**UNIVERSITY OF AGRICULTURAL SCIENCES AND VETERINARY MEDICINE "ION IONESCU DE LA BRAD" IAȘI**

**FACULTY OF VETERINARY MEDICINE**

**DOCTORAL SCHOOL OF VETERINARY MEDICINE**

**PHD FIELD: VETERINARY MEDICINE**

**SPECIALITY: PARASITIC DISEASES**



# PHD THESIS

**SCIENTIFIC COORDINATOR,**

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**-2020-**

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## *SARCOPTES SCABIEI* INFECTION IN DOGS: EVALUATION OF NEW THERAPEUTIC OPTIONS AND MODIFICATION OF THE SKIN MICROBIOME

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FACULTATEA DE MEDICINĂ VETERINARĂ

ȘCOALA DOCTORALĂ DE MEDICINĂ VETERINARĂ

DOMENIUL DE DOCTORAT: MEDICINĂ VETERINARĂ

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# TEZĂ DE DOCTORAT

## CARACTERIZAREA MICROBIOMULUI CUTANAT ȘI EVALUAREA UNOR NOI OPȚIUNI TERAPEUTICE ÎN RÂIA SARCOPTICĂ LA CÂINE

CONDUCĂTOR ȘTIINȚIFIC

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-2020-

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# ABSTRACT

Sarcoptic mange is a parasitic skin disease caused by the burrowing mite *Sarcoptes scabiei*. It affects many species of animals, including humans. The problem is that even though it has been known from ancient times, sarcoptic mange still presents some challenges in aspects such as physiopathology, diagnosis, treatment. Furthermore, the occurrence of cases resistant to some conventional molecules used for curing this skin disease has lead to a necessity in finding new substances for treatment. Moreover, the modifications of the skin barrier caused by *S. scabiei*, present a challenging aspect that needs to be studied further.

The purpose of the PhD thesis entitled "***Sarcoptes scabiei* infection in dogs: evaluation of new therapeutic options and modification of the skin microbiome**" was to bring some new information regarding the evaluation of clinical signs of sarcoptic mange in dogs, as well as to find a novel treatment for curing this skin disease, and to present some modification that occur in the skin microbiome in dogs infected with *S. scabiei*.

The objectives of the thesis were:

- to describe the clinical signs and some epidemiological aspects of dogs infected with *S. scabiei*
- to assess the efficacy of a novel molecule from the isoxazoline class, afoxolaner, in the treatment of sarcoptic mange in dogs
- to study and describe the modifications of the bacterial and fungal skin microbiome of dogs affected by sarcoptic mange.

The originality of this PhD thesis consists in:

- the use of a new clinical score (adapted from pigs with sarcoptic mange) to evaluate the degree of infection with *S. scabiei*
- the trial of a rather new, less studied molecule, represented by afoxolaner in the treatment of dogs with sarcoptic mange
- the study of both fungal and bacterial skin microbiome in dogs affected by sarcoptic mange, both before any treatment and after the first afoxolaner treatment, which represents to our knowledge, a premiere in this field.

The thesis is structured accordingly to the general rules, into two main parts- the first one describes the current state of knowledge regarding sarcoptic mange, and is made up of 4 main chapters; the second parts presents the personal contributions to the field, summed up in 5 chapters. Besides these main parts, the paper also contains introduction,

abstract, acknowledgements, list of abbreviation, list of figures, table of contents, bibliography, annexes. The current thesis contains 52 figures and 6 tables, having also 182 bibliographic sources consulted.

The first part, which focuses on the current knowledge from the literature, consists of 4 chapters and associated subchapters. The first chapter presents general knowledge regarding the *S. scabiei* mite, including the first mention in history of the mite and the disease, its classification in the animal world, the morphology of all the stages of the mite, the life cycle, evolution from egg to adult, survival capacities and how it is transmitted. It continues with some more debatable subjects such as the morphologic variability and host specificity of these mites, as well as their genetic variability.

The second chapter concentrates on describing sarcoptic mange in dogs, including: clinical signs and lesions, physiopathology of this skin disease, which still presents lots of questions, diagnosis, as well as transmission to other animals and humans.

The third chapter is specific to the control of *S. scabiei* infections, and it includes a general list of the acarcides used in human and veterinary medicine and their main characteristics, followed by specific subchapters which select the molecules used for dogs, other animals and humans. The last part addresses the important drug resistance problem.

**Chapter 4** presents a new direction in the study of sarcoptic mange in dog as it introduces the notion of skin microbiome. The subchapters include a general presentation of the skin microbiome, followed by the description of the canine skin microbiome.

The second part of the thesis, Personal contributions to the field of study, is structured into 5 chapters (Chapters 5 to 9): **Chapter 5** being assigned to the description of the purpose and objectives of the current research, the next 3 (Chapter 6 to 8) describing the studies carried out, including the description of material and methods, the results and the subsequent discussions and the last chapter (Chapter 9) being represented by the general conclusions of the paper, where all the remarks are gathered and analyzed in a final form.

**Chapter 6**, entitled "Clinical signs and epidemiology of sarcoptic mange in dogs in Moldova" starts with the description of the materials and methods used to conduct the study and it mentions: the inclusion criteria for the dogs (evocative clinical signs of mange and positive skin scrapings), the questionnaire addressed to the owner, the diagnosis method by deep skin scrapings and then identification of any form of the mite *S. scabiei* under the microscope. An interesting method was the original clinical scoring system, adapted for dogs, but initially designed for pigs infected with *S. scabiei* (Bernigaud *et al*., 2016). The score was based on evaluating the skin areas affected by mange lesions, alopecia degree, intensity of the skin erythema and crusting/scales intensity, on different parts of the body (head, trunk, legs, tail).The grades per sign were from 0 to 4, with 4 being the most severe, which resulted in a total clinical score between 0 and 60, which was assessed only at the first visit. Regarding the treatment, each dog included in the study was treated with a specific acaricid product against *S. scabiei* infection (doramectin, sarolaner, afoxolaner).

The results of this study showed that there were 48 dogs included, dogs examined from 2015 to 2018. Among them, 27 were examined in the Parasitology Clinic of the Faculty of Veterinary Medicine of Iași, Romania and 21 were examined in either private households (14) or shelters (7). We included dogs from Iasi, Vaslui and Vrancea county. They were mostly common or crossbreed dogs (n=39, 81.2%), but there were also some individuals from the following breeds: Boxer (a mother with her 7 puppies), Bull Terrier (1) and Poodle (1). Their age ranged from 1 month to 12 years, with more than half of them (n=28, 58.3%) being under 1 year. Finally, the individuals included represented almost equally both sexes, with 23 males (48% of the total) and 25 females (52% of the total). Concerning the history of the dogs, a large number were stray dogs (n=21, 43.7%), resulting in the fact that the information we had about them was limited. The zoonotic transmission of the disease was reported from only one dog, that had the most severe lesions and that was hospitalized in our clinic. The skin scrapings examined through direct examination were positive for *S. scabiei* adults, larvae or eggs. However, we observed very few parasitic elements in the samples collected.

The clinical scores of the dogs varied between a minimum of 4 and a maximum of 58, taking into consideration that the maximum score which could be obtained was 60. This represents a clear illustration of the fact that the dogs we studied had from very discrete lesions to serious, generalized lesions, which is represented in the corresponding figures from the text.

**Chapter 7** entitled "Efficacy assessment of afoxolaner in dogs infected with sarcoptic mange" onsets with the presentation of the material and methods which are similar to the ones described in Chapter 6, with the modifications that the skin sampling and clinical score were assessed at day 0 (D0) and day 28 (D28) and pictures were taken at the two time points. Also, skin scrapings were performed from 3 body sites (head, trunk and legs). The highlight of the study was the treatment protocol which consisted in the use of the commercial product NexGard® (afoxolaner), administrated orally taking into account the weight of the animal, resulting in a dose between 2.7 and 6.9 mg/kg at D0 and D28. All other dogs which were in contact were treated. For some of the dogs, we have also collected blood samples through venipuncture sampling, in order to correlate the results with some serum biochemical parameters.

The results of the study consisted in the inclusion of 16 dogs, for which the evolution of the treatment at D28 was performed by comparing the clinical score at D0 and at D28. Overall, we observed a significant resolution in clinical signs, the clinical score diminishing at D28, to 36.6% of its initial status at D0. No clinical signs of drug intolerance were observed.

The clinical efficacy of the treatment was evaluated based on the resolution of the clinical score from baseline, calculated as [(clinical score at D0 –clinical score at D28)/clinical score at D0] * 100 (Beugnet *et al*., 2016).Efficacy varied from 47% to 90%. Even though our study did not include a mite count, we did however resample skin scrapings from the 3 body sites at D28 from all dogs and did not find any mites present. The results of the efficacy of afoxolaner are in accordance with  and only two studies

carried on dogs infected with sarcoptic mange from Beugnet *et al*. (2016) and Hampel *et al* (2018), proving its high efficacy as early as D28. It is important to mention that at the time the study has been performed, afoxolaner was not an authorized molecule for the treatment of sarcoptic mange in dogs in the European Union, but has become one by the end of the year 2018.

For 10 of the dogs studied we have also collected blood samples and performed biochemical analyses (creatinine and urea nitrogen in 10 dogs; alkaline phosphatase, ALT, AST in 5 dogs) which showed an increase in the urea nitrogen in half of the dogs. However, this value was not correlated with a rise in the creatinine level, meaning that there were no important renal dysfunctions. We also observed an increase in the aspartate aminotransferase (AST).

**Chapter 8**, entitled "Modification of the skin microbiome in dogs with sarcoptic mange", is the most detailed and original part of the thesis. It begins typically, with the presentation of the materials and methods used, and it mentions: the inclusion criteria for dogs (as in the previous chapters), the specific dermatologic questionnaire adapted from human medicine which included a special part concerning the sampling sites for the microbiome study, the sampling method for the study which included 2 skin sites (leg and ear) with two samples per each site (healthy skin and mange affected skin), the samples being collected by scraping and by swabing of the skin, and the action being repeated at D0 and D28. The targeted metagenomics analyzes were performed at the NGS platform of the Institut Mondor de Recherche Biomédicale (IMRB), Créteil, France following their protocol for determining bacterial and fungal skin microbiome.

The results showed that there were 5 dogs included in the present study, all of them from the 48 described in the epidemiology study. From these 5, 3 were among the 16 dogs treated with afoxolaner .They were 2 females and one male, common breed, aged between 3 and 5 years old, from the same household. These dogs complied with the study and repeated the sampling 4 weeks after the first treatment, at D28. The other 2 dogs included were a 6 year old Boxer mother and its 5 weeks old male puppy. These dogs did not return for the resampling at D28, which means their results only reflect the situation of the skin microbiome before treatment.

In total, 32 samples were collected at D0 and D28 for the first 3 dogs and only at D0 for the Boxers. For all of the samples, we studied the sequencing of the bacterial 16S rRNA gene and the sequencing of the ITS gene. For better presentation of the results, we divided them into two main subchapters: the first one takes into consideration all 5 dogs before any treatment and compares the effect of the breed on the skin microbiome and the second one takes into account only the 3 dogs that came for the second treatment, comparing the skin microbiome before and after the first treatment with afoxolaner.

The first subchapter concerning the influence of the breed on the skin microbiome revealed for the bacterial microbiome, that the main force driving the skin microbiome is the breed, rather than the individual or the skin site. The PCoA plot, the β diversity weighted UniFrac and the β diversity unweighted UniFrac all illustrated two clusters of samples, representing the two breeds- Boxer and Common. The Shannon index which

shows species abundance and evenness of OTUs, showed a marked difference between the Common dogs that had a more diverse bacterial microbiome than that of the Boxers. However, the main genus that inhabits the skin of the Boxers infected with mange, is *Staphylococcus*, which is present in a similar abundance in the Common dogs. Studies of the skin microbiome in healthy dogs, showed that the main force driving the skin microbiome is the individual, followed by the skin site sampled and the breed and the environment in lesser measure (Cusco *et al*., 2017a, 2017b). This aspect did not apply for our study, but we must consider the fact that the number of the dogs was rather small, the number of breeds represented was only 2 and that the Boxers were closely related and probably shared the same microbiome because they were a mother and a recently weaned puppy. In addition, we compared our results with the results from healthy dogs, which can also be a problem to be interpreted. However, the differences in the skin microbiome in the two groups can easily be observed, even from the general barplot.

For the fungal microbiome, the results are the same as in the case of the bacterial microbiome, with the clustering of the samples in the two breeds, for the PCoA, the β diversity weighted UniFrac and the β diversity unweighted UniFrac. Also, the Shannon index showed an important difference between the Common dogs that had a more diverse fungal microbiome than the Boxers.

In the second subchapter, we analyzed the differences in the skin microbiome of the 3 cohabiting, Common dogs, before any treatment at D0 and after the first treatment with afoxolaner at D28.

An important aspect for the bacterial microbiome, is that we noticed that the *Staphylococcus (*mainly *S. pseudintermedius)*, which represented the most abundant genus at D0, decreased its level significantly after the treatment, which can mean that *S. scabiei* promotes the growth of opportunistic bacteria that are present normally on the skin surface. Similar results were noticed in the study of the skin microbiome of pigs with sarcoptic mange (Swe *et al*., 2014). The Shannon index shows an important change in the diversity of skin bacteria, with an increase after the first treatment with afoxolaner, which can be interpreted as a process of the healing of the skin. The PCoA plot presented two well defined groups, one before treatment and one after treatment.

For the fungal microbiome, we observed the particular case of the genus *Malassezia,* which may be associated with skin disease in dogs. We noticed that its level significantly decreases after the treatment with afoxolaner, this representing a sign of the healing and recovery of the skin barrier. The Shannon index shows a decrease after the first treatment with afoxolaner, meaning that the treated animals presented a less diverse fungal microbiome, which is rather intriguing and needs to be further investigated. In the PCoA plot and in the β diversity unweighted UniFrac we can observe the clustering of individuals in two groups, one before treatment and one after first treatment.

The last chapter, **Chapter 9**, summarizes all the conclusions of our research and creates a general view of the thesis. Summing up the most relevant conclusions of the studies, we can note that:

- The epidemiological aspects considered (age, sex) confirmed the little data which exists regarding the epidemiology of sarcoptic mange in dog
- Testing of the efficacy of afoxolaner in the treatment of sarcoptic mange in dogs, using an original clinical score, marked positive and encouraging results, even after 28 days of treatment
- The idea of observing the modifications of the skin microbiome (both bacterial and fungal) of dogs with sarcoptic mange before and after treatment with afoxolaner has been developed. This offered new and interesting results in a new, unexplored field, despite the low number of dogs included, limitation due to the financial factor.

# REZUMAT

Râia sarcoptică este o boală parazitară a pielii, cauzată de acarianul *Sarcoptes scabiei.* Această boală afectează multe specii de animale, inclusiv omul. Deși este cunoscută încă din Antichitate, râia sarcoptică ridică încă o serie de semne de întrebare legate de unele aspecte precum fiziopatologia, diagnosticul, tratamentul bolii. În plus, apariția cazurilor rezistente la unele molecule utilizate în mod tradițional pentru tratarea acestei parazitoze, a dus la necesitatea găsirii unor alte substanțe pentru tratament. Un alt aspect care necesită studierea mai detaliată, este modul în care acarienii *S. scabiei* afectează și modifică rolul de barieră al pielii.

Scopul tezei de doctorat intitulată "**Caracterizarea microbiomului cutanat și evaluarea unor noi opțiuni terapeutice în râia sarcoptică la câine**" este de a aduce noi informații privind evaluare semnelor clinice in râia sarcoptică la câine, precum și de a cerceta un tratament mai nou pentru tratarea acestei boli, dar și de a prezenta unele modificări ce apar la nivelul microbiomului cutanat la câinii infectați cu *S. scabiei.*

Obiectivele tezei sunt :

- de a descrie semnele clinice, precum și unele noțiuni de epidemiologie pentru câinii infectați cu *S. scabiei*
- de a evalua eficacitatea unei noi molecule din clasa isoxazolinelor, afoxolaner, în tratamentul râiei sarcoptice la câine
- de a descrie modificările apărute la nivelul microbiomului cutanat bacterian și fungic, la câinii afectați de această boală.

Originalitatea acestei teze de doctorat constă în:

- adaptarea unui nou scor clinic (inspirat din evaluarea porcilor infectați cu *S. scabiei*, dar modificat conform semnelor clinice care apar la câine) pentru a evalua gradul de afectare al animalului
- utilizarea unei molecule noi (afoxolaner), puțin studiate pentru tratamentul râiei sarcoptice la câine.
- studierea atât a microbiomului bacterian, cât și a celui fungic la câinii afectați de râie sarcoptică. În plus, am realizat și o comparație a microbiomului cutanat înainte de tratament și după primul tratament cu afoxolaner,fapt ce reprezintă, după cunoștințele noastre actuale, o premieră în acest domeniu.

Teza de doctorat este structurată în conformitate cu normele în vigoare, clasic, în două părți- prima parte descrie stadiul actual al cunoașterii asupra râiei sarcoptice și este structurată în 4 capitole; cea de-a doua parte prezintă contribuțiile proprii în acest domeniu și conține 5 capitole. În afară de aceste două părti, teza mai cuprinde și introducere,

mulțumiri, rezumat, listă de abrevieri, listă de figuri, cuprins, bibliografie și anexe. Lucrarea prezentă conține 52 de figuri și 6 tabele, fiind consultate si 182 de surse bibliografice

Prima parte, care descrie stadiul actual al cunoașterii in literatura de specialitate, conține 4 capitole cu subcapitolele corespondente. Primul capitol prezintă cunoștințe generale asupra acarianului *S. scabiei,* inclusiv prima menționare istorică, clasificarea sa in regnul animal, morfologia stadiilor prin care trece, ciclul de viață de la ou la adult, capacitatea de supraviețuire și modul în care este transmis. În continuare, sunt tratate subiecte care stârnesc unele controverse, precum variabilitatea morfologică, specificitatea de specie, dar și variabilitatea genetică a acestor acarieni.

Cel de-al doilea capitol se concentrează pe descrierea râiei sarcoptice la câini și menționează: semnele clinice și leziunile care apar, fiziopatologia acestei boli de piele, care încă ridică semne de întrebare, precum și transmiterea la alte animale și la om.

Cel de-al treilea capitol este specific controlului infecțiilor cu *S. scabiei* incluzând o descriere generală a acaricidelor utilizate în medicina umană și veterinară, precum și caracteristicile lor principale, urmată de subcapitole mai specifice, care prezintă moleculele utilizate pentru tratamentul râiei la câini, alte animale și la oameni. Ultima parte a capitolului,dezvoltă problema rezistenței la unele molecule.

Cel de-al patrulea capitol prezintă o nouă direcție în studiul râiei sarcoptice la câine, prin introducerea noțiunii de microbiom al pielii. Subcapitolele aferente includ o prezentare generală a microbiomului cutanat, urmate de o descriere a microbiomului cutanat canin, un domeniu în pionierat.

Cea de-a doua parte a tezei de doctorat, partea de contribuții proprii, este structurată în 5 capitole (Capitolele 5-9): **Capitolul 5** prezintă descrierea scopului și a obiectivelor lucrării, următoarele 3 capitole (Capitolele 6-8) descriu studiile realizate, incluzând descrierea materialelor și a metodelor utilizate, rezultatele obținute și discuțiile, iar ultimul capitol reprezintă concluziile generale ale tezei, unde toate remarcile și observațiile din capitolele anterioare sunt sintetizate și analizate într-o formă finală.

**Capitolul 6**, intitulat "Semne clinice și epidemiologia râiei sarcoptice la câine în regiunea Moldovei" debutează cu o descrierea a materialelor și metodelor utilizate pentru realizarea studiului și menționează: criteriile de incluziune ale câinilor (semne clinice evocative pentru râie și raclate dermice pozitive), chestionarul adresat proprietarului, metoda de diagnostic prin raclat dermic profund, urmată de identificarea microscopică a formelor de evoluție a *S. scabiei.* O metodă interesantă a fost utilizarea unui sistem de scor clinic original, adaptat pentru câini, dar care a fost inițial gândit pentru porcii cu râie sarcoptică (Bernigaud *et al*., 2016). Scorul este bazat pe evaluarea zonelor de piele afectate de leziuni, gradul de alopecie, intensitatea eritemului cutanat, precum și intensitatea crustelor și a scuamelor, rapoartate la diverse părți ale corpului (cap, trunchi, membre, coadă). Notele alocate fiecărui semn clinic au fost între 0 și 4, 4 fiind cea mai gravă, ceea ce a însumat un scor clinic total între 0 și 60, scor alocat la prima vizită. În ceea ce privește tratamentul, fiecare câine a fost tratat cu un produs acaricid specific împotriva infecției cu *S. scabiei* (doramectină, sarolaner, afoxolaner).

Rezultatele acestui studiu au arătat că au fost incluși 48 de câini, examinați în perioada 2015-2018. Dintre aceștia, 27 au fost examinați in cadrul Clinicii de Parazitologie și Boli Parazitare de la Facultatea de Medicină Veterinară Iași, iar 21 au fost examinați fie în gospodării private (14), fie în adăposturi (7). Am inclus câini din județele Iași, Vaslui și Vrancea. Majoritatea erau rasă comună sau metiși (n=39,81.25%), dar au fost și unii indivizi aparținând următoarelor rase: Boxer (o mama cu 7 pui), Bull Terrier (1), Caniche (1). Vârsta lor a variat între 1 lună și 12 ani, dar mai mult de jumătate dintre ei (n=28, 58.33%) erau câini tineri, sub un an. Indivizii incluși au fost reprezentați în proporții aproximativ egale ai ambelor sexe, cu 23 de masculi (48% din total) și 25 de femele (52% din total). În ceea ce privește istoricul acestor câini, informațiile au fost limitate în majoritatea cazurilor, deoarce un număr mare dintre acești câini erau câini comunitari (n=21, 43.7%). Transmiterea zoonotică a bolii a fost raportată de la un singur câine, cel care avea cele mai severe semne de râie sarcoptică din studiu și care a fost internat în clinica noastră. Probele de raclat dermic examinate prin examen direct la microscop, au fost pozitive pentru ouă, larve sau adulți de *S. scabiei.* Totuși am observat un număr foarte mic de elemente parazitare în probele colectate.

Scorul clinic al câinilor a variat între un minim de 4 și a ajuns până la un maxim de 58, având în vedere că scorul maxim care se putea obține era de 60. Acest lucru reprezintă o ilustrare clară a faptului că animalele incluse în studiu aveau de la leziuni foarte discrete la leziuni grave, generalizate pe tot corpul, lucru ilustrat in figurile si graficele din text.

**Capitolul 7**, numit "Evaluarea eficacității afoxolanerului în tratarea câinilor cu râie sarcoptică", începe cu prezentarea materialelor și a metodelor utilizate, acestea fiind similare celor descrise în capitolul anterior, cu modificarea că recoltarea raclatelor dermice și atribuirea scorului clinic s-a realizat la ziua 0 (Z0) și la ziua 28 (Z28), când s-au realizat și fotografii ale câinilor. De asemenea, raclatele dermice s-au realizat din trei zone ale corpului (cap, trunchi și membre). Partea inovativă a acestui studiu este protocolul de tratament care a constat în utilizarea produsului comercial NexGard® (afoxolaner), administrat oral, ținând cont de greutatea animalului, rezultând o doză cuprinsă între 2.7 și 6.9 mg/kg la Z0 cu repetare la Z28. Toți câinii aflați în contact au fost tratați. La unii câini, am colectat și probe de sânge prin puncție venoasă, în vederea corelării rezultatelor cu unii parametri biochimici din ser.

Rezultatele acestui studiu au constat în includerea a 16 câini, pentru care evoluția tratamentului la Z28 s-a evaluat prin compararea scorului clinic din Z0 cu cel din Z28.

În ansamblu, am observat o rezolvare semnificativă a semnelor clinice, scorul clinic la Z28 ajungând la 36.6% din valoare sa inițiala, la Z0. Nu au fost observate reacții de intoleranță la acest medicament.

Evaluarea eficacității clinice a acestui tratament a fost calculată și prin următoarea formulă: [(Scor clinic la Z0 –scor clinic la Z28)/scor clinic la Z0] * 100, după Beugnet *et al*.(2016). Eficacitatea a variat între 47% și 90%. Deși studiul nostru nu a inclus o numărare a acarienilor de *S. scabiei* prezenți în proba examinată (datorită numărului foarte mic de indivizi prezenți în probă), am refăcut raclatul la Z28 din cele 3 regiuni anatomice, și nu am mai observat niciun acarian. Aceste rezultate ale eficacității afoxolanerului, sunt

in concordanță cu singurele și primele studii din literatură, realizate pe câini cu râie sarcoptică de către Beugnet *et al*. (2016) și Hampel *et al*. (2018), demonstrând o foarte bună eficacitate încă din Z28. De precizat este că, în momentul realizării acestui studiu, afoxolanerul nu era moleculă autorizată în Uniunea Europeană pentru tratamentul râiei sarcoptice, dar la sfârșitul anului 2018 acestă moleculă a devenit oficial autorizată.

Pentru 10 dintre câinii incluși în studiu am colectat probe de sânge și am dozat o serie de parametri biochimici (creatinina și ureea la 10 câini; fosfataza alcalină. ALT și AST pentru 5 câini), care au arătat o creștere a ureei la jumătate dintre câinii analizați. Totuși, această valoare nu a fost corelată cu o creștere a valorii creatininei, ceea ce semnifică absența unor disfuncții renale marcante. S-a mai observat și o creștere a valorii AST.

