; the female is no longer than 300μm and a little bit wider than the male (Casarosa, 1985).

The rostrum is short and square. It has four pairs of limbs that end with a small claw, and all of them are located in the anterior part of the body called "podosoma". The posterior part of the body, called "opisthosoma", is 2/3 of the body. The vulva of the female is located in the ventral part of the body, posterior to the limbs. The male penis is located on the dorsal part of the body, next to the 2nd pair of limbs. The presence of the sex organs can help identify adults from the nymph that lack these organs. The larva has three pairs of limbs, instead of four. The egg looks like a lemon or pistachio (Albanese and Leone, 2007).

Demodex injai:

It's a long bodied species. Adult males of *D. injai* are more than twice the length of *D. canis* males, and the adult female mite is approximately 50% longer than *D. canis* female mites (Ordeix et al., 2008). It was first recognized as a separated species in Columbus, Ohio October 1996 (Desch and Hillier, 2003). So far it has been found in USA, Australia, Spain, Poland and Italy (Izdebska, 2010). Demodicosis resulting from *D. injai* has been described in several dog breeds; however, terrier dog breeds and their crosses were over-represented in several studies. It has been suggested that the West Highland White Terrier dogs may be predisposed to this demodicosis. This form of canine demodicosis is more common in adult dogs and appears to be frequently associated with coexistent allergic dermatitis (Ordeix et al., 2008).

Demodex cornei:

A form of *Demodex* mite shorter in length than *Demodex canis*. The length of the parasite is 110-130µm, significantly shorter than either male or female forms of *D. canis* (Chesney, 1999; Yukio Tamura et al., 2001). Compared to *D. canis* it has a 'stumpy' body shape. The dorsal surface of the leg region of the proterosoma is flat, while shallow transverse grooves are observed over the entire opisthosoma; the terminal end is obtuse (Yukio Tamura, et al., 2001).

First reports of the mites were in Taiwan (Chen, 1995), in Japan (Yukio Tamura et al., 2001) and Greece (Saridomichelakis, et al., 1999). At present there are reports of the presence of *D. cornei* in countries all over the world, including Italy.

Demodex is a normal component of the fauna of the skin. This species complete its entire life cycle on the host. It is transmitted from the mother to the puppies by contact during the first few hours of their lives, becoming like that a regular "guest" of the skin of dogs (Taylor et al., 2010).

They live mostly in the hair follicles and in the sebaceous glands in the dermis feeding on sebum, tissue fluids and cellular debris. Their life expectancy is between 2 to 4 weeks (Albanese and Leone, 2007). *D. injai* mites prefer the dorsal trunk (Ordeix, et al., 2008) and tend to reside within the sebaceous glands. Unlike the other canine *Demodex* species, *D. cornei* can reside in the most superficial layer of the epidermis (Tater and Patterson, 2008).

The female lays 20-24 tapered eggs inside the hair follicle. From the egg hatches a hexapod larva with limbs ending with three pronged claws. The octopod protonymphs, tritonymphs and adults are the following stages. All of these stages continue to migrate deeper in the dermis. They can't live outside of the host.

The characteristic position of the mites in the hair follicle or in the sebaceoum gland is "head upside down" (Taylor et al., 2010).

Epidemiology

Demodicosis is a common disease affecting dogs world-wide. This disease is one of the most serious canine dermatotitis (Paterson et al. 2009). *Demodex* mites have been found in most mammals (Rojas et al. 2012). They are host-specific ectoparasites (Chesney 1999), and two or more different *Demodex* species can appear in the same host, but in different ecologic niches (Mueller and Bettney 1999). *Demodex* mites are believed as normal parts of the fauna of the skin of the dogs and to be transferred to the puppies by the mother during their first days of life (Mueller et al. 2011). Their primary food source is from the secretions of follicular glands or sebaceous glands. Under normal conditions, they do not cause skin disorders (Tsai et al. 2012).

Canine demodicosis is a severe and highly prevalent dermatologic disease that occurs when mites proliferate in hair follicles and sebaceous glands and greatly increase in number. Al three recognized *Demodex* morphotypes (species) can produce clinical effects (Rojas et al 2012; Ravera et al. 2010). The disease is thought to be the consequence of a genetically mediated specific immunodeficiency that allows the proliferation of *Demodex* mites (Ravera et al. 2010; Scott et al. 2001, 2).

Demodicosis can be categorized into localized and generalized forms and the generalized form is further classified as juvenile-onset and adult onset (Rojas et al. 2012; Paterson et al. 2010; Ghubash 2006). In young animals, other parasitic infections (Plant et al. 2011), malnutrition and debilitation (Mueller et al. 2011), as well as genetics, stress, and breed appear to be contributing factors that may lead to an immune-compromised state that favors mite proliferation and development of skin disease (Ghubash 2006). In adult animals *Demodex* occurs secondary to underlying immunosuppressive conditions, chemotherapy, neoplasms, hypothyroidism or hyperadrenocorticism that may suppress the immune system sufficiently to trigger proliferation of the mites (Mueller et al. 2011; Ghubash 2006).

Clinical signs

Canine demodicosis can be separated into two categories, juvenile onset and adult onset. Demodicosis can be further subdivided into localized and generalized demodicosis. The exact pathogenesis of canine demodicosis is unknown. Juvenile demodicosis appears to be a multifactorial disease, with genetics, nutrition, stress, and breed appearing to be predisposing factors. Adult demodicosis occurs secondary to underlying immunosuppressive conditions, such as hyperadrenocorticism, neoplasia, diabetes and metabolic diseases. Localized juvenile demodicosis

is a mild form of the disease, with 90% of affected patients resolving on their own, and 10% progressing to generalized disease that requires treatment and castration. Generalized juvenile demodicosis typically occurs within the first 18 months of age and classically affects more than five body locations, an entire body region, or more than two feet (Ghubash, 2006).

In the first stages of the infection there is a moderate loss of hair on the face and legs, followed by thickening of the skin. The infection could heal spontaneously or spread on the entire surface of body. A characteristic sign is the absence of pruritus. Other symptoms are erythema, desquamation, follicular papules and comedones, hemorrhagic exudate, thick crusts. Frequently, secondary bacterial infections can be observed (Taylor et al., 2010). The most common is *Demodex canis* (Izdebska 2010), which is located in the hair follicle, sebaceous duct, and sebaceous gland (Rojas et al. 2012). *Demodex injai* lives in the hair follicle and inside the sebaceous gland (Desch and Hillier 2003) and is associated with truncal seborrhea oleosa and alopecia (Mueller and Bettney 1999; Hillier and Desch 2002). *Demodex cornei* resides in the host's stratum corneum (Rojas et al. 2012).

Diagnosis

Other diseases to take under consideration for the differential diagnosis (Albanese and Leone, 2007) are:

- Pyoderma
- Dermatophytosis
- Pemphigus foliaceus
- Sebaceous adenitis
- Leishmaniosis
- Lymphoma epitheliotropic
- Endocrinopathies

Deep skin scrapings - Deep skin scrapings are the diagnostic test of choice in suspect cases. Multiple scrapings (approximately 1 -2 cm) of affected skin are performed in the direction of the hair growth, and the skin should be squeezed during or between scrapings to extrude the mites from the deep follicles to the surface. Ulcerated areas should not be scraped because mite yields may be low in such areas. The skin is scraped until capillary bleeding occurs, which indicates that the scraping has reached sufficient depth (Mueller et al. 2011).

Although *Demodex* mites are part of the normal microfauna, it is uncommon to find also a single mite even on several deep skin scrapings. If a mite is found, this should raise suspicion and additional skin scrapings should be performed. Finding more than one mite is strongly suggestive of clinical demodicosis (Mueller et al. 2011).

Trichoscopic examinations – It is particularly useful in areas that are difficult to scrape, such as periocular and interdigital areas (Mueller et al. 2011).

Skin biopsy is used when skin scrapings and trichoscopic examinations may be negative. This may be more likely in certain body locations, such as the paws, and some dog breeds, such as shar-peis (Mueller et al., 2011).

PCR - technique that amplifies *Demodex* DNA not only on tissue samples, such as hairs, but also on formalin-fixed paraffin embedded material (Ravera et al., 2010).

Treatment and prevention

- *Amitraz* - This emulsion of water and organic solvent is a broad spectrum miticide that is a member of the formamidine family. It is an inhibitor of monoamine oxidase and prostaglandin synthesis (Ghubash, 2006). It has been approved for the treatment of canine generalized demodicosis in many countries for decades (Mueller et al., 2011).

The recommended concentration varies from 0.025 to 0.06%, with a frequency of once weekly to every 2 weeks. Clinical efficacy increases with increasing concentration and shorter treatment intervals. Amitraz rinses seem to be less efficacious in adult dogs (Mueller et al., 2011).
To increase efficacy of this medication, animals should be clipped before dipping, and should not be bathed between applications (Ghubash, 2006; Mueller et al., 2011). The rinse should be applied carefully with a sponge and soaking the skin, and allowed to air dry without rinsing.
Side effects of amitraz reported in various studies are depression, sleepiness, ataxia, polyphagia, polydipsia, vomiting and diarrhea. Amitraz anecdotally has caused headaches and asthma in owners, thus it is commonly recommended that dogs should be washed in a well-ventilated area (Mueller et al., 2011). Care should be used when using this medication in toy breeds, and one-quarter and one-half strength amitraz dips are recommended in these smaller patients. Toy breeds seem more susceptible to side effects such as lethargy, depression, and neurologic side effects. When used at label doses, Yohimbine is the reversal agent for amitraz, and can be used if side effects are excessive (Ghubash, 2006).

- *Ivermectin* - The dose recommended for treating canine demodicxosis is 0.4 to 0.6 mg/kg. It is recommended to start at the lower end of the dose range and adjust according to response to treatment (Ghubash, 2006). The use of ivermectin should be avoided in heartworm positive dogs, as animals with a high microfilaria load can have adverse reactions. The main side effects of this medication are neurological, and include changes in pupil size, behavior, ataxia, seizures, coma, and death. Collie dogs and other herding breeds are most commonly affected, but other breeds have also been reported (Ghubash, 2006; Mueller et al., 2011).
- *Moxidectin* - doses of 0.2–0.5 mg⁄ kg ⁄ day p.o. with comparable success to ivermectin. Adverse effects are similar to those of ivermectin, but more common. Moxidectin has also become available as a 2.5% spot-on formulation (Mueller, et al., 2011).
- *Milbemycin* - used at a dosage of 1.5 to 2 mg/kg/day for the treatment of canine demodicosis. Even though it is related to ivermectin, it appears to be safer and associated with fewer side effects, even in ivermectin sensitive breeds (Ghubash, 2006).
- *Doramectin* - at a dose of 0.6 mg⁄ kg p.o. or s.c. weekly may be used for the treatment of demodicosis (Mueller et al., 2011).

Otodectes cynotis

Biology

Otodectes cynotis (Acari: Psoroptidae) is an obligatory, non- burrowing mite who infects carnivores and occasionally humans (Otranto et al., 2004).

It has an egg shaped body and it's 460-530µm long. The rostrum is long and shape like a cone. The limbs are long with short pedicels and end with suckers shaped like cup, which help the mite move between the cerumen and the epidermic scales. The parasite has bristles on the back and on the abdomen, different number for each stage of life, and they function as a sensory organ. When the mite becomes an adult, the 3rd pair of limbs gains importance for walking, and the 4^{th} pair is developed. The genital open is transverse. Eggs are oval shaped.

There are a few notable differences between the male and the female (Taylor et al., 2010):

- The male has 4 pairs of long limbs that exceed the limits of the body, in front and in the back; in contrast, the 4^{th} pair of limbs of the female is rudimental and does not surpass the posterior part of the body.
- The males' abdominal lobes are poorly developed with bristles; the females' abdominal lobes are developed.
- The male has sucking cups in the end of each limb; the female has those only in the end of the anterior limbs. The posterior limbs have a long, whip-like bristles.
- The female can get to almost double the size of the male, and the form of the body remains almost like in larval and nymphal stages.

The life-cycle occurs entirely within the ear, where *O. cynotis* goes through four stages (egg, hexapod larva, proto- and trito-nymph, adult) in about 3 weeks. The females are laying 15 to 20 eggs. Their nutrition is based on skin debris. The transmission occurs by direct contact between the animals, especially between infected mothers and their litters (Taylor et al., 2010). Rarely do they live outside of the ear canal, on the body of the animal, usually on the head, feet, and the tip of the tail (Otranto et al., 2004). The copulation is between an adult male and a female deuteronymph.

Epidemiology

Ear mites of the genus *Otodectes* are frequent parasites of cats and dogs. They are regarded as the most common cause of OE in these animals all over the world (Lohse et al. 2002), with prevalence ranging from 2 to 29% in dogs (Six et al. 2000). The genus is regarded as mono-specific and the mites are attributed to a single species: *Otodectes cynotis* (Lohse et al. 2002). The mite belongs to the family Psoroptidae (Curtis 2004). It is a mite of the family Psoroptidae, which are non-burrowing surface living mites (Shanks et al. 2000, 2).

The mite infects the ear canal of carnivores, such as dogs, cats, ferrets, foxes, and occasionally also in humans (Otranto et al. 2004). Transmission is by direct contact from animal to animal and via fomites (Shanks et al. 2000). Puppies and kittens appear to be most susceptible to otoacariasis, as older animals may acquire immunity (Curtis 2004). Those mites can live off the host for several weeks (Ghubash 2006).

Clinical signs

Animals infested by *O. cynotis* most commonly develop otitis externa characterized by vertical and horizontal canal erythema and a dark brown, ceruminous exudate. In addition to otitis externa, 'ectopic' infestations of the head, neck, tail head and rarely the trunk can occur when mites escape the ear canals and papulo-crustous lesions (miliary dermatitis) may be observed (Curtis, 2003; Ghubash, 2006).

In addition, the intensive pruritus may be responsible for self-induced lesions and otoematoma (headshaking). It is possible to observe secondary infections by bacteria and *Malassezia* (Albanese and Leone 2007).

Puppies appear to be most susceptible to otoacariasis (Curtis 2003).

Diagnosis

The disease is suspected due to the animal's behavior and to the presence of dark cerumen in the ear canal (Taylor et al. 2010). Other diseases should be considerate in the differential diagnosis, as are other causes of otitis (bacteria and *Malassezia*), sarcoptic mange, allergic and fungal dermatitis (Albanese and Leone 2007).

The definitive diagnosis is based on the observation of the mites in the ear canal with an otoscope or in the cerumen that can be removed and observed on a dark surface. The mites could be observed as mobile white spots (Taylor et al. 2010). They can also be identified when checking under the microscope the cerumen. If the infection is located out of the ear canals, on the skin around, it is possible to do a superficial skin scraping. In those methods it is possible to find the mites in different stages of their life cycle (Albanese and Leone 2007).

Another method commonly used in clinics is the "Therapy trial" (Albanese and Leone 2007).

PCR was used to clarify whether *Otodectes cynotis* is a single species or different species identified as one by Lohse et al. (2002), where they found that the mites belong to a single species.

