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Clinical Presentation and Dermatopathology of Generalised Demodicosis in Canine Patients.

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Abstract

The physiology of an organism is very complex. Most if not all of the topics surrounding internal medicine are on one hand unique, but are on the other hand tightly dependent on each other's mechanisms. Thus, when one system is hindered, another system may show clinical signs. A very important example of this phenomenon is the disease caused by the *Demodex* spp. of mites that live commensally in the hair follicles. The disease caused by their overgrowth has been referred to as an "indicator disease" and is highly suggestive of a deeper, more sinister issue in the background.

This thesis explores the fundamentals of the generalised form of the disease in canine patients, from the immunological patterns discovered in the literature, to the evolution of its treatment. It also gathers information regarding the common underlying diseases and the importance of anamnesis and signalment to narrow down the list of possibilities.

Using this information, two case studies were presented; one mild and one severe. The clinical presentation of the diseases were described and the methods of diagnosis and treatment were discussed.

The more severe case was followed more closely and used information of this thesis to diagnose, explore and treat the disease and a visual scoring system was used to monitor the progress over a period of approximately 80 days. The patient made a full recovery and by using the visual scoring system, this progress could be documented and followed easily.

This thesis concludes with reiterating the theme of demodicosis as an indicator disease and emphasises the importance of treating the underlying disease as well as applying an acaricide, and how being aware of the patient's anamnesis and signalment is paramount to the successful treatment.

Összefoglaló

Az élőszervezet fenntartó folyamatai nagyon összetettek. A belgyógyászati problémák egyedülállóak a maga nemükben, de ezek nagymértékben össze is függenek egymással. Ezért, ha az egyik rendszer valamiben akadályozott, egy másik mechanizmuson keresztül láthatjuk sokszor ennek jeleit. Erre az összefüggésre egy jó példa a szőrtüszőkben természetesen megtalálható *Demodex* fajokkal összefüggő megbetegedés. Ezt az atkák elszaporodásával járó megbetegedést úgynevezett „indikátor betegségnek" nevezik, amely sokkal komolyabb, háttérben megbúvó betegségekre is utalhat. Ebben a szakdolgozatban a kutyák generalizált demodikózisának a megismerését mutatom be a szakirodalomban megtalálható immunológiai alapmintázatoktól a gyógykezelés sokféle megoldásáig. Ezen felül tárgyalom a háttérben megbúvó betegségeket és hangsúlyozom az anamnézis fontosságát abban, hogy a kiváltó okok hosszú listája csökkenthető legyen. Ezen információkat használva két esetet is bemutatok, egy kevésbé súlyos és egy súlyosabb esetet: hangsúlyt fektetve az esetek klinikai megjelenésére, a diagnózishoz használt technikákra és a gyógykezelésekre is. A súlyosabb esetnél az utána követhetőség sokkal pontosabb volt; és ez inspirálta ezt a szakdolgozatot abban, hogy mélyebben elmerüljek a diagnózis és a kezelés lehetőségeiben; ezen felül egy vizuális „scoring" rendszer is használva volt a megbetegedés kezelésének körülbelüli 80 napos folyamata során. A páciens a kezelés hatására teljesen kigyógyult a megbetegedésből és ennek folyamata a „scoring" rendszerrel jól dokumentálható és nyomon követhető volt. A szakdolgozat újragondolja a demodikózist, mint indikátor betegséget és hangsúlyozza azt, hogy az atka ellenes szerek használata mellett kezelni kell a háttérben megbújó, kiváltó megbetegedést is és hogy legfontosabb az eredményes kezelésben az, hogy tisztában legyünk a páciens pontos kórelőzményeivel.

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1. Introduction

Veterinary dermatology is a double edged sword. On one hand, the skin is one of the largest organs in the body and is essentially completely visible to the naked eye, especially by the owner. On the other hand, according to the British Small Animal Veterinary Association (BSAVA) Manual of Canine and Feline Dermatology, the collection of potential insults to the skin is extensive but the pathological response is very limited [1]. This can pose a great difficulty for clinicians for a final aetiological diagnosis which can leave both the vet and owner in a vicious cycle of symptomatic treatment but neglecting causative treatment.

The pathological responses can be divided into primary and secondary lesions, with the former being closer and more specific to the original cause [2]. However, many times these may even go unnoticed by the owner and by the time the patient presents to the clinic, they will have extensive secondary lesions and perhaps even further complications. For example, which came first, the alopecia or the pruritis? A primarily alopecic dog may eventually develop a superficial pyoderma which can cause pruritis. Moreover, a primarily pruritic dog, maybe due to food/contact allergies, will show alopecic lesions due to self-trauma [1].

This wide variety of lesions can further be explained by systematic diseases such as endocrine, autoimmune, allergic reactions or simply from an infectious agent. One major feature that could potentially be overlooked is a systemic immunosuppression [3]. As the skin is part of the first line defence against infections with a continuous barrier made of keratocytes with a network of tight desmosomes, any loss of integrity to this barrier can serve as a "portal of entry" for infectious diseases both obligate and facultative pathogens. Moreover, the skin has its own microbiome. For canines, *Staphylococcus pseudintermedius* is the principle member of the skin flora, which is a Gram Positive, Coagulase Positive *Staphylococcus* species that is considered a facultative pathogen [4].

Another and major inhabitant of the skin are the *Demodex* spp. mites that live primarily in the hair follicles of the superficial layers [5]. There is a very tightly regulated balance behind the survival and turnover of these mites so that the animal is not overwhelmed by their numbers but also benefit from their tendencies for consuming excess keratin and other debris [5].

However, when this balance is disturbed, their numbers can increase massively and can have severe consequences for the host. The immune system of the host is finely tuned to keep their numbers within the tolerable range, therefore, with any immunosuppression, the mites gain autonomy with their replication and will create lesions on a microscopic and macroscopic level [3].

The theme of this thesis is to investigate the scientific papers that have researched the physiology of the immune system that is controlling this system, and how various forms of immunosuppressed patients are under an increased risk of developing 'demodicosis'. Two real world, own cases of generalised juvenile demodicosis will also be followed from initial presentation to diagnosis, treatment, bacterial complications, and the progress of the successful treatment.

Furthermore, the microscopic and macroscopic lesions will be investigated regarding the clinical presentation of these cases and the importance of secondary bacterial infections will be emphasised as this can seriously impact prognosis. The latter is of high importance because it can mask the original conditions for an accurate cytological diagnosis, therefore the specific methods of detection will also be discussed.

Moreover, the bigger picture will be looked at from a clinical point of view as because of the fine balance with the immune system, an overpopulation of *Demodex* mites can indicate a severe immunological disease; therefore this process can be referred to as an 'indicator disease' which must be approached with managed expectations if there are no obvious causes of the immunity issue.

Iatrogenic immunosuppression therapy for autoimmune treatments, chemotherapy for cancer patients and the malpractice use of corticosteroids must also be mentioned [6]. As shelter dogs may show an overwhelming overrepresentation of the disease, the role of poor nutrition and stressful conditions, with a lack of long term prophylactic treatments will also be discussed.

As mentioned earlier that the skin has limited abilities for responding to the insults, clinical presentations for several aetiologies may be identical, and even overlap. Therefore, a list of differential diagnoses is crucially important in the discussion. Sarcoptic mange, or scabies,

caused by the agent *Sarcoptes scabei*, is a key member of the differential diagnosis and is of higher importance because of its zoonotic tendencies and its closely related pathophysiology.

However, it is very important to first discuss the *Demodex* mites themselves, in particular: their morphology, life cycle, position in the dermis and appearance in cytological smears as well as bacterial co-infections.

1.1. Parasite background

Demodex mites belong to the Order: Prostigmata, Family: Demodecidae, and Genus: *Demodex*. Among canines, the most prominent mite species are *Demodex injai, Demodex cornei* and *Demodex canis* [5].

Permanently living in the host hair follicles and various glands of the epidermis, the host immunity and mite population are in a constant balance. The first infestation occurs from dam to pup during suckling, which at this age can result in localised demodicosis in young puppies around the mouth area.

The life cycle ranges up to 20 days and males will die shortly after copulation takes place. The females however will migrate to the hair follicles where she will lay her distinctive lemon shaped eggs. The first stage larva will hatch, and from this a second stage larva; then two nymphal stages, a protonymph and a deutonymph, and finally adults [5]. Figure 1.1 illustrates the different life stages from the egg to the second nymph. Figure 1.2 illustrates the adult morphology and Figure 2 depicts the overall life cycle of the species. Figure 3 is a schematic drawing of the mites colonising a hair follicle.

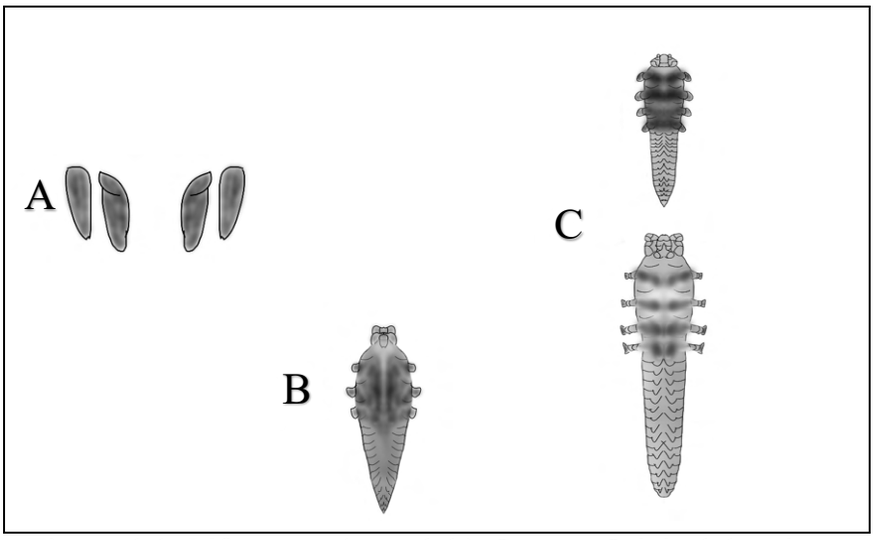

Figure 1.1: Life stages (pre-adult). Source: Original drawings.
A: Lemon shaped eggs
B: Six legged larval stage
C: Protonymph (upper) and deutonymph (lower)

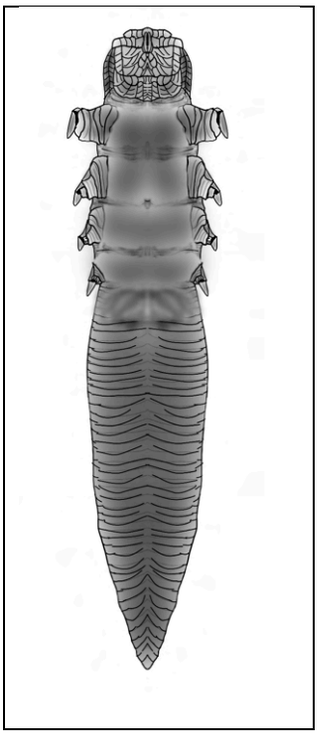

Figure 1.2: Life stages (adult). Source: Original drawings.