**Capitolul 8**, intitulat "Modificările microbiomului cutanat la câinii cu râie sarcoptică" este partea cea mai detaliată și mai originală din prezenta lucrare. Acesta debuteaza tipic, cu prezentarea materialelor și metodelor utilizate, dintre care amintim: criteriile de incluziune ale câinilor (aceleași care au fost prezentate în capitolele anterioare), chestionarul dermatologic specific adaptat din medicina umană, care include o parte specială privind locurile de recoltare pentru studiul microbiomului cutanat, metoda de recoltare, care include recoltarea din 2 locuri anatomice (ureche și membru), cu două probe recoltate pentru fiecare loc anatomic (o proba de piele sănătoasă și o probă de piele afectată de râie), probele fiind recoltate prin raclat și prin tamponare, aceste acțiuni fiind repetate la Z0 și la Z28. Analizele de metagenomică au fost realizate la platforma de NGS a Institut Mondor de Recherche Biomédicale (IMRB), Créteil, Franța, urmând protocolul laboratorului pentru determinarea microbiomului cutanat bacterian și fungic.

Rezultatele au inclus prezentarea celor 5 câini incluși în studiu, dintre cei 48 descriși în studiul epidemiologic. Dintre aceștia, 3 erau dintre cei 16 câini tratați cu afoxolaner. Este vorba despre 2 femele și un mascul, de rasă comună, cu vârsta cuprinsă între 3 și 5 ani, aparținând aceluiași proprietar. Acești câini au respectat regula studiului și au venit din nou pentru recoltarea de probe în Z28. Ceilalți doi câini incluși, au fost o cățea Boxer de 6 ani împreună cu puiul ei în vârstă de 5 săptămâni. Acești câini nu au mai revenit la recoltarea din Z28, ceea ce semnifică faptul că rezultatele lor reflectă doar situația microbiomului cutanat înainte de tratament.

În total au fost colectate 32 de probe, de la Z0 și Z28 pentru primii 3 câini și de la Z0 pentru cei 2 Boxeri. Pentru toate probele, am studiat secvențierea genei bacteriene 16S și secvențierea genei ITS. Pentru o mai bună prezentare a rezultatelor, am împărțit prezentarea în două mari subcapitole: primul ia în considerare toți cei 5 câini înaintea oricărui tratament și compară efectul rasei asupra microbiomului cutanat, iar cel de-al doilea include doar cei 3 câini cărora li s-au recoltat probe și la Z28 și compară microbiomului cutanat înainte și după primul tratament cu afoxolaner.

Primul subcapitol, care are în vedere influența rasei asupra microbiomului cutanat, a relevat, pentru microbiomul bacterian, faptul că principala forță care influențează microbiomul în studiul nostru este rasa, mai degrabă decât individul sau locul de recoltare. Graficul PcoA, cel al „β diversity weighted UniFrac", precum și cel al „β diversity

unweighted UniFrac" au ilustrat două grupuri bine definite de probe, reprezentând cele două rase- Boxer și comun. Indicele Shannon, care indică abundența speciilor și uniformitatea OTUs, a arătat o diferență semnificativă între cele două grupuri de rase, cei de rasă comună având un microbiom care curpinde o diversitate mai mare de specii bacteriene,comparativ cu cei din rasa Boxer. Totuși, principalul gen prezent în proporție majoritară la ambele rase râmâne *Staphylococcus.*

Din studiile microbiomului cutanat la câinii sănătoși, s-a observat că principala forță care influențează compoziția microbiomului cutanat este individul, urmat de locul de recoltare al probei și într-o mai mică măsură rasa și mediul de viață (Cusco *et al*., 2017a, 2017b). Acest aspect nu a fost observat și în studiul nostru, totuși trebuie să luăm în cosiderare faptul că numărul de câini incluși a fost destul de redus, numărul de rase a fost doar de două, iar Boxerii erau foarte apropiați și probabil aveau un microbiom cutanat asemănător din cauza faptului că era vorba despre o mamă și un pui recent înțărcat. În plus, am comparat rezultatele noastre cu cele ale unor câini sănătoși, ceea ce ar putea constitui un dezavantaj. Totuși, rezultatele obținute de noi arată clar o serie de diferențe nete între cele două grupuri, aspecte observate din graficele și figurile aferente.

În ceea ce privește microbiomul cutanat fungic, rezultatele sunt asemănătoare cu cele obținute la cel bacterian, în sensul că probele se grupează în funcție de cele două rase pentru graficul PcoA, cel al „β diversity weighted UniFrac", precum și cel al „β diversity unweighted UniFrac" La fel, indicele Shannon a arătat o diferență importantă între câinii de rasă comună, care aveau un microbiom fungic mult mai bogat decât cei din rasa Boxer.

În cel de-al doilea subcapitol, am analizat diferențele produse în microbiomul cutanat al celor 3 câini din rasa comună, de primul tratament cu afoxolaner.

Un aspect important observat pentru microbiomul cutanat bacterian, este cum *Staphylococcus (*în principal *S. pseudintermedius)* care reprezenta genul cel mai abundent în Z0, si-a scăzut semnificativ nivelul după tratament în Z28, ceea ce poate însemna că *S. scabiei* promovează creșterea bacteriilor oportuniste prezente în mod normal la nivelul pielii. Rezultate similare au fost obținute și în studiul microbiomului cutanat la porcii cu râie sarcoptică (Swe *et al*., 2014). Indicele Shannon a arătat o schimbare importantă la nivelul diversității bacteriene, cu o creștere semnificativă după administrarea tratamentului, aspect care poate fi interpretat ca un semn de vindecare al pielii. Graficul PCoA a prezentat două grupuri separate de probe, cele înainte de tratament la Z0 și cele după tratament la Z28.

În ceea ce privește microbiomul cutanat fungic, ne-a atras atenția cazul particular al genului *Malassezia,* care este deobicei asociat cu boli ale pielii la câine. Am observat că nivelul acestuia a scăzut după tratamentul cu afoxolaner, acesta reprezentând un semn de vindecare al barierei cutanate. Indicele Shannon a prezentat o scădere a diversității fungice după tratament la Z28, ceea ce este neobișnuit și rămâne de investigat mai în amănunt. Graficul PcoA precum și cel al „β diversity unweighted UniFrac", arată gruparea probelor în două grupuri separate, înainte de tratament la Z0 și după tratament la Z28.

Ultimul capitol, capitolul 9, însumează toate concluziile cercetării noastre și creează o viziune de ansamblu asupra rezultatelor tezei de doctorat. Sintetizând cele mai relevante concluzii ale studiului, putem nota că:

- aspectele epidemiologice luate in studiu (vârsta, sex), au confirmat puținele date existente referitor la epidemiologia râiei sarcoptice la câine
- testarea eficacității afoxolanerului în tratarea râiei sarcoptice la câine, cu metode de alocare a scorului clinic mai orginale, a marcat rezultate pozitive și încurajatoare, chiar după 28 zile de tratament
- a fost dezvoltată ideea observării și controlului microbiomului cutanat, atât bacterian, cât și fungic la câinii infectați natural cu *S. scabiei*. Acesta a oferit rezultate noi și interesante într-un domeniu puțin sau chiar deloc studiat, fapt constatat în ciuda numărului mic de câini luați în studiu, limitare provocată de factorul financiar.

# INTRODUCTION

Sarcoptic mange represents a common, highly contagious, parasitic dermatitis that affects a great number of mammals, including humans.

In humans, the World Health Organization designated scabies in 2018 as a Neglected Tropical Disease, recognizing the importance of the disease at global level, where approximately 177 to 237 million people are infected at any single time (GBD 2015 Disease and Injury Incidence and Prevalence Collaborators, 2016).

The etiological agent of sarcoptic mange is represented by the *Sarcoptes scabiei* mite, which has different varieties depending on the species of animals on which they reside (*Sarcoptes scabiei* var. *canis*-dog; *Sarcoptes scabiei* var.*hominis*- human). These mites burrow the skin and irritate it severely, leading to a specific clinical aspect in dogs, which includes erythema, yellow thick crusts, and constant pruritus (Arlian *et al*., 1995).

In dogs, it represents a common problem in big communities of animals, such as shelters, where infected animals can be brought in and the disease spread rapidly through direct or indirect contact (through fomites). Nevertheless, sarcoptic mange affects also a large number of dogs from private households, since it spreads very easily not only by direct contact with an infected animal (dog or fox), but also by indirect contamination through infected objects (hair clipping and grooming tools), or through the environment.

The diagnosis of sarcoptic mange is made by correlating the clinical presentation of the dog with positive skin scrapings (showing the presence of adults, larvae, or eggs of *S. scabiei*). Unfortunately, the number of mites that can be found in skin scrapings is usually very low, which means that in general veterinary practice, trial acaricidal therapy can be a solution in case of suspecting the disease.

The treatment for sarcoptic mange in dog is diverse, including both topical (amitraz, permethrine, lyme sulphur, fipronil) and systemic products (ivermectin, doramectin, milbemycin oxime, moxidectine, selamectin; sarolaner, afoxolaner, lotilaner; fluralaner). It is important to treat all in contact dogs and to disinfect the environment, in order to prevent the risk of reinfection. The problem of resistance has been reported, to some of the classical molecules commonly used, such as ivermectin (Terada *et al*., 2010). The authorized treatment in the European Union, in 2019, includes the molecules imidacloprid/moxidectine, selamectin, and the new molecules from the isoxazoline class: sarolaner, afoxolaner and afoxolaner/milbemycin oxime.

An interesting aspect regarding the effect of the burrowing mites on the skin of the sick dog, is the modifications that occur in the resident bacteria and fungi normally present on healthy skin. Recently, researchers started showing increased interest in the impact of the microbes (bacteria, viruses, fungi, archaea, and parasites), their gene products and metabolites in a defined habitat, this field of research being known as the study of the microbiome (Marchesi *et al*., 2015). Understanding the skin microbiome is essential, since it plays an important role in maintaining the health and functions of the skin. The studies

published so far, focus on characterizing the bacterial and fungal communities of the skin, investigated through sequencing analysis of the phylogenetic marker genes, such as the bacterial 16S ribosomal RNA (rRNA) and the fungal 18 S ribosomal RNA or internal transcribed spacer (ITS) (Hamady *et al*., 2009).

In dogs, the skin microbiome studies included both healthy and allergic/atopic dogs, in the attempt to better understand the relationship between the microbes and their contribution to skin imbalances.

The purpose of the thesis was to bring a couple of aspects into the light such as

- a new evaluating clinical score in sarcoptic mange in dogs
- a rather new treatment in the infection with *S. scabiei* in dogs
- the study of the bacterial and fungal microbiome in an old, but often overlooked skin disease, represented by sarcoptic mange in dogs.

The objectives of the thesis were:

- to describe the clinical signs and some epidemiological aspects of dogs infected with *S. scabiei*
- to assess the efficacy of a novel molecule from the isoxazoline class, afoxolaner, in the treatment of sarcoptic mange in dogs
- to study and describe the modifications of the bacterial and fungal skin microbiome in the infection with *S. scabiei* in dogs, in order to verify factors and interactions between host and parasite, because so far, most mechanisms for the host specificity remain largely unknown.

# PART I- CURRENT STATE OF KNOWLEDGE

# 1. SARCOPTES SCABIEI

## 1.1 History

In humans, infection by *Sarcoptes scabiei*, has been known from ancient times the first mention being in the Bible, where the priests were supposed to know to differentiate scabies from leprosy (Roncalli, 1987).The first one that is thought of identifying the etiological agent of scabies is Aristotle (384 BC-322 BC), who used for the first time the term "Akari" (Ramos-e-Silva, 1998). Even though some other scientists through history showed interest in these mites, it was not until 1868 that they officially established the cause of scabies with the publication of the treatise of Hebra (Hebra, 1868).

In animals, the first reports on mange appear to be in sheep, also in the Bible, where in the Leviticus it was written that sheep suffering from mange could not be offered as sacrifice. The interest in mange in sheep continued during the Roman times, with recommendation of treatment written by several authors, Virgil describing in his poetic agricultural manual Georgics, the problems caused by scabies and its treatment by tar, grease and washing in sheep (Roncalli, 1987).

In 1786, a German doctor Johann Ernst Wichmann stated in his monograph that mange in sheep, as in humans is produced by a mite. In 1812, professors from the Veterinary Faculty from Lyon collected mites from lesions of horses and cattle with mange, to observe them and to eventually describe and illustrate them (Roncalli, 1987).

## 1.2 Classification

*Sarcoptes scabiei* is an arthropod, Subphylum Chelicerata, Class Arachnida, Superorder Acariformes, Order Sarcoptiformes, Suborder Oribatida, Infraorder Desmonomata and the group (hypoorder) Astigmata (along with house dust mites), Superfamily Sarcoptoidea and Family Sarcoptidae(Zhang, 2011).

Arthropods are characterized by their jointed limbs and cuticle made of chitin, sometimes mineralized. The Phylum Arthropoda is divided infive subphyla that include insects, myriapods, crustaceans, chelicerates and trilobites. There are around 1.3 million different species of arthropods that have been described, by this making it the most numerous phylum of all living organisms (Averof and Akam, 1995; Mangowi *et al.*, 2014). The Superorder Acariformes includes mites and ticks and most of them are of medical importance, since they are parasites for humans, animals or plants. The group Astigmata is a relatively large group of slow moving, similar mites that have no tracheal system or detectable spiracles.

The Family Sarcoptidae is made up of three Subfamilies (Sarcoptinae, Teinocoptinae, and Diabolicoptinae) including 16 genera and 118 species, all of them obligate parasites of the skin of mammals (Bochkov, 2010). Their members are characterized by short legs and short capitulum.The Subfamily Sarcoptinae includes four genera: *Sarcoptes* (1 species *Sarcoptes scabiei*), *Prosarcoptes* (3 species), *Trixacarus* (3 species) and *Kutzerocoptes* (1 species).

*S.scabiei* and *Trixacarus* spp.look similar, but *Trixacarus* spp. are much smaller than *S. scabiei*, and they differ in other morphological aspects.

### 1.3 Morphology

Adults of *S. scabiei* have a characteristic oval, ventrally flattened and dorsally convex body (Fig 1.1). The mites are creamy white colour with brown legs and mouthparts. On the ventral surface of the mite's body, chitinous bars (called epimeres) can be observed, which strengthen the places where legs are inserted in the body. On the dorsal surface of the mite, there are transversely arranged thorns and 10 pairs of spines arranged on two sides, three pairs on the anterior part and seven pairs on the posterior part of the dorsal surface (Fig 1.1-1.2).The gnathosoma (capitulum) is formed of short, firm chelicerae and pedipalps (Fig1.2), that together have a rounded appearance. The anal opening is terminal in both sexes. Both females and males have short legs, with the last 2 pairs not extending beyond the body margin. Larvae have 6 legs, nymphs and adults have 8 legs, with suckers present on legs I and II in both sexes and on leg IV only in males (Fig 1.2). The suckers help the mite grip the substrate as it moves. All other legs (III and IV of females and III of males) end in long setae. The female size is 315 to 470 µm long by 225–345 µm wide, and the male size is 205 to 245 µm long by 145–170 µm wide, around two-thirds the size of the female (Cosoroabă, 2014).The adult male is distinguishable from the female by its smaller size and darker colour (Fig. 1.1).

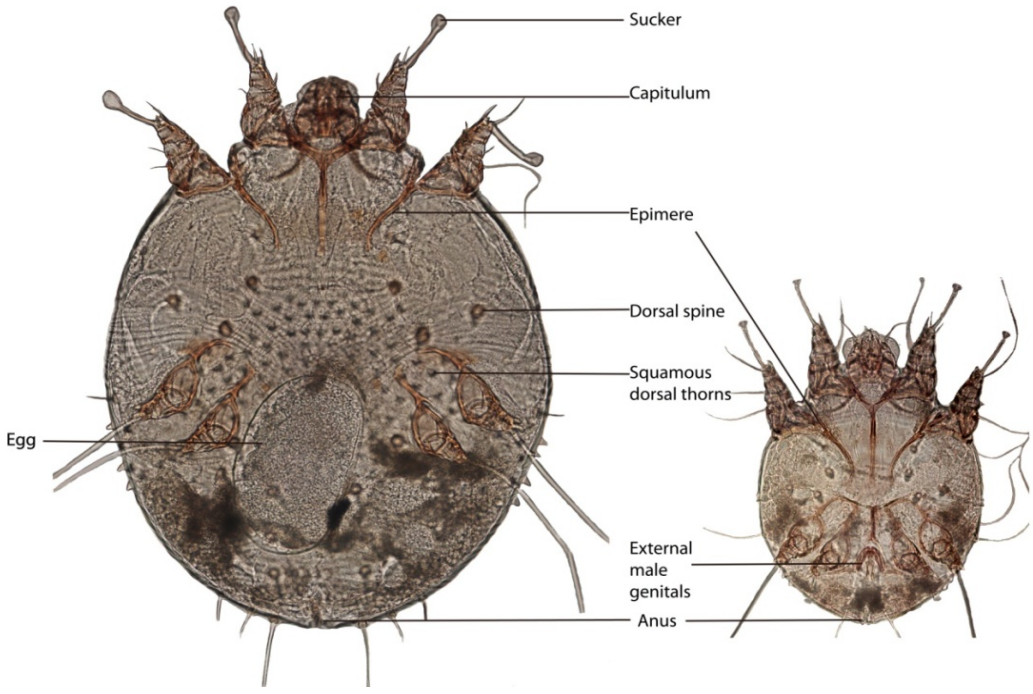



Figure 1.1- Male and female of *Sarcoptes scabiei* var. *suis* (Courtesy of Fang Fang, Parasitology, EnvA)

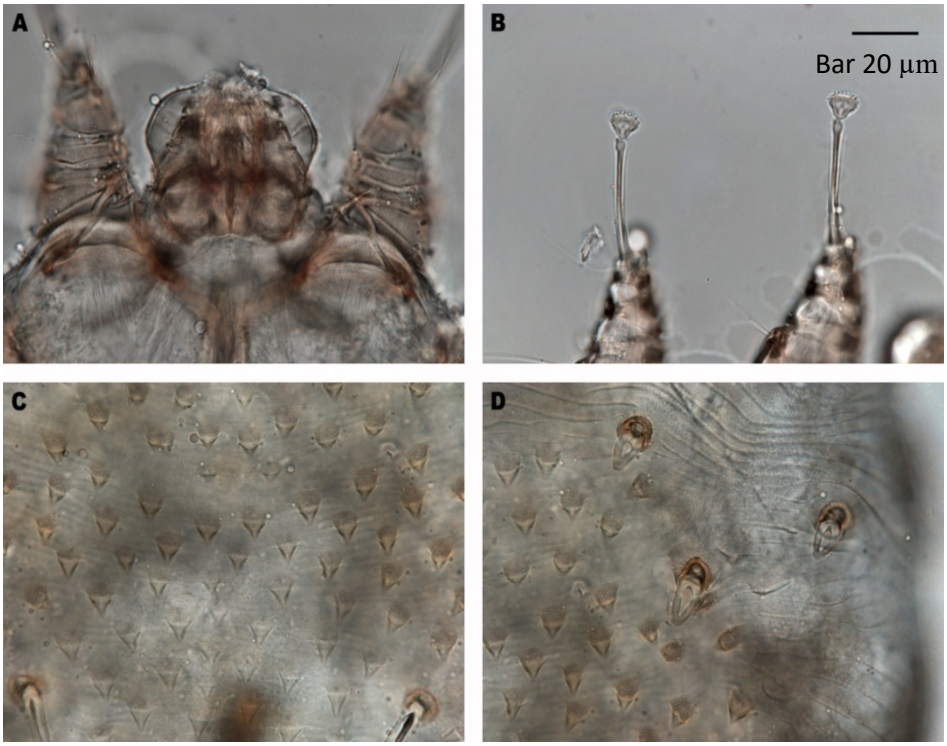



Figure 1.2- Microscopic pictures of *Sarcoptes scabiei* var. *suis* (A: capitulum. B: suckers. C: dorsal thorns. D: dosal spines) (Courtesy of Fang Fang, Parasitology EnvA, bar=20 µm)

The eggs are oval, smooth, white and glossy, with slightly tapering at the pole lying anteriorly in the female mite, and this pole is attached to the floor of the burrow by means of sticky substance. The dimensions of the eggs are 164–195 µm by 84–103 µm, and slightly increase during development (Fig. 1.3) (Heilesen, 1946).

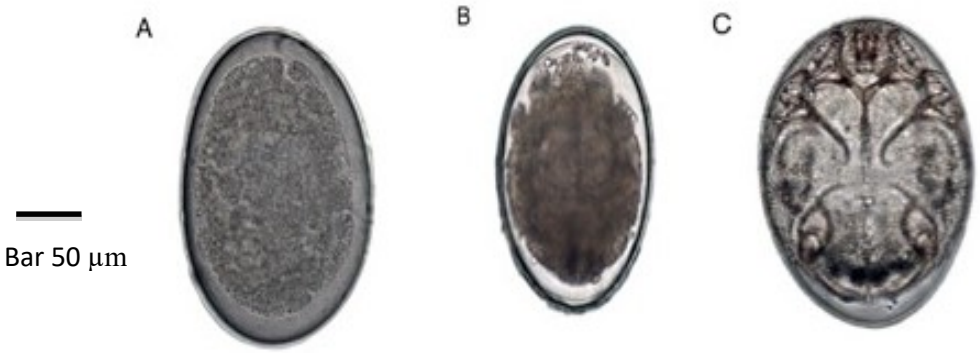



Figure 1.3- Different stages of *Sarcoptes scabiei* var. *suis* eggs

(A: newly laid egg. B: medium developed egg. C: egg about to hatch) (Courtesy of Fang Fang, Parasitology EnvA, bar=50µm)

### 1.4Life cycle

An important aspect of the life cycle of *S. scabiei* is that it takes place entirely on the skin of mammals. It begins with the coupling of the adult male and female on the surface of the skin. After mating, the male will eventually die, and the female will begin to burrow into the stratum corneum of the skin, digging with a rate of 2-3 mm/day and laying 1-3 eggs per day in the burrows for about 2 months. There is only one female, her eggs and faeces in each tunnel (Taylor *et al.*, 2016). The eggs hatch into six- legged larvae within 2 to 3 days under the condition of 35°C and 100% relative humidity (Arlian *et al.*, 2017). The larvae reach the skin surface and develop into octopod protonymphs in 3-4 days and into tritonymphs in 2-3 days and then moult into either males or females (Fig 1.4). The entire development from egg to adult takes about 14 days, more specifically, 10.1−13.2 days for the male and 9.9−13.0 days for the female of *S. scabiei* var. *canis* (Arlian and Vyszenski-Moher, 1988). All the stages of *S. scabiei* can penetrate the skin surface, consuming lysed cells and tissue fluid that oozes when digging the burrows (Hengge *et al.*, 2006). The presence of the host Ig G antibodies in the midgut of these mites suggest that they ingest host serum (Rapp*et al.*, 2006).

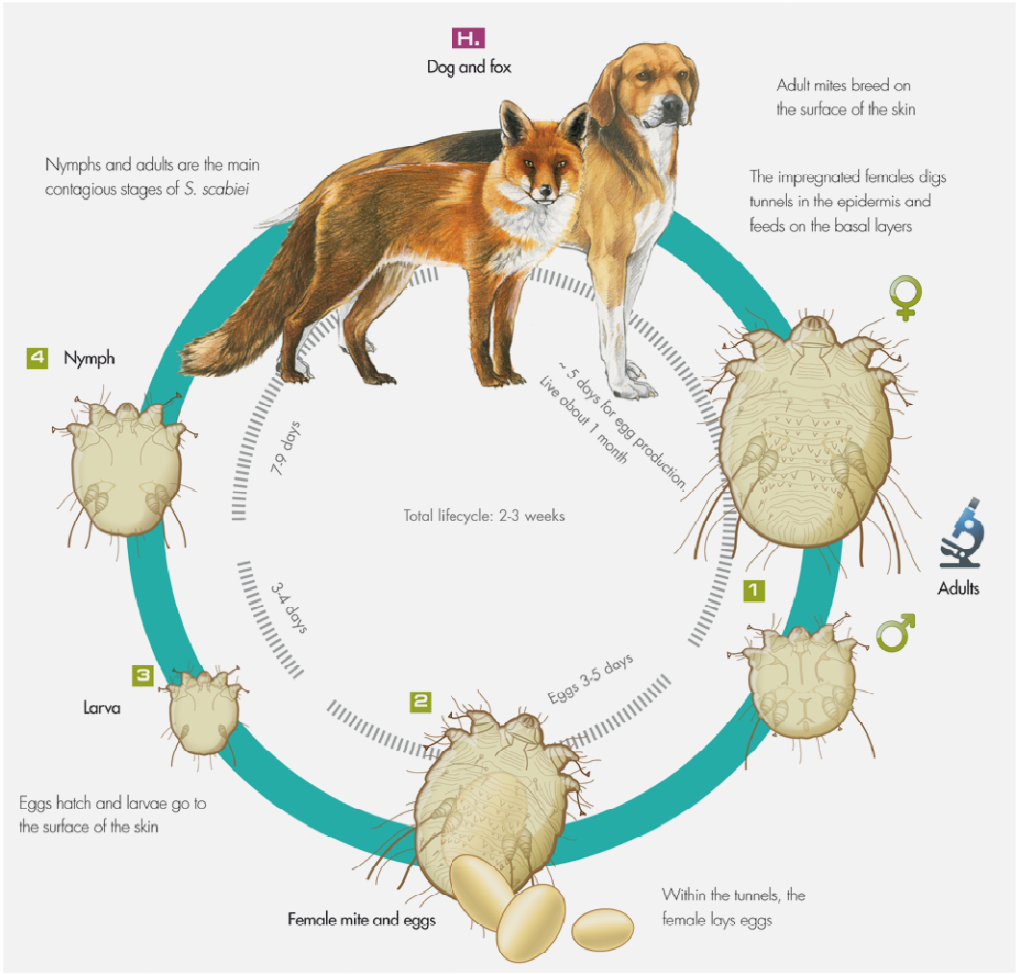



Figure 1.4- Life cycle of *Sarcoptes scabiei* (Beugnet el al., 2018)

## 1.5 Survival capacity and modes of transmission

Arlian *et al.* (1984) showed that *S. scabiei* could survive for 24 to 36 h at room conditions (21°C and 40−80% relative humidity), being capable of penetrating the skin and remaining infective. The most resistant stages are the females and the nymphs. Low temperatures (10−15°C) and high relative humidity are favourable to survival, with nymphs surviving up to 21 days at 10°C and 97% relative humidity (Arlian *et al.*, 1989a).