Treatment and prevention

Treatment of otocariasis includes the mechanical cleaning of the ear canal followed by the application of products containing acaricides, antibiotics and synthetic corticosteroids (Otranto et al. 2004), although the ear cleaning and the manipulation of the ear base recommended after the instillation of drugs are often resented by animals with aural inflammation (Six et al. 2000). As a general rule, whenever topical therapy is prescribed for otoacariosis, drug-to-mite contact is improved by pretreatment with a cerumenolytic to remove exudate from the vertical and horizontal canals (Curtis 2004). Multiple treatments are usually necessary (Six et al. 2000), for at least 10 days, to ensure that all ova have hatched and that the newly emerged larvae are exposed to the drug (Curtis 2004). Regardless of the treatment utilized, all contact animals should be treated (Ghubash 2006; Curtis 2004). No treatment has been suggested for the control of otocariasis in the

environment (kennels) where infected animals live, since there is no evidence that the transmission occurs via the environment (Otranto et al. 2004). However, since the mites are capable of surviving for several weeks to months away from the host, care should be used in treating and disinfecting the environment (bedding and grooming equipment) when treating confirmed cases of otoacariosis (Ghubash 2006; Curtis 2004).

Selamectin is labeled for the treatment of *Otodectes* at the manufacturer's recommended dose based on the weight of the patient and it has been found to be extremely effective in several studies (Six et al. 2000; Sharks et al. 2000). In these studies, one single dose was found to be highly effective. Because of the ease of treatment and wide safety margin this medicine is also recommended by Ghubash (2006).

Fipronil solution 10% was effective in controlling otoacariosis (Vincenzi and Genchi 1997). In animals that resent topical treatment, the less frequent use of a long-acting acaricide has obvious benefits and a single, otic application of two drops was effective (Vincenzi and Genchi 1997).

Topical Treatments are effective at treating *Otodectes*, although with the advent of Selamectin they have lost some of their popularity because of the lack of convenience. Some of these products are thought to work by smothering the mite (Ghubash 2006), whereas others contain miticidal agents such as thiabendazole, monosulfiram or permethrin (Ghubash 2006; Curtis 2004). These drugs have a limited residual action and require regular reapplication for at least 10 days, to ensure that all ova have hatched and that the newly emerged larvae are exposed to the drug (Curits 2004). The biggest benefit of topical products (as Tresaderm) is that they often contain a combination drugs useful for the treatment of secondary bacterial and yeast infections (Ghubash 2006).

Aural administration of ivermectin is often effective for treatment of *Otodectes*. However, because of erratic drug absorption of the aural version, ivermectin can be more effective when 0.2 to 0.3 mg/kg is given orally every 7 days for 3 treatments, or subcutaneously every 14 days for 2 treatments (Scott et al. 2001, 2). Because of the availability of safer treatments, injectable ivermectin use for *Otodectes* is only recommended when selamectin cannot be used and other treatments have failed (Ghubash 2006).

Neotrombicula autumnalis

Biology

N. autumnalis is a Trombiculid mite. Among these mites are included species known to cause skin diseases in some parts of the world (Jones, 1950). *N. autumnalis* is widespread in Europe, including Italy (Taylor, et al., 2010). Only the larva is a parasite of mammals, including cats, dogs and humans. The adults live in the external environment at depth of about 300mm below the surface of the soil (Jones, 1950). The larva is a parasite of the skin; in dogs, they are mostly found on the legs andthe genitals (Taylor, et al., 2010).

N. autumnalis larvae have an egg shaped body, 250-500µm long and a characteristic red-orange color. The rostrum is developed and pointed, with 2 clamp-shaped palps called "chelicerae". They have 3 pairs of long legs (hexapod larva) and a small dorsal pentagonal shield with 5 bristles called

"scutum". On their body and legs, long feathery bristles are present (Albanese and Leone, 2007). On each side of the scutum there are two simple eyes.

The morphology of the nymphs and adults remind the number eight. They have stigmata that open on the base of the chelicera, and the body is covered with bristles. The adults can arrive up to 1 mm long (Taylor et al. 2010).

The unfed larvae are found either upon the soil or climbing upon low-lying vegetation. Under suitable conditions they aggregate into clusters and are then more easily detected as orange patches. Development to the nymphal stage cannot take place unless the larvae obtain a meal from the superficial tissue of a vertebrate host to which they must securely attach themselves (Jones, 1950).

In Europe, endemic areas are woods, gardens and fields. They are more active in sunny areas with dry climate. The infestation is seasonal, and occurs from late summer until the end of the autumn (Albanese and Leone 2007). The hairs of a mammal, as they brush against infected soil or low-lying vegetation, are admirably suited for picking up the mites (Jones, 1950).

Clinical signs and Diagnosis

Infestation can result in pruritus, erythema, papules and excoriation leading to hair loss, though there may be considerable individual variation in response. This variation may reflect the development of a hypersensitivity reaction to the mites. Small clusters of larvae may be seen on the skin surface that may be collected and examined under the microscope for their identification. However, in some cases pruritus occasionally may continue long after the mites have left.

Treatment and prevention

Few data are available in the literature on the treatment of *N. autumnalis* infestation in dogs and cats.

Fipronil have been reported to be effective in the treatment of *N. autumnalis* (Nuttall et al. 1998).

Topical permethrin-pyriproxyfen combination was effective within one to three weeks in dermatitis caused by these mites (Smal et al. 2004).

In one recent case of dermatitis there was successful treatment with fipronil spot on, permethrin shampoo and cephalexin for two weeks (Kavitha et al. 2011).

Enviromental treatment of yards with acaricides (deltamethrin, carbaryl) may help to reduce mite abundance. However, area-wide control is usually impractical. Animals should avoid sites of known mite prevalence (Taylor et al. 2010).

MALASSEZIA AND OTITIS EXTERNA

Biology

Malassezia yeasts are commensal organisms of human and animal skin that occasionally act as pathogens (Cafarchia et al., 2005).

The genus *Malassezia* belongs to the kingdom of Fungi, phylum Basidiomycota, Hymenomycetes Class, Order Trimellales, Family Filobasidiaceae (Nobre et al. 2001).

Most of the species of *Malassezia* are lipophilic non-mycelial, unipolar budding yeasts characterised by a thick cell wall (Cafarchia et al., 2005). There are 13 species in this group (Nardoni et al. 2008). *M. pachydermatis* is the only non-lipido-dependent species. It probably adheres to the skin corneocytes by tripsin-sensitive protein adhesion molecules (Nardoni et al., 2008).

Malassezia spp. multiply by an asexual reproduction, with production of blastoconidia through a repetitive single polar process or budding, forming a round, ovoid or cylindrical cell when it separates from the mother cell. For this reason it has a particular shape of peanut, and therefore easy to recognize under the microscope. When not in a budding phase, it is possible to observe a rounded or cylindrical shape. Hyphae and pseudohyphae are absent (Nobre et al. 2001). However, in a study describing the genome and secretory proteome of *M. globosa* and *M. restricta*, authors reported that the genome of *M. globosa* contain mating type genes, providing an indication that *Malassezia* may be capable of sex (Xu et al., 2007).

Under the microscope it is possible to observe the fungus at a 1000X magnification as an isolated cell or a group of neighboring cells and/or piled up in the vicinity of the epidermal cells of desquamation. The dimension of *Malassezia* is between 3 to 5 µm in diameter (Nardoni et al., 2008).

Traditionally, lipid-dependent *Malassezia* species were thought to occur only on human skin, while *M. pachydermatis* predominated on animal skin, particularly dogs. Lipid-dependent species, however, may be isolated from a wide range of domestic and wild vertebrates (Machado et al. 2010). Although *M. pachydermatis* is mainly adapted to live on animals, it has also been reported as a causative agent of nosocomial infections in humans (Cafarchia et al., 2005). In particular, this yeast has been shown to be transmitted by human healthcare workers from their pet dogs to their neonatal patients (Chang et al., 1998).

Malassezia is frequently isolated from the external ear canal, from the skin, particularly the anal area (which could be a carriage zone), oral mucosa, vagina and eye of healthy dogs (Chen and Hill, 2005; Prado et al., 2008).

Recent paper suggested that strains of *M. pachydermatis* isolated from dogs with and without skin lesions have the ability to form biofilm at variable levels and structures (Figueredo et al., 2012). A biofilm is defined as a differentiated mass of microorganism community tightly attached on (therefore hardly removable from) a biotic or abiotic surface (Blankenship and Mitchell, 2006). The development of a biofilm allows the multiplication of the microorganisms and their protection from the host immune system (Donlan and Costerton, 2002). *Malassezia* produces enzymes, such as

phospholipases, that alter the cutaneous lipidic film, pH and proteases that induce inflammation and pruritus through proteolysis and complement activation (Nardoni et al., 2008).

Epidemiology

Malassezia dermatitis in dogs is the result of an opportunistic infection. Opportunistic infections typically arise secondary to a primary disruption in normal barrier function that creates a favorable microenvironment for *Malassezia* overgrowth (Berger et al., 2012). Skin abnormalities enhancing *Malassezia* overgrows are excessive moisture and amount of sebum or cerumen, and disruption of the epidermal barrier and intertrigo (Nardoni et al., 2008). Dogs with *Malassezia* dermatitis may have 100- to 10 000-fold the population of *Malassezia* compared with healthy dogs (Machado et al., 2010). Secondary infections with *M. pachydermatis* are frequently seen in dogs (Chen and Hill, 2005) and occasionally in cats (Ordeix et al., 2007).

Primary disorders associated with *Malassezia* dermatitis include pruritic inflammatory diseases (allergic and parasitic), primary keratinization defects, endocrinopathies, metabolic diseases and neoplasia (Berger et al., 2012).

Interactions between host and *Malassezia*, and the pathogenesis of canine *Malassezia* dermatitis, are not clearly understood (Chen and Hill, 2005); multiple mechanisms may be involved, including changes in host defenses and the cutaneous microenvironment (Maynard et al., 2011).

Otitis externa (OE) is a common problem in dogs, estimated to occur in 10 to 20% of canine patients seen by veterinarians (Scott et al., 2001). Primary causes and predisposing factors create suitable conditions for the proliferation of secondary organisms such as bacteria and yeasts in the ear canal (Rosser, 2004). The common secondary pathogens are bacteria such as *Staphylococcus intermedius* (now reclassified as *Staphylococcus pseudointermedius*), *Pseudomonas aeruginosa* and *Proteus* species and yeasts such as *M. pachydermatis* (Scott et al., 2001). *Malassezia* spp. were isolated from 41.2%-72.9% of cats and from 57.3%- 62.2% of dogs with otitis externa, mostly *M. pachydermatis* (Nardoni et al., 2007; Cafarchia et al., 2005).

Higher frequency and population size of *Malassezia* yeasts have been reported in animals with otitis compared to healthy animals, which indicates that *Malassezia* yeasts overgrow in infection sites may play a role in the pathogenesis of otitis externa (Cafarchia et al., 2005).

The most common primary causes of OE are allergies such as atopic dermatitis and adverse food reactions (Zur et al., 2011). Keratinization disorders, either primary, as in idiopathic seborrhea or secondary, as in hypothyroidism and sex hormone imbalance, are also common primary causes affecting the secretions of the ceruminous and sebaceous glands lining the ear canal (Rosser, 2004). Anatomical changes in the ear canal such as conformational abnormalities are commonly represented among the predisposing factors (Zur et al., 2011). Ear parasites and foreign body, such as plant, hair or hardened secretions are predisposing factors as well (Machado et al., 2010).

Opportunistic bacterial or fungal infection in humans and dogs can occur when organisms that are normal resident flora or transient flora become pathogenic due to alteration of the host's immune system, and can be seen with congenital or acquired immune dysfunction, with immunosuppressive

therapy or with chronic disease, such as diabetes mellitus and hyperadrenocorticism (Short et al., 2009; Greene and Chandler, 2006). Additionally, in humans and less commonly in dogs, prolonged antibiotic treatment which alters the normal resident flora of the body can predispose to opportunistic fungal infection (Greene and Chandler, 2006; Munguia and Daniel, 2008).

Of the breeds that are genetically affected by conformational abnormalities, German shepherd dogs and cocker spaniels are prone to seborrhea, German shepherd dogs have high moisture levels in their ear canals, cocker spaniels have pendulous pinnae, shar-peis have hypoplastic and stenotic ear canals and poodles have a high density of hair in their ear canals, etc. (Zur et al., 2011; Rosser, 2004; Yoshida et al., 2002).

The age, sex and sampling period are not commonly considered to be predisposing factors for *Malassezia* dermatitis or otitis in dogs (Cafarchia et al., 2005), though it is more often diagnosed in dogs between 1 to 3 years of age (Nardoni et al., 2008; Maynard et al., 2011).

In many cases of OE, more than one kind of microorganism is either isolated by bacterial culture or found in cytology smears (Graham-Mize and Rosser, 2004).

Clinical signs

Primary lesions of *Malassezia* dermatitis are commonly associated with pruritus. The pruritus may vary from mild to severe, and the severity is related to the importance of predisposing factors (Nardoni et al., 2008). Secondary changes might be erythema, alopecia, excoriations, seborrhea, lichenification, hyperpigmentation, excessive scaling (Machado et al., 2010; Berger et al., 2012).

Malassezia dermatitis in dogs may be localized or generalized. The classical areas involved are neck, axillae, abdomen, pinnae and external ear canal, lips and perianal area. Dogs with generalized lesions have a rancid odor (Nardoni et al., 2008; Negre et al., 2009).

In rare cases, *M. pachydermatis* can cause a folliculitis which resembles that caused by Staphylococci (Morris 1999).

Another disorder, that may be the only clinical manifestation of infection *M. pachydermatis*, or more often can be understood in a context of widespread dermatitis, is paronchia (inflammation of the nail bed). The characteristic symptom is a continuous chewing and licking of the paws that in the long run leads to a brownish-red coloration of the nails and the hair of the fingers, and to the production of a dark brown waxy exudate between the inter-digital spaces with inflammation of the soft tissues adjacent (Morris 1999).

The disease is often recognized during warm period, at the time at which allergic dermatitis are generally diagnosed (Nardoni et al., 2008).

Lipid-dependent species have been associated with pathologies in humans including pityriasis versicolor, folliculitis, seborrheic dermatitis, otitis and even fungaemia found in new born infants (Duarte and Hamdan, 2010).

The clinical symptoms observed in dogs with otitis are: severe itching, erythema, thickening of the epidermis of the ear canal with possible stenosis of the same until its complete obliteration in severe cases, and a significant production of earwax. The exudate that is found *Malassezia* otitis is very characteristic, not only for the look that recalls the consistency of a jam, and the color that ranging from brown to yellow, but for the unmistakable smell of rancid or may remember the yeast (Nobre et al., 2001; Morris, 1999).

Diagnosis

Initial diagnosis of canine *Malassezia* dermatitis relies on compatible clinical signs and demonstration of high *Malassezia* numbers on skin (Maynard et al., 2011). Clinical signs of *Malassezia* dermatitis are variable and may mimic many dermatoses, therefore the differential diagnosis includes many pruritic dermatoses characterized by erythema, hyperpigmentation and seborrheoa together with all underlying dermatological diseases of the yeasts overgrowth (Nardoni et al. 2008).

Several techniques, such as cytology, fungal culture and histopathology, are used to detect yeasts, but which, if any, are the most efficient is largely unknown. When cytological examination is negative, a fungal culture is necessary to exclude or confirm *Malassezia* dermatitis (Cafarchia et al., 2005). Cytological examination allows to rapidly observe yeasts and to "quantify" their number (Nardoni et al., 2008). It is considered very unlikely that significant organisms will be retrieved by culture if they are not seen on cytological examination (Ginel et al., 2002).