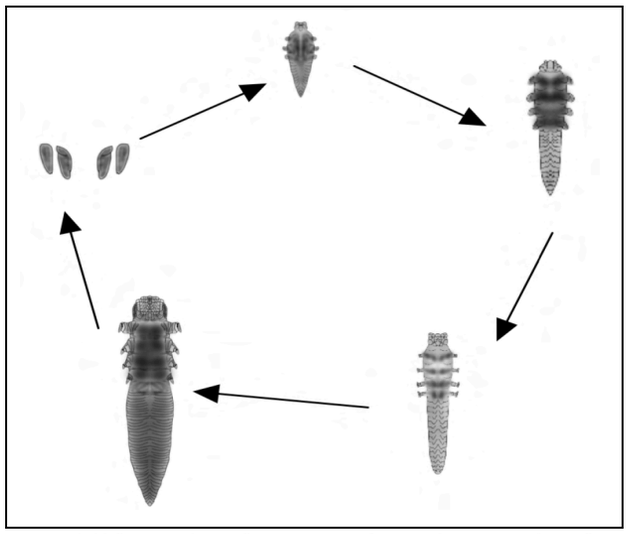

Figure 2: Illustrated life cycle of a *Demodex* mite species from egg to adult.
Source: Original drawings.

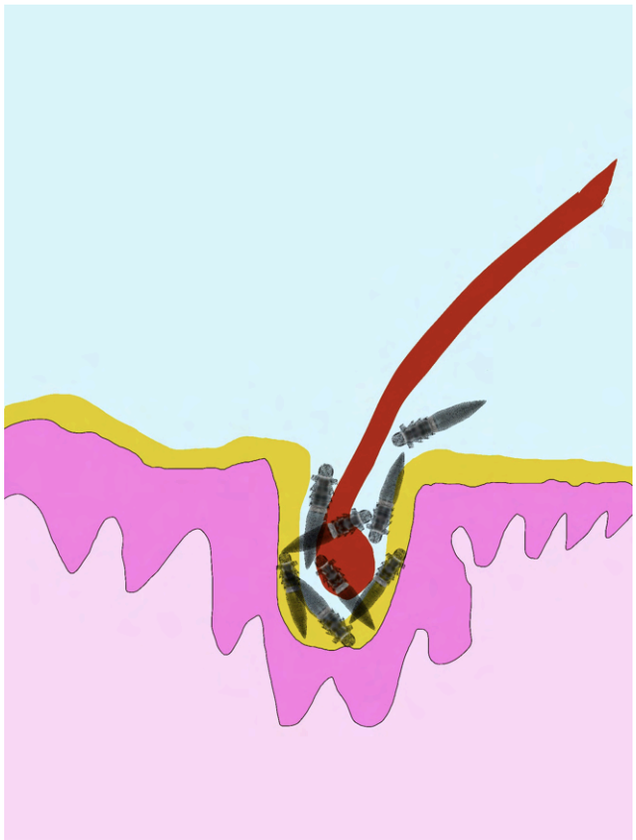

Figure 3: Schematic drawing of *Demodex* mites colonising a hair follicle.
Source: Original drawings.

As it is evident in the above images, these mites are long cigar shaped creatures with eight legs in nymphal and adult stages but only six in the larval stage (i.e. hexapod larvae). Their invasiveness is superficial and is usually confined to the hair follicle or other adnexal tissues. This is why when searching for suitable specimens, a deep skin scrape and/or trichography is essential to gather enough material to find the mites [1].

The difficulty of this technique coupled with their usually sparce colonisation, most demonstrations of the samples in healthy animals will be negative. However when the animal is compromised, the population increase of these creatures drastically improves the probability of detection. A native/unstained preparation of a *Demodex* mite can be seen in Figure 4. This sample was taken from a seven month old female dog suffering from a severe generalised case of demodicosis and deep pyoderma. Technique used was trichography to minimise further trauma and pain to the patient. Note the high amount of cellular debris also seen in picture B, and a potential hexapod larva in picture E.

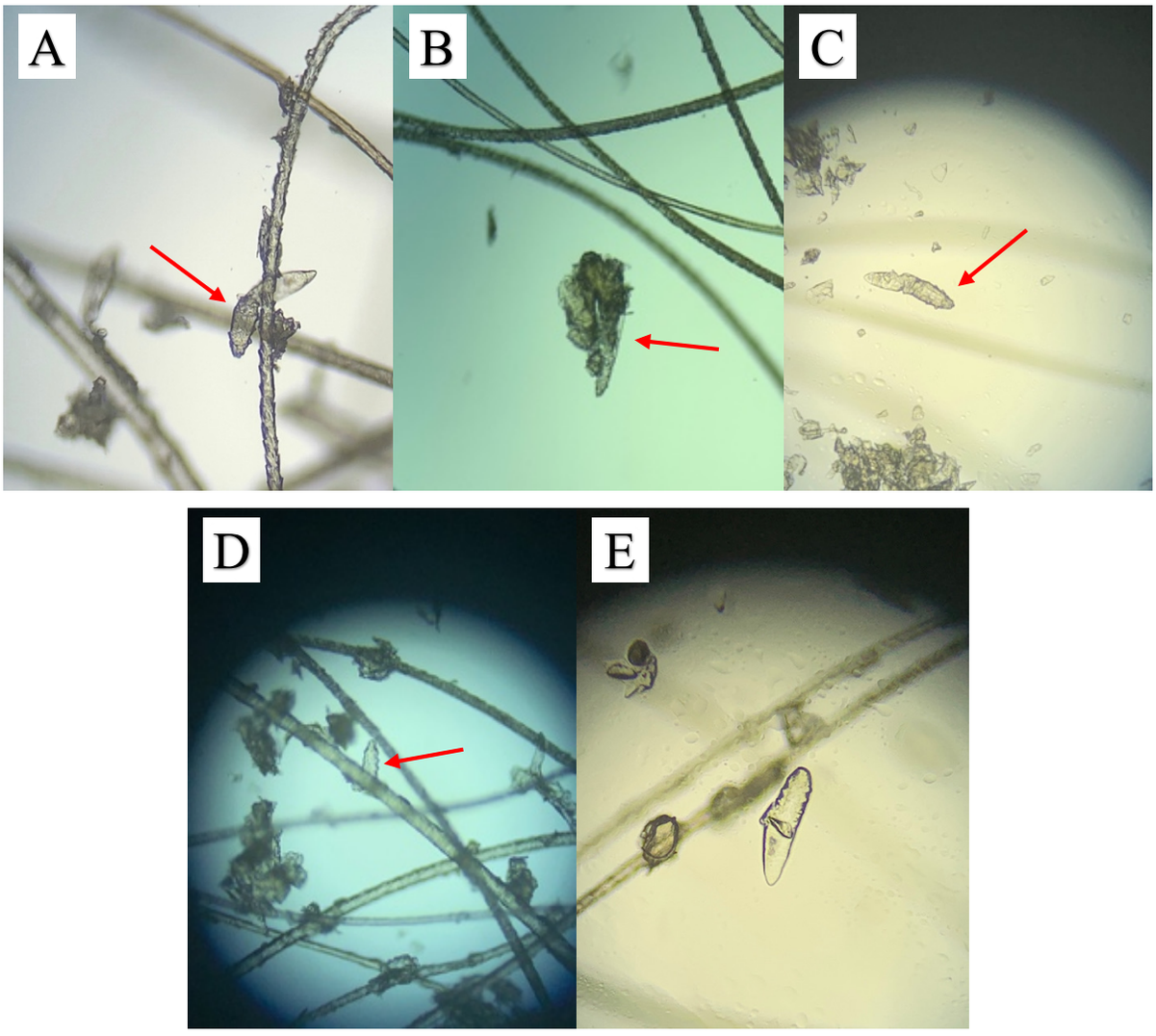

Figure 4: Cytological confirmation of a *Demodex* mite infestation of a canine patient. Source: Original pictures from own research.

Histopathological sections have no real clinical value in the diagnosis of the disease but may be incidental findings in other cutaneous biopsy preparations. Furthermore, in the next

section, a patient will be presented which also suffered from a deep pyoderma and upon a culture and sensitivity test, she was confirmed to be infected with *Staphylococcus* sp., *E. coli,* and *Klebsiella* sp.. The culture smears taken from a clinical case illustrate the characteristics of the bacterial infection and which specific antimicrobial drugs can be used. In a hospital setting, the clinician involved in the case can also use cytological examinations to predict the nature of the bacterial infection; whether cocci or rods are shown will lead them to a more specific diagnosis. However, a culture and sensitivity test is the gold standard method for diagnosis and choosing treatment options. Culture and sensitivity tests should always be encouraged and the prescription of any antibacterial medication should be carried out with utmost prudency.

In the following chapters, the most important aspects of the disease will be discussed. From the physiological conditions that lead to an infection, how to treat the infection, what are the current and future trends of bacterial pyodermas in canines, to the application of this information to two case studies of generalised demodicosis in canines; one milder and one more extreme.

In essence, the objective of this thesis is to summarise the most important details of canine generalised demodicosis and how the dermatopathology of the infection is presented in a clinical setting. It will also explore using real world examples of how using this information, can help with the understanding of the disease, a more efficient diagnosis and a more successful therapeutic plan for the patient.

2. Literature review

While this area of veterinary dermatology may appear to be quite narrow, the subject matter and research has been extensive. Moreover, one aspect that is showing an increased importance in this field is the possibility of antimicrobial resistance. Therefore, this chapter will collect all the relevant papers on the canine demodicosis and the importance of any emerging resistant pathogens. The discussion surrounding the Literature Review will be broken down by facet and the papers presented will aim to investigate their aspects.

2.1. Demodicosis immunopathological aspects

Kumari et al. (2017) state that the relationship between *Demodex* infection and the immune system is very significant but that no study was conducted as of yet to prove this relationship [7]. However, their paper analysed the data of interleukin-10 (IL-10), an immunosuppressive cytokine, on the patient and was able to show that there is a very high increase in the observed levels. Moreover, they also researched the cholinesterase level in the patients and were able to show that the relationship with the mites and the immune system may have a relationship with the cholinergic system as well.

In contrast to this result, Gasparetto et al. (2018) made a similar study of cytokine reactions by using flow cytometry and used control groups as a baseline along with localised and generalised *Demodex* patients [8]. While Kumari et al. (2017) witnessed an increase in the IL-10 level, Gasparetto et al. (2018) had conflicting results and concluded that there was no relative change among the groups with the IL-10 level. However, interestingly, with the localised *Demodex* group, the results were able to show that there was an increase in interleukin-6 (IL-6), a cytokine that is produced during tissue damage. This may suggest that there is a stark difference among individuals and studies, or a highly fluctuating cytokine response during the phases of pathogenesis. Further studies on these results should be conducted.

In light of these results, it should be mentioned that Singh and Dimri (2014) focused their study on the lymphocytic behaviour of the disease but also saw an elevated IL-10 level [6]. In addition to this, they concluded that oxidative stress during the pathogenesis can create these discrepancies between patients and their levels of cytokine responses.

Singh and Dimri (2014) further investigated the conversion of the host to the presence of the *Demodex* antigens and queried how such an immunosuppression could complicate the seroconversion to these antigens. They stated that the *Demodex* mites have specific mechanisms which allow them to downregulate the CD4+ (T-helper; Th) cells either by exhaustion of the immune system, or by an increased rate of apoptosis. The downregulated level of Th cells would allow the mites to multiply without hindrance.

However, since these creatures are facultative pathogens, they should not start this process alone, otherwise their levels would be a constant threat to the host. Instead, any immunosuppression whether systematically or iatrogenic use of certain lymphocytic drugs (corticosteroid or chemotherapy) may be the first domino required for this process; this was not mentioned in the study.