Transmission of *S.scabiei* can occur through direct contact between individuals, or indirectly, through fomites (Burkhart *et al.*, 2000). Observations about life cycle demonstrated that all life stages of *S.scabiei* leave the burrow frequently, reach the surface of the skin and may fall from the host (Arlian *et al.*, 1984). A survey conducted by Arlian *et al.* (1988a), in the homes and nursing homes of *S. scabiei* infected patients confirmed the presence of mites in fomites. A special case is the one of patients or animals with crusted lesions, infected by thousands of mites (CDC, 2011; Chosidow *et al.*, 2000; Walton *et al.*, 1999b). In 1990, Kim *et al.*, reported a case of medical staff infected from the contamined medical instruments used for a scabies patient with crusted lesions.

## 1.6 Morphological variability and host specificity

### 1.6.1 Morphological variability

There is a constant question around the idea that the genus *Sarcoptes scabiei* contains one species with different varieties or several species, because strains from different hosts are morphologically similar. The majority of scientists agree with Fain (1968,1978) that the genus *Sarcoptes* contains only one valid, but variable species with numerous varieties regardless of variations in size, host specificity and anatomical characteristics (Fain, 1968; Fain, 1978; Walton*et al.*, 1997). Fain divided *S. scabiei* varieties into three main groups of strains, depending on variation in dorsal field spines, ventrolateral spines and the bare area in the dorsal field of scales: **1)**Strains with a bare area in most or in all the specimens. This group contains strains completely devoid of ventrolateral scales (strains from humans, dromedaries, camels, cabiais, peccaries, wild sheep, gibbons) and strains having ventral scales in all specimens (strains from domestic and wild pigs) or in some specimens (strains from a chimpanzee, a goat from South Africa, some African antelopes, a tapir from the Vienna zoo, horses from USA and SouthAfrica) **2)**Strains with most of the specimens devoid of a bare area. This group contains strains completely devoid of ventrolateral scales (strains from cattle in Belgium and Holland) and strains with ventrolateral scales present in all the specimens (strains from dogs, ferrets, polecats, foxes, llamas, sheep and goats from Austria, chamois, red deer, mountain dogs) or in almost all the specimens (wombats, chimpanzees, strains from horses from Mayaguez and from Holland) **3)**Intermediate forms. This group contains strains with intermediate characteristics, for both the bare area and the ventrolateral scales, which prevents Fain from putting them in either of the 2 preceding groups. (Fain, 1978).

### 1.6.2 Host specificity

*Sarcoptes scabiei* infects humans and mammals and has the largest variety of hosts among all the permanent parasitic mites (Currier *et al.*, 2011). It is commonly accepted that each variety is adapted to a specific host (or a small number of closely related hosts) and there is little evidence of interbreeding between varieties or strains (Neveu-Lemaire, 1938). Cases of human contamination following contact with an infected animal, have been reported occasionally, but the disease seemsgenerally self-limiting, with no evidence of long-term reproduction on the accidental host (Delafond, 1862; Morsy *et al.*, 1994).

However, there is a description of a case of a 14−year−old girl with crusted scabies due to *S. scabiei* var. *canis* and living with three severely infected dogs. Furthermore, several members of her family developed self−limiting rashes after sleeping with her. Also, a naive dog was successfully infected with mites from the girl (Ruiz-Maldonado*et al.*, 1977).

Animal-derived strains of *S. scabiei* which infect humans usually come from dogs (Aydıngöz and Mansur, 2011; Beck, 1965; Charlesworth and Johnson, 1974; Emde, 1961; Smith and Claypoole, 1967), but can also come from camels, horses, pigs, goats, sheep, chamois, ferrets, foxes, wombats, lions, buffaloes and llamas.

Genetic and phylogenetic studies demonstrated conflicting results regarding the host specificity of *S. scabiei*. DNA fingerprinting technique, hypervariable satellite markers, and sequencing of mitochondrial 16S DNA (mtDNA 16S) or mitochondrial cytochrome *c* oxidase subunit 1 (mtDNA *cox 1*) were used.

First studies suggested that human and dog mites were genetically distinct in North Australia sympatric populations (or Panama) and that gene flow between the scabies mite population was extremely rare (Walton *et al.*, 1997; Walton *et al.*, 1999a; Zhao *et al.*, 2015).

Other studies found no interspecific differences among *Sarcoptes* mites collected from different host species and that they were genetically similar to certain human mites (Andriantsoanirina *et al.*, 2015a, b).

The choice of the genetic marker may have played a role in the conflicting results. Mofiz *et al.* (2016) analyzed the entire mitochondrial genome of thousands of *Sarcoptes* mites from humans and pigs and observed that they were very similar, stating that only limited genetic differences may separate human and animal varieties. Fraser *et al.* (2019) performed extensive molecular typing of the *S. scabiei* mtDNA *cox 1* from infected wombats, koalas, red foxes and dogs from Australia. The study revealed 16 haplotypes among Australian animals, sharing between 93.3% and 99.7% sequence similarity. They concluded that the origin and emergence of *Sarcoptes* mites into Australian marsupials could be the spillover from canids.

All in all, available data show that a strict ‘host taxon’ law cannot be assumed and that several subpopulations of mites might exist within a single host species.The mechanisms for the host specificity of *S. scabiei* remain largely unknown. They may be attributed to many factors and interactions between hosts and parasite, such as differences in dietary and non−dietary properties of the host skin environment; ability of the host to mount an immune response; antigenicity of the parasite; and resistance of the mites to the host immune response (Arlian, 1989b).

## 1.7 Genetic variability of *S. scabiei* var. *canis*

The research work based on the genetics of *S. scabiei* is quite limited compared to classical epidemiological studies. This limitation comes from the difficulty of having appropriate skin samples, with a sufficiently large number of mites, and managing to extract enough genetic material from each of these mites (Alasaad, Rossi *et al.*, 2009; Alasaad, Soglia. *et al.*, 2009). In order to clarify the taxonomic status, population dynamics and epidemiology of *S. scabiei* var. *canis* infection, several molecular markers have been used (1) microsatellite DNA; (2) *12S rRNA, 16S rRNA* and *COX1* gene of mitochondrial DNA (mtDNA); (3) the second internal transcribed spacer (ITS2) of the *rRNA* gene (Table 1.1).

Table 1.1-Genetic characterization of *S. scabiei* var. *canis* with information of markers, origin of the mites and conclusions

| **Markers** | | **Origin of the mites (host/country)** | **Conclusions** | **Reference** |
|---|---|---|---|---|
| **microsatellite DNA** | Sarms 1,15,20 | 712 scabies mites from humans and dogs/Ohio,Panama and Australia | *S. scabiei* from dogs and humans clustered by host species rather than by geographic location | Walton *et al.*, 1999a |
| **mtDNA** | *16S rRNA* and *Cox1* | Humans, dogs, chimpanzees, wallabies and wombats / Panama, Australia, USA, Sweden | Substantial divergence between human and other animal−associated mite populations; they may not have shared a common mitochondrial ancestor since 2−4 million years ago | Walton *et al.*, 2004 |
| | *16S rRNA* and *Cox1* | Dogs, humans / China | *Cox1* was suitable DNA barcode for phylogenetic study of *Sarcoptes* mites but not 16S rRNA | Zhao *et al.*, 2015 |
| | *Cox1* | Humans, pig, dog/ France | *S. scabiei* mites in humans are distributed into three genetically distinct clades | Andriantsoa nirina *et al.*, 2015a |
| | *12S rRNA* | Wombats, dogs and humans / Australia Humans and dogs/France | *S. scabiei* was introduced to Australia with people and/or their dogs | Skerratt *et al.*, 2002 Andriantsoa nirina *et al.*, 2015b |

| **Markers** | | **Origin of the mites (host/country)** | **Conclusions** | **Reference** |
|---|---|---|---|---|
| **rRNA gene** | ITS2 | Dogs, pigs, cattle, foxes,lynxes, wombats, dromedaries and chamois/ Germany | No association between mite haplotype and host species observed | Zahler *et al.*, 1999 |

# 2. SARCOPTIC MANGE IN DOGS

## 2.1 Clinical signs and lesions

Sarcoptic mange is an intensely pruritic, non-seasonal dermatitis that can affect dogs of all ages, breeds and sexes (Beugnet *et al.*, 2018).

The condition has the following characteristic, almost constant clinical signs: pruritus, presence of crusts, diffuse or localized alopecia. The distribution of lesions involves the head (the muzzle, the ears), the abdomen, the feet, the elbows, the hocks (ESCCAP Guideline, 2018). In the classic evolution of the disease, the dorsal part is usually spared. The lesions are erythematous, with papules and vesicles with a grey-yellowish crust that will form the "mangy buttons" that can be noticed when touching. Because of the intense pruritus, self-trauma lesions can appear, followed by lichenification, excoriation and alopecia. A characteristic area affected by sarcoptic mange in dogs is the pinnal area, which may have a crusted, sandy look. Furthermore, infected dogs usually present a positive pinnal-pedal reflex, meaning that when the margin of the ear is being rubbed, dogs make a scratching movement with their hind leg. This reflex can orientate the diagnostic of sarcoptic mange (Miller *et al.*, 2012).

In case there is no onset of treatment for sarcoptic mange, the disease developsand can extent all over the body. The dog will refuse to eat, it will lose weight, and lymphadenopathy can occur.

In young dogs, the clinical signs are somehow different: there are more scales but the pruritus is mild, the alopecia zones are hard to see and"mangy buttons" are rare (Nuttall *et al.*, 2009; Cosoroabă, 2014).

The primary lesions that appear on the skin where the female mite begins burrowing are erythematous and crusted papules. They appear before or even simultaneously with the increasing of itching. Secondary lesions are represented by crusts, excoriations, hyperpigmentation and lichenification. Besides these lesions, there are also some associated dermatological findings that include: seborrhoeic problems, pyoderma, alopecia (Jasmine, 2011).

Histological examination of skin biopsies reveals acanthosis, parakeratosis, spongiosis, with dense eosinophilic dermal infiltrate in the epidermis.In the dermis, superficial perivascular and sometimes diffuse infiltrate of lymphocytes and histiocytes, sometimes with neutrophilsand less often eosinophils, can be observed. (https://www.pathologyoutlines.com/topic /skinnontumorscabies.html).

## 2.2 Physiopathology

In the initial stages of the infection, *S. scabiei* mites are capable of suppressing the immune response of the host (4-6 weeks after the contact with the mites). It is believed that the capacity of modulating the immune response of the host was developed, due to the long evolution of *S. scabiei* with its hosts (Bhat *et al.*, 2017). This includes anti-inflammatory, anti-

immune and anti-complement activities (Arlian and Morgan, 2017). As the number of parasites increases, the host will start presenting a hypersensitivity reaction (both type 1-immediate and type 4-delayed) resulting in skin inflammation, which will cause a cascade of reactions that can be split into two categories: physiological and behavioural.

Physiological changes include changes in metabolism, thermoregulation, immunology, biochemistry, body and skin condition.

Behavioural shifts are represented by pruritus, home range, dispersal and circadian rhythmicity (Overskaug, 1994; Borchard, 2012; Cross *et al.*, 2016).

Martin *et al.* (2018) investigated the changes related to sarcoptic mange in wombats and concluded that:

1) sick wombats had higher metabolic rates, because of thermal loss through the zones with alopecia, than healthy wombats,
2) sick wombats spent more time inactive (for short periods of time, alternating with short periods of foraging, resulting in the insufficiency and inefficiency of both activities), which means they don't meet their needs for food intake, resulting in the depleting of their fat stores. Furthermore, an imbalance in the fatty acids composition was observed, which may be associated with persistence of chronic inflammation.

From the immunological point of view, after the number of mites has increased, the mast cells, activated by the complement system or immunoglobulin E, and eosinophils will start the immune response. In classic scabies in humans, the T cell-mediated response differs from the one from crusted scabies. The Th1 response is predominant in classic scabies, while in the crusted form, it is a Th2 nonprotective allergy-like response. In crusted scabies, CD81 T cells are major constituents of T cell dermal infiltrates which can lead to keratinocyte apoptosis, followed by epidermal hyperproliferation. There are several hypotheses regarding the way these mechanisms lead to the itch sensation in scabies, although there is almost no specific data that reveals the pathophysiology of the itch (Jannic *et al.*, 2018).

*Sarcoptes scabiei* burrows the skin, gaining access to the intercellular fluid in the epidermis and therefore diffusing soluble substances containing antigenic compounds (enzymes, hormones, and excretory materials) from the parasites into the liquid bathing the epidermis and dermis cells. These substances will trigger a response from the host's tissues cells, such as: fibroblasts, mast cells macrophages, lymphocytes, Langerhans cells and other dendritic cells, endothelial cells of the microvasculature, keratinocytes (Arlian *et al.*, 2003)

The pruritus that usually accompanies *S. scabiei* infection causes severe scratching, resulting in excoriations that will become the entrance gates for bacteria, leading to secondary bacterial infections. The SMIPP and SMS complement inhibitors in mite faeces may play a role in the survival and growth of bacteria in the skin, as mites leave fecal pellets in the burrow behind them as they feed and dig further. These components might inhibit the complement attack of the host's skin/ plasma on bacteria, promoting the multiplication of bacteria and superinfection of the skin (Mika *et al.*, 2012) (Swe *et al.*, 2014a). In humans, this can lead to local or general complications (bacteremia,cellulitis, lymphadenopathy, abscesses) or post-streptococcal complications (rheumatic heart disease, acute glomerulonephritis,

acuterheumatic fever). The most frequent bacteria to be involved in these complications are group A *Streptococcus* (GAS) and *Staphylococcus aureus* (Hay *et al.*, 2012).

Another important aspect regarding the pathogeny of *S. scabiei* is the oxidative stress that it produces in the host's body. This process is involved in the physiopathology of many human and animal parasitic diseases and it occurs when the free radicals (which are highly produced during inflammatory events) exceed the antioxidant systems of the body (Chandramathi *et al.*, 2009). Beigh (2016) examined the effect of sarcoptic mange on liver function and oxidative stress in dogs. He reported elevated levels of liver injury biomarkers (ALT and AST) and bilirubin, and lower levels of albumin, glucose and cholesterol, meaning that the liver function was altered. Furthermore, the activities of the superoxide dismutase and catalase were very low and the malondialdehyde level was significantly higher, compared to normal, healthy, dogs, proving a significant alteration in the oxidant/antioxidant balance. This topic was also investigated in other animals affected by sarcoptic mange: in pigs (Dimri *et al.*, 2014) and in goats (De *et al.*, 2010). A study from Singh *et al.* (2010) demonstrated that the rate of apoptotic leukocytes was higher in infected dogs than in healthy dogs, suggesting that mites excrete/secrete some substances that induce peripheral blood apoptosis in leukocytes. This may be favourable to *S. scabiei* as it can escape the host's immune response and proliferate at its will.

### 2.3 Diagnosis

First of all, the history of the dog, the clinical aspect and the nonseasonal, constant, pruritus can orientate the diagnosis. Also, the pinnal−pedal reflex test can be used, although non-specific. Between 75% and 90% of dogs with mange and ear lesions have a positive pinnal-pedal reflex. The test can be negative if ear lesions are not present (Mueller *et al.*, 2001).

In order to have a positive diagnosis, *S. scabiei* mites, eggs or their faeces need to be found under direct examination under microscope. Deep skin scrapings from several characteristic sites (pinnae, hooks, elbows) must be performed, with a scalpel until capillary bleeding results.The scrapings are then put on a slide with lactophenol or mineral oil, macerated and spread in the liquid, then covered with a coverslip and examined with the 10X objective. Also, if the skin scrapings are heated gently, the mites become active and will be easier to notice. For wildlife diagnosis, if the host is dead, pieces of affected skin can be removed and put on a Petri dish, and with the help of the heat from the stereomicroscope, the mites will be stimulated to come out from the skin (Samuel *et al.*, 2001).

Fecal examination may be interesting to perform, as it can show mites and mite eggs swallowed during grooming.

In Europe, an enzyme−linked immunosorbent assay (ELISA) commercial test exists for the detection of Ig G antibodies to *S. scabiei* in dogs (Sarcoptes−ELISA 2001® Dog,AFOSA, GmbH, Germany) and pigs (Sarcoptes−ELISA 2001® Pig,AFOSA, GmbH, Germany). The sensitivity and the specificity of the test for dogs were 92.1% and 94.6%, respectively, after 3 weeks infection (Bornstein *et al.*, 1996; Lower *et al.*, 2001).

In early infestations (it takes up to four weeks to develop an adequate titer), false negative results can occur, and false-positive tests can appear in atopic dogs sensitized to *Dermatophagoides* spp. house dust mites, which share common antigens with *S. scabiei* (Schumann *et al.*, 2002).

It is rather frequent to not find any sign of mite presence, especially in dogs that are intensely pruritic, have had multiple washings before, or have had the disease for a long time. Trial acaricidal therapy is the best solution in the case sarcoptic mange is highly suspected. The treatment should start as soon as possible. If the pruritus gets more intense after the acaricidal treatment, this is a good sign, as the mites die and produce a hypersensitivity of the skin. Finally, if the clinical signs disappear after the end of the treatment, then the diagnostic of mange can be confirmed.

### 2.4 Transmission to other animals or humans

*Sarcoptes scabiei* var. *canis* mites can infect rabbits permanently and temporarily it can affect cats, pigs, calves or goats for a period between 4 and 13 weeks (Arlian *et al.*, 1988b). Furthermore, the mites from foxes and dogs can be interchanged as they seem morphologically identical (Samuel 1981; Bornestein 1991; Soulsbury *et al.*, 2007).

A number of studies reported the transmission of *S. scabiei* from animals to people. In 2011, Aydıngöz and Mansur concluded that the human infection with *S. scabiei* var. *canis* is one of the most frequent zoonoses because of the close contact between dogs and their owners.

In humans, the zoonotic form produced by *S. scabiei* var. *canis,* differs clinically from the primary disease produced by *S.scabiei* var. *hominis.* The incubation period is usually shorter between 24−96 h after contact with the infected animal, whereas in human scabies it takes 4−6 weeks (Walton and Oprescu, 2013). Also, the distribution of the lesions is atypical on the body. In human scabies, the patients will have intense pruritus, erythemic papules or vesicles, mainly on the trunk, forearms and thighs (Beck, 1965; Charlesworth and Johnson, 1974; Tannenbaum, 1965), while in the zoonotic type of mange, the papules are small, also pruritic, but appear only at the contact zone with the infected animal. In the end, the severity of the zoonoses is very low, since it is transitory and self-limiting, lasting for four to five weeks (Faust *et al.*, 1962).

# 3. CONTROL OF *SARCOPTES SCABIEI* INFECTION

Scabies (in humans) and sarcoptic mange (in animals) are highly contagious diseases, with a potential risk of long mite survival in the environment, meaning that the treatment needs to take into consideration treating the infected patient or animal, the environment, and also all the individuals the patient or animal had contact with (Buffet and Dupin, 2003).

## 3.1 Acaricides

### Pyrethroids

Pyrethrins are derived from the chrysanthemum flowers, being botanical insecticides. The chemical, synthetic compounds derived structurally from these natural insecticides, are called the Pyrethroids. They are considered to be the safest class of insecticides/acaricides available. There are many different products registered for the control of arthropods in veterinary medicine and agriculture (Soderlund *et al.*, 2002).

Pyrethroids have a mechanism of action on the arthropods nerves. They modify the kinetics of voltage−gated sodium channels, which mediate the transient increase in the sodium permeability of the nerve membrane, causing prolonged depolarization of nerve−cell membranes and disrupting neurotransmission (Soderlund and Bloomquist, 1989).

One of the main highlights of the pyrethroids, is permethrin(Fig 3.1), discovered in 1973. It is a drug that can be used during pregnancy or on babies over the age of 2 months, for this reason being added on the World Health Organization's List of Essential Medicines as an effective and safe drug needed in health system (WHO Model List of Essential Medicines - 20th List). Systemic absorption of topical permethrin is minimal.

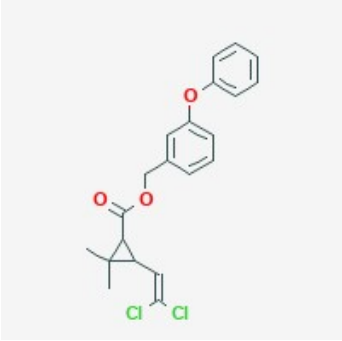



Figure 3.1-Permethrin molecular structure (from https://pubchem.ncbi.nlm.nih.gov/)

### Macrocyclic Lactones

Macrocyclic lactones (MLs) represent a family of broad-spectrum antiparasitic drugs, widely used in veterinary and human medicine for the treatment of arthropods and nematodes. This group includes two distinct chemical families: milbemycins (moxidectin, milbemycin

oxime) first described in 1974 and avermectins (ivermectin, abamectin, doramectin, eprinomectin, selamectin) first described in 1975. They are both a fermentation product of the actinomycete *Streptomyces* spp.

Doramectin, which is structurally closer to abamectin than ivermectin, is produced by chemical modification of *S. avermitilis* (McKellar and Benchaoui, 1996; Shoop *et al.*, 1995).

Ivermectin is produced during fermentation of *Streptomyces avermitilis,* being considered a semi−synthetic derivative of the natural avermectins. It is a mixture of two chemically modified avermectins (Fig. 3.2) containing at least 80% of 22-23 dihydroavermectin $B_{1a}$ and less than 20% 22-23 dihydroavermectin $B_{1b}$(Campbell, 1989). For the discovery and development of ivermectin, Omura and Campbell received the Nobel Prize in Medicine in 2015 (Omura *et al.*, 2014).

It is a product widely used in both veterinary and human medicine. In animals, it is used against endoparasites- gastro-intestinal nematodes, but also against ectoparasites-arthropods including lice, fleas, mites. It is the most used antiparasitic drug in animals, and since its release in the 1980s, over 5 billion of doses of ivermectin have been sold through the world (Omura, 2008).

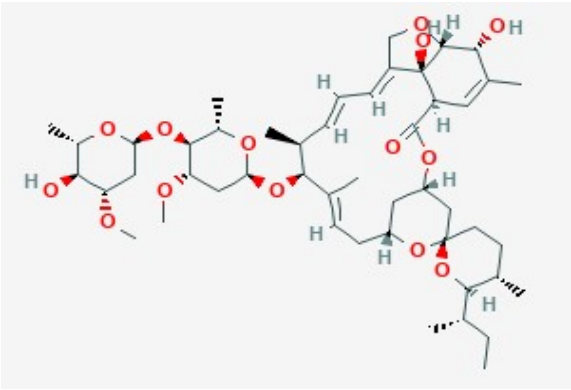


Figure 3.2- Ivermectin molecular structure (from https://pubchem.ncbi.nlm.nih.gov/)

Ivermectin is metabolized in the liver and excreted in faeces (98%) and urine (1%).In humans, the concentration in the blood is maxim around 4 hours after administration. The plasma half-life is between 12 and 56 h (Campbell, 1989). Toxicity of ivermectin is usually rare in animals and in dogs, except for collies or sheepdogs because of their multiple drug–resistant gene ABCB1 mutations (Curtis, 2004).

Moxidectin has its origins from chemical modification of nemadectin, a fermentation product of *Streptomyces cyanogriseus* (Fig 3.3). It is a highly lipophilic drug, with low susceptibility to transport by ABC transporters, making it largely distributed to all body parts, accumulating in fat tissue, which will stock the drug as a reservoir (Lifschitz, 1999) (Lespine, 2005).

Moxidectin is considered to be the macrocyclic lactone with the longest period of activity. It is a well tolerated drug with low toxicity in both humans and animals (Ménez, 2012).

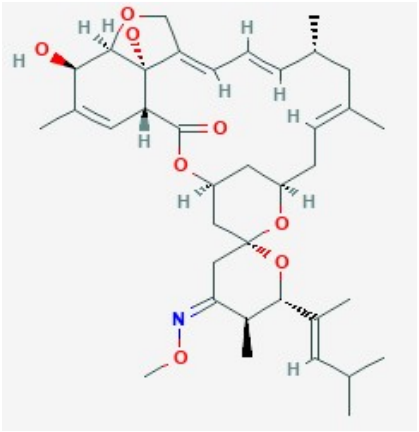


Figure 3.3- Moxidectin molecular structure (from https://pubchem.ncbi.nlm.nih.gov/)

In animals it is registered for the treatment of internal and external parasites in dogs, cats, cattle, sheep, horses, goats.

As far as scabies in humans is concerned, moxidectin is studied in a phase II trial recently founded by the Global Health Investment Fund.The efficacy of the moxidectin is of course related to its superior pharmacokinetic characteristics: rapid absorption, larger distribution and longer persistence in plasma, but also in the skin (Bernigaud *et al.*, 2016).