Many techniques are available to obtain material from the skin, such as direct impression smear, Scotch test, scrape smear and swab smear. Impression smear and Scotch test seem to be the best techniques if the skin surface is flat or greasy, and the swab smears should be more useful for cytological examination of the external ear canal (Nardoni et al., 2008).

Diagnosis of *Malassezia*-associated otitis is performed by otoscopic and cytologic examination. Erythema and increased moisture in the ear canal are indicators for an inflammatory process, while hyperplasia of the ear canal wall also indicates chronicity (Hensel et al., 2009).

In order to examine the ear exudate in dogs with ceruminous or exudative OE, rolling of exudate in a thin layer on glass slides with a cotton-tipped swab may be the preferred method (Aspı´roz et al. 2010). Heat fixing does not seem to increase the number of *Malassezia* on cytology of ear swab samples for cytological evaluation. Slides or actate tape may be stained with Diff-Quick, May Grünwald-Giemsa, Giemsa or new methylene blue (Nardoni et al., 2008).

Diff-Quick coloration is performed by consecutive immersion of the slide in three different reagents, which are methanol (fixative), eosin (acidophilus dye), methylene blue (basophilic dye). (Cafarchia et al. 2005, 1; Nobre et al. 2001; Morris 1999).

The May Grünwald-Giemsa stain is performed as follows: the slides are treated with methyl alcohol for 5 minutes, and then for the next 40 minutes with Giemsa solution of the reagent with 1:10 dilution. Finally, it will be necessary to rinse with distilled water and dried at room temperature (Cafarchia et al. 2005, 1; Nobre et al. 2001; Morris 1999).

Microscopic examination with an oil immersion lens in 1000X magnification reveals the yeasts, free or adhered to keratinocytes (Nardoni et al., 2008).

The minimum number of yeasts that indicates the possibility of a true *Malassezia* dermatitis is unknown. As a general guide, 1-2 organisms per field (1000X) in several fields in the presence of typical clinical signs are suggestive of *Malassezia* dermatitis (Nardoni et al., 2008).

Malassezia otitis is considered positive if more than 10 cells morphologically identifiable as *Malassezia* yeasts are found in five random fields at 400X magnification (Cafarchia et al., 2005).

Since *Malassezia* spp. is a normal component of the cutaneous flora of the dog, the number of colonies (growing in cultures like mDixon agar, modified Leeming, Notman agar for all species, and selective medium for dermatophytes is in use for the growth of *M. pachydermatis*) might be an indication, but just a positive culturing does not indicate the disease (Nardoni et al., 2008).

Currently, the identification of *Malassezia* species is based on phenotypic and molecular characteristics (Machado et al., 2010).

Several molecular methods, including sequencing of the large subunit (LSU) and internal transcribed spacer 1 (ITS-1) of nuclear ribosomal RNA, and chitin synthase 2 gene (CHS2), have been proposed for taxonomic and⁄or epidemiological purposes (Sugita et al., 2004; Gupta et al., 2004). Currently, polymerase chain reaction–restriction endonuclease analysis (PCR-REA) of the LSU rRNA gene33 is regarded as a reliable and rapid method to distinguish *Malassezia* species (Machado et al., 2010).

Isolates of *Malassezia* spp. may be characterized using random amplified polymorphic DNA (RAPD) techniques and with the use of different primers, some intra-species variations may be seen (Duarte and Hamdan, 2009).

The genetic heterogeneity between *Malassezia* strains isolated from animals and humans has not been completely studied. The most important argument to consider the RAPD–PCR as a clearly useful procedure for grouping *Malassezia* species according to their animal origin may be attributed to the nosocomial and occasionally, fatal infections in neonates caused by the zoophilic species *M. pachydermatis* (Duarte and Hamdan, 2009).

Dogs with *Malassezia* dermatitis have greater concentration of specific IgG than normal subjects, whereas atopic dogs, with or without concurrent *Malassezia* dermatitis, have higher levels of specific IgG and IgE then non atopic dogs with *Malassezia* dermatitis or normal dogs (Nardoni et al., 2008).

The response to treatment with specific antifungal therapy is considered the best tool for a definitive diagnosis (Nardoni et al., 2008).

Treatment and prevention

Malassezia dermatitis in dogs can be topical, systemic or both (Negre et al., 2009). Systemic antimicrobial agents are efficacious in many situations, but may be associated with systemic

adverse effects and increased costs. In addition, there is concern about antibiotic resistance following the use of systemic antibiotics. Topical therapy provides an alternative approach to skin infections (Mueller et al., 2012). The choice of the pharmacological intervention should be adapted to the severity of the dermatitis and the achievability of the intervention (Negre et al., 2009).

Shampoos are generally indicated when haired skin and/or large areas on the animal are affected. The suitability of shampoo therapy for a particular patient will depend on a number of factors. Species, breed, temperament, pet–owner relationship, climate, season and shampoo technique may all affect treatment outcome (Mueller et al., 2012). Chlorhexidine has also been used in association with miconazole with good efficacy, particularly against *M. pachydermatis* in vitro (Young et al., 2012) and in vivo (Bond et al., 1995).

There is good evidence for recommending the use of 2% miconazole nitrate + 2% chlorhexidine gluconate shampoo twice a week for 3 weeks (Negre et al. 2009). In a recent study (Maynard et al. 2011) about the difference of the efficacy of 2 shampoos - 3% chlorhexidine (CHX) shampoo and 2% miconazole-2% chlorhexidine (MIC/CHX) shampoo, both shampoos were highly effective in controlling *Malassezia* overgrowth, 28 days of topical treatment with either shampoo were required for full efficacy in most dogs, both products also greatly ameliorated canine *Malassezia* dermatitis. Mild scaling and pruritus was reported in four dogs treated with 3%CHX versus no dogs with 2%MIC/CHX, although these side effects were benign and transient and overall tolerance was considered good by veterinarians. This is the first report of a potential product-related adverse event with the use of a chlorhexidine shampoo (Maynard et al., 2011).

Topical preparations are best suited for treatment of areas where the pet cannot easily lick the product off. If the affected skin is easily reached, preventive measures, such as E-collar should be undertaken after application. In cases where extensive areas are affected, shampoos, sprays or solutions are better suited than gels, creams and ointments. Exudation can prevent the active ingredient from reaching the infection and lead to treatment failure if the area is not cleaned before applying the drug (Mueller et al., 2012).

In fungal cells, the fixation of miconazole on cytochrome P450 inhibits the synthesis of ergosterol, triglycerides, phospholipids, chitin and oxidative and peroxidative enzymes. All these modifications perturb numerous functions of the yeast membrane, leading to accumulation of hydrogen peroxides, which asphyxiate the fungal cell (Negre et al., 2009).

There is also evidence of the efficacy of benzoyl peroxide at 2–3% against cutaneous bacterial and yeast infections (Mueller et al., 2012).

A recent study suggested that both twice-weekly and once-daily dosing of oral terbinafine may be efficacious for the treatment of *Malassezia* dermatitis in dogs (Berger et al. 2012).

Cafarchia and others reported that isolates from animals with lesions may be less sensitive to some antifungal agents (Cafarchia et al., 2012). Difference in susceptibility could be also due to the fact that prolonged exposure to antifungal drugs may induce resistance (Juseus et al. 2011). As a consequence, the antifungal therapy in *Malassezia* infections requires careful appraisal of choice of drugs especially in cases of unresponsiveness to antifungal treatment or recurrent infections (Cafarchia et al., 2012). However, most cases of *Malassezia* otitis that fail to respond to topical

antifungals are likely to be due to poor recognition ⁄ management of underlying conditions rather than to the involvement of 'true' resistant strains of the yeast (Peano et al., 2012).

The frequency of recovery in dogs with otitis externa is about 58.8% (Lyskova et al. 2007; Hariharan et al. 2006). The best therapeutic response is achieved when both *M. pachydermatis* and bacteria are removed by topical treatment (Aspı´roz et al. 2010).

Topical treatment is the preferred method of treating otitis externa caused by *Malassezia* due to the direct contact with the pathogens which can be found in the cerumen and on the surface of the ear epithelium (Hensel et al.,2009). Different azole derivatives, such as clotrimazole (CTZ), miconazole (MCZ) and thiabedazole (TBD), are widely used in topical formulations as suspensions that usually also contain glucocorticoids and antibacterial agents (Peano et al, 2012).

Topical miconazole, a synthetic imidazole, has a broad antifungal spectrum and was more effective in the treatment of *Malassezia* in otitis externa than other topical ear solutions containing antifungals. It also showed some activity against gram-positive bacteria such as *Staphylococcus aureus* (Hensel et al., 2009).

In a study (Lyskova et al. 2007), *M. pachydermatis* was susceptible to all anti-mycotics tested (amphotericin B, bifonazole, ciclopiroxolamin, econazole, fluconazole, itraconazole, ketoconazole, clotrimazole, miconazole, nystatin and pimaricin), with the exception of fluconazole.

Gentamycin at high concentrations used in commercialized media (100mg/ml) effectively inhibits the growth of *M. pachydermatis* on SDA. Growth is also inhibited at other concentrations used in the market (40mg/L). Therefore, topical gentamycin could be an efficacious treatment for disease related to *M. pachydermatis*, and this can be especially convenient in infections where this yeast appears together with bacteria (Aspı´roz et al., 2010).

MATERIALS AND METHODS

In the period between March 2011 and September 2012, skin and wax samples from 87 dogs of various age and breed showing clinical signs of dermatitis and/or otitis externa, were collected (Table 1). Animals were referred to the teaching animal hospital of Pisa University (37) and to other private veterinary clinics (23) located in Pisa municipality or hosted in kennels (27) located in Pisa and Florence districts.

Cases of dermatitis were represented by focal lesions on a small area of the skin or spread on different areas of the skin (head, abdomen, tail, etc.). The skin was the only damaged organ or also other organs, such as the external ears, the eyes, and other organs were involved. Lesions and clinical signs observed in examined dogs are shown in Tables 1, 2 and 3.

- **Clinical examination**

Animals (59) referred to a veterinary clinic or to the veterinary hospital were clinical examined. The first phase of the clinic examination was the signaling of the animals as breed, age and sex (Tab. 1).

The second phase was the anamnesis of the animals, including the reason of the visit, the presence of other concomitant diseases, a remote and close patient history and treatments and drugs that were/are being used.

The third phase was the actual examination of the body of the animal starting with the examination of the pinnae for the evaluation of changes of the skin and/or presence of cerumen close to the ear canal. By mean of an otoscope, the ear canal was examined for pathological changes, such as incomplete eardrum, erythema, quantity and color of ear cerumen. Then eyes, mouth, head, back, chest, abdomen, limbs and the tail are then examined for signs of conjunctivitis, changes of the mucosa, dandruff, erythema, alopecia, etc. The digital pads and the areas between the fingers, a common place for pruritus and erythema, and around the genitals were also examined. Changes of odor and any change of the animal behavior, mainly for signs and localization of pruritic or pain lesions, were reported.

- **Sampling and Parasitological analysis**

According to lesions found during the clinical examination, various kinds of samples were collected. If it was not possible to examine the material in the same day it was collected, it was placed in the refrigerator at 4°C and examined within 24 hours.

- In order to diagnose ***S. scabiei***, superficial skin scrapings of the affected area/s were collected. The hairs from these areas were cut and a scalpel blade was used to collect skin scrapings. The collected material was mounted on a glass slide with a few drops of paraffin oil, covered with a coverslip and examined under the microscope in magnification of 100-400X. If skin scrapings were large and/or thick, few drops of 10% NaOH solution were added and left to stand for 30 minutes and then examined to the microscope. In other cases the scotch test was used: this method consists in sticking the scotch to the hair and skin of the infected area and removing it. The scotch was taped to a glass slide and examined under the microscope at 100-400X magnification.

Considering the difficulty to isolate *S. scabiei* (Albanese and Leone 2007; Bornstein 1991), in some cases the diagnosis was based on the symptoms and on the recovery after using the specific therapy, even with the negativity of the samples that were examined with the previous technics.

- In order to diagnose ***Cheyletiella***, there was no need to do the skin scraping, since the mites live on the outer layer, thus the scotch test and dandruff microscopical examination were performed. In this latter case, few drops of paraffin oil were added on a slide glass to the collected material before it was examined under the microscope.

- In order to diagnose ***Demodex***, the following methods were used: 1) Trichoscopic examination – from the borders of the lesions and from different lesions. The hair was placed on the glass slide with few drops of paraffin oil, covered with a covering slip and the roots of the hairs were examined under the microscope at 400X magnification. 2) Deep skin scraping – since these mites lives in the derma, it was important to arrive there when doing the scraping (Fig. 1). To increase the chances to find the mites, several skin scrapings from different lesions, were collected. The collected material was placed on a glass slide with paraffin oil, covered with the cover slip and examined under the microscope at 400X magnification.

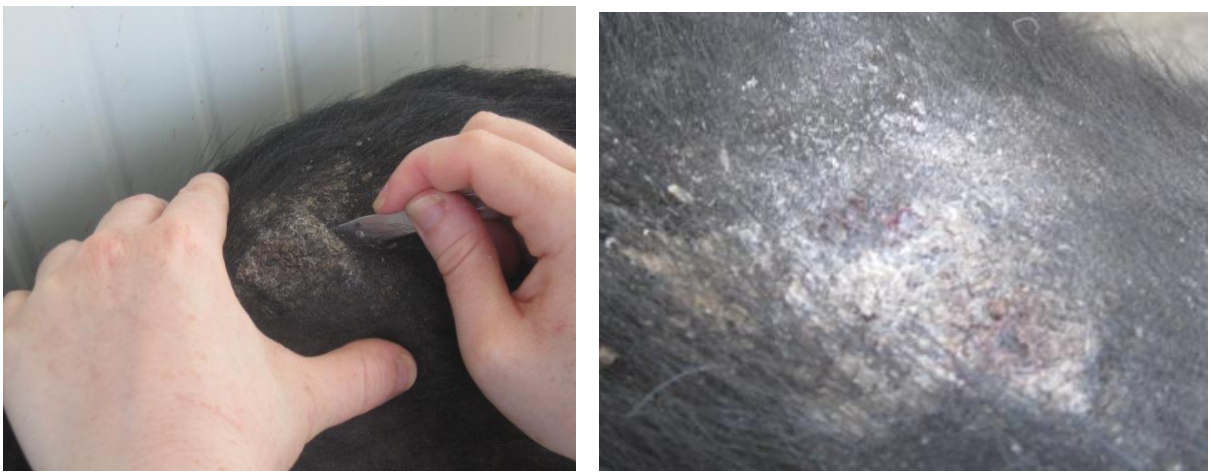


Figure 1. Deep skin scraping showing red dots.

- For **otitis externa** and in order to find mites and *Malassezia* the method used was different. In these cases ear swabs from both ears were taken.