In an earlier study, Signh et al. (2010) also looked at the relationship of CD4+ and CD8+ (T-killer; T-cytotoxic; Tc) cells and was again able to show the decrease of CD4+ levels in *Demodex* patients [9]. Moreover, generalised patients had a significantly lower level than the localised patients, which were again lower than the healthy control patients. This shows a clear correlation between CD4+ levels and either severity of the disease and/or time lapse of the pathogenesis.

Apart from the previously mentioned cytokine and T-cell pathways, Ferrer et al. (2014) used transgenic mice models to illustrate the innate immune response elicited by the presence of the mites [10]. They concluded that there are toll-like-receptors that are able to detect the chitin compounds on the outer surface of the mites. This in turn will then keep the population within the normal limits. However, they also specified that there is a genetic predisposition for young puppies to develop the juvenile form of the disease but the primary immune defect is still unknown. Genetic testing is required in this field. Moreover, this study also reinforced the findings that a generalised demodicosis can result in not only a high IL-10, but also a lowered interleukin-2 (IL-2).

As the previous study emphasised the need for further genetic testing of innate immunity is required, Kumari et al. (2018) investigated the Toll-like receptor (TLR) expression in dogs showing no, local and generalised manifestation of demodicosis [11]. Their study analysed namely TLR-2, -4, and -6 and the expression of the genes by the use of Polymerase Chain

Reaction (PCR). It was found that a high expression of TLR-2 and a lower expression of TLR-4 and TLR-6 was significant among the animals showing either the local or generalised form when compared to the healthy control dogs, with further variety among severity of clinical forms. It was also speculated that one mechanism used by the mites was to downregulate the expression of the TLR-4 and TLR-6 genes. Moreover, the genetic heritability of this gene pattern cannot be overlooked, especially if the clinical form is presented in breeding kennels.

One could argue that an investigation into the genetic expression of these TLR in the same animals in a later age to see how they change in the course of the dog's life, and what are the consequences of this pattern if it persists into adulthood. It is possible that these dogs will be more prone to infectious agents or immune mediated diseases later in life; further research is needed in this matter.

The relationship between the immune system and the endocrine system is tightly interwoven and irregularities in one or both of these systems can be indicated by lesions in the skin. Pinsenschaum et al. (2019) studied a large pool of dogs from Europe and Australia and found that with clinical presentation of Adult onset demodicosis, approximately 40% of those patients had also a concurrent disease [12]. The most significant being Cushing's syndrome, hypothyroidism, and leishmaniasis, leading them to conclude that if presented with a case of demodicosis in adulthood, then an investigation into the patient's blood hormone parameters and the current epidemiological situation for *Leishmania* is highly warranted.

Interestingly, Soman et al. (2020) also investigated the impact of the mites on the immune system but went further than Kumari et al. (2018) and compared the cutaneous cell expression of the same three TLR on the blood mononuclear cells along with the pattern of cytokine levels [1]. Their findings supported those presented by Kumari et al. (2018) however they also discovered that the previously known elevations in IL-10 and TGF-beta were not present in the skin lesions themselves, allowing them to conclude that the mites elicit different immunopathological responses systemically and locally.

For the systemic response, one study by Tani et al. (2002) investigated the mRNA expression levels among the peripheral mononuclear cells in the blood [13]. With their investigations, they found that the most significant results were found in the mRNA expression of

interleukin-5 (IL-5) and transforming growth factor-beta (TGF-beta). While the previous studies mentioned above saw a rise in IL-10 and IL-6, this study found no such increase in the mRNA expression levels from the Peripheral Blood Mononuclear Cells (PBMCs), indicating further sources of these inflammatory cytokines.

As for the IL-5 and TGF-beta, Tani et al. (2002) was able to differentiate those patients with generalised form from those with the localised form of the disease; the blood concentration of TGF-beta was drastically higher in the generalised form. This can be considered evidence of different mechanisms of disease onset or progression. Moreover, the levels of IL-5 and TGF-beta were shown to decrease post-treatment which allowed Tani et al. (2002) to speculate the use of these parameters in a clinical setting for monitoring the disease and the treatment and for evaluating the prognosis.

The above research on the clinical form of the disease clearly illustrated the relationship between the immune system of the patient and the extent of the lesions. While in some studies one could argue that the level of cytokines are merely a response to the disease itself rather than a mechanism to develop, it is clear that with the level of TLR expression that the disposition of these clinical forms are coded in the genes and the possibility of heritability needs to be emphasised more. Also, the adult onset of clinical signs in patients that are immunosuppressed from an endocrinological disorder or an infectious agent clearly highlights the opportunistic behaviour of the mite population.

2.2. Treatment options for demodicosis

When a clinician is presented with a demodicosis case, it is of upmost importance to take into consideration the signalment and anamnesis of the patient, namely its age, breed, living standards, regular vaccinations and flea/worm treatments, or any medications given, especially those that are immunosuppressive such as chemotherapeutic or catabolic steroids.

With the constant development of new formulations becoming available on the market, there are a wide variety of treatment options for the clinician to choose from. In this section, a handful of journals and studies that analysed the efficacy of these drugs for the purpose of treating *Demodex* will be presented. Since however there has been many new drugs introduced, the time span of these studies is wide.

In 1994, Duclos et al. analysed the efficacy of amitraz dips as a treatment option in dogs with adult onset demodicosis [14]. As mentioned earlier, adult onset demodicosis can be construed as an indication of an endocrinology defect; namely Cushing's disease and hypothyroidism. In this study, many of the subjects fell into one of these two categories, with a few being treated for allergy related conditions with corticosteroids. The study showed that sole treatment with amitraz was only successful in a handful of cases and that most patients also required treatment for the underlying condition. Interestingly, of those patients that were treated for the primary condition, most of them also required amitraz treatment as well. This highlights the importance of the mite-host balance and that once the mites reach a certain point in their population, they will require direct treatment in order to regain control; just treating the primary condition may not be sufficient.

Amitraz dips are shown to be effective, and Mueller (2004) outlines that the best protocol is to administer the treatment every 7 to 14 days until negative cytology [15]. However this can prove to be inconvenient and time consuming to the owner so other treatments were also offered, namely ivermectin or milbemycin oxime as daily oral medication or moxidectin spot on treatment. While all these drugs are very effective, Mueller et al. summarised the previous findings in 2012 in order to provide a concise plan to clinicians [16]. While the drugs were again mentioned, the caution regarding milbemycin oxime and the multidrug resistance 1 (MDR1) mutant breeds such as collies and shelties were highlighted and also emphasised the treatment for underlying conditions as well.

In another study, Mueller et al. (2012) stated that the most important pre-existing conditions were endoparasites and malnutrition in young patients and endocrinology and chemotherapy in older patients and should be investigated accordingly if demodicosis is diagnosed [2]. Moreover, the need for treatment, they said, would extend beyond the visual and microscopic recovery for fear of relapse and that the most important clinical consequence of the infestation itself is a deep pyoderma. This latter condition would need extensive antimicrobial therapy either topically or orally, and should follow a resistance test.

In the ten years that followed this study, a new group of ectoparasiticides, called isoxazolines, arrived onto the market. These drugs: sarolaner, fluralaner, and afoxolaner to name a few had revolutionised the treatment of ectoparasite infections and prevention and are now the most widely used by owners.

In 2016, Six et al. tested the drug sarolaner in the treatment of generalised demodicosis in 16 dogs. Their study not only showed that after one oral treatment, live mite counts were down by over 97% within two weeks and by day 30, they were down by 99.8%. In comparison to the former treatment options of weekly spot on moxidectin, with added imidacloprid, the mite counts were also reduced but at a much slower rate than those found with sarolaner. This study emphasises the high efficacy and the rapid resolution of mite population within the affected patients and since there were no adverse reactions within any of the treated groups, it provides owners with a quick and simple treatment option for these infections. Moreover, the new drugs' spectrum spans beyond mites and into fleas and ticks as well.

To support these findings, a very similar study was carried out by Beugnet et al. (2016) but in this study, the tested drug was afoxolaner. It was also compared to the moxidectin and imidacloprid combination [17]. In the case of afoxolaner, the oral treatment was given on days 14, 28 and 36 and, with deep skin scrapings once per month, they were able to quantify the reduction in mite population over the treatment period. The efficacy mimics the results presented by Six et al. (2016) with sarolaner as the mite population was reduced by 99.2% on day 28 and reached 100% on day 84 [18].

In comparison to the moxidectin and imidacloprid formulation which also showed lower efficacy than its newer counterpart with a mite reduction of less than 90%. Interestingly, the trend for mite reduction also showed a decreasing efficacy on days 56 and 84 for moxidectin and imidacloprid.

Afoxolaner was also shown to be effective in reducing the severity of skin lesions caused by the mites themselves [19]. As the mite population is drastically reduced, so too does the clinical pruritis and erythema triggered by their proliferation. This trial of mite reduction has been demonstrated in many studies and despite having a slight deviation in the data presented, all studies concluded that the isoxazoline group was superior in both efficacy and speed.

Moreover, when the former drug formulations are no longer showing significant improvements, these drugs have proven to maintain their potency and ability to reduce the mite populations. Morita et al. (2018) presented a case whereby an adult onset demodicosis

triggered by hyperadrenocorticism and hypothyroidism did not respond to treatment with doramectin (similar formulation to moxidectin) and was unable to be given amitraz dips due to a developing diabetes mellitus [20]. In this study, the patient was given one dose of fluralaner and was completely cured of the mite infestation. This was also interesting to compare to the results shown in the 1994 journal by Duclos et al. [14] regarding the older formulations used to treat adult onset demodicosis, which concluded that some patients did not respond completely to the treatment unless the underlying disorder was also controlled.

This is yet another benefit of these new drugs and can again emphasise their superiority over the older treatments. It is however crucial to mention that with the study carried out by Morita et al. (2018) that the patient developed a transient erythema and papule dermatitis on the abdominal region post fluralaner treatment, but no other adverse reactions were reported. Perego et al. (2019) however stated that there needs to be more clinical trials to analyse the efficacy of all these treatments, and new formulations, and especially in vivo reports of more than 5 patients as anything less will lose statistical merit [21]. However despite this statement, they concluded that enough evidence existed to recommend sarolaner, fluralaner, moxidectin, milbemycin oxime, doramectin and ivermectin as effective treatments, selective to each patient following a risk analysis.

On a molecular level using real-time PCR, it is also possible to track the dwindling mite population post treatment. Djuric et al. (2019) used this very technique to show the speed and efficacy of the miticidal drugs and concluded that after one single dose of fluralaner, there was 1000 times decrease in the level of mite DNA detected on day 112 [22]. However, this did not reach a zero level. While PCR has a higher sensitivity and specificity than most tests, especially in the sensitivity of skin scrapes and cytology, it must be stated however that it is not without its drawbacks. Djuric et al. (2019) states that the level of mite DNA detected on day 112 may be due to the presence of dead mites as this test cannot differentiate living and dead organisms, or it could be detecting the very small number of mites that survived the treatment [22]. Either way, the patients had been cleared of any clinical symptoms caused by the mite population as the new level, whether this was indeed zero or not, was tolerated by the host.

While the previous articles presented isoxasolines as oral therapies, Fourie et al. (2019) investigated whether a topical spot on treatment of the same active compound would show

the same results [23]. One single dose of topical fluralaner was administered and deep skin scrapings were carried out at monthly intervals. The results were very similar to those presented in the aforementioned reports that used the oral formulations, indicating that the pharmacokinetics of fluralaner is not entirely dependent on the oral absorption.

Interestingly, when comparing the spot on fluralaner to moxidectin and imidacloprid spot on solution, the mite reduction of the latter treatment showed extremely poor results and even reached 0% efficacy on day 84 (Fourie et al., 2019). This study used 16 dogs with generalised demodicosis and split them evenly into two groups.