The MLs actions upon parasites are based on their high affinity for glutamate−gated chloride channels (GluCls) that is limited to invertebrates (Kiki-Mvouaka *et al.*, 2010; Wolstenholme and Rogers, 2005). GluCls have various functions in invertebrate nervous systems like the control and modulation of locomotion, the regulation of feeding, and the mediation of sensory inputs (Wolstenholme, 2012). Secondary target of MLs are the GABA receptors, resulting in somatic muscle paralysis of nematodes (Beech *et al.*, 2010; Brown *et al.*, 2012).

**Isoxazolines**

The isoxazolines belong to a new chemical class of acaricides and insecticides, introduced in the 2000s, and include afoxolaner (Nexgard®), fluralaner (Bravecto®), sarolaner (Simparica®) and lotilaner (Credelio®) (Fig. 3.4). For the moment, the available commercial products are presented in oral formula (for all the drugs), spot on (fluralaner) administration in dogs (all drugs) and cats (fluralaner and lotilaner) (Dumont, 2014; Letendre, 2014).There is also a drinkable formulation of fluralaner for chicken (Exzolt®). Isoxazolines exhibit insecticid and acaricid activities, having a large spectrum, and being effective against some ectoparasites such as fleas, ticks and mites (Weber, 2016; Shoop, 2014).The isoxazolines also have interesting pharmacokinetic properties. They are rapidly absorbed, from 2 to 6 h. They have long plasma half-lives, for example both fluralaner and afloxolaner have around 15 days half life (Kilp *et al.*, 2014; Letendre *et al.*, 2014). Furthermore, fluralaner could be quantified in plasma (> 10 ng/mL) for up to 112 days after a single oral administration (Kilp, 2014).

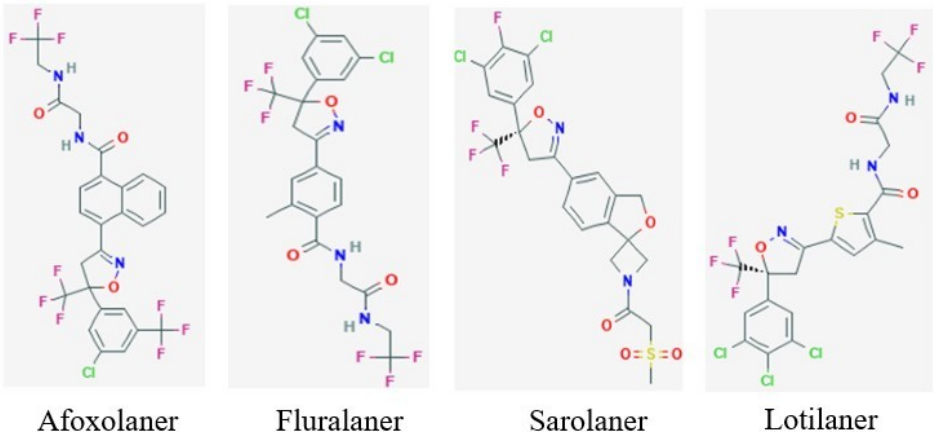



Figure3.4-Molecular structure of isoxazoline−derived compounds
(from https://pubchem.ncbi.nlm.nih.gov/)

The isoxazolines compounds are lipophilic, unionized, small and highly bound to plasma proteins. It has been demonstrated that they act on specific GABA/glutamate receptor inhibiting the chloride ion channels of arthropods (García-Reynaga *et al.*, 2013; Gassel *et al.*, 2014; Shoop *et al.*, 2014).

Fipronil also inhibits the chloride ion channels, but in comparison with it, the isoxazolines exhibit higher potency toward GABA chloride channels. In addition to that, it is unlikely to have cross−resistance to isoxazolines on arthropods that show resistance to commonly used acaricides or insecticides (Shoop *et al.*, 2014; Weber and Selzer, 2016; Zhao and Casida, 2014a).

Afoxolaner has been studied in the last years, in order to better understand its pharmacokinetic proprieties as well as its efficiency in eliminating certain ectoparasites in dogs. A study regarding afoxolaner's pharmacokinetic, revealed that following oral administration of the minimum effective dose of 2.5 mg/kg, the substance was rapidly absorbed, with maximum plasma concentration at 2 to 6h after treatment. The terminal plasma half-life was 15.5 ± 7.8 days, and oral bioavailability was 73.9%. Following the hydroxylation of afoxolaner, one major metabolite results, which will be eliminated in the dog's plasma, urine and bile. These proprieties confirm that the afoxolaner products are suitable for monthly administration, because of the fast absorption and long term half-life (Letendre *et al.*, 2014).

The use of afoxolaner in treating sarcoptic mange in dogs was mentioned in two studies. The study from Beugnet *et al*, 2016, included 20 dogs naturally infected with sarcoptic mange (10- control group, 10- treated group).The treatment with afoxolaner (Nexgard®) was given at a monthly interval, at the labeled dose, for 2 months, and it was observed that even after the first month, there was a complete cure of mites (from an average of 166.9 to 0 in the treated group at both D28 and D56) and in 2 months, the clinical signs resolved in the treated dogs. The other study from Hampel *et al.*, 2018, considers both afoxolaner (Nexgard®) or afoxolaner/milbemycin oxim (Nexgard Spectra®) for the treatment of 65 dogs with sarcoptic mange, monthly, for 2 months. The efficacy of the treatment, illustrated in reduction of geometric mean live Sarcoptes mite counts was 98.9% and 99.7%

for NexGard® treated (n = 38) and 99.6% and 100% for NexGard Spectra®-treated dogs (n = 27) at one month and two months after treatment initiation. Both treatments marked a rapid significant improvement in papules, crusts and pruritus at one month and two months after treatment. In conclusion, afoxolaner or afoxolaner/ milbemycin oxim have remarkable effects on mange mites.

Sarolaner has also been studied for its effect on *S.scabiei* in dogs, in a complex study from Becskei *et al.*, 2016, including both a field and a lab study.

The field study included a comparison between the effects of orally administrated sarolaner to 75 infected dogs versus the spot on consisting of imidacloprid/moxidectin administrated to 45 dogs with mange, both treatments repeated at D0 and D30.The parasitological curerate was 88.7% and 100% in the sarolaner group and 84.6% and 96.0% in the imidacloprid/moxidectingroup, on Days 30 and 60, respectively, showing that sarolaner was non-inferior to imidacloprid/moxidectin at any of the time.

The lab study consisted of 44 dogs with sarcoptic mange, divided into 22 dogs receiving placebo treatment and 22 dogs receiving sarolaner treatment on D0 and D30. By D14, 77% of the sarolaner treated group was free of mites, 100% were free on D30 and D60, and only one live mite present being found on one dog on D44.

Two other studies observed the efficacy of fluralaner on sarcoptic mange in dogs, using both the spot on and the oral formulation.

Romero *et al.* (2016), performed a study on 17 dogs with sarcoptic mange, which were all treated with fluralaner, orally, once at D0. At D7, 7 dogs were negative for mites and by D14, all the dogs were negative from mites and remained so until the end of the study at D28. Furthermore, the clinical scores of the dogs improved visibly starting with D14.

Taenzler *et al.*(2016), conducted a study on 29 dogs naturally infected with scabies mites, and dived them into 3 groups: the first one with 9 dogs which were given oral fluralaner at D0, the second one with 11 dogs treated topically with fluralaner at D0 and the third group with 9 dogs treated topically with saline solution. At D28, the dogs were controlled through skin scrapings, resulting in 100% mite free dogs in the first two groups and the clinical scores of the lesions diminished.

Taking into consideration the good treatments results these molecules have, they tend to become a promising treatment for a wide range of external parasites, including *S. scabiei* in humans and animals.

**Other molecules with acaricid activity**

The formamidines include chlordimeform and amitraz (Hollingworth, 1976). In invertebrates, toxicity is mediated by activating an octopamine−dependent adenylate cyclase (Nathanson, 1985). Formamidines are a class of chemicals used as acaricids in agriculture and veterinary medicine.

The organophosphates work by inactivation of the acetylcholinesterase (AChE), which is essential to nerve function in insects (Fukuto, 1990). Organophosphates are a group of elements used as pesticides, insecticides, acaricides (Senthilkumaran, 2015).

The phenylpyrazoles were introduced on the market in the early 1990's, as pesticides, insecticides and acaricides for veterinary and agricultural use. This class includes substances such as fipronil and pyriprole, important for their effectiveness against a number of external parasites such as fleas, ticks, mites, lice and flies. They are lipophilic, allowing a rather long residual effect against external parasites, especially in the topical formula, where the substance is deposited in the sebaceous glands of the skin. Their mechanism of action is by inhibiting both GABA− and L− glutamate−gated chloride channels (Bloomquist, 2003; Narahashi *et al.*, 2010).

Botanical extracts have come to the interest of scientists lately as alternative acaricides, since the use of synthetic products is becoming more and more problematic.

Topical tea tree oil (5%) combined with benzyl benzoate (25%), has been used in treating human scabiesin Australia, being not only efficient, but even better with the addition of tea tree oil, as it reduced the irritation caused by benzyl benzoate (Currie *et al.*, 2004).

Apart from tea tree oil, other botanical extracts that have a promising scabicidal effect have been tested. In dogs, a neem (*Azadirachta indica*) seed based product was used for treatment, resulting in 80% of dogs cured after 14 days consecutive treatment (Abdel-Ghaffar *et al.*, 2008). Another study showed that the use of 1% oil of citronella (*Cymbopogon nardus*) and tea tree, 10% ethanol extract of hogweed (*Heracleum sosnowskyi*), tansy (*Tanacetum vulgare*) and wormwood (*Artemisia absinthium*) reduces *in vivo* mite infection levels to less than 10% of the pretreatment level of mange in pigs (Magi *et al.* 2006).

In vitro studies involving botanical extracts have been performed on *S. scabiei* mites. The most recent one, from 2016, elaborated by Fang *et al.*, studied and compared the effect of 10 essential oils on *S. scabiei* var. *suis* mites,through contact and through fumigation. The efficacy order for the in contact method was: clove> palmarosa > geranium > tea tree > lavender > manuka > bitter orange > eucalyptus >Japanese cedar. In fumigation bioassays, the efficacy order was: tea tree (killed all mites in 4 min) > clove> eucalyptus > lavender > palmarosa >geranium > Japanese cedar > bitter orange > manuka.

### 3.2 Control of sarcoptic mange in other animals

In animals, there are many different medicines that can be used, on and off label, for the treatment of sarcoptic mange. The available therapy we have today includes subcutaneous injection, oral products and topical products (pour−on, spot on, spray or dip). The choice depends on the animal species, age, cost, number of in contact animals. Also there are some precautions to take into account when giving the treatment: amitraz should not be used in pregnant or nursing bitches or puppies younger than 3 months. Ivermectin is contraindicated in collies, sheepdogs and their crosses, causing tremors, salivation, ataxia, depression, mydriasis, coma and even death (Curtis, 2004). For farm animals (cattle, pig, sheep) topical solution can be used such as: phoxim, amitraz, deltamethrin, eprinomectin or subcutaneous injections with solution like: ivermectin, doramectin,moxidectin.

It is important, in order for the treatment to succeed, that every in contact animal of the same species as the diagnosed patient, is also treated and that the environment is disinfected.

### 3.3 Control of sarcoptic mange in dogs

In dogs, treatments can be topic or systemic. There is a wide range of substances that have good results in treating sarcoptic mange, both authorised and unauthorised for the use in dog.

The topical therapy is more constraining for the owner, as it requires regular applications all over the body of the animal, several days apart, for 2 biological cycles of *S. scabiei*. Furthermore, if it is a multiple dog household, it can be very time consuming. The scabicidal substances include: lyme sulphur, amitraz (which has a special issue in handling, especially by diabetic owners, as the vapours can cause transient hyperglycaemia) (Folz *et al.*, 1984), permethrines (Miller *et al.*, 2012), fipronil (Curtis *et al.*, 1996; Bordeau *et al.*, 2000).

The systemic therapy is more convenient and easier for the owner.

The macrocyclic lactones are widely used:ivermectin (orally/ pour on/subcutaneous injection, not to be given to collies or sheepdogs because of their multiple drug–resistant gene ABCB1 mutations, which will lead to neurotoxic side effects) 0.2-0,4 mg/kg every 7 days orally, or every 14 days sc for 4–6 weeks (Paradis, 1998);doramectin (subcutaneous injection); milbemycin oxime (orally), 2 mg /kg every 7 days on 3–5 occasions, success rates ranging from 71 to 100% have been reported (Miller *et al.*, 1996; Bergvall, 1998); moxidectin (orally/subcutaneous injection/spot on with imidacloprid) 37 of 41 dogs given 0.2–0.25 mg/kg either orally or by sc injection weekly for 3–6 weeks were cured but side effects such as urticaria, angioedema and ataxia were observed in 7 of the animals (Wagner *et al.*, 2000); selamectin (spot on) 6–12 mg/ kg on two occasions, 30 days apart (Six, 2000).

The new category of isoxazolines has also had important positive results in the systemic treatment of sarcoptic mange in dogs. Laboratory and field studies have been performed, concerning the efficacy of afoxolaner (Beugnet *et al.*, 2016) (Hampel *et al.*, 2018), or other isoxazolines molecules such as fluralaner or sarolaner (Taenzler *et al.*, 2016) (Romero *et al.*, 2016) (Becskei *et al.*, 2016) against infection with *S. scabiei* in dogs (Table 3.1).

Table 3.1-Authorised treatment for sarcoptic mange in dog in the European Union

| **Molecule** | **Formulation/dosage** | **Frequency** |
|---|---|---|
| Afoxolaner | Tablet, 2.7-6.9 mg/kg | Monthly, for 2 months |
| Afoxolaner/milbemycin oxime | Tablet 2.50-5.36 mg/kgafoxolaner 0.50-1.07 mg/kg milbemycin oxime | Monthly, for 2 months |
| Sarolaner | Tablet, 2-4 mg/kg | Monthly, for 2 months |
| Selamectin | Spot on, minimum 6 mg/kg | Monthly, for 2 months |
| Imidacloprid/moxidectin | Spot on, minimum 10 mg imidacloprid /kg ; 2,5 mg moxidectin /kg | Monthly, for 2 months |

### 3.4 Control in humans

In human medicine, a variety of treatments for sarcoptic mange exists, although not as many as in the veterinary field. These medicines have topical or oral formulations, the choice depending on the patient, the age, the toxicity, the cost, the extent of superinfection of the skin. At the moment, the permethrin and ivermectin seem to remain the best options in scabies treatment in humans. Rosumeck *et al.* (2018), included 15 randomized controlled trials that compared permethrin against ivermectin for people (1,896 participants in total) with scabies of all ages, males and females. Their conclusion was that there was no difference detected in the efficacy of permethrin compared to systemic or topical ivermectin.

Other drugs recommended include: benzyl benzoate (emulsion), crotamiton (cream or lotion, used on scabies nodules in children) or sulphur ointment (oldest drug used for scabies).

### 3.5 Drug resistance

The treatment failure can occur because of a variety of factors such as: incorrect application of drugs, reinfection and drug resistance. *S. scabiei* has been reported resistant in some cases to drugs such as: permethrin, ivermectin, lindane, crotamiton (Thomas *et al.*, 2015; Roth, 1991). In various countries, resistance to pyrethroids (*kdr*–knockdown resistance) have been reported in several arthropods (Heukelbach and Feldmeier 2004).Scabies treatment failures have been reportedly caused by the increasing resistance to permethrin (Pasay *et al.*, 2006).

Ivermectin treatment in both humans and animals, has been studied and there are reports of clinical treatment failure in the last years (Currie *et al.*, 2004; Terada *et al.*, 2010). Mounsey *et al.*, published in 2009, a 10 year old study of in vitro sensitivity tests performed on these mites, and concluded that they are becoming more and more tolerant to ivermectin in remote Aboriginal communities across northern Australia (Chosidow, 2012).

In dogs, there is one report of sarcoptic mange refractory to ivermectin treatment, in two dogs that were in the same household. After 2 oral ivermectin treatments (0.3 mg／kg) at 14 day interval, and a ivermectin subcutaneous injection on D28 (0.3 mg／kg), at D35 they still had live mites and the clinical score deteriorated, the skin lesions becoming more extended. It was then decided to change the molecule, so the autors administrated fipronil in spray (3 ml/kg) and in spot on (0.67 ml/dog) at 14 days interval for 3 treatments. After the first week, the dogs' pruritus improved significantly. At the third treatment the lesions were completely resolved (Terada *et al.*, 2010).

The mechanism that gives *S. scabiei* resistance is complex, and the importance of this phenomen is still uncertain. Studies performed in laboratories showed that acaricide resistance is likely mediated by sodium channel mutations (for permethrin) (Pasay *et al.*, 2008), byP−glycoprotein mediated efflux (for ivermectin) (Mounsey *et al.*, 2010), and increased activity of metabolic enzymes, such as esterases, cytochrome P450 and glutathion S−tranferases (for permethrin and ivermectin) (Pasay *et al.*, 2008, 2009).

# 4. SKIN MICROBIOME

## 4.1 General presentation

The skin covers and protects the body from the external factors. It is the first line of defense of the body and it sustains microorganisms that can be commensals (nonpathogenic permanent residents), transients (temporary residents) or pathogenic (only the microbe benefits, while the host is harmed) (Solcan, 2011) (Fig 4.1). Usually, the skin pathogens are at first commensals, but because of a microbial dysbiosis, a genetic shift or a decrease in the immune status, they become pathogen.

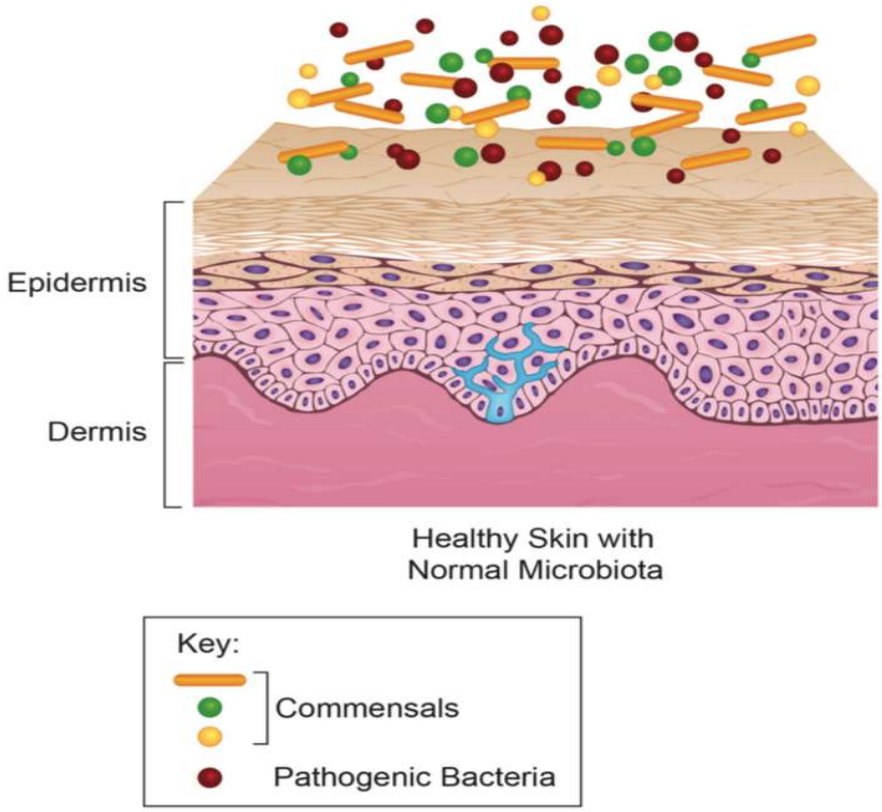



Figure 4.1-Current model of relationship between skin barrier and skin microbiota (from https://www.researchgate.net/publication/319663057_The_Role_of_Cutaneous_Microbiota_Harmony_in_Maintaining_a_Functional_Skin_Barrier/fulltext/59f529c5458515547c21cfc9/The-Role-of-Cutaneous-Microbiota-Harmony-in-Maintaining-a-Functional-Skin-Barrier.pdf)

In recent years, researchers started showing increased interest in the impact of the microbes (bacteria, viruses, fungi, archaea, and parasites), their gene products and metabolites in a defined habitat, this field of research being known as the study of the microbiome.The microbiota is a collection of microbes in a defined microenvironment, and its characterization is often based on conserved genes found on bacteria and fungi (Marchesi *et al.*, 2015). This latter term is more limited and is now preferred instead of microflora, which refers to microscopic plants.

So far, multiple body sites have been studied such as: gastrointestinal, urogenital tract, nasal passage, oral cavity, and more recently the skin. The conclusion was that each body site has its own microbial community, which is maintained in balance in a healthy organism (Rodrigues Hoffmann, 2017).

The skin microbiome plays an important role in maintaining the health and functions of the skin, making its understanding essential. The studies elaborated so far, aimed at characterizing the bacterial andfungal communities of the skin, investigated through sequencing analysis of the phylogenetic marker genes, like the bacterial 16S ribosomal RNA (rRNA) and the fungal 18 S ribosomal RNA or internal transcribed spacer (ITS) (Hamady *et al.*, 2009).

The link between the microbiome and the immune system is very tight, as they need to maintain a balance and avoid excessive immune and inflammatory responses. Basically, there must be two opposite reactions in balance. The first is for the body to respond rapidly and effective against the external factors that aggress it, and secondly to refrain from attacking its own microbiota (Tizard *et al.*, 2018).

The skin microbiome is influenced by many factors, with the individual being the major one to differentiate the microbiome among populations (Gao *et al.*, 2007; Ursell *et al.*, 2012). Age, diet, hygiene, sex, lifestyle also influence the composition of the microbiome of healthy skin (Costello *et al.*, 2009; Griece *et al.*, 2008; Song *et al.*, 2013).

The body site is another important factor that shapes the microbiome, the human skin being composed of compartmentalized regions which share within them common physiological features (Barnard *et al.*, 2017).

Generally, the human skin can be divided into dry, sebaceous and moist micro-environments. The most diverse microbiome can be found on dry skin, phyla Actinobacteria and Firmicutes, with lower proportions of Bacteroidetes and Proteobacteria being represented. Lower diversity is observed in the sebaceous areas where Actinobacteria and Propionibacterium are present. The skin microbiota of moist areas is dominated by phyla Actinobacteria (*Corynebacterium* spp.) and Firmicutes (*Staphylococcus* spp.) (Costello *et al.*, 2009; Griece *et al.*, 2009).

The only disadvantage of using these culture independent methods of detecting microbes is that they cannot discriminate between live and dead microbes. It its estimated that most of the microorganisms detected through next generation sequencing (NGS) are probably inactive or even dead (Cangelosi *et al.*, 2014).

Fungi represent an important part in defining the microbiome of the skin. The mycobiome is defined as the fungal habitat, including fungi," their genomes and the surrounding environmental conditions" (Marchesi *et al.*, 2015). The link between bacteria and fungi communities may give insight in many skin disorders like seborrheic or atopic dermatitis.

In the past, the study of the mycobiome at community levels was difficult, because cultivating skin fungi required heterogeneous culture conditions. Now, with the advances in DNA sequencing technologies, research in this field can be easily performed (Jo *et al.*, 2017, Han *et al.*, 2018).

The initial cultivating methods for determining the skin mycobiome, showed that on healthy skin, the main genus present was *Malassezia*, and occasionally *Candida* and many other genus (Mok *et al.*, 1984).

The new studies using NGS method, examined the skin mycobiome of people from different geographical regions, and found out the same predominance of *Malassezia* on most skin sites, except the feet (Findley *et al.*, 2013; Leung *et al.*, 2016).

Song *et al.* (2013) studied the effect of family cohabitation on the skin microbiome of the members. His results demonstrated that family membership reflects in the diversity of the microbiome, meaning that members of the same family had similar bacterial diversity across all body sites. He went further into the idea of cohabitation and analyzed the relationship between the skin microbiome of dogs and their owners and observed that the similarity between the two is high. Furthermore, the microbiome of the owner is more similar to their own dog, than to other dogs in general. Adults, who have dogs, have a greater diversity of bacteria on their skin, especially on the hand and forehead.

The first study which investigated changes in the skin microbiome induced by the parasitism with *S. scabiei*, was made by Swe *et al.* (2014) in pigs.

The study included 15 pigs, divided into 3 groups: 5 healthy in the control group, 5 infected with *S. scabiei*, and 5 infected with *S. scabiei* and treated with dexamethasone.

The sampling place was the inner sebaceous surface of the pinnae, and the samples were taken before infection, during the infection and after the treatment.

The infection occurred in week 0, the doramectin treatment on week 16 and the final samples were taken on week 21.

The results showed that during week 7- week 10, both infected groups had a predominance of *Staphylococcus (*which shifted from the harmless *Staphylococcus hominis* to

the pathogen *Staphylococcus chromogenes).* At week 10, when severe crusts appeared in the pigs from the infected groups, the microbiome became dominated largely by *Corynebacterium,* and the community diversity was markedly reduced. At week 21, when the clinical signs of sarcoptic mange resolved, all the three groups seem to recover their microbiome diversity, with the maintenance of higher *Staphylococcus* numbers in pigs that suffered from mange. The population of *Streptococcus* remained almost unchanged through all the trial in all the three groups. The healthy pigs manifested a decrease in the diversity of the microbiome, as they grew up, changing from a complex and diverse microbiome in their youth to two dominating genera at week 21, *Lactobacillus* and *Streptococcus* (Swe *et al.*, 2014).

The most recent study regarding the influence of sarcoptic mange on the skin microbiome was conducted in wild canids, by DeCandia *et al.* (2019).