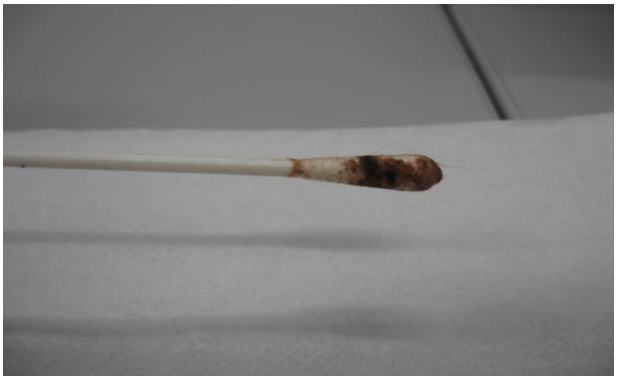


Figure 2. Ear swab from a dog with *Malassezia spp.*

The swab was smeared on a glass slide, colored with the Diff-Quick stain and checked for *Malassezia* under the microscope. The Diff-Quick stain was performed in the following way: First the glass slide was immersed in methanol for about one minute, and then washed with water. Then eosin stained for 1 minute and washed with water. The last phase was to put it for a minute in a tiazinic colorant and wash it again. When the glass slide was dry it was examined under the microscope at 400X and 1000X magnifications. Since *Malassezia* is normally present on the skin and in the external canal of the ears of healthy dogs, the presence of at least 10 *Malassezia* at the examination of 10 fields under the microscope at 400X magnification were indicative of positivity. Pieces of cerumen collected from the swab were placed on a glass slide with vaseline oil, mounted with a cover slip, and then examined for the presence of mites (***Otodectes cynotis***, ***Trombicola autumnalis*** (only larva), ***Demodex***).

Table 1: Breed, age and lesions of 87 dogs examined for parasitic dermatitis and/or otitis externa

				Lesions		
	Age (years)	Breed	Sex	Body	Ear	Ownership
1	8.5	Crossbred	male	+	-	Kennel
2	8	Crossbred	male	+	-	Kennel
3	12	crossbred	male	-	+	Kennel
4	0.83	Maremma sheepdog	male	-	+	Private
5	10	Labrador	female	-	+	Private
6	8	Bracco	female	+	-	Private
7	10	English Setter	female	-	+	Private
8	8.5	crossbred	female	-	+	Kennel

9	2	Boreder Collie	male	+	-	Private
10	0.5	Pug	female	+	+	Private
11	15	Crossbred	female	-	+	Kennel
12	9	Crossbred	male	-	+	Kennel
13	0.5	Cocker	female	-	+	Private
14	15	Crossbred	male	-	+	Kennel
15	8	Crossbred	male	-	+	Private
16	4.5	crossbred	male	-	+	Kennel
17	4	Golden Retriever	female	-	+	Private
18	6	Crossbred	male	-	+	Kennel
19	11	Crossbred	female	-	+	Private
20	5.5	German Shepherd	male	+	-	Private
21	7.5	Crossbred	male	-	+	Kennel
22	6	Crossbred	female	-	+	Kennel
23	0.16	Golden Retriever	male	+	-	Private
24	10	W.H.K.G	female	-	+	Private
25	1	Crossbred	female	+	-	Kennel
26	2.5	Crossbred	male	+	+	Private
27	3	Bulldog	male	-	+	Private
28	8	Crossbred	female	+	+	Kennel
29	7	Crossbred	male	-	+	Kennel
30	4.5	Crossbred	female	-	+	Kennel
31	8	Crossbred	female	+	-	Kennel
32	6.5	Crossbred	male	-	+	Kennel
33	6.5	Crossbred	female	+	-	Kennel
34	4	W.H.K.G.	female	-	+	Private

35	5	German Shepherd	male	-	+	Private
36	5.5	Crossbred	female	+	-	Kennel
37	8	Crossbred	male	-	+	Kennel
38	10	Crossbred	female	-	+	Kennel
39	2	Crossbred	male	-	+	Kennel
40	10	English Setter	male	-	+	Private
41	1	English Setter	female	-	+	Private
42	8	Crossbred	male	+	-	Kennel
43	7	English Setter	male	-	+	Kennel
44	2	Setter	male	+	-	Private
45	8	Crossbred	male	+	-	Kennel
46	15	Poodle	female	+	-	Private
47	14	W.H.K.G.	female	-	+	Private
48	1	Golden Retriever	female	-	+	Private
49	3	Crossbred	female	-	+	Private
50	10	Crossbred	male	-	+	Kennel
51	11	W.H.W.T.	male	-	+	Private
52	4	Cane Corso	male	-	+	Private
53	12.5	Jack Russell Terrier	male	+	-	Private
54	10	Crossbred	female	-	+	Kennel
55	0.5	Bulldog	female	+	-	Private
56	2	French Bulldog	female	+	-	Private
57	1.5	French Bulldog	female	+	-	Private
58	8	Bichon Frise	female	+	-	Private
59	7	W.H.W.T.	female	+	-	Private
60	0.5	Collie	female	+	-	Private

61	12	Belgian Shepherd	male	+	-	Private
62	0.25	Miniature Poodle	male	+	-	Private
63	8.5	Segugio Italiano	female	+	-	Private
64	0.16	Cavlier King Charles	male	-	+	Private
65	1.5	Lagotto	male	+	-	Private
66	0.7	American Staffordshire	male	+	-	Private
67	13	Siberian Husky	male	+	-	Private
68	0.58	French Bulldog	female	+	-	Private
69	1.5	Weimaraner	female	+	-	Private
70	0.58	Boston Terrier	male	+	-	Private
71	3	Springer Spaniel	female	+	-	Private
72	6.5	Volpino Italiano	male	+	-	Private
73	3	Corso	male	+	-	Private
74	0.5	Poodle	male	+	-	Private
75	3.5	Collie	male	+	-	Private
76	0.25	Maltese	male	+	-	Private
77	6.5	Siberian Husky	female	+	-	Private
78	2	Breton	male	+	-	Private
79	10	Setter	male	+	-	Private
80	8	Crossbred	male	+	-	Private
81	1	Pincher	female	+	-	Private
82	9	Maltese	female	+	-	Private
83	1.5	Crossbred	male	+	-	Private
84	4	Boxer	male	+	-	Private
85	8	Collie	female	+	-	Private
86	2.5	Hovawart	male	+	-	Private

87	9.5	Crossbred	male	+	-	Private

Table 2. Clinical signs observed in 50 examined dogs affected by dermatitis.

	Lesions of the skin		Type of lesions													
	Localized	Generalized	Alopecia	Change of the hair	Comedones	Crusts	Darkning of the skin	Excoriation	Erythema	Hypotricosis	Lichenification	Papules	Pruritus	Scales	Seborrea	Ownership
1	-	+	+	-	-	+	+	-	-	-	-	-	+	+	-	K
2	-	+	+	-	+	+	-	-	-	-	+	+	+	+	-	K
3	-	+	+	-	+	+	-	-	+	-	-	+	+	-	-	P
4	-	+	+	-	-	+	-	-	+	+	-	-	+	+	-	P
5	+	-	+	-	-	+	-	-	-	-	-	-	+	-	-	P
6	-	+	+	-	-	+	-	+	+	-	-	-	+	-	-	P
7	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	P
8	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	K
9	-	+	-	-	+	+	-	-	-	-	-	+	+	-	-	P
10	+	-	+	-	-	-	+	-	+	-	-	-	-	-	-	K
11	+	-	-	-	-	-	-	-	-	+	-	-	+	+	-	K
12	+	-	-	+	-	-	-	-	-	-	-	-	+	-	-	K
13	-	+	+	-	-	-	-	-	+	-	-	+	+	+	-	K
14	-	+	-	+	-	-	-	-	-	-	-	-	+	-	-	K
15	+	-	+	-	-	-	-	-	-	-	-	-	+	+	-	P
16	-	+	+	-	+	+	+	+	+	-	+	+	+	-	-	K
17	+	-	+	-	-	-	-	+	-	-	-	-	+	-	-	P
18	+	-	-	-	-	-	-	-	+	-	+	-	+	-	-	P
19	-	+	+	-	-	-	-	-	+	+	-	-	+	-	-	P
20	-	+	+	-	-	-	-	-	+	-	-	-	+	+	-	P
21	-	+	-	-	-	-	-	-	+	+	-	-	+	-	-	P
22	-	+	+	-	-	-	-	-	+	+	-	-	+	-	-	P
23	-	+	-	+	-	-	-	+	+	-	-	-	+	-	+	P
24	+	-	+	-	+	+	-	-	+	+	-	-	+	-	-	P

25	-	+	+	-	-	-	-	+	+	-	-	-	+	-	-	P
26	+	-	-	+	-	-	-	-	-	-	-	-	+	+	-	P
27	-	+	-	-	-	-	-	-	+	-	-	+	+	-	-	P
28	+	-	+	-	-	-	-	-	+	-	-	+	+	-	-	P
29	-	+	-	-	-	-	-	-	-	+	-	+	+	-	-	P
30	-	+	+	-	-	+	-	-	+	+	+	+	+	-	-	P
31	-	+	+	-	+	-	-	-	+	-	-	-	+	-	-	P
32	-	+	+	-	-	-	-	-	+	+	-	-	+	-	-	P
33	-	+	+	-	-	-	-	-	+	+	-	-	+	+	+	P
34	-	+	-	-	-	+	-	-	+	+	-	-	+	+	-	P
35	-	+	-	-	-	-	-	-	+	-	-	+	+	-	-	P
36	-	+	+	-	-	+	-	-	-	-	-	+	+	-	-	P
37	-	+	-	-	-	+	-	-	+	-	-	+	+	-	-	P
38	-	+	-	-	-	+	-	-	+	-	-	+	+	+	-	P
39	+	-	-	-	-	-	-	+	-	+	-	-	+	-	-	P
40	-	+	+	-	-	+	+	-	+	-	-	+	+	+	-	P
41	-	+	-	-	-	+	-	+	+	+	-	+	+	-	-	P
42	-	+	+	-	-	+	-	-	+	-	-	+	+	-	-	P
43	+	-	-	-	-	-	-	-	+	-	-	+	+	+	-	P
44	-	+	-	-	-	-	-	-	+	-	-	-	+	-	-	P
45	-	+	-	-	-	+	-	-	+	+	-	+	+	+	-	P
46	-	+	-	-	-	+	-	-	+	-	-	+	+	+	-	P
47	+	-	-	-	-	-	-	-	+	-	-	-	+	+	-	P
48	-	+	+	-	-	+	-	-	+	-	-	+	+	-	-	P
49	-	+	-	-	-	+	-	-	+	-	-	+	+	-	-	P
50	-	+	+	-	-	+	-	-	+	+	-	-	+	-	-	P

P: Private K: Kennel

Table 3. Clinical signs observed in 40 examined dogs affected by otitis externa.

	Ears lesions		Clinical signs							
	Bilateral	Unilateral	Abundant wax	Dark wax	Bad smell	Lesions of the pinnae	Headshaking	Pruritus	Chronic otitis	Owners hip
1	-	+	+	+	+	-	-	-	+	Kennel

2	+	-	+	+	-	+	+	-	-	Private
3	+	-	+	+	+	-	-	-	-	Private
4	+	-	+	-	-	-	-	-	-	Private
5	+	-	+	-	-	-	-	+	-	Kennel
6	+	-	+	-	-	-	-	-	-	Private
7	+	-	-	+	-	-	-	-	+	Kennel
8	+	-	+	-	-	-	-	+	+	Kennel
9	+	-	+	-	-	-	-	-	-	Private
10	+	-	+	-	-	+	+	+	+	Kennel
11	+	-	+	+	+	-	-	+	-	Private
12	+	-	-	+	-	-	-	-	-	Kennel
13	+	-	-	+	-	-	+	-	+	Private
14	-	+	+	+	+	-	+	-	+	Kennel
15	+	-	+	+	-	-	-	+	+	Kennel
16	+	-	-	+	-	-	-	+	+	Kennel
17	+	-	-	+	-	+	-	+	+	Kennel
18	+	-	+	+	+	-	+	+	+	Private
19	+	-	+	+	+	+	-	+	-	Private
20	+	-	-	+	-	-	-	-	-	Private
21	+	-	+	-	-	-	-	+	-	Kennel
22	+	-	+	+	-	+	-	+	+	Kennel
23	+	-	-	+	-	-	-	+	-	Kennel
24	+	-	+	+	+	-	-	+	+	Kennel
25	+	-	+	-	-	-	-	-	-	Private
26	+	-	+	+	-	+	-	-	-	Private
27	+	-	+	-	+	-	-	+	-	Kennel
28	+	-	-	-	-	+	-	+	+	Kennel
29	+	-	+	+	-	-	-	-	+	Kennel
30	+	-	+	-	+	-	+	-	+	Private
31	+	-	+	-	-	-	+	-	-	Private
32	+	-	-	-	-	-	-	-	+	Kennel

33	+	-	+	-	-	-	-	-	+	Private
34	+	-	+	+	+	-	+	-	+	Private
35	+	-	-	+	-	-	-	-	+	Private
36	+	-	+	+	+	-	-	+	-	Kennel
37	-	+	+	-	-	-	-	+	-	Private
38	+	-	+	+	-	-	-	+	+	Kennel
39	+	-	+	+	-	+	+	+	+	Kennel
40	+	-	+	+	-	-	-	+	-	Private

Data analysis

The prevalence of isolated parasites was estimated as the number of positive dogs/total number of cases of dermatitis or otitis externa. Type of symptoms and/or lesions, age, sex and breed were considered as putative risks factors. The dogs were divided in three different age groups: 0-2 (including 2), 2-7 (including 7) and higher than 7 years old. Data were preliminarily analysed using a $\chi 2$ test with the Yates correction or a Fisher test (R package, R Development Core Team, 2011), when appropriate. Results were considered significant when the null hypothesis had a probability less than $P<0.01$ or $P<0.05$.

RESULTS

In the present study, 87 dogs with dermatitis and/or otitis were examined for the search of parasites. Forty-seven dogs were affected by dermatitis, 37 dogs by otitis and 3 by both dermatitis and otitis. Obtained results are summarized in Table 4.

Table 4. Skin parasites, lesions and concomitant diseases found in 87 dogs of different breed, age and origin, examined for parasitic dermatitis and/or otitis externa. Treatment used at the time of the visit is also reported.