To compliment these results, Petersen et al. (2020) carried out very similar clinical trials on 124 dogs with fluralaner oral tablet and moxidectin/imidacloprid spot on solution [24]. In this trial, the criteria to prove efficacy was, they believed, to be over 90% reduction of mite population by days 56 and 84 of the trial. Where fluralaner achieved this criteria, the moxidectin/imidacloprid did not.

This growing trend of dwindling efficacy among the moxidectin/imidacloprid spot on may suggest either a developing resistance to the drugs or the standard for mite reduction was set too high for this formulation to achieve and the miticidal ability of these drugs was never at this level. However, Rohidich et al. (2022) conducted another similar clinical trial comparing fluralaner to moxidectin and imidacloprid and reported the latter spot on treatment to show efficacy by day 56 and 84 [25]. Whereas the results with the isoxazoline group are maintained throughout all these studies, the variety in the results given by the moxidectin/imidacloprid would dampen the confidence of these drugs, and given the ease of administration of its superior rival, the use of the spot on formulation is surely expected to become obsolete for treating canine patients.

2.3. Demodicosis infestation in a clinical setting

A dermatological case presented to the clinician can come in the form of mild to intense pruritis, erythema and scaling with evidence of pustules and furunculosis. This section will use the BSAVA manual on the clinical pathology seen with *Demodex* infections in canines, and with the help of original images of real case studies, the clinical pathology mentioned in the BSAVA manual will be illustrated. Jackson and Marsella (2021) illustrate the differences between localised and generalised demodicosis cases in the small animal practice [1]. While

localised infections may be mild and usually only occur in young puppies and require no treatment, they must also be taken seriously due to the potential of an underlying disease or even the progression to a generalised form. It was stated in this manual that until the 1980s, some cases of generalised infection were severe enough to warrant euthanasia. However, with the development of new treatments such as the isoxazolines, the treatments have become far more successful. In the manual, it was reiterated the three main species affecting the canine patients were *Demodex canis, Demodex injai* and *Demodex cornei*. All three species inhabit the follicles and sebaceous glands but have been reported in other organs, faeces and even blood due to ingestion and lymphatic drainage [2].

Clinical signs appear where the mites are more active. This results in the damage to the hair follicle leading to hypotrichosis, comedomes and alopecia; these symptoms are generally restricted to the face and limbs with localised forms. Generalised forms are more widespread and appear to show more intense lesions around the body, with certain areas, especially the limbs showing various degrees of hyperpigmentation. With pedal lesions (‘pododemodicosis’), the clinician must be vigilant in assessing the situation carefully as the patient will lick the area intensely, greatly increasing the probability of a deep pyoderma or hot spot to develop. Differential diagnoses in this case is the psychogenic mediated acral lick dermatitis, atopic dermatitis and/or allergic dermatitis. As a sidenote, *D. injai* behaves differently to the other two species as it is located more superficially and more so on the dorsum, resulting in a milder alopecia but more intense pruritis [2].

Once the mites proliferate enough, the hair follicle can rupture under the protective cutaneous layers, introducing the mites, debris and bacteria into the subcutaneous environment. This is known as a furunculosis, and secondary bacterial pyodermas are very likely. The BSAVA manual (2021) highlights these main clinical symptoms: Epidermal collarettes, crusting, scaling, seborrhoea and pustules, which are all direct consequences of the folliculitis/perifolliculitis and furunculosis. If these lesions extend into a large area of the skin the animal will show pyrexia, anorexic, lethargy and have a generalised lymphadenomegaly. Histologically around the damaged hair follicle, a lymphoid mural folliculitis will be seen, where ‘mural’ indicates the involvement of mainly the outer root. The term “red-mange” is used when there is an intense erythematous inflammation to the skin.

The following cases presented illustrate the different lesions a clinician may see with different severity of the disease.

2.3.1. Case 1: Milder form of generalised demodicosis1.

A six month old Jack Russell Terrier, female, presented to the clinic with no owner. Found wandering the streets of Cork County, Ireland. Surrendered to Gilabbey Veterinary Hospital in February 2022. Patient had generalised erythematous inflammation, with patchy alopecia, pododermatitis, ocular crusts and scattered comedomes in the inguinal region. There were no signs of bacterial pyoderma. A skin scrape confirmed the presence of *Demodex* mites in large numbers. It was then discovered the co-infection with parvoviral enteritis and despite best efforts, the patient did not survive. The case is presented in Figures 5, 6 and 7, with a different part of the patient's body showing the particular lesions. Figure 8 is a microscopic skin scrape that confirmed the presence of the *Demodex* mites.

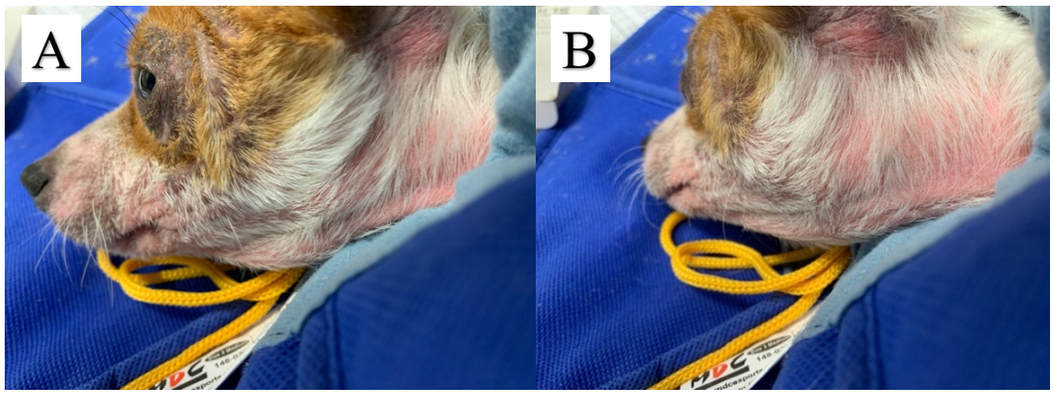

Figure 5: A) Visible erythema on the nasal plane, and pharyngeal region. Ocular Crusts also seen. B) More visualisation of the intense erythema.
Source: Original pictures from own research.

[1] All cases presented are original and collected from a veterinary setting in which the author worked. No copyright infringement occurred and pictures taken were taken from rescue animals.

Figure 6: Abdominal and inguinal erythema with scattered comedomes. Source: Original pictures from own research.

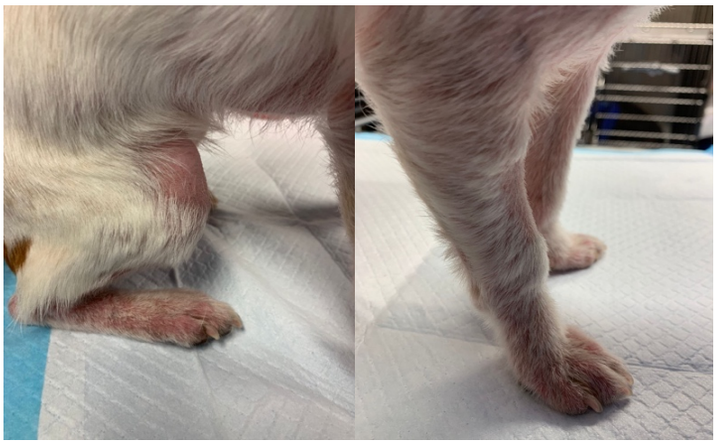

Figure 7: Intense erythema involving the limbs and pododermatitis. Source: Original pictures from own research.

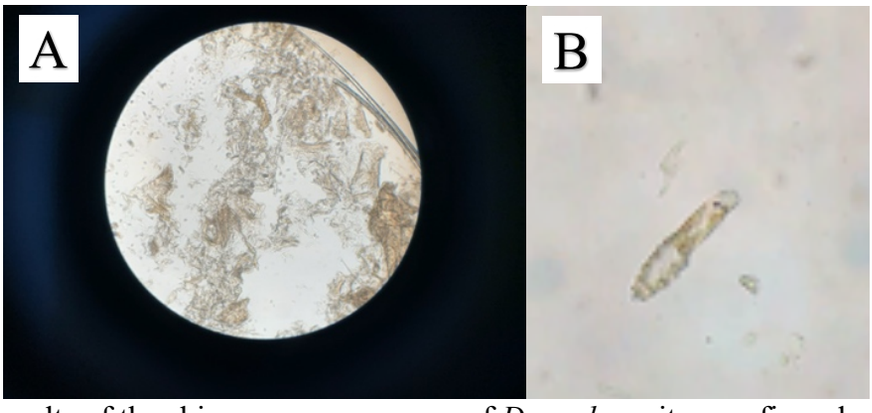

Figure 8: Results of the skin scrapes: presence of *Demodex* mites confirmed under A) low and B) high magnification. Source: Original pictures from own research.

2.3.2. Case 2: Severe form of generalised demodicosis[2].

A seven month old mixed breed, female, dog was rescued by the Foundation of Animal Protection, Füzesabony (FAPF) from the streets of Hungary. Patient was extremely depressed, lethargic, had pyrexia with dangerous levels of dehydration and malnourishment. Upon clinical exam, the patient was showing extreme signs of crusting pustules, alopecia, erythema and extensive bacterial pyoderma. A skin scrape was performed and confirmed the presence of *Demodex* mites in large numbers. The animal was treated for the *Demodex* infection and pyoderma. The next section will follow her case report in more detail, but in this section, the gross pathological lesions to the skin will be discussed. The main lesions are presented in Figures 9, 10 and 11, showing the severity and extent of the condition.

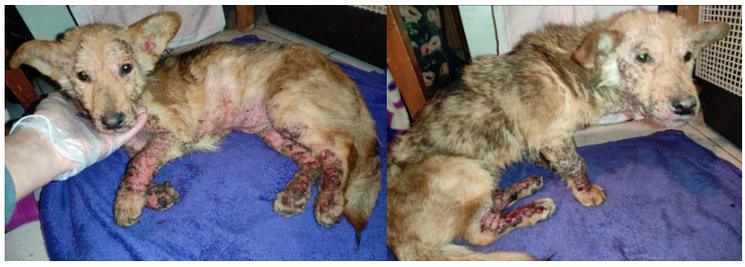

Figure 9: Severe lethargy, depression and malnutrition and the overall presentation of the condition. Source: Original pictures from own research.

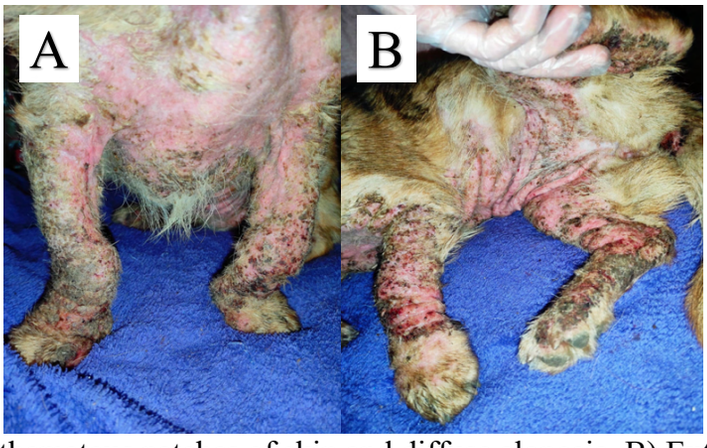

Figure 10: A) Erythematous patches of skin and diffuse alopecia. B) Extensive crusting on the pectoral, abdominal and inguinal region as well as the limbs, with evidence of pustules. Source: Original pictures from own research.