The animals included were coyotes (8 infected, 4 uninfected), red foxes (6 infected, 5 uninfected) and gray foxes (1 infected, 1 uninfected) and samples were taken from 5 body sites.

The results show that the samples from uninfected animals tend to cluster around individuals rather than body site. For the mange infected animals, they observed that the bacterial diversity is lower than in healthy animals, and they present an increased level of opportunistic bacteria such as *Staphylococcus pseudintermedius* and *Corynebacterium* spp.

### 4.2 Canine skin microbiome

The microbiome of both healthy and allergic/atopic dogs has been studied during the last years, in the attempt to better understand the relationship between the microbes and their contribution to skin diseases.

Rodrigues- Hoffmann's *et al.* (2014) study first used the sequencing of the 16 S rRNA gene to determine the diversity of bacteria found on dog skin. The cohort included 12 healthy dogs and 6 allergic dogs, and the sampling sites were 12 for the healthy dogs and 4 for the allergic dogs, including haired skin and mucosal surfaces.

The most abundant taxa detected across all the sampled sites were Proteobacteria, followed by Firmicutes, Actinobacteria, Bacteroidetes, and Cyanobacteria. The first factor observed to influence the variability between samples is the individual, followed by the skin site (higher number of species on the haired skin vs. the poorly haired skin, mucous surfaces). Among all these sites, the dorsal aspect of the nose was on average the most diverse, being colonize primarily with the genus *Moraxella*.

The main difference noticed when comparing healthy skin to allergic skin microbiome is the lower species number in allergic dogs.There were a large number of bacteria previously

uncultured or rarely found, emphasizing that the new sequencing techniques are more precise than culture-based methods (Rodrigues- Hoffmann, 2014).

Bradley *et al.* (2016) performed a longitudinal study with 14 dogs diagnosed with atopic dermatitis and 16 healthy dogs sampled from 4 different skin sites. The atopic dogs were found to have a less diverse microbiome, which is consistent to other studies and to observation from human medicine. Furthermore, the species that were the most abundant in atopic dogs were *Staphylococcus pseudintermedius* and *Corynebacterium*. After antimicrobial treatment, the skin microbiome began to change, to become more diverse, the relative abundance of *Staphylococcus* spp.became lower and the skin barrier function normalized.

Another interesting study regarding the skin microbiota of atopic dogs was conducted on 8 atopic dogs challenged with HDM (house dust mite). After they had been hypersensitized with HDM, they developed focal skin lesions on the inoculation site. The samples were collected from the affected and contralateral areas, prior to and after challenges. The results were that no important changes in the skin microbiome in richness or diversity have been noticed between the different timepoints evaluated. The observed differences were between the lesional and non-lesional skin, with the lesional skin showing incresead number of *Corynebacterium* and *Staphlylococcus* (*Staphylococcus pseudintermedius* confirmed by PCR) which went on for three weeks after mite challenge and two weeks after the recovery of the skin (Pierezan *et al.*, 2016).

Further studies have been conducted by Cusco *et al.* (2017 a; 2017 b) which included bigger cohorts of dogs, only healthy ones. The results suggested that the microbiome is mainly individual specific, but it is also influenced by skin site, temporality and sex. The environment seems to play an important part too, so one of the studies included only dogs that cohabited, were the same cross breed, almost the same age, and had the same diet. The results were the same as in the previous studies, showing that even in homogenous cohorts, the individual is the one driving the microbiome, followed by the skin site.

The fungal microbiome of the dog skin has also been studied in 10 healthy dogs and 8 allergic dogs, sampled across various sites of the skin (Meason-Smith *et al.*, 2015).

The results were that the fungal microbiome of dog skin is highly diverse, and as in bacterial microbiome, more diverse than in humans. The NGS results revealed that the skin was colonized predominantly with fungi within the phyla Ascomycota and Basidiomycota. Within the phylum Ascomycota, the most abundant genera found on dog's skin were Alternaria, Cladosporium and Epicoccum.

In this study, an overall low abundance of *Malassezia* was observed, although it is the most common fungal genus cultured from dog skin. Only a few dogs presented relatively higher abundances of *Malassezia* spp. on different body sites.

The fungal microbiome is more similar across skin sites within the same individual, than it is at the same body site between dogs.

The conclusion of the study was that the allergic dogs had lower fungal diversity than the healthy ones, which is in contrast with what happens to the fungal microbiome of allergic humans that increases in diversity. The fungi found on the skin are ubiquitous, meaning they are likely acquired from the environment, so further studies must be performed to see if these fungi are transient or truly indigenous.

# PART II-PERSONAL CONTRIBUTIONS

# 5. OUTLINE AND OBJECTIVES OF THE THESIS

The authorized treatment in the EU for *S. scabiei* infection in dogs has been recently enriched with the addition of novel molecules from the isoxazoline class.

The interest in these molecules is high, since they are highly efficient against fleas, ticks and mites (Beugnet *et al.*, 2014a, 2014b; Dumont *et al.*, 2014; Letendre *et al.*, 2014; Beugnet *et al.*, 2016; Lebon *et al.*, 2018; Hampel *et al.*, 2018). Moreover, adverse effects and resistance are being reported to conventionally used acaricides (ivermectin), making the use of alternative molecules more interesting.

Also, *S. scabiei* seems to affect the microbiome of the skin in dog, generating a disequilibrium which justifies the degree of secondary infection.

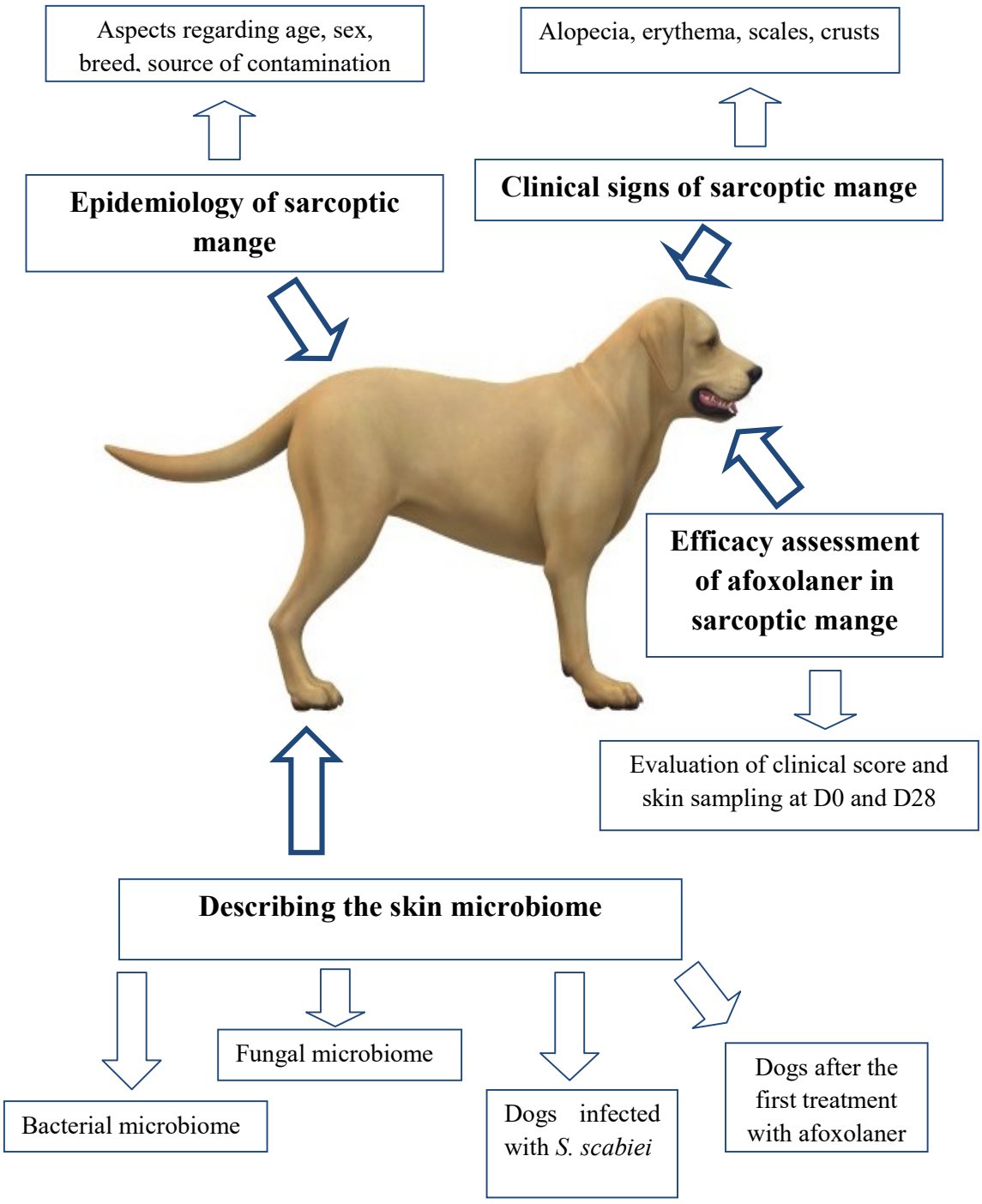
The main objectives of the thesis were (illustrated in the image below):

- to describe the clinical signs and epidemiology of dogs infected with *S. scabiei*
- to assess the efficacy of afoxolaner, a molecule from the isoxazoline class, in the treatment of sarcoptic mange in dogs
- to describe the modifications of the skin microbiome of dogs affected by sarcoptic mange.

The first part of the experimental study described the clinical signs and epidemiology of *S. scabiei* infected dogs in Moldova.

The second part evaluated the efficacy of afoxolaner in dogs with sarcoptic mange.

The third part of the research concentrated on concomitant modifications of the skin microbiome in dogs affected by sarcoptic mange, before treatment with afoxolaner and 28 days after the first treatment with the molecule.




**Outline and objectives of the thesis**

# 6. CLINICAL SIGNS AND EPIDEMIOLOGY OF SARCOPTIC MANGE IN DOGS IN MOLDOVA

Sarcoptic mange in dogs is a common dermatitis, especially in non-controlled, stray dogs that develop evocative clinical signs. It is often a problem in shelters or in big communities of animals, where dogs are closely in contact with each other and disinfection is not always done properly. However, there are cases of infection with *S. scabiei* mites in privately-owned dogs, the contamination occurring by direct contact with an infected animal (dog or fox) or through fomites (hair clipping tools).

## 6.1 Materials and methods

The dogs included in the study had to present evocative clinical signs of mange (pruritus, crusts, alopecia) and to have positive results in the skin scraping test.

A questionnaire regarding the history of the dog was addressed to the owner, including the presentation of the animal, the breed, the sex, the age, if there were other in contact animals (especially dogs), if cutaneous problems appearedin the owner or in other members of the house.

Deep skin scrapings from different body sites presenting lesions suggestive of sarcoptic mange (alopecia, crusts and scales) have been performed with a scalpel until capillary bleeding resulted. An area of approximately 4 $cm^2$ was sampled from different body sites. The skin scrapings were then put on a slide with lactophenol or mineral oil, macerated and spread in the liquid, then covered with a coverslip and examined with the 10X objective of the microscope (Fig 6.1).

Adults and nymphs of *S. scabiei* were identified based on morphological aspects, including size, and general characteristics (oval shaped, short legs, dorsal part with thorns and spines and ventral part with epimers) (Fig 6.1-left). Larvae were identified based on their number of 6 legs and resemblance to adult mites, and eggs were identified by size and shape (Fig 6.1- right).

At enrolment in the study, the dogs received a general health evaluation.

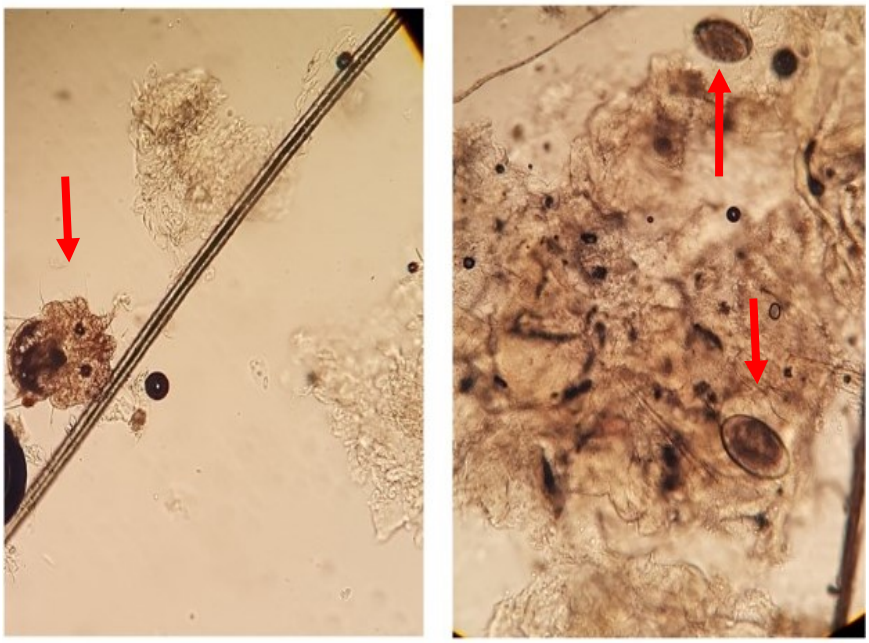


Figure 6.1-Microscopical aspect of skin scrapings positive for *S. scabiei*- left-adult; right-eggs (X10)

Concerning the dermatological examination, an original clinical score adapted for dogs, but initially designed for pigs infected with *S. scabiei* was used (Bernigaud *et al.*, 2016).

The score was based on evaluating the skin areas affected by mange lesions, alopecia degree, intensity of the skin erythema and crusting/scales intensity, on different parts of the body (head, trunk, legs, tail) (Fig. 6.2).

The grades per sign were from 0 to 4, with 4 being the most severe, which resulted in a total clinical score between 0 and 60. The scores were assessed only at the first visit.

Regarding the treatment, each dog included in the study was treated with a specific acaricid product demonstrated to be efficient against *S. scabiei* infection.

The treatment consisted in the administration of either afoxolaner (Nexgard®) twice at a monthly interval (2.7-6.9 mg/kg), either sarolaner (Simparica®) twice at a monthly interval (2-4 mg/kg), or doramectin (Dectomax®) 0.2 mg/kg twice at 14 days interval.

The only authorized product in the E.U. (from the 3 products mentioned above) for the treatment of sarcoptic mange in dogs at that period of time, was sarolaner (Simparica®).

The owners were informed and agreed to the treatment protocol. All in contact dogs were also treated.

The follow up of the dog was scheduled every two weeks for the doramectin treated dogs, over a period of one month.

Also, for the dogs treated with sarolaner or afoxolaner it was scheduled every month for a period of two months.

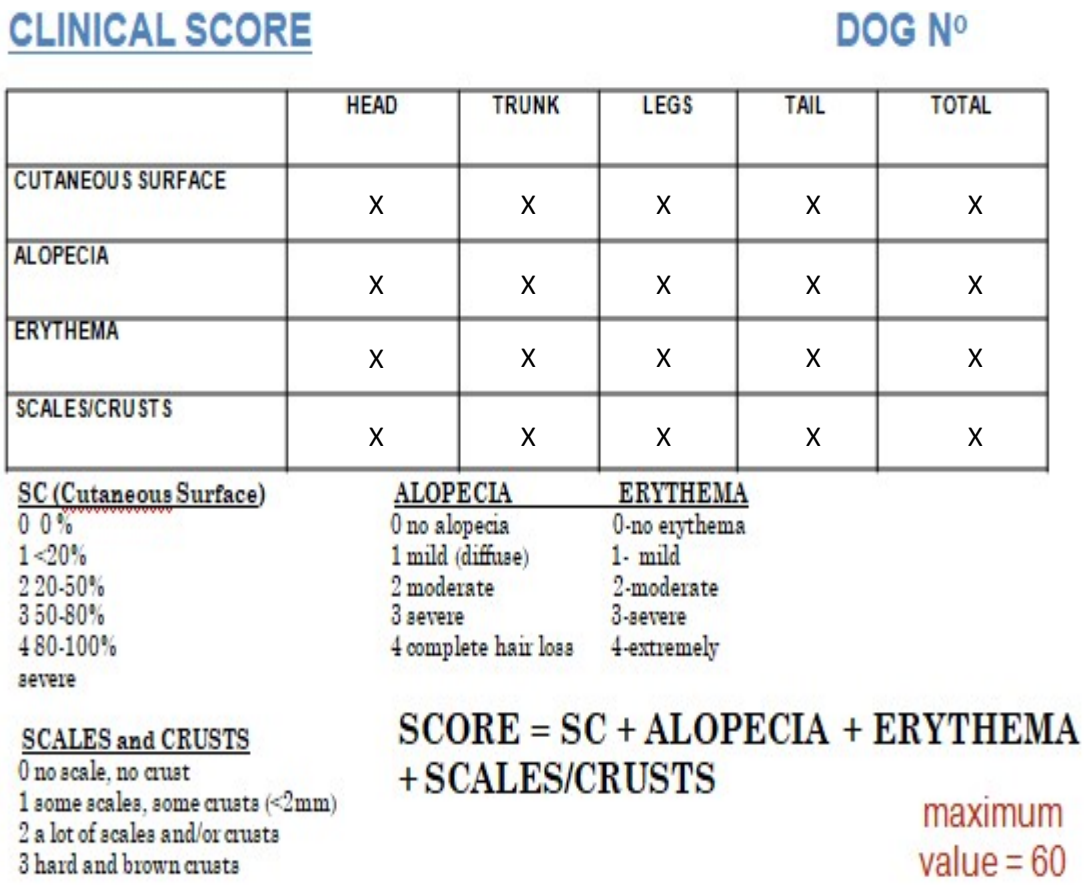



Figure 6.2-Clinical scoring system for dogs infected with sarcoptic mange

## 6.2 Results

There were 48 dogs included in the study, dogs examined between 2015 and 2018. Among them, 27 were examined in the Parasitology Clinic of the Faculty of Veterinary Medicine of Iași, Romania, and 21 were examined in either private households (14) or shelters (7).

They were mostly common or crossbreed dogs (n=39, 81.2%), but there were also some individuals from the following breeds: Boxer (a mother with her 7 puppies), Bull Terrier

(1) and Poodle (1). Their age ranged from 1 month to 12 years, with more than half of them (n=28, 58.3%) being under 1 year. Finally, the individuals included represented almost equally both sexes, with 23 males (48% of the total) and 25 females (52% of the total).

Concerning the history of the dogs, a great number were stray dogs from shelters or in foster families (n=21, 43.7%), resulting in the fact that the information we had about them was limited. We also studied two households that had more than five dogs.

The first one was a countryside household in Vaslui County, that came to the attention through the help of an animal protection association.At the moment when the owner noticed the first clinical signs, there were a total of 19 dogs with ages between 2 months and 10 years present in the yard of the house.

The dogs were left to roam freely, they did not have individual housing and they could also get out in the street. The source of the disease remains unknown. Finally, we could catch and sample 14 dogs. The other dogs that could not be caught for sampling were also given the treatment.

The second one was also a countryside household, near Iasi. There were 8 dogs present on the site, a Boxer mother with her 1 month old 7 puppies.The owner noticed the lesions on the mother's skin 2 months ago, after a fox with skin lesions had wandered through their yard.

Regarding the source of infection, apart from this latter case, it is largely unknown, since a large number of animals were former stray dogs.

The 7 dogs (14.5% from the total number) that belonged to the shelters, were from 2 public shelters near Iași (Tomești shelter and Miroslava shelter), and from one private shelter.

Furthermore, the zoonotic transmission of the disease was reported from only one dog, A10, that had the most severe lesions and that was hospitalized in our clinic.

The infection occurred in one of the clinical staff that manipulated the dog during the treatment, and manifested through papules on the arm and forearm, and pruritus.

The skin scrapings examined through direct examination were positive for *S. scabiei* adults, larvae or eggs. However, we observed very few parasitic elements in the samples collected.

The clinical scores of the 48 included dogs varied between a minimum of 4 and a maximum of 58, taking into consideration that the maximum score which could be obtained was 60. Furthermore, we observed that the males had slightly higher clinical scores than the females (Fig 6.3).

Fig. 6.3 shows a clear illustration of the fact that the dogs we studied had from very discrete lesions to serious, extended lesions all over the body.The arithmetical mean of all the clinical scores was around 22.

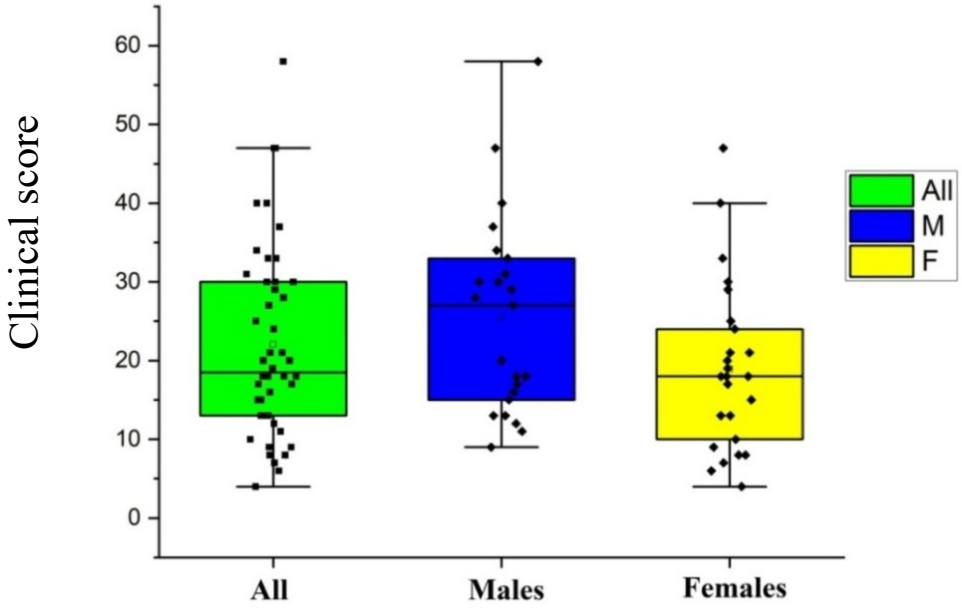



Figure 6.3 - Clinical score of the dogs

The most scored sign was the extent of mange on the cutaneous surface followed closely by the degree of alopecia, illustrated in the boxplot graphic from Fig. 6.4. The clinical aspects of these signs are shown in Fig. 6.5-6.8.

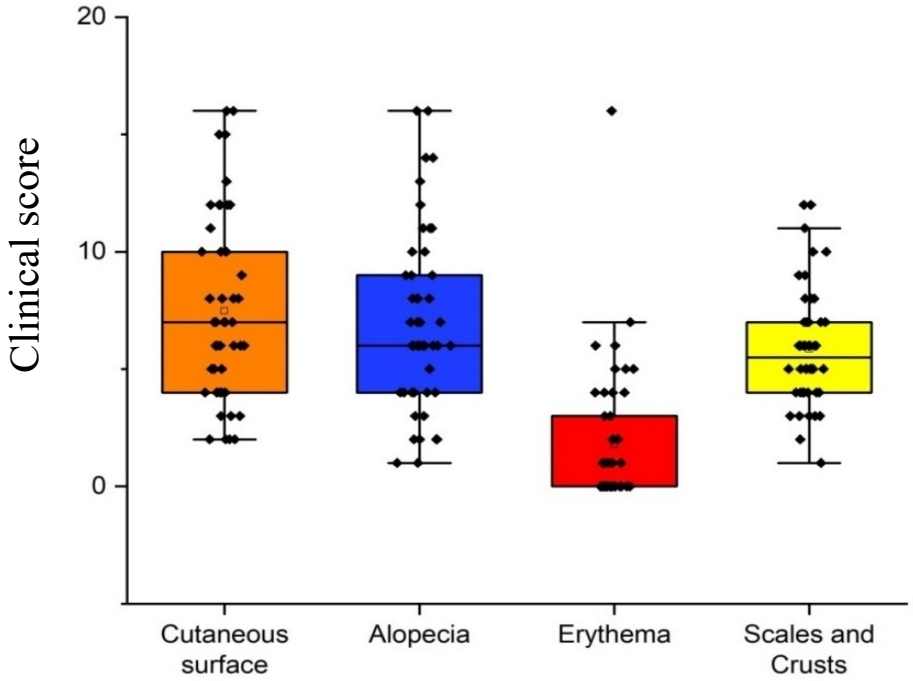



Figure 6.4- Clinical score per signs for the dogs

The most affected body part was the head, especially the ears (Fig 6.5-6.6).

Regarding the treatment correct administration and follow-up, only the 16 dogs treated with afoxolaner respected the requirements stated.