				Lesions					
	Age (years)	Breed	Sex	Body	Ear	Origin	Concomitant disease	Results	Current therapy
1	8.5	Crossbred	Male	+	-	Kennel	-	*D. canis*	-
2	8	Crossbred	Male	+	-	Kennel	Leishmaniosis	*D. canis* + *D. cornei*	Allopurinol, Denamarin, Peridan and Ivomec
3	12	Crossbred	Male	-	+	Kennel	-	*Malassezia*	Cephalosporin
4	0.83	Maremma sheepdog	Male	-	+	Private	-	*Malassezia* +*O. cynotis*	-
5	10	Labrador	Female	-	+	Private	-	*Malassezia* + *Bacteria*	-
6	8	Bracco	Female	+	-	Private	-	*D. canis*	-
7	10	English Setter	Female	-	+	Private	-	*Malassezia*	-
8	8.5	Crossbred	Female	-	+	Kennel	-	Negative	Otomax
9	2	Boreder Collie	Male	+	-	Private	-	*S. scabiei*	-
10	0.5	Pug	Female	+	+	Private	-	*D. canis*	Amitraz
11	15	Crossbred	Female	-	+	Kennel	-	Negative	Posatex
12	9	Crossbred	Male	-	+	Kennel	-	*Malassezia*	-
13	0.5	Cocker	Female	-	+	Private	-	Negative	Otomax

14	15	Crossbred	Male	-	+	Kennel	Ehrlichiosis, deformed pinnae (otohematoma)	*Malassezia*	Local Ivomec in the ears
15	8	Crossbred	Male	-	+	Private	-	*O. cynotis*	-
16	4.5	Crossbred	Male	-	+	Kennel	-	*Malassezia*	-
17	4	Golden Retriever	Female	-	+	Private	-	*Malassezia*	-
18	6	Crossbred	Male	-	+	Kennel	-	*Malassezia*	Ear wash, Cephalosporin
19	11	Crossbred	Female	-	+	Private	-	*Malassezia, Bacteria*	Kartileg, Omegapet, Ear wash
20	5.5	German Shepherd	Male	+	-	Private	-	*S. scabiei*	-
21	7.5	Crossbred	Male	-	+	Kennel	-	Negative	Posatex
22	6	Crossbred	Female	-	+	Kennel	-	*Malassezia*	-
23	0.16	Golden Retriever	Male	+	-	Private	-	Negative	-
24	10	W.H.K.G	Female	-	+	Private	-	*Malassezia + Bacteria*	Surolan
25	1	Crossbred	Female	+	-	Kennel	-	*D. canis + D. injai*	Stronghold
26	2.5	Crossbred	Male	+	+	Private	-	Negative	Clorexyderm Oto
27	3	Bulldog	Male	-	+	Private	-	*Malassezia*	-
28	8	Crossbred	Female	+	+	Kennel	-	*Malassezia*	Surolan
29	7	Crossbred	Male	-	+	Kennel	-	*Malassezia*	Local Ivomec to the ears + Posatex + Cortisone

30	4.5	Crossbred	Female	-	+	Kennel	-	*Malassezia*	-
31	8	Crossbred	Female	+	-	Kennel	Intestinal adherences	Negative	-
32	6.5	Crossbred	Male	-	+	Kennel	-	*Malassezia*	Ear wash
33	6.5	Crossbred	Female	+	-	Kennel	Neurological problems	*S. scabiei*	-
34	4	W.H.K.G.	Female	-	+	Private	Gave birth recently	*Malassezia*	-
35	5	German Shepherd	Male	-	+	Private	-	*Malassezia*	-
36	5.5	Crossbred	Female	+	-	Kennel	-	*D. canis*	Ivomec, Deroxen
37	8	Crossbred	Male	-	+	Kennel	-	*Malassezia + Bacteria*	Surolan
38	10	Crossbred	Female	-	+	Kennel	-	*Malassezia*	-
39	2	Crossbred	Male	-	+	Kennel	-	*Malassezia*	Clorexyderm Oto
40	10	English Setter	Male	-	+	Private	-	*Malassezia*	-
41	1	English Setter	Female	-	+	Private	-	*Malassezia*	-
42	8	Crossbred	Male	+	-	Kennel	-	Negative	-
43	7	English Setter	Male	-	+	Kennel	-	Negative	Clorexyderm Oto
44	2	Setter	Male	+	-	Private	-	Negative	-
45	8	Crossbred	Male	+	-	Kennel	-	*D. canis + D. cornei*	-
46	15	Poodle	Female	+	-	Private	-	*D. canis*	-
47	14	W.H.K.G.	Female	-	+	Private	Cushing syndrome	*Malassezia*	-
48	1	Golden Retriever	Female	-	+	Private	Bilateral renal dystrophy	*Malassezia*	-

49	3	Crossbred	Female	-	+	Private	-	*Malassezia*	-
50	10	Crossbred	Male	-	+	Kennel	Cardiopathic	*Malassezia*	-
51	11	W.H.W.T.	Male	-	+	Private	-	Negative	Surolan
52	4	Cane Corso	Male	-	+	Private	-	*Malassezia + T. autumnalis*	-
53	12.5	Jack Russell Terrier	Male	+	-	Private	-	*D. canis*	Amitraz + Ivomectin
54	10	Crossbred	Female	-	+	Kennel	Deformed pinnae (otohematoma)	*Malassezia + O. cynotis*	-
55	0.5	Bulldog	Female	+	-	Private	-	*D.canis*	Interceptor, Advocate
56	2	French Bulldog	Female	+	-	Private	-	*D. canis*	-
57	1.5	French Bulldog	Female	+	-	Private	-	*D. canis*	-
58	8	Bichon Frise	Female	+	-	Private	Allergies	*S. scabiei*	Tinset, Gentalin Beta
59	7	W.H.W.T.	Female	+	-	Private	Borreliosis	*D. injai*	-
60	0.5	Collie	Female	+	-	Private	-	*D. canis*	Tresaderm, Amitraz
61	12	Belgian Shepherd	Male	+	-	Private	-	Negative	-
62	3 months	Miniature Poodle	Male	+	-	Private	-	Suspected Demodicosis	-
63	8.5	Segugio Italiano	Female	+	-	Private	Pyoderma	*D. canis*	-
64	0.16	Cavlier King Charles	Male	-	+	Private	-	*O. cynotis*	-

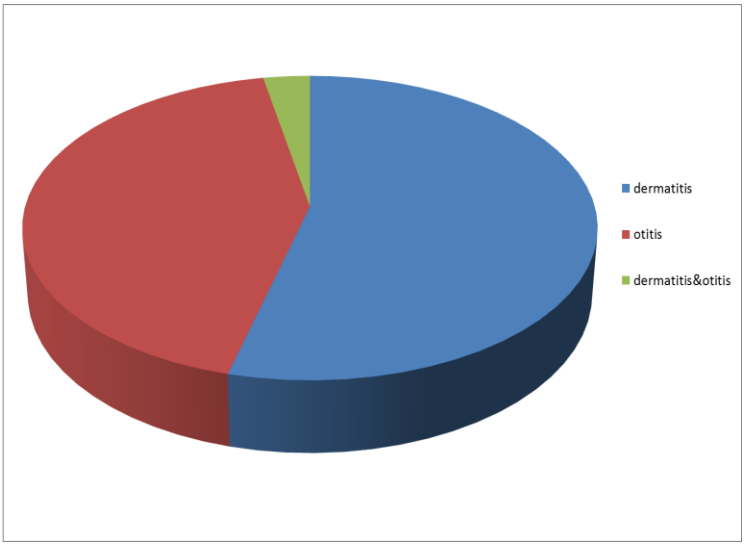
65	1.5	Lagotto	Male	+	-	Private	-	*D. canis*	-
66	8 months	American Staffordshire	Male	+	-	Private		*D. canis*	Cefacure, Amuchina, Clorexyderm shampoo
67	13	Siberian Husky	Male	+	-	Private	Pyoderma + Metacarpal fistula + Perianal glands adenomas +	*D. canis*	Cefalexin, Ivomec, Amitraz
68	0.58	French Bulldog	Female	+	-	Private	-	*D.canis*	Stronghold
69	1.5	Weimaraner	Female	+	-	Private	-	Suspected demodicosis	-
70	0.58	Boston terrier	Male	+	-	Private	-	Suspected demodicosis	-
71	3	Springer Spaniel	Female	+	-	Private	-	*S. scabiei*	-
72	6.5	Volpino Italiano	Male	+	-	Private	-	*S. scabiei*	Ivermectin
73	3	Corso	Male	+	-	Private	-	*S. scabiei*	-
74	0.5	Poodle	Male	+	-	Private	-	*S. scabiei*	-
75	3.5	Collie	Male	+	-	Private	-	*S. scabiei*	Advocate spot on
76	0.25	Maltese	Male	+	-	Private	Monorchidism	Negative	Ivermectin
77	6.5	Siberian Husky	Female	+	-	Private	-	Suspected Sarcoptic mange	Stronghold
78	2	Breton	Male	+	-	Private	-	Suspected Sarcoptic mange	Ivermectin, Prednisone
79	10	Setter	Male	+	-	Private	-	Suspected Sarcoptic mange	Ivermectin

80	8	Crossbred	Male	+	-	Private	-	Suspected Sarcoptic mange	Ivomec
81	1	Pincher	Female	+	-	Private	-	Negative	-
82	9	Maltese	Female	+	-	Private	-	*S. scabiei*	-
83	1.5	Crossbred	Male	+	-	Private	-	Suspected Sarcoptic mange	Stronghold
84	4	Boxer	Male	+	-	Private	-	*S. scabiei*	-
85	8	Collie	Female	+	-	Private	-	*S. scabiei*	-
86	2.5	Hovawart	Male	+	-	Private	-	Suspected Sarcoptic mange	Ivomec
87	9.5	Crossbred	Male	+	-	Private	-	Suspected Sarcoptic mange	Ivermectin

Data Analysis

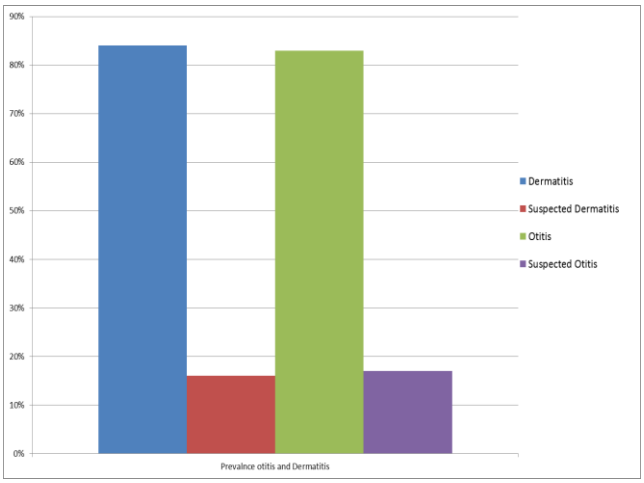
As shown in Graph 1, 54% of examined dogs resulted affected by dermatitis, 43% by otitis, while 3% showed both dermatitis and otitis.

Graph 1: Percentage of otitis and/or dermatitis in 87 examined dogs.



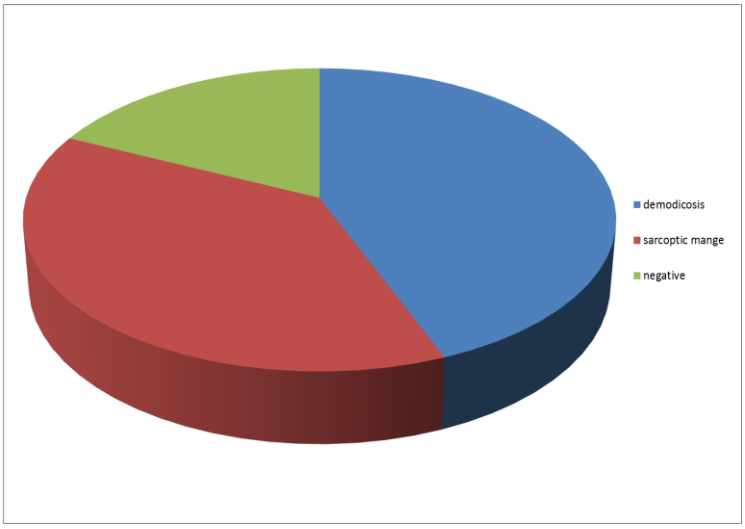
As shown in Graph 2, the prevalence of parasitic dermatitis was 84% (42/50 dogs with dermatitis) and that of parasitic otitis was 82.5% (33/40 dogs with otitis). The prevalence of suspected cases of parasitic dermatitis and of parasitic otitis was 16% and 17.5%, respectively (Graph 2). The dogs were considered suspected of parasitic otitis/dermatitis when no other cause was diagnosed and animals responded to treatment against parasitic arthropods or against *Malassezia*.

Graph 2. Prevalence (%) of parasitic otitis, of parasitic dermatitis and of suspected parasitic otitis and dermatitis.



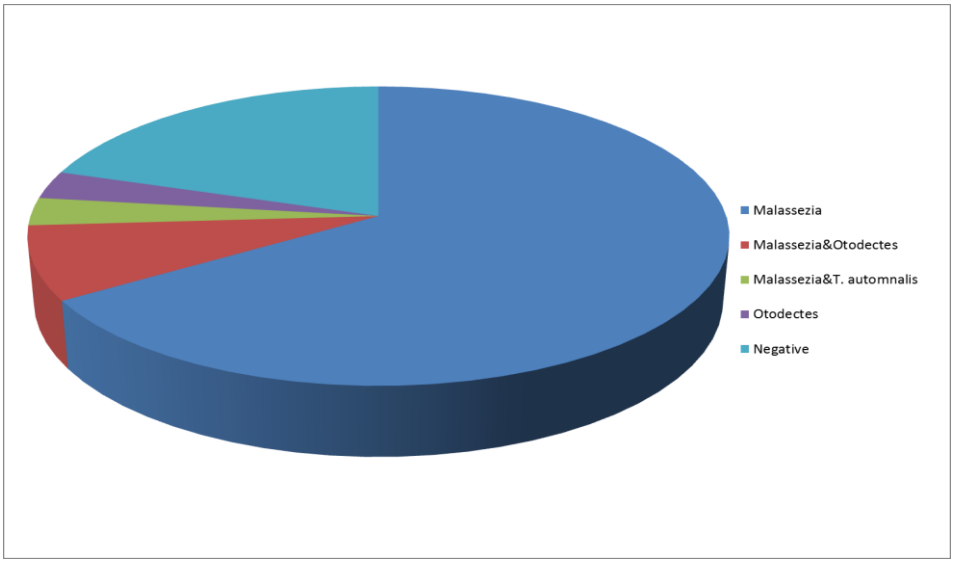
Among dogs with dermatitis, prevalence rates of 44% (22/50), 38% (19/50), and 18% (9/50) resulted for demodicosis, sarcoptic mange and dogs without a confirmative diagnosis, respectively (Graph 3).

Graph 3. Prevalence (%) of parasites found in dogs with dermatitis.



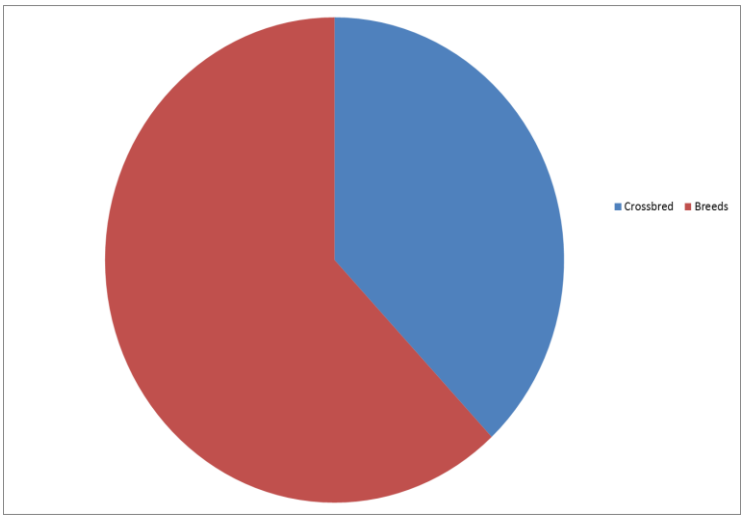
The prevalence of parasites diagnosed in parasitic otitis is showed in Graph 4. The number animals diagnosed with *Malassezia* was 27 (67.5%), that of animals diagnosed with *Malassezia* + *O. cynotis* was 3 (7.5%), while a dog (2.5%) was diagnosed with *Malassezia* + *T. autumnalis* and a further dog (2.5%) with *O. cynotis*. Eight (20%) dogs resulted negative for parasites (Graph 4).

Graph 4. The prevalence (%) of the different diagnosis of parasitic otitis



As evidenced in graph 5, among examined dogs there were 33 crossbred dogs and 54 dogs of different pure breeds.