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[2] All cases presented are original and collected from a veterinary setting from a charity case. Pictures of a rescue dog were taken by a charity worker in FAPF and were freely usable without copyright infringement.

Source: Original pictures given to author from FAPF, with thanks.

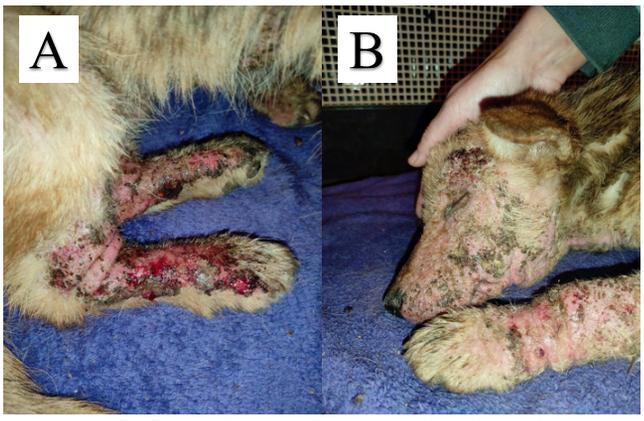

Figure 11: A) Erosive and ulcerative regions on the dorsal aspect of the hindlimbs. Presumably due to the self-trauma caused by the intense pruritis. B) Diffuse facial erythema and crusting. Source: Original Pictures from own research.

2.4. Pyoderma risk associated with the severe form of generalised demodicosis.

With the extent of lesions seen in the previous cases, the likelihood of the patient to develop a pyoderma both influences the prognosis and the treatment plan. A bacterial pyoderma is of critical importance to diagnose and treat as the animal will have to battle both the mite population as well as the invading bacteria, making the prognosis for recovery much more complicated. In this section, the importance of bacterial pyodermas in canine patients will be investigated. Plus, due to the paramount importance of the topic, the prevalence of anti-microbial resistance in relation to common species/genera of common bacteria found on canine skin will be discussed.

According to Lowenstein (2011), a common bacterial infection in canine patients are seen with the introduction of *Staphylococcus* species, namely *S. pseudintermedius* [5]. Gortel (2013) emphasises the importance of recognising a pyoderma and differentiating the condition from other deep ulcerating dermatological conditions such as pemphigus foliaceous, juvenile cellulitis, and lymphocytic pododermatitis [27]. A variety of pyodermas can occur from bullous impetigo to furunculosis caused by *Demodex* infection. For the clinician, it is essential to be able to differentiate the aforementioned conditions from the pyoderma.

Gortel (2013) explains that generally, histopathology can differentiate an autoimmune disease from a pyoderma, however the general rule for skin biopsies would be to treat a

possible co-existing bacterial infection prior to sample taking in order to reduce the possibility of misdiagnosis. Hence, a diagnosis *ex juvantibus* for pyoderma is possible if the patient responds well to the antimicrobial therapy and the lesions diminish. This study concluded that histopathology is required to diagnose the immune mediated skin diseases, however, a cytological sample should be conducted to first rule out the bacterial influence of the sample. Going one step further, this method could also detect the presence of demodectic mites. Moreover, it is always recommended that a culture and sensitivity test be carried out to reduce the emergence of bacterial resistance.

Antimicrobial resistance is one of the most threatening dangers to the medical field. Not only does it impact the veterinary field of therapeutics but the potential for zoonotic transmission is critical. Cain (2013) emphasises that *Staphylococcus* species pose one of the greatest threats to the area of antibiotics and this began with the emergence of methicillin resistant strains carried on the *mecA* gene. These genes alter the protein binding site for all beta-lactam antibiotics such as penicillin, cephalosporins and carbapenems [28]. Furthermore, a multidrug co-resistance has been shown especially to Clindamycin, fluroquinolones and tetracyclines (Cain, 2013). Leading the author to conclude that dispensing of antimicrobials should be carried out with strict prudency, and sensitivity tests based on empirical evidence should shape the therapeutic plan.

The field of treating canine pyodermas has changed dramatically with the occurrence of antimicrobial resistance. Loeffler and Lloyd (2018) emphasised how the current approach to managing the canine pyoderma needs to be updated with the consideration of the emerging resistant strains and the growing legislation that restricts the veterinary practitioner from prescribing certain classes of antibiotics [29]. One major point that was emphasised by Loeffler and Lloyd (2018) was that pyodermas are “always secondary to underlying disease” and that “reoccurrence is likely unless such disease is corrected”.

The risk of pyoderma with an untreated generalised demodicosis is strong. Taking into account the conclusions by Loeffler and Lloyd (2018), eliminating the demodicosis is of primary importance when treating the bacterial pyoderma that accompanied the parasitic infection.

With bacterial infections being secondary, it is important to note that while certain resistant strains may exist on an animal, there seems to be no influence on the pathogenicity of the bacteria itself. Naziri and Majlesi (2023) investigated the prevalence of *S. pseudintermedius* and its methicillin resistant counterpart (Methicillin Resistant *Staphylococcus pseudintermedius*; MRSP) among a pool of healthy dogs and dogs with skin infections [30]. They discovered that there is no significant difference between healthy and sick dogs between the prevalence of the two strains, leading to conclude that MRSP carriers exist among the healthy dog population and that if any of these animals were to develop a bacterial pyoderma with MRSP, the clinician would have a greater challenge in the therapeutic plan. Furthermore, they also discovered that the biofilm producing strains of *S. pseudintermedius* were higher in the MRSP strains which influences the resistance to other antibiotics such as erythromycin, oxacillin and tetracyclines (Naziri and Majlesi, 2023).

Biofilm producing ability can be considered a virulence factor for the *S. pseudintermedius* bacteria and the ability of the particular strain can vary by study. For example, the above journal reported this ability in 81.1% of isolates, while Singh et al. (2013) reports detection occurred in 96% of isolates, but reported no significant difference between the MRSP and the MSSP (Methicillin Sensitive *S. pseudintermedius*) [31]. This contraindicates the previous study's results concluding that the variety can indeed be extremely wide.

The timely detection of MRSP infections could enhance the prognosis and van Duijkeren et al. (2011) conclude that better diagnostic methods such as serial dilution or molecular analysis should be used over the disk diffusion testing as this can produce a large amount of false results [32]. Interestingly, this study also discovered that there was a strong correlation between MRSP carriers and those dogs that had received antimicrobial therapy within 30 days prior to testing, suggesting that research into the long term carriage should be conducted. Moreover, the presence of MRSP in a veterinary clinic is overwhelmingly high and that post-operative infections are generally positive for MRSP or MSSP, indicating the importance of sterility in theatre and recovery areas.

While the accidental infections can occur, van Duijkeren et al. (2011) state that skin infections such as pyoderma and otitis externa are the most common conditions seen with MRSP infection. Hence, this reinforces the conclusion by Loeffler and Lloyd (2018) that the underlying condition will need to be the primary focus of the clinician and to reduce the risk

of unsuccessful treatments and reoccurring pyodermas that can widen the resistance spectrum [29].

Prevalence of the MRSP strains are of strict importance as the zoonotic potential exists between the spread of the resistant genes from MRSP to *S. aureus*, a commensal bacterial species more common to human skin. With the increasing incidences of MRSA (Methicillin Resistant *Staphylococcus aureus*), it is of crucial importance that MRSP is also monitored closely and effectively [33]. While the zoonotic potential of the MRSP strains are of minor concern, the genes responsible could be potentially spread between species of *Staphylococcus* bacteria [32].

Surveillance on a large scale was recommended by Moodley et al. (2014) when they wrote a literary review of the MRSP occurrence from 1980 to 2013 [33]. In this review, they concluded that many inconsistencies existed in the reported data and that a more concise database was needed for reporting MRSP along with incidences where other potentially dangerous pathogens are showing signs of resistance in a veterinary setting.

Worldwide, MRSP is a growing concern for clinicians presented with canine pyodermas. Naziri and Majlesi (2023) reported that of the *S. pseudintermedius* carrier dogs, approximately 12% of healthy dogs and 18% of dogs with clinical pyoderma were positive for MRSP [30]. This incidence is consistent in the dogs studied in Spain with 8% [34], Canada with 12.1% [35], China with 12.7% [36], and Japan with approximately 11-12% beta-lactam resistance in the isolates [37]. However the situation is much worse in Thailand with 28.3% [37], South Africa with 83.8% [38] and Australia with 63.2% [39]. Moreover, many of these isolates showed high biofilm production and the antimicrobial resistance was not restricted to the beta-lactams; antimicrobial resistant mutants were observed in most of these studies.

The veterinary clinician dealing with canine pyodermas is facing an increasing challenge with the rise in antimicrobial resistance and the narrowing spectrum of antibiotics legally available for the use in companion animal medicine. Therefore, alternative methods of dealing with the infection rates must be explored. As mentioned earlier, the main objective should be based on the primary cause of the disease and if antibiotic treatment is warranted,

then a culture and sensitivity test should be carried out prior to long term therapy. Moreover, the use of topical antiseptics cannot be understated.

According to the BSAVA Manual on Canine and Feline Dermatology (2021), chlorhexidine 2-4% shampoo/spray/mousse has a broad spectrum of antibacterial properties and may even be effective against MRSP infections [1]. Benzoyl peroxide is a keratolytic agent and can be useful in the cleansing of follicular debris, found with the demodicosis infections. As a new recommendation, the manual states that with surface and superficial pyodermas, antiseptic topical therapy could prove to be completely adequate in the treatment of bacterial infections of the skin. This both reduces the reliance on systemic antibiotics but also the chance for resistance to develop. Systemic antibiotics should now be reserved for deep pyodermas, such as those associated with the severe furunculosis caused by demodicosis, but there is also evidence to show that the topical use of antiseptics in conjunction with the systemic therapy can reduce the time to recovery of these patients. Resistance to antiseptics is suggested to be unlikely due to the sensitive nature of the *Staphylococcus* spp. structure and the high concentration used in the available products, but close monitoring and good veterinary practice should always be ensured when dispensing this medication.

3. Objectives/Questions

The previous section summarised the most important aspects of a confirmed *Demodex* case in the clinic. In this section, the clinical case of a severe generalised demodicosis infection will be presented and, using the information collected above, what important aspects must be considered. In essence, this section focuses mainly on the important questions a clinician must have when presented with a demodicosis case.

Firstly, the role of the anamnesis is one of the most important facets to consider: is the animal old or young, stray or owned etc. Then, taking this into account, the question of why the animal is infected must be asked. Secondly, it must be emphasised that the differential diagnosis list for a demodicosis case is quite long, however, the role of sarcoptic mange in the differential diagnosis list cannot be overlooked due to its highly infectious nature to other animals and also humans. Thirdly, when the animal is confirmed to be positive for demodicosis, any possible underlying conditions should be investigated for and treated accordingly. Lastly, the scoring of the case presented based on severity of the lesions should shape the prescribed treatment plan. The case study 2 presented in section 2.3 above will be used henceforth to illustrate this procedure.

3.1. Signalment and Anamnesis

Like most diseases, a list of differentials can be drastically reduced based on age, breed, sex, background and recent history. While these are not always a full exclusion, the basis of probability can help the clinician to steer the diagnostic plan in a certain direction. For example, a dog that was born in Scotland and that has never travelled to the Mediterranean would be very unlikely to be positive for *Leishmania*. Similarly, an older dog showing a symmetrical pattern of non-pruritic alopecia is more likely to have hypothyroidism than atopic dermatitis from an environmental allergen [1]. Thus, when presented with a suspected *Demodex* case, the clinician should consider the age of the animal. Young animals can show juvenile and local form of the disease and not require treatment, whereas an adult dog showing local signs of demodicosis would warrant a full blood endocrinology parameter check.