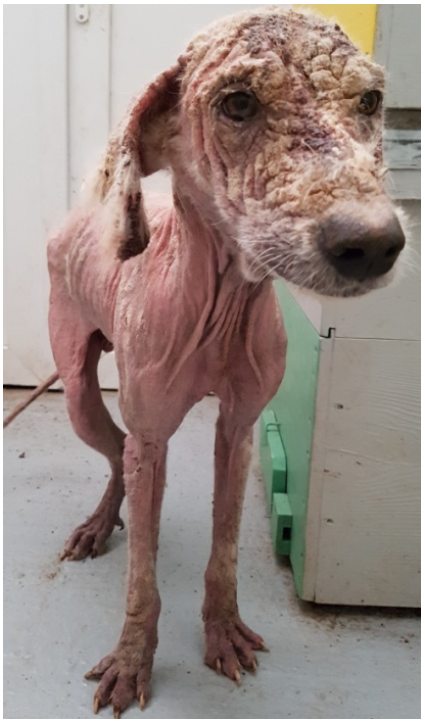


Figure 6.5- Clinical aspect- Extent of mange affected skin and crusts

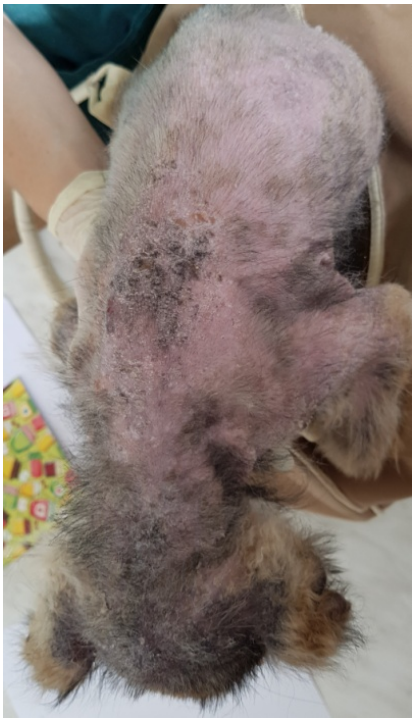


Figure 6.6- Clinical aspect- Erythema

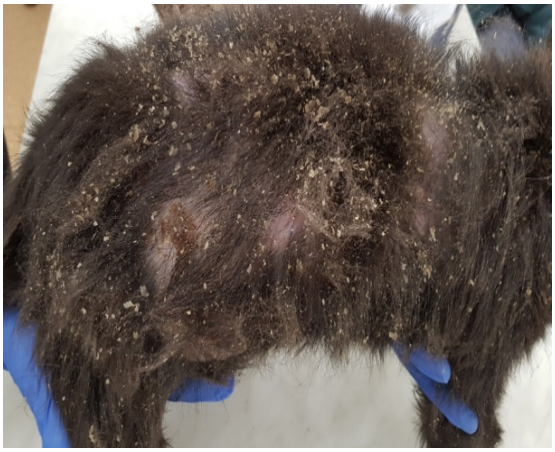


Figure 6.7 -Clinical aspect-Scales

Figure 6.8- Clinical aspect- Alopecia

## 6.3 Discussion

The number of dogs included in this study conducted between 2015 and 2018, was 48, which is similar to the epidemiological study Feather *et al.* performed in dogs in 2010, where they investigated retrospectively 42 dogs.

Including a larger number of dogs infected with *S. scabiei* could be done by a stronger association with the shelters in Moldova, even though the problem is that in most shelters, dogs are euthanized after 14 days, which makes conducting a long study rather difficult. A solution could be to contact private shelters, but they are usually very sensitive to the animal welfare, and avoid being implicated in experimental studies.

Another possibility would be to find more attractive ways to make the owner of the dog come back for the follow up (free treatment, other free analysis).

Regarding the diagnosis of *S. scabiei* infection, the absolute confirmation resides in the positivity of the skin scraping which shows different life stages of the mite, or its faeces. The problem, however, consists in the low number of mites generally observed in the samples (Miller *et al.*, 2012).

An enzyme−linked immunosorbent assay (ELISA) commercial test exists for the detection of Ig G antibodies to *S. scabiei* in dogs (Sarcoptes−ELISA 2001® Dog,AFOSA, GmbH, Germany). The sensitivity and the specificity of the test for dogs were 92.1% and 94.6%, respectively, after 3 weeks infection (Bornstein *et al.*, 1996; Lower *et al.*, 2001). In early infestations (it takes up to four weeks to develop an adequate titer), false negative results can occur, and false-positive tests can appear in atopic dogs sensitized to *Dermatophagoides* house dust mites, which share common antigens with *S. scabiei* (Schumann *et al.*, 2002). However, we did not use this method of diagnosis in our study, because of the low availability of the product in Romania and its high price.

Furthermore, different types of PCR tests can be performed to confirm the mite infection (Angelone-Alasaad *et al.*, 2015), but their cost are high, and the necessary materials are expensive, this being the reason why we did not use this diagnosis method. In perspective, it would be interesting to complete the results using these methods.

The techniques used to evaluate the clinical score in our study, were similar to those used in other studies, including evaluation of the alopecia, inflammation (erythema), crusts and scales. The score we used in the study had however a unique feature in evaluating the extent of the scabies affected skin. A negative aspect of our study was that we could not include a pruritus score, pruritus being an almost constant clinical sign in the evolution of sarcoptic mange in dog (Beugnet *et al.*, 2016; Taenzler *et al.*, 2016; Romero *et al.*, 2016; Becskei *et al.*, 2016; Hampel *et al.*, 2018).

The treatment protocol included only one authorized molecule at that time, for the treatment of sarcoptic mange in dogs, sarolaner. The reason why we did not give this treatment to all animals and decided to use the doramectin, was because of economic reasons, since most dogs were stray dogs, the persons taking care of them could only afford a limited sum of money for the treatment. As for the use of afoxolaner, we received a sponsorship from the Boehringer Ingelheim, consisting of money for the research and Nexgard® products for

different body weights of dogs, which resulted in a free treatment offered to some of the dogs infected with mange.

The predisposing factors that influence dogs on contacting sarcoptic mange are important for the diagnosis and control of the disease. In our study we analyzed age and sex.

From our study we observed that age is the most recurrent variable that seems to cluster around young dogs. The percentage of dogs less than one year examined was around 58.3%, which is similar to the findings published in other studies (Feather *et al.*, 2010; Chen *et al.*, 2014). Furthermore, they suggested that young dogs are more likely to be infected with *S. scabiei* mites because of their less developed immune system, their behavior of sniffing everything around them, their very sociable behavior, but also because of their close contact between each other and with their mother.

The gender distribution had no particular value since the males represented 48% of the studied population and the females slightly more with 52% of the population. This finding is consistent to other studies that state that sex was not a predisposing factor for contacting sarcoptic mange (Feather *et al.*, 2010; Curtis *et al.*, 2003).

Breed is not a variable to be interpreted in our study, since most dogs were common or crossbreeds.

Even though not a predisposing factor, but a source of contamination, we observed that contact with foxes could explain infection with *S. scabiei* mites, as we reported in the Boxer household, aspect observed also in 2010 by Feather *et al.*

On the frequency of the signs observed, alopecia, crusts and scales had the highest values, aspect that we also observed (Beugnet *et al.*, 2016; Taenzler *et al.*, 2016; Becskei *et al.*, 2016; Feather et. al., 2010).

Regarding the most affected body site of the dog, we found it was the head, especially the ears, which corresponds to the classic clinical signs which define the infection with *S. scabiei* (Miller *et al.*, 2012; Feather *et al.*, 2010; Kennis, 2004).

The results of the study are published in the BDI journal Lucrări Științifice USAMV Iași, seria Medicină Veterinară, Ed. "Ion Ionescu de la Brad" Iași, 2019, with the following authors and title: Raluca MÎNDRU, Constantin ROMAN, Andrei C. LUPU, Gabriela V. MARTINESCU, Larisa M. IVĂNESCU, Dumitru M. ACATRINEI, Ilie BODALE, Jacques GUILLOT, Liviu D. MIRON, *Epidemiology and clinical presentation of dogs infected with sarcoptic mange*.

# 7. EFFICACY ASSESSMENT OF AFOXOLANER IN DOGS INFECTED WITH *S. SCABIEI*

From the dogs previously described, some were included in an open pre-treatment versus post-treatment study (afoxolaner).

## 7.1 Materials and methods

This study was conducted using the same materials and methods as described in the previous chapter.

The differences consisted in the fact that skin sampling and clinical score were assessed at D0 and D28 and pictures were taken at the two time points. Also, skin scrapings were performed from 3 body sites (head, trunk and legs).

Furthermore, the most important part of the study was the treatment protocol which consisted in the use of the commercial product Nexgard® (afoxolaner), administrated orally according to commercial registration labeling, taking into account the weight of the animal, resulting in a dose between 2.7 and 6.9 mg/kg. The treatment was administrated at day 0 (D0) and at day 28(D28). All other in contact dogs were equally treated. The owners of the dogs consented in the off label use of the product, as at the time of the study, the molecule was not approved yet for the treatment of *S. scabiei* in dogs.

For some of the dogs, we have also collected blood samples through venipuncture sampling, using blood collection needle (pen type), and vacuum blood collection tubes with Clot Activator, for serum. The tubes were refrigerated and the serum was separated through centrifugation and then sent to a laboratory for biochemical analyses (urea nitrogen, creatinine, alkaline phosphatase, AST, ALT).

## 7.2 Results

The experimental unit was the individual dog. The dogs remained in the same environmental conditions as before the clinical signs of mange appeared, with the exception of one dog, which was hospitalized in our clinic (A10).

Finally, 16 dogs took part in this experiment, which were consulted and clinically evaluated at D0 and D28 (Table 7.1**)**.

They were mostly Common breed dogs, weighting between 2 kg and 35 kg, with an age range between 2 months and 7 years. There were equal number of males and females.

Table 7.1-Characterization of the dogs included in the study

| ID | Breed | Weight | Age | Sex | Location | In contact dogs |
|---|---|---|---|---|---|---|
| **A1** | Common | 14 kg | 4 Y | M | Vaslui (owner) | 19 dogs |
| **A2** | Common | 6 kg | 3 Y | F | Vaslui (owner) | 19 dogs |
| **A3** | Common | 6 kg | 6 Y | M | Vaslui (owner) | 19 dogs |
| **A4** | Common | 6 kg | 5 Y | F | Vaslui (owner) | 19 dogs |
| **A5** | Common | 10 kg | 6-7 Y | F | Vaslui (owner) | 19 dogs |
| **A6** | Common | 4 kg | 4 Y | F | Vaslui (owner) | 19 dogs |
| **A7** | Common | 8 kg | 3-4 Y | F | Vaslui (owner) | 19 dogs |
| **A8** | Common | 7 kg | 6-7 Y | F | Vaslui (owner) | 19 dogs |
| **A9** | Common | 2 kg | 4 m | M | Vaslui (owner) | 19 dogs |
| **A10** | Cross breed | 12 kg | 2 Y | M | Vrancea(stray dog) | / |
| **A11** | Common | 20 kg | 4 Y | M | Iasi, Public Shelter | / |
| **A12** | Common | 2 kg | 2m | M | Iasi (owner) | 1 dog |
| **A13** | Cross breed | 10 kg | 4 m | M | Suceava (owner) | 0 |
| **A14** | Common | 2 kg | 2m | M | Iasi Public Shelter | / |
| **A15** | Common | 18 kg | 4 Y | F | Iasi Public Shelter | / |
| **A16** | Cross breed | 35 kg | 5 Y | F | Iasi (owner) | 0 |

Among these 16, 9 of them belonged to the same countryside household in Vaslui County, which has been previously described (A1 to A9) (Fig 7.1).

Figure 7.1- Clincal aspect of dogs A6 (left) and A9 (right) at D0 (up) and at D28 (bottom)

An interesting case is A10, a stray dog from Vrancea County, which was brought to our clinic with severe skin lesions due to the infection with *S. scabiei*. The history of the dog was unknown. He was hospitalized for a long period of time, approximately 4 months until his fur was fully grown (Fig 7.2).

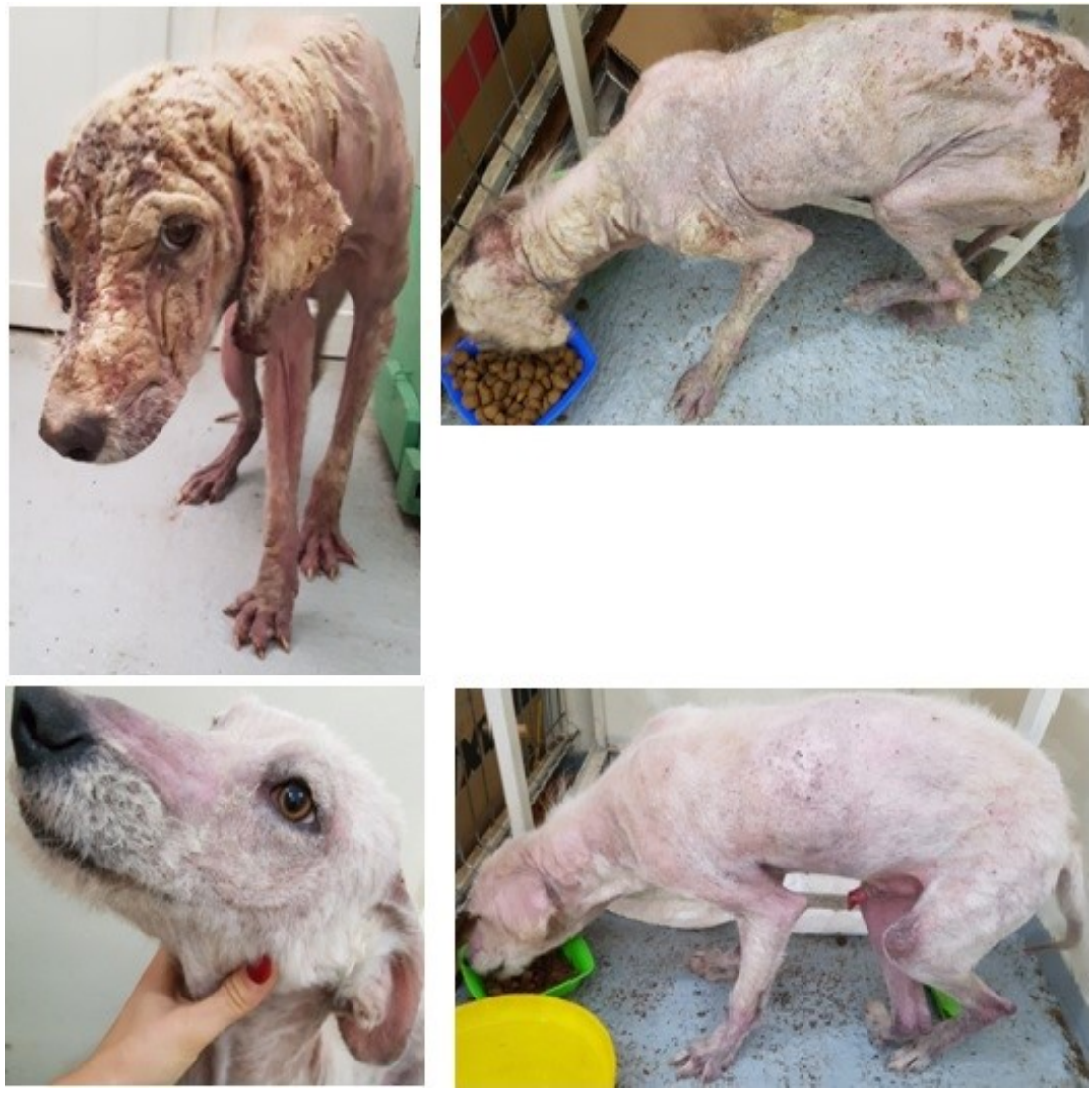


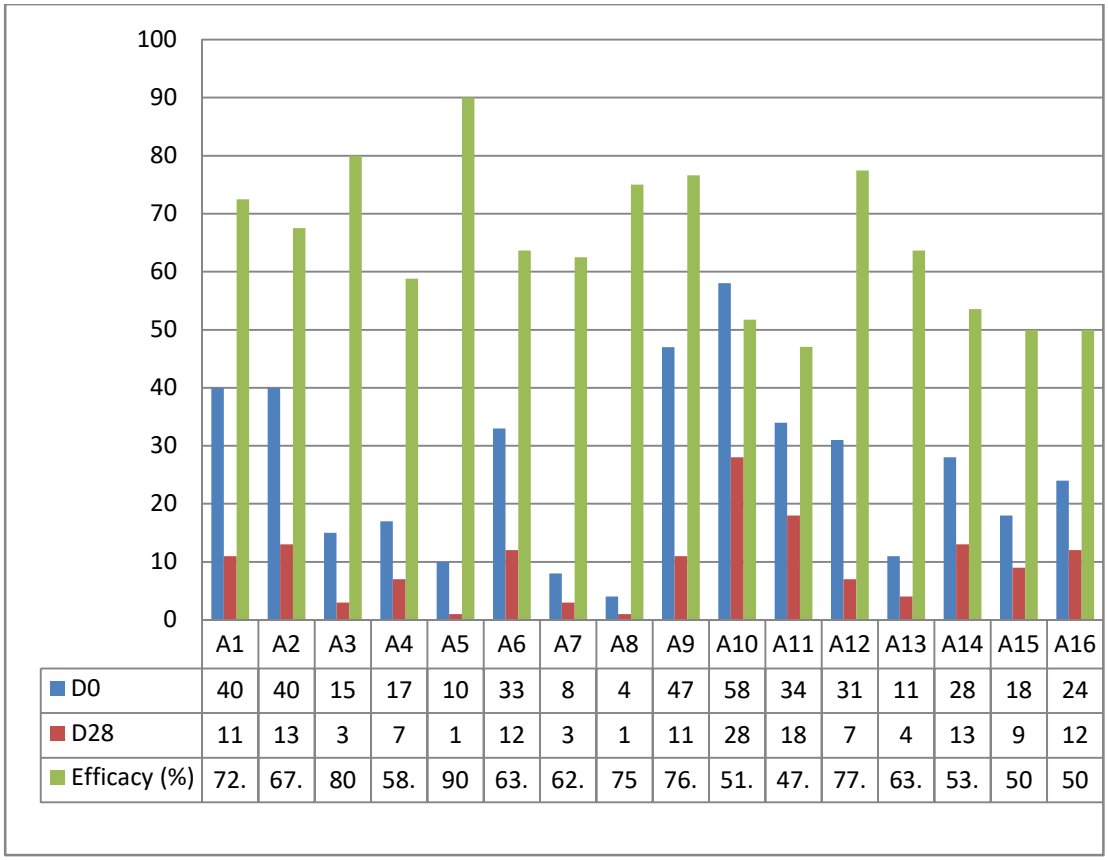
Figure 7.2- Clincal presentation of dog A10, at D0 (up) and at D28 (bottom)

Furthermore, we had included 3 dogs from Public shelters, an adult male (from Tomești shelter, A11) and from another shelter, one pupy and his mother (A14, A15).

Also, we had 2 dogs from different households from Iași, a puppy (A12) and an adult female (A16) infected with *S.scabiei*.

The evaluation of the efficacy of the treatment at D28 was performed by comparing the clinical score for each dog at D0 and at D28. Overall, we observed a significant resolution in clinical signs, the clinical score diminishing to 36.6% of its initial status (Fig 7.3). No clinical signs of drug intolerance were observed.

Maximum theoretical score is 60, and the dog A10 almost reached it, having a resolution of the clinical signs of 51.8% from the initial score by D28 (Fig 7.3). The scores were highly variable across individuals, because we included dogs that were highly affected by sarcoptic mange like A10, and dogs that showed very little signs of the disease like A8 (Fig 7.3).




| | A1 | A2 | A3 | A4 | A5 | A6 | A7 | A8 | A9 | A10 | A11 | A12 | A13 | A14 | A15 | A16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D0 | 40 | 40 | 15 | 17 | 10 | 33 | 8 | 4 | 47 | 58 | 34 | 31 | 11 | 28 | 18 | 24 |
| D28 | 11 | 13 | 3 | 7 | 1 | 12 | 3 | 1 | 11 | 28 | 18 | 7 | 4 | 13 | 9 | 12 |
| Efficacy (%) | 72. | 67. | 80 | 58. | 90 | 63. | 62. | 75 | 76. | 51. | 47. | 77. | 63. | 53. | 50 | 50 |

Figure 7.3- Clinical score of the dogs studied, at D0 and D28 and clincal efficacy of the treatment (%)

The clinical efficacy of the treatment was evaluated based on the resolution of the clinical score from baseline, calculated as [(clinical score at D0 –clinical score at D28)/clinical score at D0] * 100. Efficacy varied from 47% to 90% (Fig 7.3)

Furthermore, the total score per sign and the average score per sign was evaluated at D0 and D28 and compared in the following Figure 7.4.

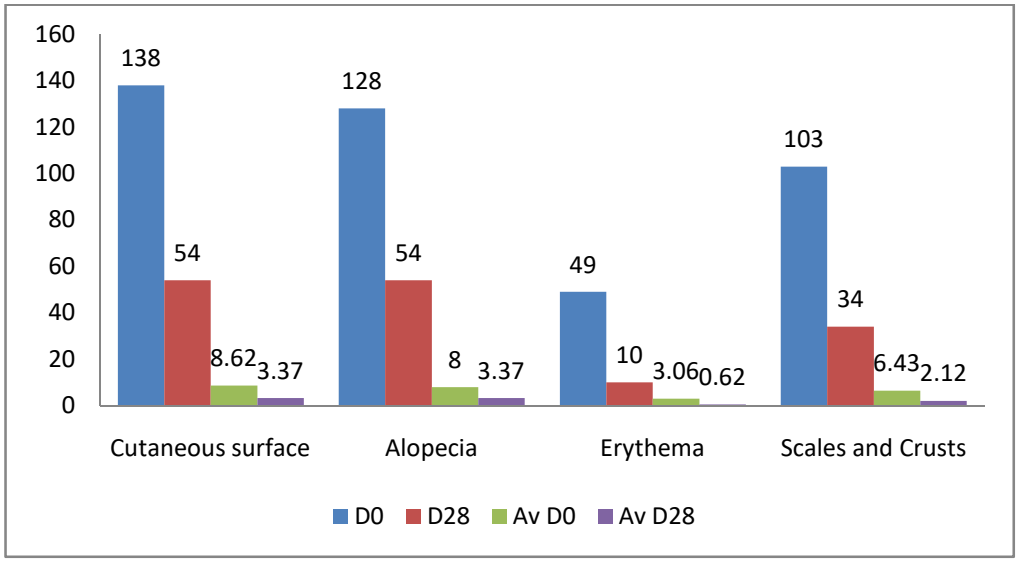



Figure 7.4- Total score per sign and average score per sign for the dogs studied

We can notice a significant improvement by D28 of all clinical aspects. This aspect is also presented in Fig 7.5 and Fig 7.6.

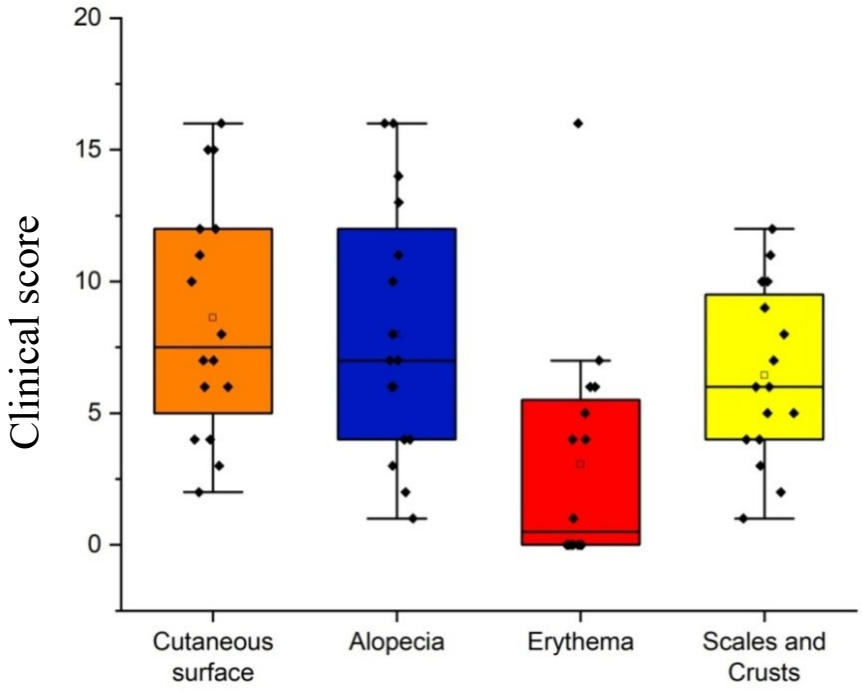



Figure7.5- Clinical score of the dogs, per clinical signs at D0

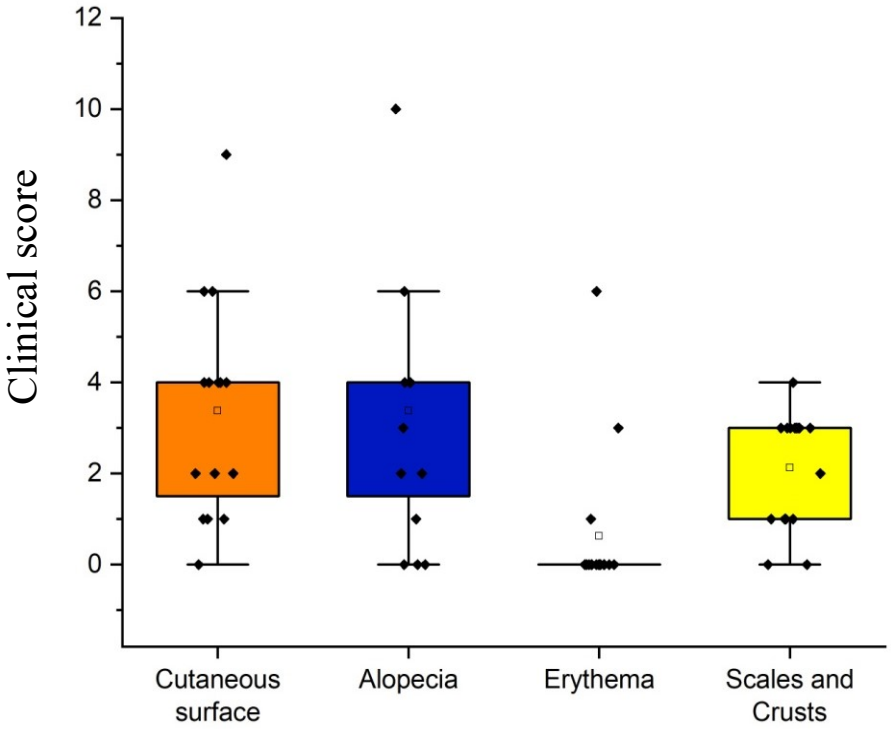



Figure7.6- Clinical score of the dogs, per clinical signs at D28

Even though our study did not include a mite count, we did however resample skin scrapings from the 3 body sites at D28 from all dogs and did not find any mites present.