Graph 5: Prevalence (%) of crossbred and of purebreed dogs.



From statistical analysis, sex did not show any influence on parasitic dermatitis and/or otitis ($P>0.05$). Although the origin of the dogs (owned or kenneled) had no influence on parasitic dermatitis, prevalence of otitis was significantly higher in dogs from kennels than in dogs of private owners ($P<0.01$). Parasitic dermatitis was significantly prevalent among purebred dogs than among crossbreds ($P<0.01$), while parasitic otitis was significantly prevalent among crossbred dogs ($P<0.01$). The age was a significant factor for parasitic dermatitis, with a significantly higher prevalence among the 0-2 years age group ($P<0.05$). However, age was not found to be an important factor for parasitic otitis ($P>0.05$).

With regard to parasitic diseases, the origin of the dogs (privately owned or kenneled) had a significant influence only on sarcoptic mange, since this parasitic disease resulted prevalent ($P<0.05$) in privately owned dogs, while no influence on the presence of *Demodex, Malassezia* or *O. cynotis* ($P>0.05$) was found. Age resulted related to demodicosis and sarcoptic mange: demodicosis resulted significantly more frequent among animals of the 1st and 3rd groups of age ($P<0.05$), while sarcoptic mange among animals of the 2nd age group ($P<0.01$).

Table 5 summarises clinical signs showed by and parasites isolated from the 50 dogs with parasitic dermatitis.

Table 5. Clinical signs showed by and parasites isolated from dogs affected by parasitic dermatitis (42/50) or found negative for parasites

	Clinical form		Type of lesions													Origin	Results
	Localized	Generalized	Al	Ch	Co	Cr	Da	Ex	Er	Hy	Li	Pa	Pr	Sc	Se		
1	-	+	+	-	-	+	+	-	-	-	-	-	+	+	-	K	*D. canis*
2	-	+	+	-	+	+	-	-	-	-	+	+	+	+	-	K	*D. canis D. cornei*
3	-	+	+	-	+	+	-	-	+	-	-	+	+	-	-	P	*D. canis*
4	-	+	+	-	-	+	-	-	+	+	-	-	+	+	-	P	*S. scabiei*
5	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	P	*D. canis*
6	-	+	+	-	-	+	-	+	+	-	-	-	+	-	-	P	*S. scabiei*
7	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	P	Negative
8	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	K	*D. canis D. injai*
9	-	+	-	-	+	+	-	-	-	-	-	+	+	-	-	P	Negative
10	+	-	+	-	-	-	+	-	+	-	-	-	-	-	-	K	Negative
11	+	-	-	-	-	-	-	-	-	+	-	-	+	+	-	K	Negative
12	+	-	-	+	-	-	-	-	-	-	-	-	+	-	-	K	*S. scabiei*
13	-	+	+	-	-	-	-	-	+	-	-	+	+	+	-	K	*D. canis*
14	-	+	-	+	-	-	-	-	-	-	-	-	+	-	-	K	Negative
15	+	-	+	-	-	-	-	-	-	-	-	-	+	+	-	P	Negative
16	-	+	+	-	+	+	+	+	+	-	+	+	+	-	-	K	*D. canis D. cornei*
17	+	-	+	-	-	-	-	+	-	-	-	-	+	-	-	P	*D. canis*
18	+	-	-	-	-	-	-	-	+	-	+	-	+	-	-	P	*D. canis*
19	-	+	+	-	-	-	-	-	+	+	-	-	+	-	-	P	*D. canis*
20	-	+	+	-	-	-	-	-	+	-	-	-	+	+	-	P	*D. canis*
21	-	+	-	-	-	-	-	-	+	+	-	-	+	-	-	P	*D. canis*
22	-	+	+	-	-	-	-	-	+	+	-	-	+	-	-	P	*S. scabiei*
23	-	+	-	+	-	-	-	+	+	-	-	-	+	-	+	P	*D. injai*
24	+	-	+	-	+	+	-	-	+	+	-	-	+	-	-	P	*D. canis*
25	-	+	+	-	-	-	-	+	+	-	-	-	+	-	-	P	Negative
26	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-	P	Suspected Demodicosi

																	s
27	-	+	-	-	-	-	-	-	+	-	-	+	+	-	-	P	*D. canis*
28	+	-	+	-	-	-	-	-	+	-	-	+	+	-	-	P	*D. canis*
29	-	+	-	-	-	-	-	-	-	+	-	+	-	-	-	P	*D. canis*
30	-	+	+	-	-	+	-	-	+	+	+	+	+	-	-	P	*D. canis*
31	-	+	+	-	+	-	-	-	+	-	-	-	+	-	-	P	*D. canis*
32	-	+	+	-	-	-	-	-	+	+	-	-	+	-	-	P	Suspected Demodicosis
33	-	+	+	-	-	-	-	-	+	+	-	-	-	+	+	P	Suspected Demodicosis
34	-	+	-	-	-	+	-	-	+	+	-	-	+	+	-	P	*S. scabiei*
35	-	+	-	-	-	-	-	-	+	-	-	+	+	-	-	P	Suspected Sarcoptic mange
36	-	+	+	-	-	+	-	-	-	-	-	+	+	-	-	P	*S. scabiei*
37	-	+	-	-	-	+	-	-	+	-	-	+	+	-	-	P	*S. scabiei*
38	-	+	-	-	-	+	-	-	+	-	-	+	+	+	-	P	Suspected Sarcoptic mange
39	+	-	-	-	-	-	-	+	-	+	-	-	+	-	-	P	Negative
40	-	+	+	-	-	+	+	-	+	-	-	+	+	+	-	P	Suspected Sarcoptic mange
41	-	+	-	-	-	+	-	+	+	+	-	+	+	-	-	P	Suspected Sarcoptic mange
42	-	+	+	-	-	+	-	-	+	-	-	+	+	-	-	P	Suspected Sarcoptic mange
43	+	-	-	-	-	-	-	-	+	-	-	+	+	+	-	P	Suspected Sarcoptic mange
44	-	+	-	-	-	-	-	-	+	-	-	-	+	-	-	P	Negative
45	-	+	-	-	-	+	-	-	+	+	-	+	+	+	-	P	*S. scabiei*
46	-	+	-	-	-	+	-	-	+	-	-	+	+	+	-	P	Suspected Sarcoptic mange
47	+	-	-	-	-	-	-	-	+	-	-	-	+	+	-	P	*S. scabiei*
48	-	+	+	-	-	+	-	-	+	-	-	+	+	-	-	P	*S. scabiei*
49	-	+	-	-	-	+	-	-	+	-	-	+	+	-	-	P	Suspected Sarcoptic

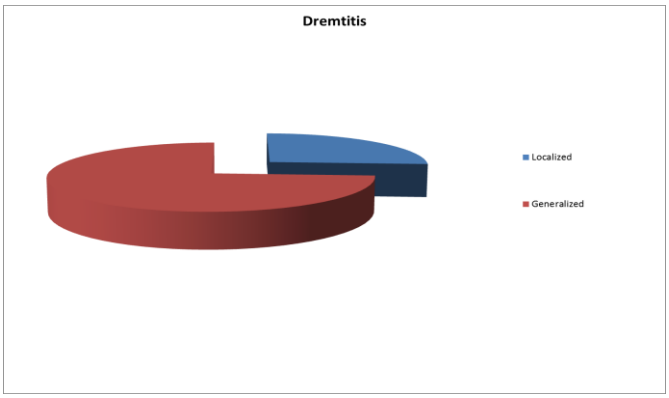
																	mange
50	-	+	+	-	-	+	-	-	+	+	-	-	+	-	-	P	Suspected Sarcoptic mange

Al: Alopecia Ch: Change of the hair Co: Comedones Cr: Crusts Da: Darkening of the skin Ex: Excoriation Er: Erythema Hy: Hypotricosis Li: Lichenification Pa: Papules Pr: Pruritus Sc: Scales Se: Seborrhea P: Private K: Kennel

Data Analysis

As shown in Table 5, cases of dermatitis were localized or generalized. The prevalence of localized dermatitis was 26% and that of generalized dermatitis was 74%, as visualized in Graph 6.

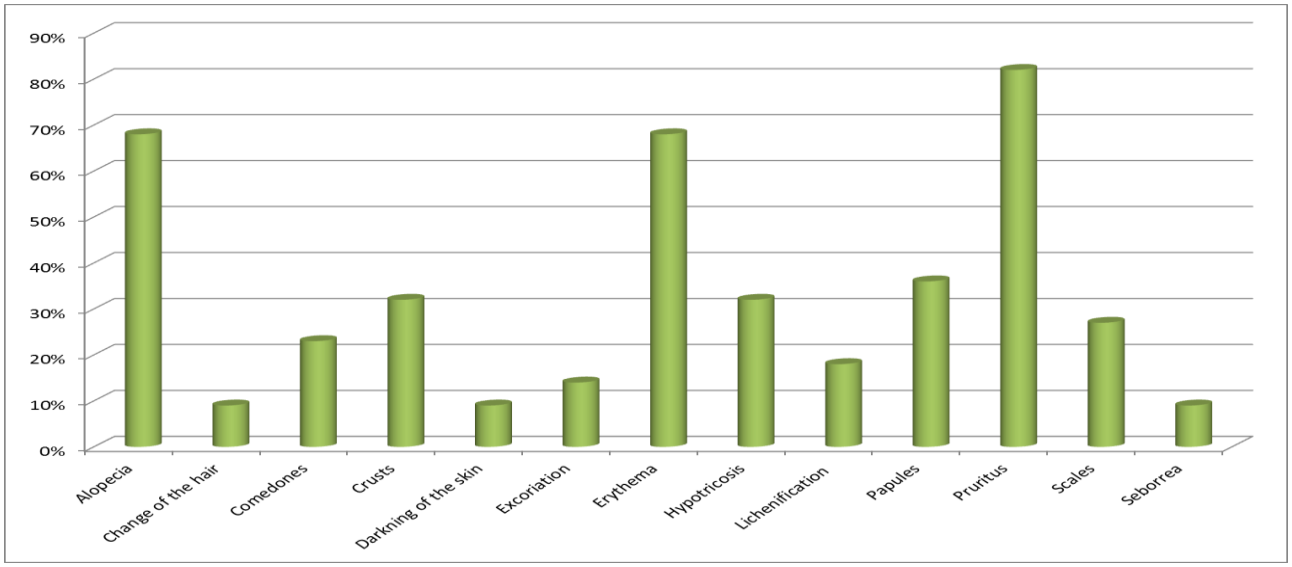
Graph 6. Prevalence (%) of localized and generalized dermatitis



From statistical analysis neither demodicosis nor sarcoptic mange resulted related to one or the other form dermatitis (localized or generalized) (P>0.05).

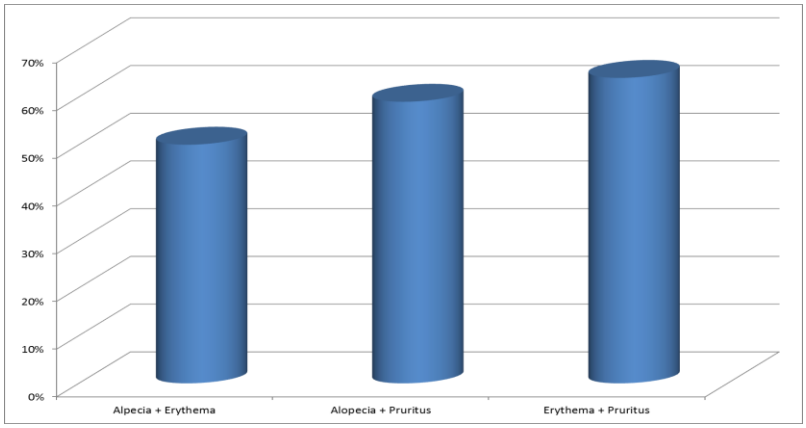
In Graph 7 are shown the frequencies of the various clinical signs found in the dogs that were diagnosed with demodicosis.

Graph 7. Frequency (%) of the different signs found in the dogs that were diagnosed with Demodicosis



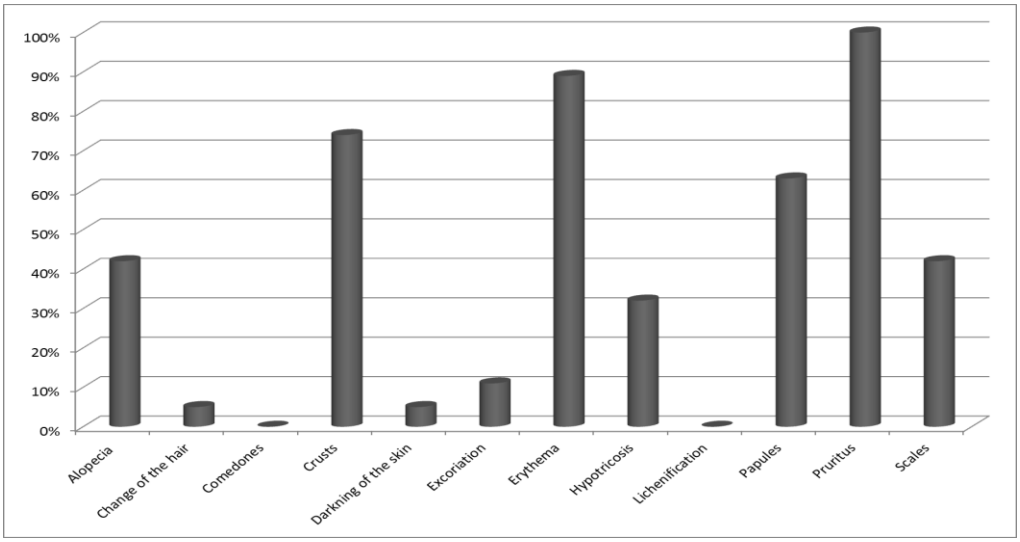
The frequency of the different combinations of the three most common symptoms observed in dogs affected by demodicosis, namely pruritus, alopecia and erythema, was also evaluated (Graph 8).

Graph 8. Frequency (%) of the associations of the 3 most common symptoms found in dogs with demodicosis.



In Graph 9 are shown the frequencies of the various signs found in the dogs that were diagnosed with sarcoptic mange, while Graph 10 shows the frequency of the different combinations of the three most common symptoms observed in dogs affected by sarcoptic mange (not including pruritus), namely crusts, erythema and papules.

Graph 9. Frequency (%) of signs found in the dogs that were diagnosed with sarcoptic mange.



Graph 10. Frequency (%) of the associations of the 3 most common symptoms found in dogs with sarcoptic mange

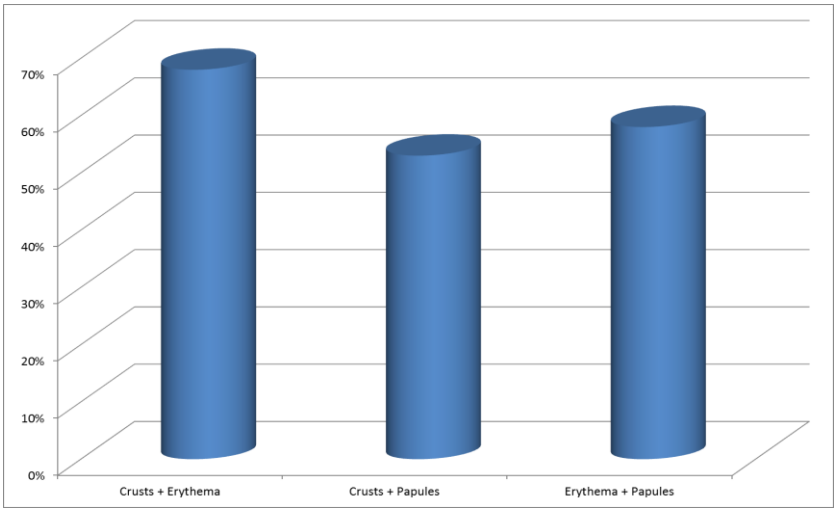


Table 6. Clinical signs observed in 40 examined dogs affected by *otitis externa*.