However when it comes to a generalised form of the disease, the age and background need to be considered together. An adult dog showing generalised signs would indeed need this

endocrinology work up but it is also possible that the sole cause could be animal negligence. Therefore, the case and background as a whole need to be considered. If the animal is young, stray and malnourished, an underlying immunological disease needs to be considered, but again the sole cause of these symptoms can be due to stress and poor nutrition. High roundworm count, seen with *Toxocara* spp. or *Toxascaris leonina*, which are more common in young animals, can lead to an exhausted immune system and thus, opportunity for *Demodex* mites.

A chronic stress causes high blood corticosteroid levels which are known to have immunosuppressive properties, coupled with poor nutrition and no prophylactic treatment is a prime opportunity for the *Demodex* mites to proliferate; as in the cases presented in section 2.3 above. Iatrogenic immunosuppression can also occur from heavy or long term use of corticosteroids and if mismanaged, can cause long lasting endocrinological damage to the patient; this is essentially creating the profile of a hyperadrenocorticism patient via the use of these drugs. The same could also be stated for other immunosuppressive agents such as long term usage of cyclosporin, azathioprine or other chemotherapeutic agents. Therefore, a careful documentation of recent history is just as essential.

3.2. Important differential diagnoses

In general, before handling the patient, a full history would be taken. However, when the history is not available such as in the case of a stray animal, it is first and foremost the responsibility of the clinician to rule out anything that could be harmful to other animals and humans. For example, dermatophyte species of fungi that cause ringworm in dogs can be transmitted to humans as well as other animals. Flea allergic dermatitis, lice and *Cheyletiella species* can also be considered for this list, especially since these parasites are also shown to have zoonotic tendencies [1, 5].

However, the main differential diagnosis to consider is the Sarcoptic mange infection, caused by the *Sarcoptes scabei*, a purely infectious mite that lives superficially under the stratum corneum of the skin and can cause a serious pruritis in the host, and unlike the *Demodex* mites, this particular species is a pure obligate pathogen and is zoonotic. The following case will emphasise the devastating effects an untreated sarcoptic mange infection on a wild fox.

Case 3: Sarcoptic mange in a wild fox.

In Ireland, scabies would be considered uncommon in an urban setting with pets receiving regular prophylactic treatments but in the countryside, the population of wild foxes can easily transmit the scabies mite to one another and other animals, including stray dogs. The following case will show how severe this mite infection can be on an animal. This young, male fox, found in Ireland in June 2022, was suffering from severe malnutrition, dehydration, a generalised scaling and lichenification with cause unknown. The author used gloves and other personal protective equipment when handling this animal. Upon discovery of the fox, the animal showed very little signs of life and poor to grave prognosis so he was humanely euthanised. A post mortem skin scrape and trichogram confirmed the scabies mites in an extremely large number. Figure 12 is an antemortem picture taken to show the overall condition of the animal. Figures 13 and 14 are post mortem pictures showing the extent and condition of the skin. Figure 15 shows microscopic images taken from a skin scrape which confirms the presence of scabies mites.

This case is presented here to emphasise that if these lesions occurred in a stray dog, it is almost indistinguishable from a demodicosis infection. Therefore, extra vigilance should be taken when similar lesions appear.

Figure 12: Antemortem presentation of the fox. Note the level of malnourishment, dehydration and the extent of alopecia and crusting. Source: Original Pictures from own research.

Figure 13: Post mortem, up close view of the lesions surrounding the head. Note the extent of the crusting and lichenification around the nasal planum and the ulcerated pinna. Source: Original pictures from own research.

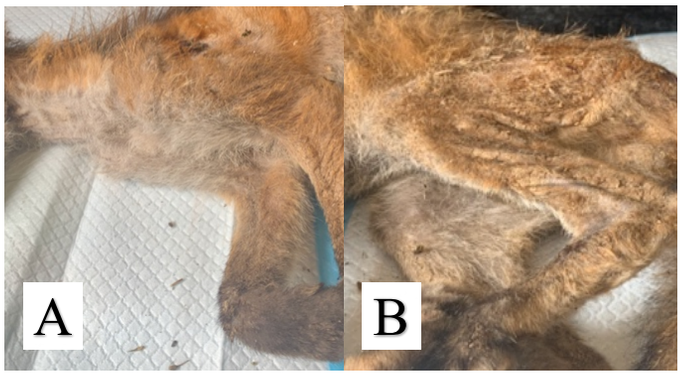

Figure 14: Post mortem view of the alopecia, scaling and crusting of the A) neck and forelimbs, and B) hindlimbs, abdomen and tail. Source: Original pictures from own research.

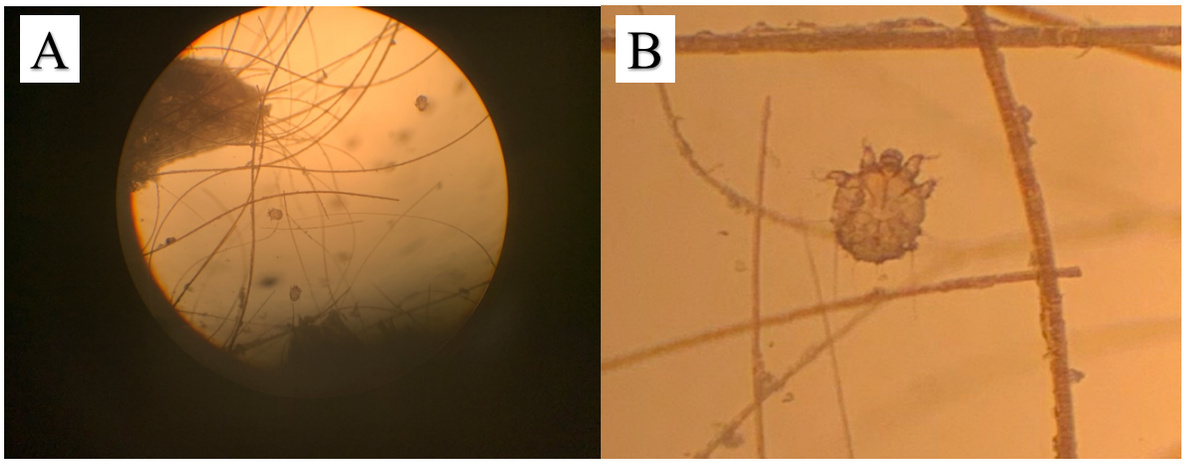

Figure 15: A) Skin scrape confirming the presence of the sarcoptic mange mites under a light microscope (40x magnification). B) Morphology of an adult scabies mite under a light microscope, with high magnification. Source: Original pictures from own research.

3.3. Possible underlying conditions

As it was mentioned previously, this section will just reiterate the importance of the possible underlying conditions that can contribute to the development of the demodicosis and if not treated correctly, the *Demodex* infection will persist or the patient will relapse in the future.

In young animals, hygiene, nutrition and a good immune system are paramount. Infections such as parvoviral enteritis, can considerably increase the chances of developing this skin disease and as such, proper management of vaccinations, antiparasitic medication and other hygiene related aspects must be kept up to date.

Adult animals developing demodicosis should warrant an endocrinological investigation and also a complete blood count (CBC) to check if the white and red blood cell parameters are within normal limits; some types of neoplasia in the bone marrow could alter the level of white blood cells, leading to an exhausted immune system.

Biochemistry and urinalysis would also be recommended as routine tests to check the function of vital organs such as kidney and liver function, and if necessary, diagnostic imaging. And finally, as a general rule, travel history, vaccination status and nutrition records should be reported as these can carry significant weight on the direction of the case work up.

3.4. Lesion severity scoring

With a confirmed demodicosis case, regardless of underlying issue, a judgement on severity must be made which will help in both shaping the treatment plan and tracking the progress. Dengler et al. (2021) devised a visual observation scale based on severity of the lesion in regards to erythema, pustules, crusts and alopecia, in 36 areas of the body [40]. Each area was evaluated separately for each lesion and given a 6 point scale as follows: “none (0), mild (1-2), moderate (3-4), and severe (5-6); with the total score being the sum of values given. At the end of the evaluation, the skin scrapes were used to estimate the total mite count. Both of these parameters were used by two clinicians under blind conditions and found a high degree of correlation with their results.

During this study, the patients were given oral doses of ivermectin and their progress was monitored by re-evaluation using this scale. They discovered that using these scoring scales with a linear model, a clear reduction of both scores were observed. Moreover, the statistical

significance between a reduction in one scale and the other scale was significant, therefore, the clinical scoring system can prove to be an effective and simple way to determine progress and treatment results [40]. Table 1 presented below is an example of this system being used to evaluate the lesions seen before with Figure 10 (A).

Table 1: Example of Clinical Scoring using Figure 10 (A) (Source: Clinical Scoring Scale full credit to Dengler et al., (2021)).

(Body Part): Pectoral	None (0)	Mild (1-2)	Moderate (3-4)	Severe (5-6)
Erythema				X
Pustules				X
Crusts				X
Alopecia				X

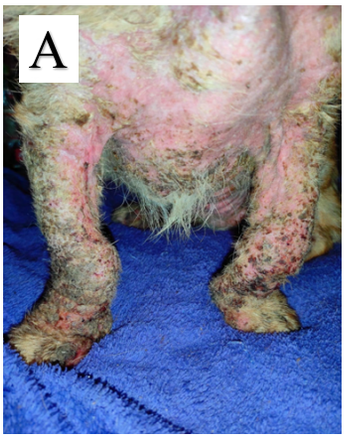

Figure 10: A) Erythematous patches of skin and diffuse alopecia. Source: Original pictures from own research.

Using this scale in the clinic can help track the patient's progress leading to a better overall picture and provides validation that the treatment plan is working successfully. The involvement of pyodermas in the case can potentially complicate the scoring system, but effective medication and treatment options should minimise this interference.

4. Methods used in a clinical setting to detect mite infections

Case study 2 from section 2.3 was used in the field research of this thesis. A proper diagnostic approach was undertaken to ensure that effective treatment options were allocated and the patient was given the best chance possible to recover. The directions outlined in the BSAVA manual of Canine and Feline Dermatology (2021) explain the process behind the mite collection tests. Blood samples were also taken and the patient was tested using blood snap tests for *Dirofilaria immitis*, *Babesia* sp., *Anaplasma* sp., and *Erlichia* sp.; each of these tests were negative.

According to the BSAVA manual (2021) [1], the *Demodex* mite lives deep in the hair follicle and thus requires a deep skin scrape for evaluation. Gently squeezing the skin prior to scraping can provide extra benefit for detection. The scrape is to continue until the first spot of blood appears and then quickly evaluated under the microscope. Alternatively, a hair pluck, or trichogram, can be performed if the patient is non-compliant or the clinician is otherwise unable to perform the skin scrape on the tissue. In case study 2, the patient's skin was considered too severe for a deep skin scrape that only a trichogram was carried out. Mites can be observed under a light microscope using 40x magnification.

It was stated in the manual that the best areas for detection are the lesions showing the most alopecia and/or comedome rich regions as the activity of the mites will worsen the clinical symptoms. It was also noted that with the deep nature of the mites, they can also be detected with histopathology using a punch biopsy. However, given the invasive nature of this technique and the high sensitivity of the former techniques, punch biopsies would not add much diagnostic value to the case.