For 10 of the 16 dogs studied (A1-A10), we have also collected blood samples and performed biochemical analyses (creatinine and urea nitrogen in 10 dogs; alkaline phosphatase, ALT, AST in 5 dogs) (Table 7.2).

Table 7.2-Results of the biochemical analyses performed in dogs A1-A10

| **Dog ID** | **Serum Biochemical Parameters analyzed** | | | | |
|---|---|---|---|---|---|
| | Creatinine(normal 0.5-1.7mg/dl, after Merck) | Urea nitrogen (normal 8-28 mg/dl,after Merck) | Alkaline phosphatase (normal 1-114 U/L, after Merck) | ALT (normal 10-109 U/L, after Merck) | AST (normal 13-15 U/L, after Merck) |
| A1 | 0.85 mg/dl | 22.4 mg/dl | 72 U/L | 24 U/L | 35 U/L |
| A2 | 0.74 mg/dl | 31.3 mg/dl | - | - | - |
| A3 | 0.72 mg/dl | 30.3 mg/dl | - | - | - |
| A4 | 0.63 mg/dl | 23.9 mg/dl | 109 U/L | 75 U/L | 34 U/L |

| Dog ID | Serum Biochemical Parameters analyzed | | | | |
|---|---|---|---|---|---|
| | Creatinine(normal 0.5-1.7mg/dl, after Merck) | Urea nitrogen (normal 8-28 mg/dl,after Merck) | Alkaline phosphatase (normal 1-114 U/L, after Merck) | ALT (normal 10-109 U/L, after Merck) | AST (normal 13-15 U/L, after Merck) |
| A5 | 0.77 mg/dl | 42.1 mg/dl | 38 U/L | 35 U/L | 35U/L |
| A6 | 0.57 mg/dl | 35.5 mg/dl | - | - | - |
| A7 | 0.8 mg/dl | 27.4 mg/dl | - | - | - |
| A8 | 0.59 mg/dl | 38.5 mg/dl | - | - | - |
| A9 | 0.29 mg/dl | 18.4 mg/dl | 111 U/L | 27 U/L | 22 U/L |
| A10 | 0.44 mg/dl | 40.2 mg/dl | 28 U/L | 24 U/L | 37 U/L |

### 7.3 Discussion

The number of the dogs included in the present study was 16, which is a number comparable to that of the first study of afoxolaner in dogs infected with *S. scabiei*, which included 20 dogs from South Africa divided into a treatment group (treated with the product Nexgard® at D0 and D28 according to body weight) and a control group (Beugnet *et al.*, 2016). The most recent study from Hampel *et al.* (2018), included 65 dogs from Portugal and Germany, among which, 38 were treated with Nexgard® at D0 and D28 and 27 withNexgard Spectra® (afoxolaner and milbemycin oxime) at D0 and D28 according to body weight.

Regarding the diagnosis method used in the two studies on the efficacy of afoxolaner on *S. scabiei* mites in dogs, it consisted on identifying positive skin scrapings from 5 different body sites/dog. Comparing to our study, the diagnosis method used was the same, but the number of body sites included was less in our research (3 compared to 5).

The clinical score we used included an original aspect by evaluating the extent of the skin affected by sarcoptic mange. Also, we evaluated the degree of erythema present on the skin. What our study has in common with the other two studies mentioned, is the evaluation of the alopecia (hair loss) and crusts and scales. However, a downfall is that we did not include a pruritus score, this clinical sign being an important evaluating factor for the efficacy of the studied molecule, as shown by the two studies.

Furthermore, the evaluation of the dogs was made at D0 and D28 in our experiment, comparing to the evaluation made by the other authors at D0, D28 and after the second treatment at D56.

Concerning the treatment, afoxolaner, a member of the isoxazoline family, is a relatively newly introduced molecule, a safe drug, effective against fleas, ticks and most recently approved for the treatment of other external parasites, such as mites (*Demodex* spp. *and S. scabiei,* 2018) (Beugnet *et al.*, 2014a, 2014b; Dumont *et al.*, 2014; Letendre *et al.*, 2014; Lebon *et al.*, 2018). At the moment of our study, the product was not officially approved for the treatment of sarcoptic mange in dogs.

The final results of these studies are similar to our findings, showing a very high efficacy of afoxolaner. In both studies, mite counts were used as primary criteria in evaluating the success of the treatment.

Even though we did not include this step in our study, we did observe a complete disappearance of the mites at D28.

Furthermore, in these studies the efficacy was assessed also by comparing the score of the characteristic lesions in sarcoptic mange at the beginning of the trial D0, at the time of the second administration of afoxolaner D28, and at the end of the treatment at D56. The clinical signs were already significantly improved at D28, which is consistent with our findings.

The results of the blood analyses of the 10 dogs (Table 7.2) showed an increase in the urea nitrogen in half of the dogs. However, this value was not correlated with a rise in the creatinine level, meaning that there were no important renal dysfunctions. We also observed an increase in the aspartate aminotransferase (AST).

Kido *et al.* (2011) performed a study on racoon dogs affected by sarcoptic mange. Their conclusions were that the debilitated, weakened racoons presented a rise in some biochemical parameters of the blood, especially in urea nitrogen, AST, bilirubin, sodium, chloride and phosphor, compared to the non-debilitated racoon dogs affected by sarcoptic mange. We can corelate these results with the fact that the dogs present in our study, especially the young and old ones, had a rather poor condition.

Another study regarding this subject, from Beigh *et al.*(2016), examined the effect of sarcoptic mange on liver function and oxidative stress in dogs. They reported elevated levels of liver injury biomarkers (ALT and AST) and bilirubin, and lower levels of albumin, glucose and cholesterol, meaning that the liver function was altered. This is consistent with our findings, regarding the high level of AST.

The present study is published in the ISI Proceedings of the International Scientific Congress ”Life sciences, a challenge for the future”, 2019, p.426-431, with the following authors and title: MÎNDRU Raluca,ROMAN Constantin, LUPU C. Andrei, Gabriela MARTINESCU, Larisa IVĂNESCU, ACATRINEI M. Dumitru, GUILLOT Jacques, MIRON Liviu, 2019 - *Efficacy Assessment of Afoxolaner (Nexgard®) in Dogs Naturally Infected with Sarcoptes scabiei.*

Furthermore, part of these results was presented in the 27th Conference of the World Association for the Advancement of Veterinary Parasitology (WAAVP), which took place from the 7th to the 11thof July 2019 in Madison, WI, USA. The title of the oral presentation was *Clinical Efficacy of Afoxolaner in Dogs Naturally Infected With Sarcoptes Scabiei and Concomitant Modifications of the Skin Microbiota,* and the authors were Raluca MÎNDRU, Andrei Cristian LUPU, Constantin ROMAN, Charlotte BERNIGAUD, Amaury BRIAND, Frédéric BEUGNET, Katja FISCHER, Liviu MIRON, Jacques GUILLOT.

# 8. MODIFICATION OF THE SKIN MICROBIOME IN DOGS WITH SARCOPTIC MANGE

The skin microbiome consists of the microbes (bacteria, viruses, fungi, archaea, and parasites), their gene products and metabolites in a defined habitat (Marchesi *et al.*, 2015). It plays an important role in maintaining the health and functions of the skin, making its understanding essential.Furthermore, the resident bacteria/fungi present on the surface of the skin protect the host by restricting the access of pathogenic bacteria/fungi. The *S. scabiei* mites burrow the skin of animals, causing a breach in the skin barrier that can possibly promote the development of opportunistic bacteria/fungi, leading to secondary pyoderma. The concomitant modification of the skin microbiome in dogs infected by *S. scabiei* mites has not been studied until present.

## 8.1 Materials and methods

The dogs included in the study had to present evocative clinical signs of mange (pruritus, crusts, alopecia) and to have positive skin scraping tests.

A specific dermatological questionnaire adapted from the human medicine was addressed to the owner, including age, sex, habitat, history of other dermatological diseases, treatment received in the last month

A skin scraping test was then performed by the method described in the previous chapter to confirm the presence of *S. scabiei* mites.

The questionnaire continued with the clinical exam concentrating on sarcoptic mange lesions, severity of the lesions, associated pyoderma, and severity of pyoderma, other type of skin lesions. The last part consisted of the microbiome study, showing the precise sampling spots that have been used (Annex 1).

The dogs were also given a general health exam.

The sampling method for the microbiome study was divided into 2 parts: the scraping and the swabbing of the skin. Firstly, it was important that the dog's skin had not been washed or sterilized before the sampling procedure and that the operator wore gloves.

For the scraping, a green 7 mm Kai® disposable dermal curette was used to scrape an approximately 2cm$^2$ area of the epidermal layer on 1 scabies-infected site and its controls in healthy skin (ears area). The scraping needed to be done not too deep to provoke bleeding, and not too far away from the scabies lesions, to avoid diluting the sample with healthy skin bacteria. The scraping was done vertically, horizontally and in both diagonals for a 30 s' time.

The scraping was repeated on another anatomical site in both scabies infected site and control in healthy skin (leg area). The curettes and gloves were changed between each sample.

After the collection of material, the curette was introduced in a 2 ml tube (containing 200 µL of lysis buffer) and stirred for at least 15 s.

For the swabbing- a sterile cotton swab was dipped in a plastic falcon tube containing BHI+ 50% glycerol. All the areas from which scraping had been made needed to be swabbed

with different swabs. The swab was then put back into the falcon tube with the wood shift broken to fit the tube.

The tubes were labeled with the dog ID, the place of the sampling and the date and put in the freezer at -20 degrees Celsius. The exact places where the samplings have been made at first visit were noted on a dog silhouette present at the end of the questionnaire.

The owners agreed that the dogs would be seen and sampled again at D28, when a second questionnaire regarding the evolution of the lesions would be completed (Annex 2).

**Targeted metagenomics protocol**

These analyses were performed on the metagenomics platform, NGS Platform, InstitutMondor de Recherche Biomédicale (IMRB), Créteil, France.

**-Bacterial microbiome -16S metagenomic sequencing**

The operational method was developed starting from Illumina "16S Metagenomic Sequencing Library Preparation ", Document #15044223 Rev.B of 27 November 2013 and adapted to the analyses of skin microbiome. This protocol is usually used on the metagenomic platform. We selected from the 9 hypervariable regions coding the 16 S rRNA gene, a fragment containing the regions V3-V4, fragment which is validated through the studies on the skin microbiome (Zeeuwen *et al.*, 2012).

a) Amplicon PCR

Amplification was made with the kit KAPA HiFi HotStart Ready Mix 2x, using the following protocol (Table 8.1-8.2).

Table 8.1-Composition of mix for PCR

| Mix PCR | Volume (25 µl in total) |
|---|---|
| KAPA HiFi HotStart Ready Mix 2x | 12.5 µl |
| Primer Forward 1µM* | 3 µl |
| Primer Reverse 1µM** | 3 µl |
| DNA pure extract | 6.5 µl |

Table 8.2-Temperature protocol for PCR

| Temperature | Time | Cycles |
|---|---|---|
| 95°C | 3 min | 1 cycle |
| 95°C<br>53°C<br>72°C | 30 s<br>30 s<br>30 s | 35 cycles |
| 75°C | 5 min | 1 cycle |
| 4°C | ∞ | ∞ |

***V3V4341F modified:**
5'- TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGAGGCAGCAG -3'
****V3V4785R modified:**
5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACCAGGGTATCTAATCC-3'

b) PCR Clean Up 1

This step is made by using the Hamilton® automate. It uses AMPure XPR (Beckman Coulter®, ref: A63881 Agencourt AMPure XP, 60mL) beads for purifying the 16S V3-V4 amplicons from free primers and primer dimers.

c) Indexing the samples

This step consists of indexing the samples (dual indices) with the help of the kit Nextera XT Index Kit®, in order to identify the samples during the analytical demultiplexing. Dual indexing was used. 2.5 µl of Amplicon PCR product was transferred to a new plate with 96 wells. For easy handling, an IlluminaTruSeq Index Plate Fixture® rack was used. 2.5 µl of each Index 1 primer (i7) was put in each column and 2.5 µl of each Index 2 primer (i5) was put in each row. Then, 12.5 µl of KAPA HiFi HotStart ReadyMix 2x®(Roche ref: 07958935001) was added to each well containing the Amplicon PCR product and Index primers and also 5 µl of milliQ water.

After that, we centrifuged the plate at 1000 x g, at 20°C, for 1 min. The total volume from each well was 25 µl. The amplification program of the thermal cycler was similar to that of the amplicon PCR (Table 8.2), except for the time of amplification (8 cycles) and the hybridization temperature (55°C).

d) PCR Clean Up 2

This step is made by using the Hamilton® automate. It uses AMPure XPR beads for cleaning the final library before quantification.

e) Normalization and pooling

- Verifying the quantity and quality of the samples

A measurement of samples' absorbance on the Varioskan® was made. Starting from the concentrations indicated by the Varioskan®, a calculation of the DNA concentration (in nM), based on the size of the DNA fragments, was performed.

We used the following formula:

$$\frac{(concentration\ in\ ng/\mu l)}{(660g/mol \times average\ library\ size)} \times 10^6 = concentration\ in\ nM$$

-the average library size for V3-V4 is 580 bp

Once we had the concentrations, we calculated the volume of Tris-HCl 10mM, pH8.0, with 0.1% of Tween 20®, necessary for normalizing the samples, with the following formulas:

$$\frac{(Initial\ concentration\ in\ nM \times Initial\ volume\ in\ \mu l\ )}{Final\ concentration\ in\ nM} = Final\ volume\ in\ \mu l$$

$$Final\ volume - Initial\ volume = \ Volume\ of\ Tris - HCL$$

The final concentrations were of 20 nM for the 16S meta V3-V4 library. The initial volume represented the volume of sample, which was 5 µl.

- Normalization of samples

We prepared Tris-HCl pH 8.0, at 10 mM , with 0.1% Tween 20® (9890 µl of milliQ water, 100 µl of Tris-HCl 1M, pH 8.0, 10µl of Tween 20®). We transferred 5 µl of each sample to a new MIDI plate® and added the previously calculated volume of Tris-HCl 10 mM, pH 8.0, with 0.1% Tween 20® in order to normalize the concentration of the sample in each well of the plate at 20 nM.

- Pooling design

The samples from the 16S meta library were normalized at 20 nM. Then, the samples were brought together. Once the pool was formed, we diluted the samples at 4 nM, by making a dilution of $1/5^{e}$ (20 µl of pool at 20 nM, 80 µl of Tris-HCl 10 mM, pH 8.0, with 0.1% Tween 20®).

A control library was also formed. The Phix control was diluted from 10 nM to 4 nM (10 µl of Phix 10 nM, 15 µl of RSB (Resuspension Buffer), or Tris-HCl 10 mM, pH 8.0, with 0.1% Tween 20®) and added at the 4 nM samples pool (5µl of Phix at 4 nM).

f) Sequencing on MiSeq Illumina

- Denature DNA

The following volumes were combined: 5 µl of pooled final DNA library at 4 nM and 5 µl of NaOH 0.2N, then vortexed and centrifuged at 280 g for 1 min. An incubation of 5 min at room temperature is necessary to denature the DNA into simple strands. 990 µl of pre-chilled HT1 was added to 10 µl denatured DNA. Adding the HT1 results in a 20 pM denatured library in 1 mM NaOH.

- Diluting the denatured DNA

A dilution of the denatured DNA at the desired concentration of 8pM, was made by combining 240 µl of the denatured library at 20 pM with 360 µl of pre-chilled HT1. The mix was vortexed and centrifuged at 280 g for 1 min.

The reagent cartridges were loaded after deposing 600 µl of the 8 pM dilution.

- Launching the MiSeq sequencer

The flow-cell, the Buffer (PR2), the reagent cartridge (MiSeq Reagent Kit v3, 600 cycles PE (Reference: 15043895) and the sample sheet were then loaded. The duration of the sequencing was of 56 hours. The expected depth was about 30000 sequences per sample.

**-Fungal microbiome-sequencing of the internal transcribed spacer (ITS)**

- Extraction and controls

An unbiased DNA-RNA extraction procedure was applied to all samples before performing targeted metagenomics. Briefly, pre-extraction combining bead beating, chemical cell disruption with detergent, and Proteinase K lysis was followed by extraction using DSP virus-pathogen kit on QiaSymphony (Qiagen, Hilden, Germany). A negative control was tested in each run and positive controls were used to evaluate the performance of the metagenomics techniques.

- Targeted metagenomics

Targeted Metagenomics included the study of two amplicon libraries: two ribosomal fungal internal transcribed spacer (ITS) regions ITS1 and ITS2 (Sitterle *et al.*, 2017). Each amplicon was prepared from 5 µL extract following the "16S Metagenomic Sequencing Library Preparation protocol" provided by the manufacturer (Illumina, San Diego, CA, USA). For each library, the quality was evaluated using a D1000 ScreenTape on a TapeStation (Agilent, Santa Clara, CA, USA) and the quantity using the Quant-it dsDNA Assay kit (ThermoFischer, Waltham, MA, USA) on a Mithras LB 940 (Berthold Technologies, Bad Wildbad, Germany). All libraries were normalized to 4 nM, pooled, and denatured before pair-end sequencing (v3, 2 x 300 bp) on a MiSeq device (Illumina, San Diego, CA, USA). The targeted fungal regions were sequenced according to the manufacturer's instructions.

**Bioinformatics analyses**

The analyses and quality control steps were performed with Snakemake® (version 5.4.5) in 3 stages:

- Selection of sequences for the following analyses, keeping sequences and bases of sufficient quality.

We eliminated also the non specific amplified sequences.

-Chimeras were eliminated

-Creation of OTUs (operational taxonomic units) for the diversity analyses using QIIME® (version 2019.1). A dereplication was performed, then the OTUs were formed, taking into account the sequencing errors.

The OTUs were identified using Blast+ (version 2019.1) and the data base SILVA (version 128).

The diversity analyze was made using:

-the alpha diversity measured with quantitative indicators (Number of OTUs, Chao1) and qualitative indicators (Shannon and Simpson scores). The groups were compared with the Kruskall-Wallis test.

The alpha diversity (whitin sample) is a measure that calculates the number (richness) and distribution (evenness) of taxa within a sample. The observed OTUs is a richness metric, showing the number of observed species within a sample.

The Shannon diversity is a metric, considering evenness and abundances of different OTUs (Lozupone & Knight, 2008).

-the beta diversity measured with one quantitative indicator (weighted Unifrac) and one qualitative indicator (unweighted Unifrac). The groups were compared using the PERMANOVA test.

The beta diversity (between samples) measures used to estimate shared OTUs (microbial species) between different samples and/or subjects. Data are often presented as Principal Coordinates Analysis (PCoA) plots (used to show dissimilarities). These can be

phylogenetic based, which take into account microbial evolution (Unifrac distance metric) or OTU based (Lozupone & Knight, 2008).

UniFrac measures the phylogenetic distance between sets of taxa in a phylogenetic tree. Its important role is to determine whether communities are significantly different, to compare communities simultaneously using clustering and ordination techniques (Lozupone & Knight, 2005).

**Data interpretation program**

"Calypso is an easy-to-use online software, allowing non-expert users to mine, interpret and compare taxonomic information from metagenomic or 16S rDNA datasets. The software is free for academic use.

Calypso has a focus on robust multivariate statistical approaches that can identify complex environment-microbiome associations, whereby differences in microbial composition can be attributed to multiple environmental variables. Powerful visualization techniques are provided, generating high quality figures that can readily be used in scientific publications. Calypso enables, at all taxonomic levels and for large taxonomic datasets, quantitative visualizations and comparisons of composition (e.g. bubbleplots, interactive hierarchical trees, Krona plots and heatmaps), parametric and non-parametric statistical tests, univariate and multivariate analysis, supervised learning, factor analysis, multivariable regression, network analysis, and diversity estimates. To render the data suitable for analysis by standard statistical procedures, Calypso can transform and normalize community profiles using various methods, including log transformation and total-sum normalization. Since Calypso does not require scripting or programming skills, it enables all microbiology researchers to routinely apply advanced statistical analysis in their work" (Zakrzewski *et al.*, 2016, http://cgenome.net/calypso/).

For analyzing the data in Calypso, a data normalization and transformation was required. This was made using total sum normalization (TSS) combined with square root transformation (Hellinger transformation). TSS normalizes count data by dividing feature read counts by the total number of reads in each sample. The method converts raw feature counts to relative abundance.

## 8.2 Results

There were 5 dogs included in this study, all of them from the 48 described in the epidemiology study. From these 5, 3 were among the 16 dogs treated with afoxolaner (A1-Fig 8.1; A2-Fig 8.2, A4-Fig 8.3).

They were 2 females and one male, common breed, aged between 3 and 5 years old, from the same household from Vaslui county. These dogs complied with the study and repeated the sampling 4 weeks after the first treatment, at D28.

Figure 8.1- Clinical aspect of dog A1 at D0 (up) and at D28 (bottom)

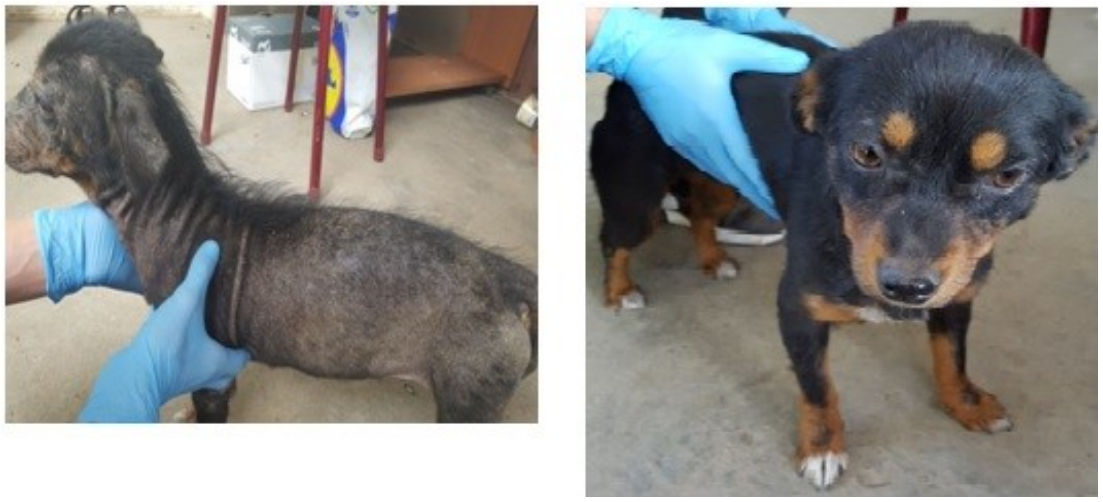


Figure 8.2- Clinical aspect of dog A2 at D0 (left) and at D28 (right)

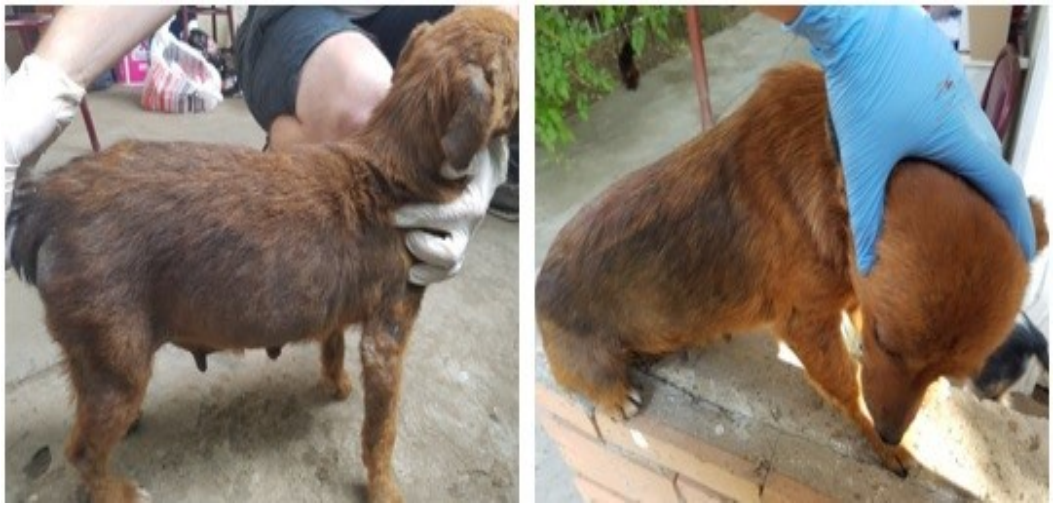


Figure 8.3- Clinical aspect of dog A4 at D0 (left) and at D28 (right)

The other 2 dogs included were a 6 year old Boxer mother (A29-Fig 8.4) and its 5 weeks old male puppy (A23-Fig 8.4), which belonged to a household with 6 other puppies. These dogs did not return for the resampling at D28, which means their results only reflect the situation of the skin microbiome before treatment.