	Affected Ears		Clinical signs							Origin	Results
	Bilateral	Unilateral	Ab	Da	Ba	Le	He	Pr	Ch		
1	-	+	+	+	+	-	-	-	+	K	*Malassezia*
2	+	-	+	+	-	+	+	-	-	P	*Malassezia, Otodectes*
3	+	-	+	+	+	-	-	-	-	P	*Malassezia*
4	+	-	+	-	-	-	-	-	-	P	*Malassezia*

5	+	-	+	-	-	-	-	+	-	K	Negative
6	+	-	+	-	-	-	-	-	-	P	*D. canis*
7	+	-	-	+	-	-	-	-	+	K	Negative
8	+	-	+	-	-	-	-	+	+	K	*Malassezia*
9	+	-	+	-	-	-	-	-	-	P	Negative
10	+	-	+	-	-	+	+	+	+	K	*Malassezia*
11	+	-	+	+	+	-	-	+	-	P	*Malassezia, Otodectes*
12	+	-	-	+	-	-	-	-	-	K	*Malassezia*
13	+	-	-	+	-	-	+	-	+	P	*Malassezia*
14	-	+	+	+	+	-	+	-	+	K	*Malassezia*
15	+	-	+	+	-	-	-	+	+	K	*Malassezia*
16	+	-	-	+	-	-	-	+	+	K	Negative
17	+	-	-	+	-	+	-	+	+	K	*Malassezia*
18	+	-	+	+	+	-	+	+	+	P	*Malassezia*
19	+	-	+	+	+	+	-	+	-	P	Negative
20	+	-	-	+	-	-	-	-	-	P	*Malassezia*
21	+	-	+	-	-	-	-	+	-	K	*Malassezia*
22	+	-	+	+	-	+	-	+	+	K	*Malassezia*
23	+	-	-	+	-	-	-	+	-	K	*Malassezia*
24	+	-	+	+	+	-	-	+	+	K	*Malassezia*
25	+	-	+	-	-	-	-	-	-	P	*Malassezia*
26	+	-	+	+	-	+	-	-	-	P	*Malassezia*
27	+	-	+	-	+	-	-	+	-	K	*Malassezia*
28	+	-	-	-	-	+	-	+	+	K	*Malassezia*
29	+	-	+	+	-	-	-	-	+	K	*Malassezia*
30	+	-	+	-	+	-	+	-	+	P	*Malassezia*
31	+	-	+	-	-	-	+	-	-	P	*Malassezia*
32	+	-	-	-	-	-	-	-	+	K	Negative
33	+	-	+	-	-	-	-	-	+	P	*Malassezia*
34	+	-	+	+	+	-	+	-	+	P	*Malassezia*
35	+	-	-	+	-	-	-	-	+	P	*Malassezia*

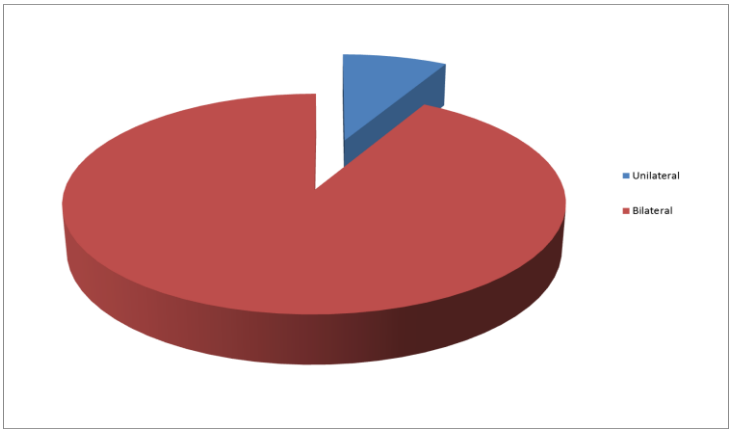
36	+	-	+	+	+	-	-	+	-	K	*Malassezia*
37	-	+	+	-	-	-	-	+	-	P	Negative
38	+	-	+	+	-	-	-	+	+	K	*Malassezia, T. autumnalis*
39	+	-	+	+	-	+	+	+	+	K	*Malassezia, Otodectes*
40	+	-	+	+	-	-	-	+	-	P	*Otodectes*

Ab: Abundant wax Da: Dark wax Be: Bed smell Le: Lesions of the pinnae He: Headshaking Pr: Pruritus Ch: Chronic otits P: Private K: Kennel

Data Analysis

As shown in Graph 11, the frequency of monolateral otitis was 8% and that of bilateral otitis was 92%.

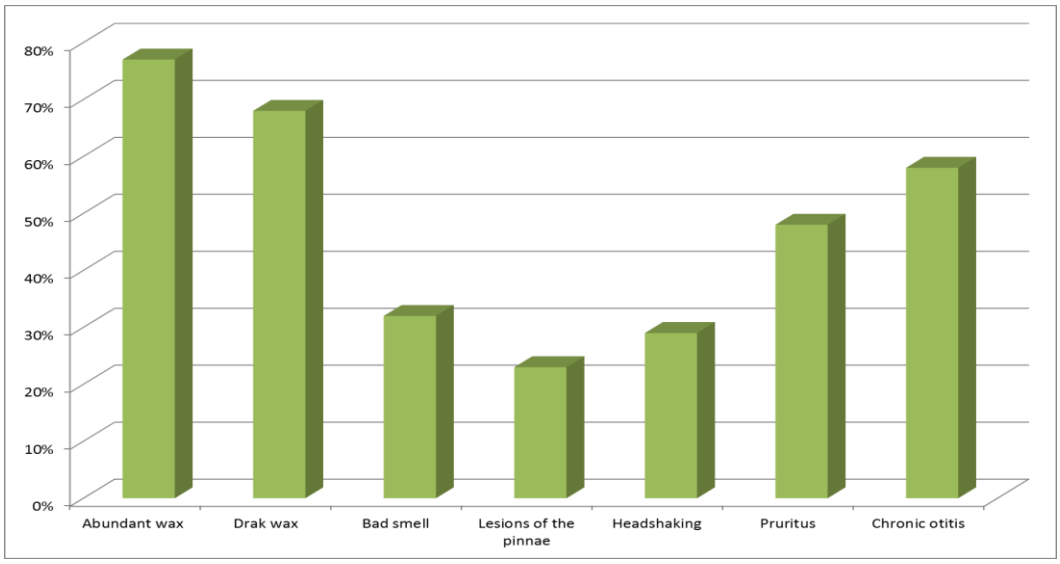
Graph 11. Frequency (%) of monolateral and bilateral parasitic otitis.



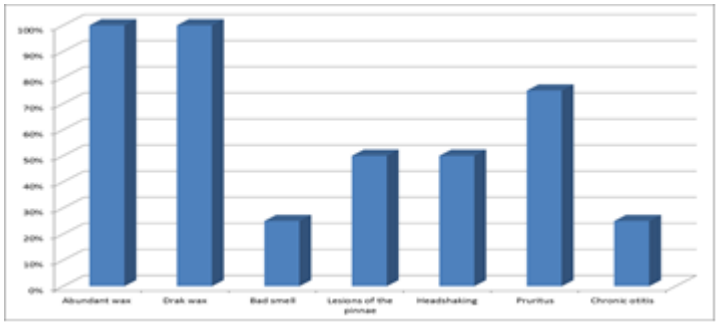
Since there were 3 dogs out of 40 showing monolateral otitis and only 4 cases of otitis by *O. cynotis*, it was not possible to analyze these data statistically.

In Graph 12 are reported the frequencies of the different symptoms found in dogs with *Malassezia*.

Graph 12. Frequency (%) of the different symptoms found in dogs with *Malassezia*



Graph 13. Frequency (%) of the symptoms found in dogs with *O. cynotis*



In this study all 3 known morpho-types of *Demodex*, namely *D. canis* (figure 3), *D. injai* (figure 4) and *D. cornei* (figure 3), were isolated, as well as eggs (figure 5). In figures 6, 7 and 8 we also found sarcoptic eggs (figure 6), a larva that hatches from the egg (figure 7) and adults of *S. scabiei* (figure 8). There were also found a larva of *T. autumnalis* (figure 9), various stages of life of *O. Cynotis* (larva in figure 10), eggs of *O. cynotis* (figure 11) and *Malassezia* (figure 12).

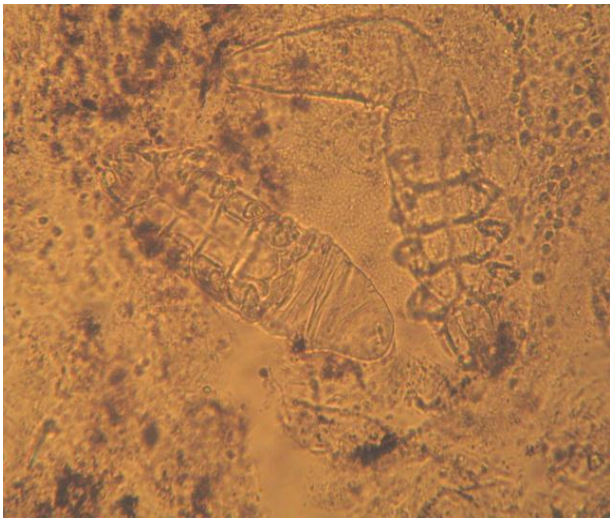


Figure 3. *D. canis* and *D. cornei*

Figure 4. *D. injai*

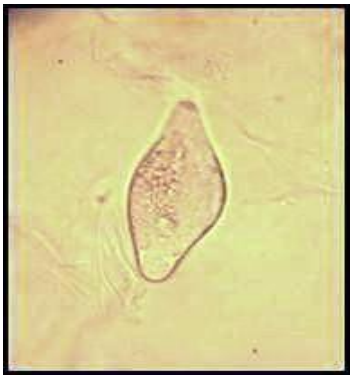


Figure 5. Egg of *Demodex*

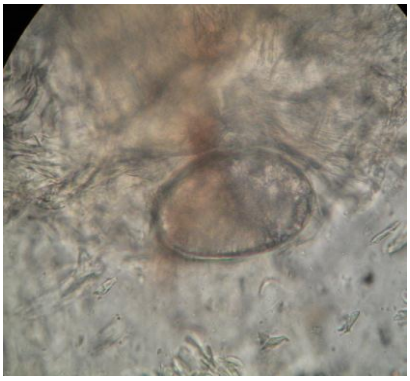


Figure 6. Egg of *S. scabiei*

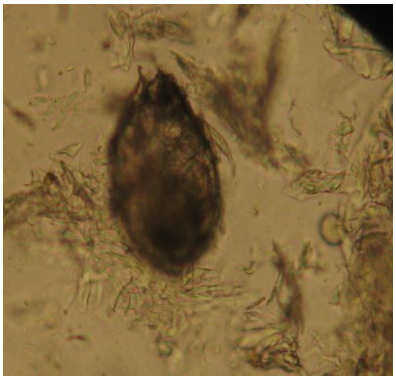


Figure 7. Larva of *S. scabiei* hatching from the egg

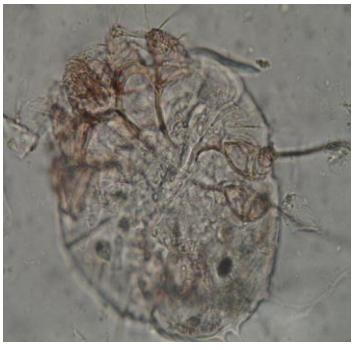


Figure 8. A female of *S. scabiei*

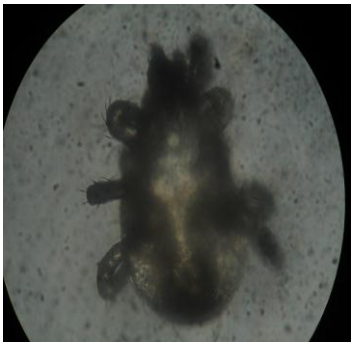


Figure 9. Larva of *T. autumnalis*

Figure 10. Larva of *O. cynotis*

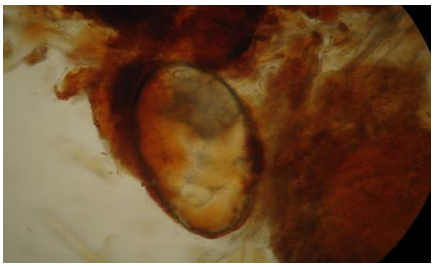


Figure 11. Egg of *O. cynotis*

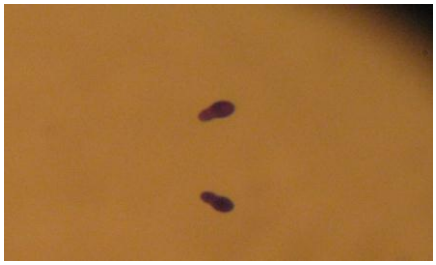


Figure 12. *Malassezia*

In the following pictures some sick dogs are shown: in figure 13 a dog with a generalized **demodicosis** and in figure 14 a dog with a local **demodicosis**. In figure 15 a dog with a generalized **sarcoptic mange**.