Moreover, given the nature of the patient's history, a test for sarcoptic mange was also carried out. Unlike the deep skin scrape seen with the *Demodex* mites, the scrape for sarcoptic mange mites requires finding a lesion and scraping at the very edge of this lesion, or beyond [1]. They explain that this is because the scabies mite will burrow into the skin, under the stratum corneum, and travel along under the surface laying its eggs. These eggs and faeces cause the hypersensitivity reaction to the host and thus, the mite will have already travelled beyond this area. Trichograms are not recommended for detection of sarcoptic

mites. Mites can be observed under a light microscope using 40x magnification, using paraffin and a cover slide.

The detection of the scabies mites by physical methods may yield false negative results due to its low sensitivity, therefore, a serum enzyme-linked immunosorbent assay (ELISA) test is available and proven to be statistically sensitive and specific by Curtis (2001), if the patient is given enough time for seroconversion [41].

Finally, a deep pyoderma was diagnosed in this patient and due to the extreme nature of the lesions, systemic antibiotic usage was recommended and oral medication was prescribed on the basis of a culture and sensitivity test. In the next section, the results of the above tests will be presented along with the chosen medication.

5. Results of tests and treatments

In this section, the results obtained from the laboratory tests will be presented along with a brief discussion of these results. Secondly, the official diagnosis of the demodicosis infection, and possible underlying causes. Thirdly, a closer look at the pyoderma in regards to the species of bacteria that are causing the lesions and the results of the sensitivity test will be presented. Then, a brief explanation of the chosen medication plan going forward based on the results of the previous tests and the information presented above in the literature review. And finally, an illustrative report will show the progress of the patient over a certain time period using a visual scoring system similar to Dengler et al., (2021) [40].

5.1. Clinical exam and Laboratory results

Patient presented with very poor prognosis, drank well but had inappetence. Bloody faeces was apparent and samples were tested for *Parvovirus*, canine *Coronavirus* and *Giardia*; all results were negative. Patient had a severe level of dehydration and pyrexia and was lethargic. Blood samples were taken for routine haematology, electrolyte and biochemistry profile tests, along with laboratory tests for other blood infections.

5.1.1. Haematology and blood infection tests

A typical finding in patients with this level of inflammation and infection is a neutrophilia, showing a high degree of relative lymphocytosis, consistent with a chronic inflammatory response and overtime resulting in an exhausted lymphocyte count. This pattern is consistent with the immunological processes explained by the literature previously discussed. Packed Cell Volume (PCV) and Haemoglobin concentration were dangerously low with levels reaching 22.3% and 73g/l, respectively; explainable by the blood loss through the faeces. However, with the level of dehydration observed, the real value was likely to be lower. All other parameters were within normal limits.

The patient's blood was tested for *Dirofilaria immitis* microfilaria, *Anaplasma phagocytophilum*, Haemotropic *Mycoplasma* spp., *Babesia canis* and *Babesia gibsoni*. All these tests had negative results.

5.1.2. Biochemistry and Electrolytes

Nothing drastic to report. Alkaline phosphatase was slightly elevated but this could be explained by either the age of the animal as an isoenzyme exists within the skeletal system, or it could also be induced by the chronic stress of the patient with high levels of corticosteroid. Albumen was considerably low, consistent with the malnutrition; and the albumen/globulin ratio was also in the low range, possibly also due to the increased globulin levels, indicating a chronic inflammatory response. C-reactive protein was four times the upper threshold of normal, confirming that there was an intense inflammatory reaction. Electrolytes showed no significant changes.

5.1.3. Dermatological tests

The results of the trichogram for this patient confirmed the presence of *Demodex* mites in large numbers. No lice, fleas or scabies mites were detected but personal protective equipment was still utilised. The use of an ultraviolet lamp also showed positive results, consistent with certain species of fungal infections [1]. The *Demodex* mite infestation was explained by presumption that the patient was under severe chronic stress, dehydration and malnutrition, and given the background, was not able to receive adequate veterinary treatment for the condition before it advanced to this level of severity. No other conclusion was made regarding the cause.

The results from the culture and sensitivity test of the pyoderma came back positive for three species of bacteria, namely: *S. aureus*, *Escherichia coli*, and *Klebsiella oxytoca*. While *S. aureus* is not the main species of *Staphylococci* found in canines, van Duijkeren et al. (2021) mentioned that many laboratories would not make the differentiation between this species and *S. pseudintermedius*, thus diagnoses the test as positive for *S. aureus* [31]. The sensitivity tests gather all information based off a culture growth under conditions in which different antibiotic compounds are added. Those bacterial cultures which fail to grow alongside the antibiotics are deemed antibiotic susceptible, those which grow at a moderate rate are moderately susceptible, and those cultures where no growth was inhibited are deemed resistant.

In this case study, many antibiotic groups were effective against the three strains but the pharmacokinetics to the skin are suboptimal for many of these classes of drugs. Thus, cephalexin antibiotics were chosen based on their high distribution into the skin despite *S.*

aureus being the only strain fully susceptible. E. coli was moderately susceptible and *Kl. oxytoca* was resistant. However, topical antiseptics would be important to mention as they can contribute to the treatment in different mechanisms despite having a resistant strain to the chosen systemic [1].

5.2. Treatment plan

With all this taken into account, the animal was first stabilised at the clinic for approximately 7 days. She was given astringents for the bloody diarrhoea, fluids, and Vitamin B complexes. Moreover, she was given oral medication to combat the mites and also oral antibiotics to reduce the bacterial load on the skin, oral anti-fungal medication as well as an array of topical treatments, probiotics and immune stimulating supplements.

- Nexgard Spectra (afoxolaner, with milbemycin oxime) was prescribed for the *Demodex* infection.
- Cephalexin was chosen as the best choice of antibiotics, given the spectrum of sensitivity of two out of three bacterial species and the excellent pharmacokinetics of this drug class for dermatological conditions, and the complimentary topical treatment to combat the resistant strains.
- Itraconazole was chosen for the suspected fungal infection.
- Chlorhexidine 2% Spray was prescribed twice daily for a full body treatment with a complimentary Leniderm mousse to help revitalise the damaged areas.
- Oral probiotics and Immunovet supplements were also added.

This course of treatment was given with weekly monitoring by the veterinarian to check the progress and effectiveness of the treatment plan. Patient was discharged into foster care and would continue treatment as prescribed.

5.3. Visual progress reports[3]

As mentioned above, the patient was sent into foster care and treatment continued with regular check-ups. In this section, the visual scoring of the lesions will be presented over a

[3] Source of Pictures: Rescue day pictures given to author by FAPF, subsequent progress pictures are original and from own research.

time period of a number of weeks, starting from her arrival into foster care. These images will help to illustrate the improvements made with the chosen treatment plan. Figure 16 are images taken of the patient when rescued by the charity. We know from the subsequent tests that this is the damage caused by the presence of the *Demodex* mites. Figure 17 shows a select few areas of the patient during the recovery period, namely week 1 and 2 of foster care. Figure 18 shows the further progression witnessed during weeks 3 and 4, and Figure 19 presents the subsequent 6 weeks that followed. This visual timeline highlights how the most severe lesions were able to gradually diminish and the skin was able to regenerate when the patient was given the correct treatment plan.

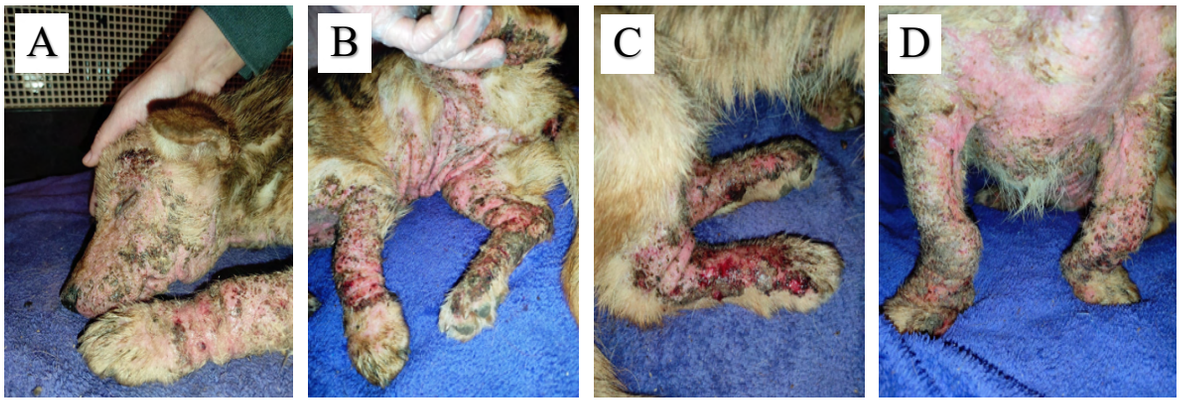

Figure 16: Rescue day photographs of the patient: A) Face, B) inguinal region, C) dorsal pedal region, D) pectoral region. (Source: FAPF charity).

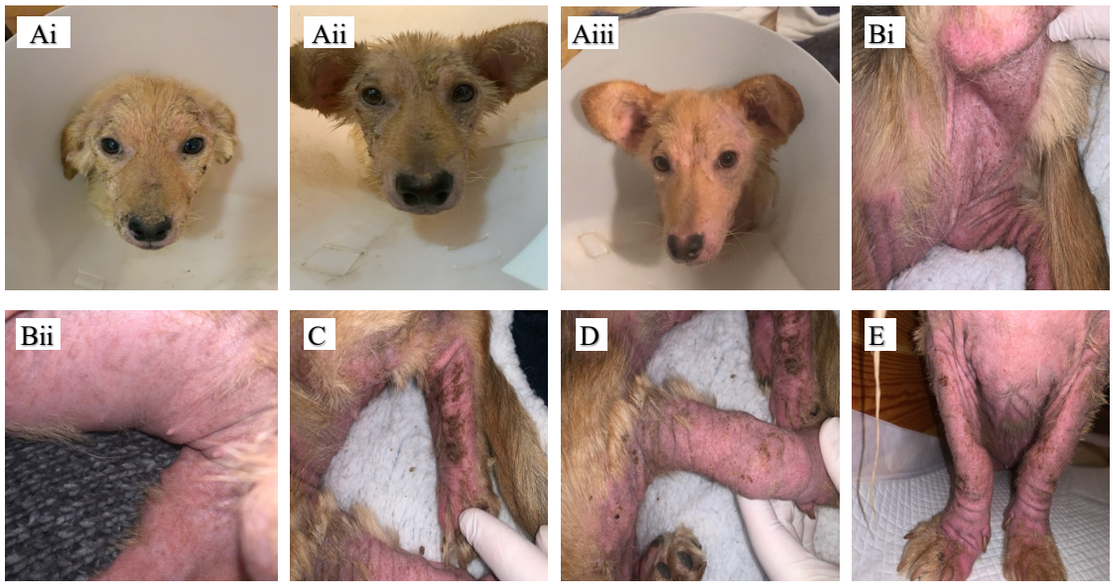

Figure 17: Photographs of the patient during week 1 and 2 of recovery. Ai-iii) Face, Bi-ii) inguinal region, C) dorsal pedal region, D) antebrachium, E) pectoral region and forelimbs. Source: Original pictures from own research.

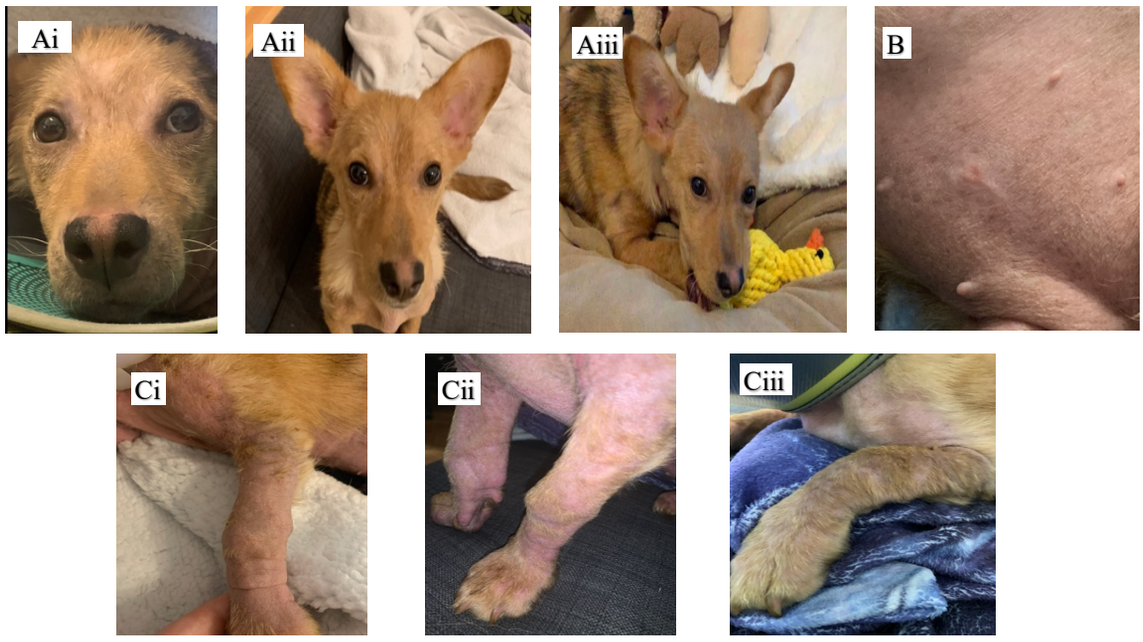

Figure 18: Photographs of the patient during week 3 and 4 of recovery. Ai-iii) Face, B) umbilical region, Ci-iii) antebrachium. Source: Original pictures from own research.

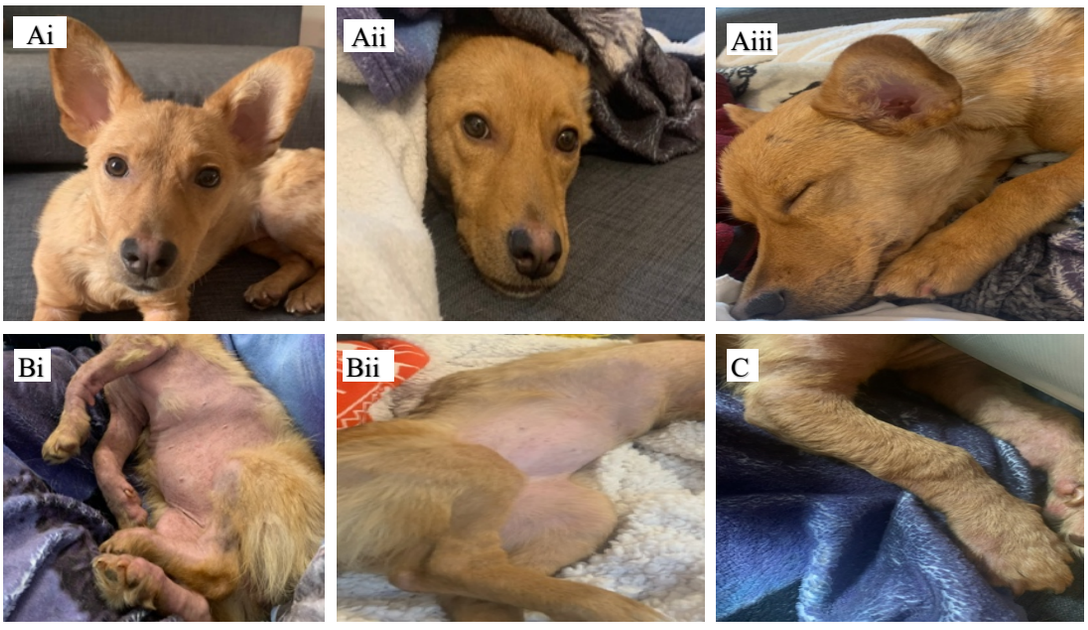

Figure 19: Photographs of the patient during week 5 to 10 of recovery. Ai-iii) Face, Bi-ii) abdomen, C) antebrachium. Source: Original pictures from own research.

The above examples illustrate the recovery process of three different body parts, namely the face, the abdomen and the extremities. The severe erythema, crusting and pustules gradually heal and the fur begins to grow back. It is evident from the pictures how rapid this process can happen given the correct treatment regime and is consistent with the literature regarding treatment efficacy of the isoxazolines.

6. Discussion/Conclusions

Throughout this thesis, the theme of *Demodex* being a secondary infection was emphasised in detail. How the patient's own background history and recent medical therapy are major factors to consider when facing a potential *Demodex* case. Moreover, it was mentioned that in adulthood, *Demodex* can be a signal to the clinician that underlying endocrine or immunological diseases are present and while there are many other clinical signs to these conditions, the treatment of the *Demodex* must also be included in the therapy.

While the clinical symptoms of demodicosis can mimic other conditions, a simple skin scrape or trichogram can aid in the diagnosis of the condition. An imbalance in the immunity of the host will allow the mites to proliferate and create these clinical symptoms, thus earning the name of "indicator disease". Furthermore, overuse of immunosuppressive agents can also shift the balance in favour of the mites and if a patient is receiving this treatment, the clinician must always be vigilant if this patient starts to develop alopecia or erythema.

Inflammatory cytokines are parameters that were analysed in the studies discussed in the literature review. While some discrepancies existed, the IL-6 and IL-10 levels were shown to be consistently elevated. Furthermore, the analysis of the CD4+ and CD8+ showed that there is a downregulation of these lymphocytes during an infection. Kumari et al. (2018) presented the expression of the TLR in dogs that were infected versus those that were healthy and the results show that a high TLR-2 and low TLR-4 and TLR-6 were consistent across all the subjects, and the change over time of these genetic expressions and the heritability of this genetic pattern should be investigated [11].

When the treatment options were discussed, there was an overwhelming superiority of the isoxazoline group when compared to the more traditional methods of treatment. Seen also with the above case study, the afoxolaner drug was administered once every 30 days and, along with the supplementary treatment, the clinical symptoms dramatically improved within a few weeks. This will emphasise the importance of these new, inexpensive and highly efficacious drugs in the long term prophylaxis and treatment of ectoparasites.

The gross pathological lesions in the clinical setting were presented and a comparison of a mild versus a severe case illustrated the descriptive nature of these lesions. While some

patients, especially juvenile dogs, will only show a localised form of the disease and not require treatment, some dogs may progress into a more generalised state and over time, these symptoms can worsen and develop into a secondary bacterial infection.

Moreover, the influence of the bacterial co-infection on the prognosis, and the impact resistant strains of bacteria can have on the available treatment options were emphasised in detail. MRSP being the biggest threat to animal health when battling a pyoderma was evaluated and how this strain of bacteria can vary across continents; leading some studies to conclude a better monitoring system is warranted for further research. Taking these aspects into account, the BSAVA manual (2021) recommended that oral systemic antibiotic usage should be reserved for patients that are presenting with deep pyodermas only and that superficial and surface infections should only require topical treatment, thus widening the scope of treatment while minimising the opportunity for further resistance [1]. This was also illustrated in the successful treatment of the above patient despite the presence of bacterial strains resistant to the chosen systemic antibiotic.

In a clinical setting, the importance of having a differential diagnosis in place will not only help the clinician to steer the case work up properly, but it will also allow them to highlight the potential zoonotic agents that could be present. Sarcoptic mange was presented after confirmation in a wild fox and the lesions compared to those seen with demodicosis. While a negative skin scrape may not be conclusive of sarcoptic mange, there are other alternative tests available; but caution should always be practiced if the tests are doubtful. The tests for demodicosis and sarcoptic mange slightly differ and were explained in detail by Jackson and Marsella (2021) in the BSAVA Manual of Canine and Feline Dermatology [1].

Thus, with the confirmed test samples, Dengler et al. (2021) devised a visual scoring system based off four key dermatological symptoms present with a demodicosis case, which would evaluate the severity of the lesions seen [40]. This would allow the clinician to monitor the progress of treatment and make alterations where necessary. Using a visual scoring system allowed the author to monitor the progress of the patient over a span of several weeks and based on the selected treatment, was able to see a drastic improvement over this period, which was consistent with the available literature.

To conclude this discussion, a brief reiteration of the main points is needed and a recommendation going forward will help illustrate these concepts. Firstly, a regular monitoring of all patients for skin lesions by their owners is paramount. Regular usage of prophylactic acaricides, such as the isoxazoline group, can help to keep the infectious and commensal ectoparasites under control. Moreover, higher risk animals should always be monitored and checked regularly for clinical symptoms. Those patients most at risk of developing a case of demodicosis are shelter animals, endocrinology patients, patients with long term use of corticosteroids, patients receiving chemotherapy, and patients who suffer from an underlying immunological disorder.

It is vital that if a pyoderma develops, it is treated alongside the *Demodex* infection, and with the current trend in multidrug resistant bacteria, a culture and sensitivity test is highly recommended. Not only will this aid the clinician to prescribe the best treatment for the animal, but it will also reduce the likelihood of further resistance. Moreover, the pharmacokinetic aspects of each antibiotic should be considered, as this will influence the concentration of the antibiotic that accumulates in the desired target tissue. Topical therapy is also highly recommended in severe cases to aid the bactericidal effects of the systemic antibiotic treatment and to reduce the recovery time. A more rapid recovery is beneficial for the animal, the vet and the owner and if patient and owner compliance can be ensured, it can provide an extremely valuable tool in the treatment of these infections.

The skin is the only organ in which the owner can see, feel and smell on a regular basis, even when they aren't necessarily looking for symptoms. Therefore, any new patterns of alopecia, scaling or pruritis should be investigated by the veterinarian. The role of underlying diseases were discussed in detail in regards to demodicosis, therefore, if lesions start to appear over time, the clinician should always search for the cause and not just treat the symptoms.

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Thesis progress report for veterinary students

Name of student: Cormac Kiely

Neptun code of the student: Z8L4NZ

Name and title of the supervisor: Szekeres Sándor PhD

Department: Department of Parasitology and Zoology

Thesis title: Clinical Presentation and Dermatopathology of Generalised Demodicosis in Canine Patients

Consultation – 1st semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2023	01	10	Thesis topic meeting	
2.	2023	01	31	Literature progress	
3.	2023	02	21	Literature progress	
4.	2023	03	28	1st version of the Thesis	
5.	2023	05	03	Literature	

Grade achieved at the end of the first semester: excellent (5)

Consultation – 2nd semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2023	09	13	2nd version of the Thesis	
2.	2023	09	20	3rd version of the Thesis	
3.	2023	10	10	4th version of the Thesis	
4.	2023	10	26	5th version of the Thesis	
5.	2023	10	29	Final version	

Grade achieved at the end of the second semester: excellent (5)

The thesis meets the requirements of the Study and Examination Rules of the University and the Guide to Thesis Writing.

I accept the thesis and found suitable to defence,

.....
signature of the supervisor

Signature of the student: .....

Signature of the secretary of the department: .....

Date of handing the thesis in..... .....