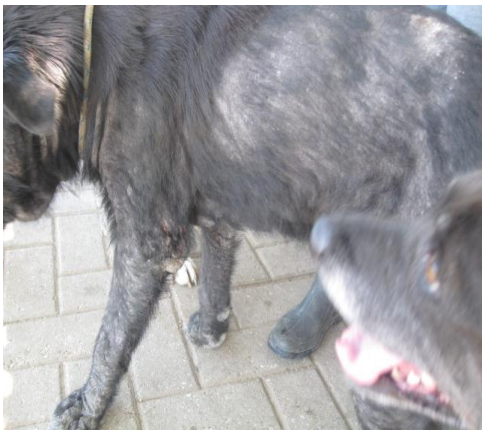


Figure 13. Generalized Demodicosis

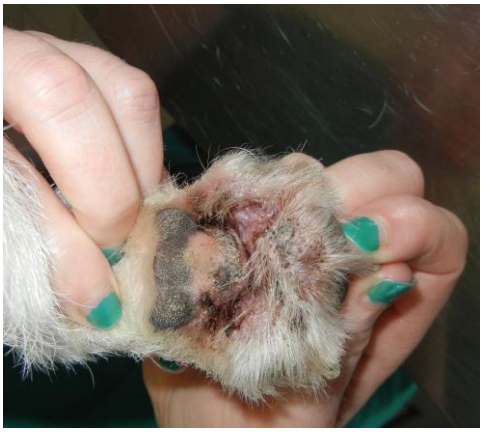


Figure 14. Localized Demodicosis

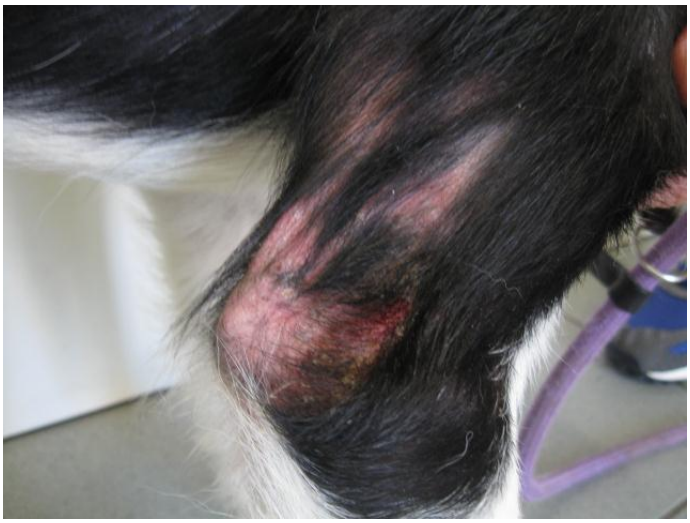


Figure 15. Sarcoptic mange

DISCUSSION

In a population of 87 randomly selected dogs of different breed, sex and origin (owned or kenneled) and affected by dermatitis and or otitis externa, the evaluation of the prevalence of parasitic dermatitis, of parasitic otitis and of each isolated parasite and the determination of possible associated factors represented the main aims of this study. Among examined animals, 54% showed symptoms of dermatitis, 43% had symptoms of otitis and 3% had both. Prevalence of parasites in dogs affected by dermatitis (84%) and otitis externa (82.5%) resulted very high showing that parasites are accounted for the majority of the diagnoses of these diseases confirming results from previous studies (Hill et al., 2006).

With regard to associated factors, prevalence of parasitic dermatitis resulted significantly higher among purebred dogs than among crossbred dogs ($P<0.01$), while parasitic otitis resulted significantly prevalent among crossbreds ($P<0.01$). The high prevalence of parasitic dermatitis among purebreds confirm data reporting a higher frequency of parasites resulted prevalent in this study in some dog purebreds, such as of *S. scabiei* among Labrador, Border collie and Jack Russell Terrier (Feather et al, 2010) or of *D. injai* and *D. canis* among terriers (Ordeix et al. 2008; Plant et al., 2011). Furthermore, especially in adults demodicosis is secondary to immune-suppressive diseases (Ravera et al. 2010; Scott et al. 2001, 2) and some breeds may be genetically predisposed to some of them (Watson et al., 2006). As described by Peano and collaborators (2012), the high prevalence of otitis among crossbreds might be due to poor recognition and⁄or management of underlying conditions, like concomitant disease that predisposed to otitis externa, maintaining good hygiene of the ear canal, especially in predisposed dogs. Most of the crossbred animals examined in this study lived in kennels where the cleaning of the ears is not frequently applied and the treatment of other concomitant diseases is not always ideal. Indeed, while the origin of the dogs (owned or kenneled) had no significant influence on parasitic dermatitis, a significant prevalence of otitis was found in this study in dogs from kennels than in dogs from private owners ($P<0.01$).

Sex did not show any influence on parasitic dermatitis and/or otitis ($P>0.05$). This result agrees with findings of other studies (Feather et al. 2010; Zur et al. 2011; Rodriquez-Vivas et al. 2003).

In this study, the age was a factor associated with parasitic dermatitis, since a significant higher prevalence among the 0-2 years age group dogs ($P<0.05$) was found. These data agree with previous reports (Chee et al, 2008; Aujla et al., 2000) in which a higher frequency of parasitic dermatitis was found among young dogs, up to a year old. In accordance with the study of Zur and others (2011), the age was not found to be associated with parasitic otitis ($P>0.05$) in this study. However, in other studies a significant higher prevalence among younger dogs (Girao et al., 2006) or dogs older than 2 years was found (Perrucci et al., 2008). Those different results might be due to epidemiological factors, such as weather, seasonal variations, geographical location, innate resistance, as well as the examined breeds since morphology of the pinnae and predisposition to allergies may represent predisposing factors (Zur et al., 2011; Rosser, 2004; Yoshida et al., 2002). For example, in our study we had 2 Golden Retrievers, a mother (4 years) and a daughter (1 year old). Other component that might have influenced results of the present study was the age of the kenneled dogs with OE, all of them adults, mostly elderly dogs. About 58% of the dogs with OE

and examined in this study had a chronic recurrent otitis, but we have no information regarding to their life pre-kennel.

As stated above, 84% out of cases of dermatitis were diagnosed as parasitic dermatitis based on finding *D. canis, D. cornei, D. injai* and *S. scabiei* mites or their eggs at parasitological examination. However, also the remaining cases were suspected to be parasitic dermatitis based on clinical signs and on their response to a "therapy trial", as indicated in other studies (Albanese and Leone, 2007; Mueller and Bettenay, 1999).

Out of the cases of parasitic dermatitis, 44% were diagnosed with demodicosis and 38% were diagnosed with sarcoptic mange. This data agree with results of other studies (Rodriguez-Vivas et al. 2003; Choi et al. 2000) who also found that demodicosis was the most common disease, but disagree with results of other studies, in which sarcoptic mange resulted the most common disease (Aujla et al. 2000; Chee et al. 2008). In fact in the study of Aujla and others (2000) the prevalence of sarcoptic mange was 29.5% and the one of demodicosis was only 6%. It is difficult to explain those differences, but it might be attributed to some epidemiological factors, such as weather, seasonal variations, geographical location, innate resistance, and particularly the age of the animals examined. Although sarcoptic mange is a non-seasonal disease condition (Terada et al., 2010), in hunting dogs it could be more frequent during the hunting season, especially if they have contact with foxes that represent important vectors of the mites (Bornstein 1991; Bond, 1998). Even though in our study there were no reports of infections of the owners, *S. scabiei* var. canis has a zoonotic potential (Aydıngöz and Mansur 2011; Jofré et al., 2009). Thus, the high frequency (38%) of this mite in dogs affected by dermatitis may pose a public health risk.

D. canis is a normal inhabitant of canine skin and the disease is thought to be the consequence of a genetically mediated specific immunodeficiency that allows the proliferation of the *Demodex* mites (Ravera et al. 2010). Therefore different breeds and the age of dogs examined in each study could influence the prevalence rates. The origin of the dogs had a significant influence on sarcoptic mange, with a higher prevalence of this parasitic dermatitis found in dogs with a private ownership ($P<0.05$), but there was no influence of the style of life on the presence of demodicosis ($P>0.05$).

Also the age was found to be correlated to sarcoptic mange. Indeed, although in our study the dog age ranged from 6 months to 10 years, the highest prevalence was found among dogs 2-7 years old (2nd age group). This result disagree with data reported Feather and others (2010), in which 40% of cases of sarcoptic mange were found in dogs less than 2 years old. These different data are hard to explain, but it is probably due to some peculiar epidemiological factors of different geographic area that may influence results. Other important factors could also be the style of life, e.g. hunting dogs that could be more common in some areas than in others.

Age was also correlated to demodicosis since prevalence of this disease was significantly higher in the 1st age group (0-2 years). This data agree with those reported in other studies (Nayak et al 1997; Rodriguez-Vivas et al.2003; Tsai et al 2011) in which significantly higher rate of this disease in dogs up to 2 years of age was found. In particular, in the study of Nayak and others (1997) about 83% of dogs with demodicosis were younger than 2 years old. *D. canis* is a normal component of the fauna of the skin of dogs and is transferred to the puppies by the mother (Mueller et al. 2011). Demodicosis overgrows appears to be caused by a wick immune system, which is age correlated

(young and elderly dogs have wicker immune system), and could also be caused by stress, nutrition and predisposed breeds (Ghubash, 2006). The prevalence of the localized dermatitis was 26% and the one of the generalized dermatitis was 74%, with no correlation of *S. scabiei* and *Demodex* spp. to a specific clinical form of dermatitis. According to various studies (Arlian et al. 1996; Pin et al. 2006), the local form of the sarcoptic mange is usually described as having a tendency for lesions to spread over the body. About demodicosis, Nayak and collaborators (1997) found a significant prevalence of the localized demodicosis with 65% of the dogs, and only 35% had the generalized form. In our research, most of the cases of dermatitis were diagnosed in the veterinarian hospital of the University of Pisa, where usually complicated cases arrive. Since the localized form is usually self-curing (Paterson et al. 2009; Mueller et al. 2011), there is a possibility that dogs with this form simply were less frequently presented.

In this research pruritus was the most common symptom of dogs with *S. scabiei*, as reported in previuos studies (Feather et al.2010). However, in our research 100% of the dogs affected with these mites presented pruritus, while in the study of Feather et al. (2010) it was 74%. Erythema was the 2nd most common symptom in our research with 89% of the dogs, then crusts (74%) and papules (63%), while alopecia and scales were less frequent (42%). Those frequencies are completely different from those reported by Feather and collaborators (2010), as alopecia (43%), crusts (40.5%), erythema (33.3%), and scaling (23.8%). Curtis (2004) and Pin and collaborators (2006) described the disease as intensely pruritic, papulocrustous dermatosis, a description that fit in our findings.

All of the dogs presented at least one of these symptoms, usually a combination of 2 or more, but never all of them together. The same results were found also at the Feather and others study (2010).

In this study the most common symptom of demodicosis was pruritus, with 82% of the cases. The next two symptoms were alopecia and erythema, with 68% each. Afterwards there were papules (36%), crusts (32%) and scales (27%). All of the dogs presented at least one of these symptoms, usually a combination of two or more, but never all of them. Both pruritus and papules may indicate the involvement of secondary bacterial infections and the presence of pyoderma. Though according to Rodrigues and others (2012), the most common symptoms of demodicosis are alopecia and erythema, also pruritus, scales, crusts, can be observed. However, as in this study, in the study of Nayak and collaborates (1997) the most common symptoms were pruritus, alopecia and erythema.

A seven years old West Highland White Terrier found infected by *D. injai* in this study showed seborrhea and grey hair. The signalment (breed and age) and the main symptom (seborrhea) were the same reported in previous studies (Ordeix et al. 2008; Carlotti et al. 2005; Hillier and Desch 2002), which suggests the predisposition of this breed to this species of *Demodex*. *D. injai* lives mostly in the sebaceous glands. In fact, at histological examination Ordeix and collaborates (2008) found hyperplasia of the sebaceous glands.

According to Izdebska (2010), *D. canis* is the species with the greatest prevalence, while the other two species (*D. injai* and *D. cornei*) are far less common. Indeed, in this study the prevalence of *D. canis* was 82%, that of *D. injai* 5%. One dog was infected with both *D. canis* and *D. injai* (5%), and 2 dogs with both *D. canis* and *D. cornei* (9%). In the study of Izdebska (2010), the prevalence of *D. canis* was 42%, of *D. injai* 5% and of *D. cornei* 7%, and one case with both *D. canis* and *D. cornei*.

Recently there were raised some questions whether *D. cornei* and *D. injai* are truly different species or not. The fact that both cases of *D. cornei* in our study were correlated with *D. canis* might suggest that *D. cornei* is not a new species, but a short formed *D. canis*. On the other hand, findings from this study confirm the peculiarity of *D. injai* infection reported in other studies (Ordeix et al. 2008; Carlotti et al. 2005; Hillier and Desch 2002) suggesting that this mite may actually be a different species. However, from results of recent studies (Rojas et al. 2012; Bourdeau 2011) it emerged that both *D. injai* and *D. cornei* may not be different species, but different variants of *D. canis*.

Out of the 40 cases of otitis, 82.5% resulted as primary or secondary parasitic otitis. Most of the dogs with otitis (67.5%) had only *Malassezia*, 7.5% had *Malassezia* and *O. cynotis*, 2.5% had *Malassezia* and *T. autumnalis*, 2.5% had only *O. cynotis* and 8% were negative. Most of the animals found negative for parasites were already treated before sampling. These results differ from results obtained in the study of Mircean and others (2008) in which their mycotic otitis and otoacariasis showed the same prevalence (26%). However, our data are similar to those found in the same area considered in this study (Perrucci et al. 2008) in which a higher prevalence of *Malassezia* spp. (40%) and a lower prevalence of *O. cynotis* (10%) were found, although in the same study all of the dogs with mites were diagnosed with *Malassezia* spp. as well. Considering that in the present study some dogs were already treated with local acaricides at the moment of the examination, while others were treated with antifungal drugs, prevalence of these parasites may be underestimated. However, differences from other studies may also depend on different epidemiological factors, as well as the breeds of dogs included in all these studies (morphology of the pinnae, predisposition to allergies, etc.). *Malassezia* spp. is commonly a secondary cause of otitis externa (Scott et al. 2001); this means that *Malassezia* overgrowth imply the presence of a primary condition able to change the normal environment inside the external ear canal (Rosser 2004; Zur et al. 2011). The most common primary causes of otitis externa are allergies, such as atopic dermatitis and adverse food reactions (Zur et al. 2011). Therefore, the presence of breeds or subjects more predisposed to these diseases could influence results. Keratinisation disorders, either primary, as in idiopathic seborrhea or secondary, as in hypothyroidism and sex hormone imbalance, are also common primary causes affecting the secretions of the ceruminous and sebaceous glands lining the ear canal (Rosser 2004). In our study a dog had Cushing syndrome, one dog recently gave birth, and one dog with a bilateral renal dystrophy. Anatomical changes in the ear canal such as conformational abnormalities are commonly included among the predisposing factors (Zur et al. 2011). Two positive dogs in this study showed a mal-conformation of the pinnae of both ears after having chirurgical interventions to treat otohematoma. Otohematoma in turn, is often caused by headshaking, one of the symptoms of otitis externa. As mentioned above, some breeds are more predisposed, such as German shepherd dogs and cocker spaniels that frequently show seborrhea, German shepherd dogs have also a high moisture levels in their ear canals, while cocker spaniels also have pendulous pinnae. Shar-peis have hypoplastic and stenotic ear canals and poodles have a high density of hair in their ear canals, etc. (Zur et al., 2011; Rosser, 2004; Yoshida et al., 2002). In our study, most of the dogs had at least one predisposing factor.

The prevalence of the monolateral otitis was 8% and that of bilateral otitis was 92%. These data are similar to those of Mircean et al. (2008), in which 94% bilateral and 6% monolateral external otitis

are reported. The origin of the dogs had no influence on the presence of *Malassezia* or *O. cynotis* (P>0.05).

The most common symptoms of dogs with otitis and *Malassezia* were abundant (77%) and dark (68%) wax, followed by pruritus (48%), bad smell (32%), headshaking (29%) and lesions of the pinnae (23%). All dogs had at least one symptom, usually a combination of two or more, but never all of them together. In addition, 58% of affected dogs showed recurrent otitis externa. These data agree with that reported by Saridomichelakis and others (2007), in which it was found that *Malassezia* is more commonly associated with the presence of dark and semiliquid than yellowish and semiliquid ear canal exudate; in addition, a 63% of dogs showed chronic-recurrent otitis externa.

The most common symptoms of dogs with *O. cynotis*, a primary cause of otitis externa, were in the following order: abundant wax and dark wax (100% each), pruritus (75%), head shaking and lesions of the pinnae (50% each), and bad smell (25%). However, these results are not representative since only four dogs with *O. cynotis* were found in this study. In order to have more accurate results it is necessary to have a study with more dogs with *O. cynotis*.

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