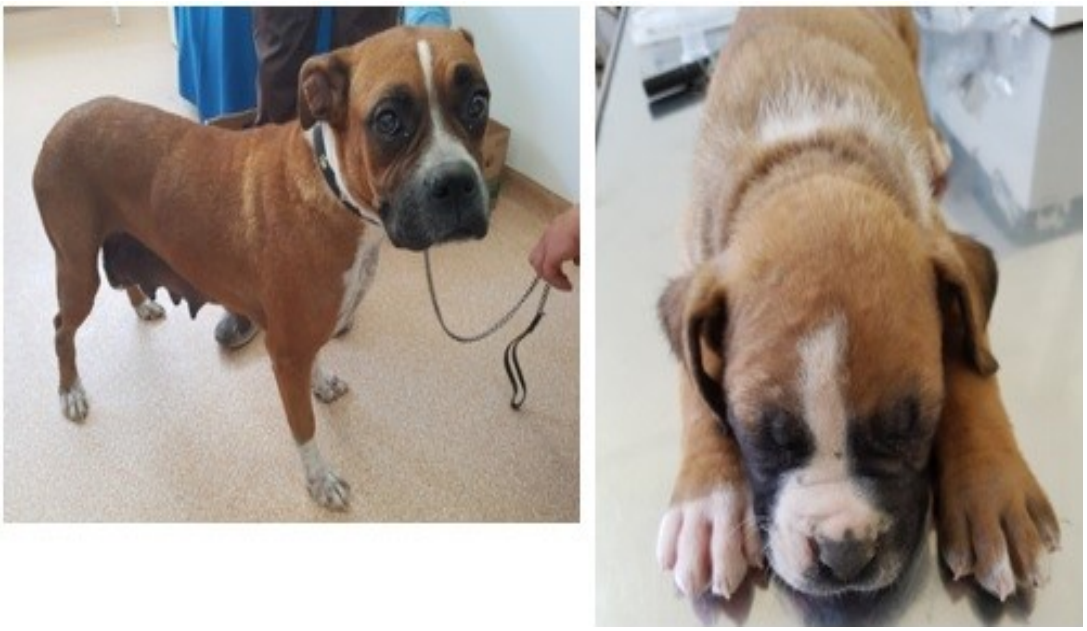


Figure 8.4- Clinical aspect of dog A29 (left) and A23 (right) at D0

At the general examination, no modification of the health status was observed.

Concerning the dermatological questionnaire at the first visit, we noted that all dogs received no treatment one month before the sampling took place, they had other in contact dogs and they also had flea infestation. Regarding the extent of the sarcoptic mange, the first two dogs had a great severity of lesions, affecting the entire body, while the other 3 had moderate degree of lesions. None of the dogs had associated pyoderma.The pruritus score was not assessed.

At the second visit, which took place only for the first 3 dogs, we observed that the lesions were still visible, but they were very mild (Fig 8.1-8.3).

In total, 32 samples were collected from 2 body sites (ear and leg-1affected skin site+ its control in healthy skin), at D0 and D28 for the first 3 dogs and only at D0 for the Boxers.

For all of the samples, we studied the sequencing of the bacterial 16S rRNA gene and the sequencing of the ITS gene.

We divided the presentation of the results in two main subchapters, based on the main differences observed in the changing of the microbiome. We first compared the dogs by grouping them into the two main breeds (Common breed and Boxer) studying the microbiome before any treatment, at D0. Secondly, we included only the dogs that received treatment, and compared their results before and after the first treatment with afoxolaner (D0 and D28).

### 8.2.1 Comparison of breed

a. Bacterial microbiome

Quality of samples

The total number of raw sequences analyzed was 2 536 321 (approximately 76 858 per sample). The quality of the samples was appropriate. 99.93% (99.75-100%) of the OTUs were identified up to genus. In fact, the general characteristics of both analyze and technique, usually allowed us to identify up to genus level.

We observed that the composition of the bacterial skin microbiomein the Boxers were different than that of the other 3 dogs studied, which were Common breed (Fig 9.5). Even though the main genera present are similar between the 2 groups, it is the relative abundance that differed, the Boxers showing greater abundance of: *Bacteroides, Psychrobacter, Corynebacterium, Lactobacillus, Conchiformibius, Brachybacterium, Brevibacterium, and Streptococcus.*

Furthermore, another difference is that the Boxers had a lower number of bacteria than the common dogs, from the following genera: *Pseudomonas, Nocardioides, Paracoccus,Pontibacter, Chitinophagaceae, Planomicrobium, Mycoplasma, Knoellia.*

However, the main genus that inhabits the skin of the 2 Boxers infected with mange is *Staphylococcus*, which is present in a similar abundance in the Common dogs.

However, we must also take into consideration the strong connection between the two Boxers, mother and little puppy (5 weeks old), who were in close contact, resulting in a very similar microbiome.

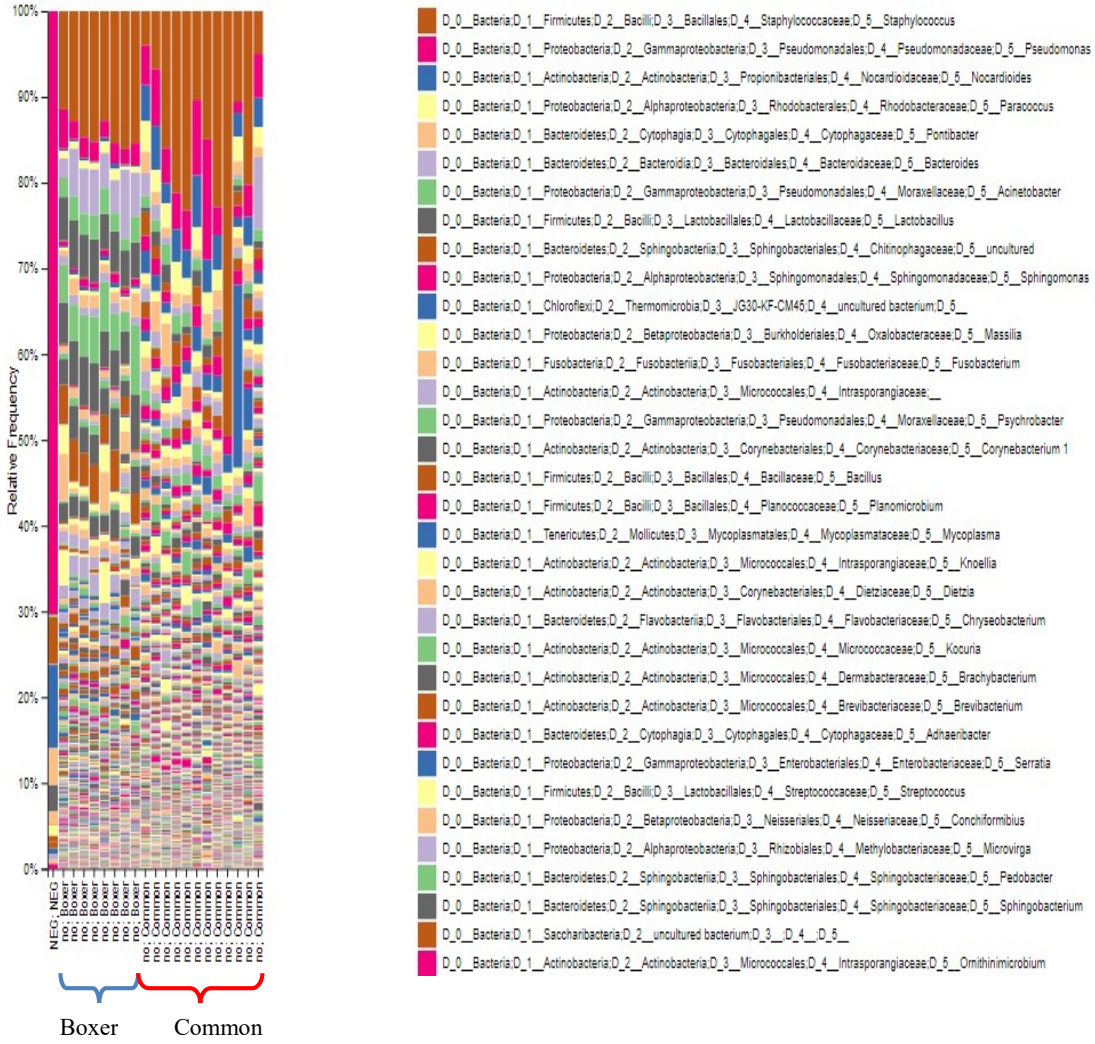



Figure 8.5- General barplot of the main bacteria present in Boxer and Common dogs

The rarefaction analysis, which evaluates speciesrichness in the samples, revealed a slight difference between the two studied breeds, with a higher richness of observed OTUs in Common dogs than in Boxers (Fig 8.6).

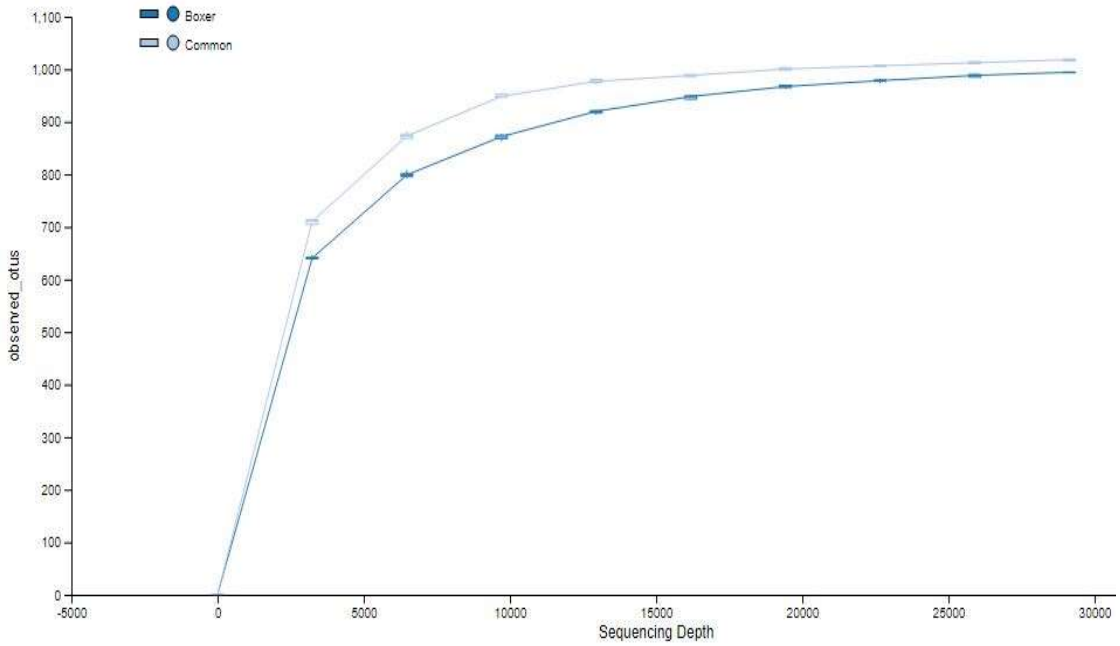



Figure 8.6-16 S- Alpha rarefaction

In this case, the Shannon index showed greater microbial community diversity in Common dogs, than in Boxers (Fig 8.7).

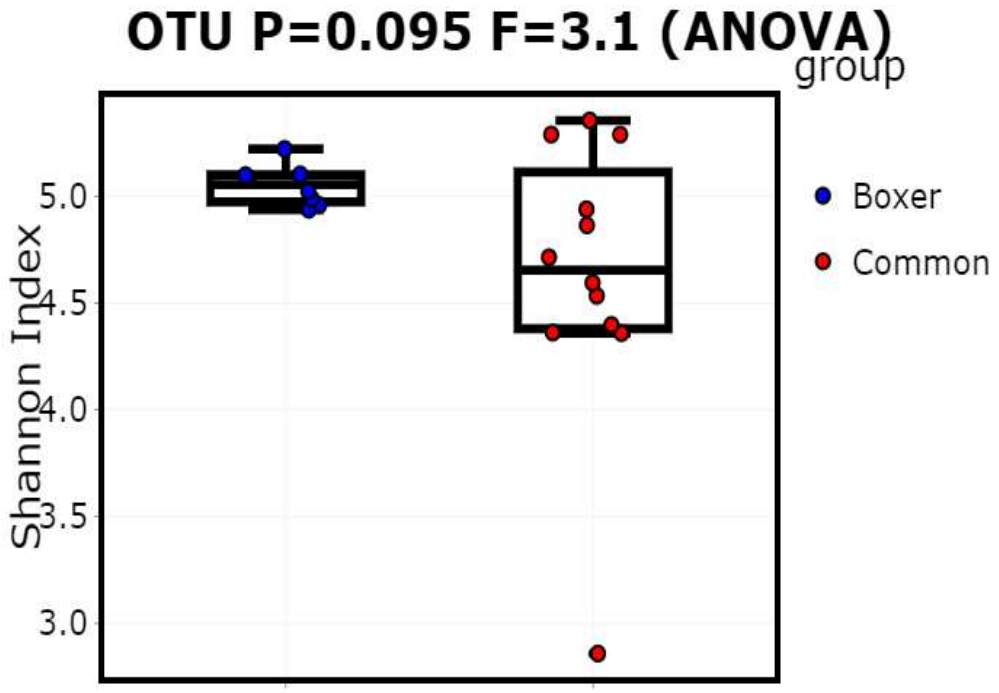



Figure 8.7-16 S- Shannon index

The PCoA plot presents individual or group differences and we can easily distinguish the clustering of individuals in the two main breeds- one group for Boxers and one group for Common breed (Fig 8.8).

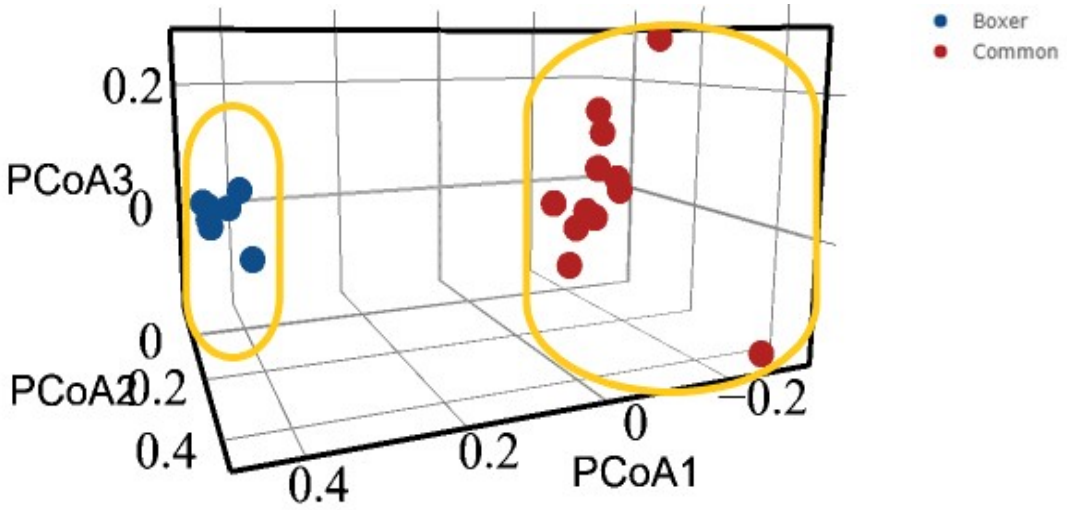



Figure 8.8-16 S-PCoA graphic

The β diversity weighted UniFrac (quantitative) takes into account the relative abundance of species/taxa shared between samples. We can observe a clear separation of the two groups representing different breeds (Fig 8.9).

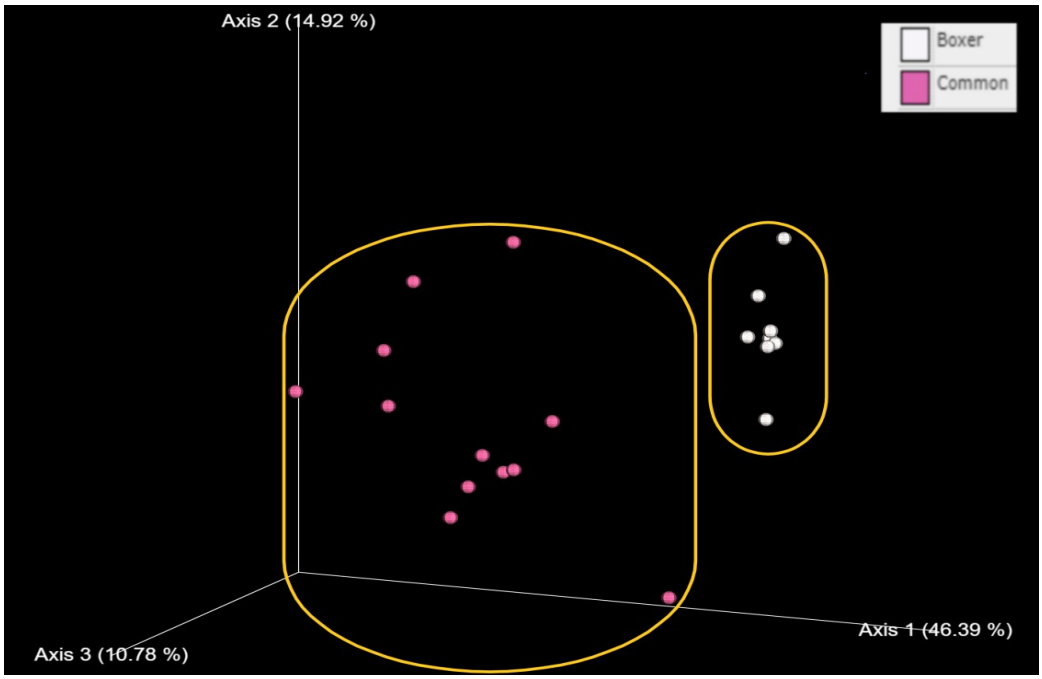



Figure 8.9- 16 S- β diversity weighted UniFrac

Regarding the β diversity unweighted UniFrac (qualitative), it only considers the presence or absence of species/taxa shared between samples, without taking into consideration the abundance (http://www.metagenomics.wiki/pdf/definition/alpha-beta-diversity). The similarities appeared mainly because the addition of rare taxa rather than abundant taxa (Fig 8.10). We can observe the same clear cluster of the samples into the group of common dogs and into the group of Boxers.

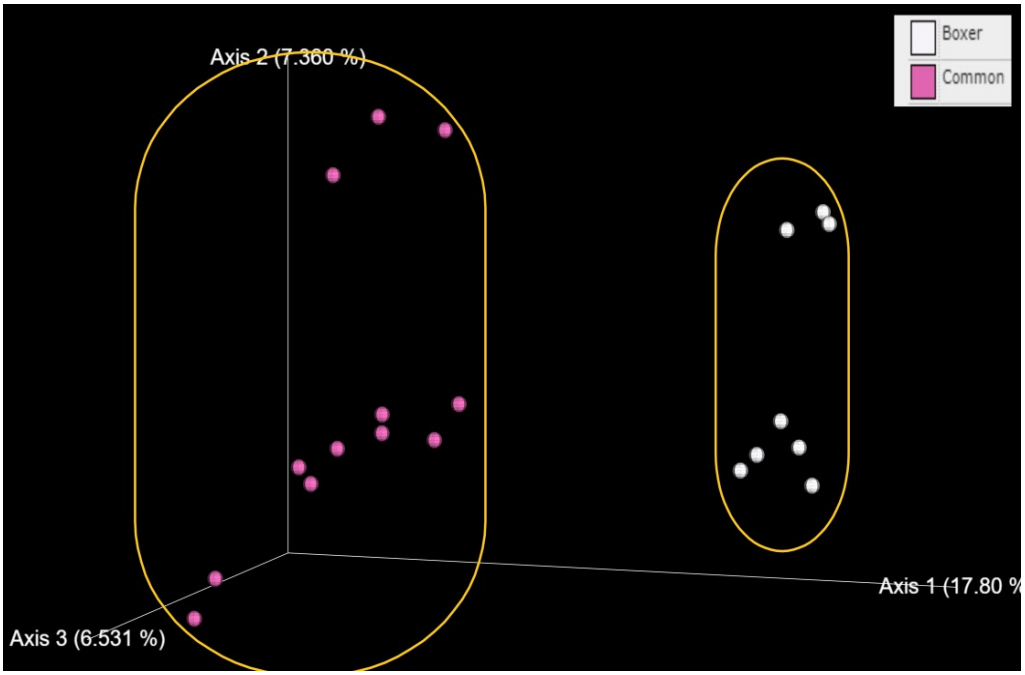



Figure 8.10-16 S- β diversity unweighted UniFrac

b- Fungal microbiome

Quality of samples

The total number of raw sequences analyzed was 3 174 268 (approximately 96207 per sample). The quality of the samples was appropriate. 96.34% (86-100%) of the OTUs were identified up to genus. In fact, the general characteristics of both analyze and technique, usually allowed us to identify up to genus level.

We observed that the composition of the fungal skin microbiome is similar between the two breeds studied, yet it is the relative abundance of the species that offers the difference between the Boxers and Common dogs (Fig 8.11).

The main phylum present was similar between the 2 groups, with a higher relative abundance of Ascomycota, followed by Basidiomycota. Also, one of the most abundant genera was *Cladosporium*, which had similar abundance within the two mentioned groups.

However, the difference between the two breeds consisted in the fact that the Common dogs presented a greater abundance of the following genera: *Alternaria, Malassezia, Fusarium*, genera represented in minor abundance in Boxers.

Furthermore, the Boxers had a greater abundance of fungi from the order *Hypocrales*, the genus *Aspergillus*, genus *Kazachstania*, genus *Cystofilobasidium,*order Saccharomycetales, genus *Papilliotrema*, family Microascaceae, and genus *Wallemia*. These fungi were present in very low numbers in Common dogs (Fig 8.11).

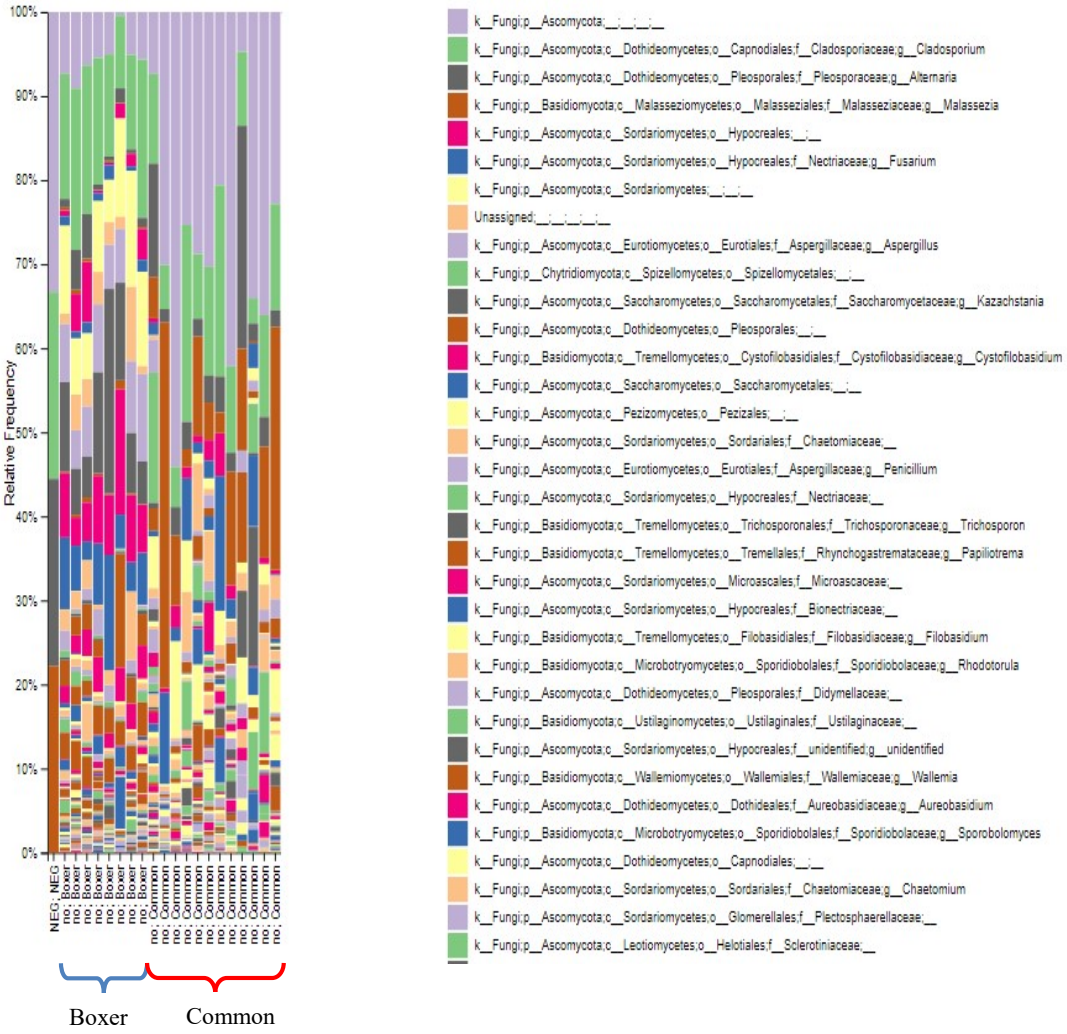



Figure 8.11-General barplot of the main fungi present in Boxer and Common dogs

The PCoA plot presents individual or group differences and we can clearly observe the clustering of individuals in the two main breeds- one group for Boxer and one group for Common breed. The fact that the individuals in the Boxer group are very close to each other can be explained by the idea that that the two Boxers studied were in a very close relationship, as a newly weaned puppy and his mother (Fig 8.12).

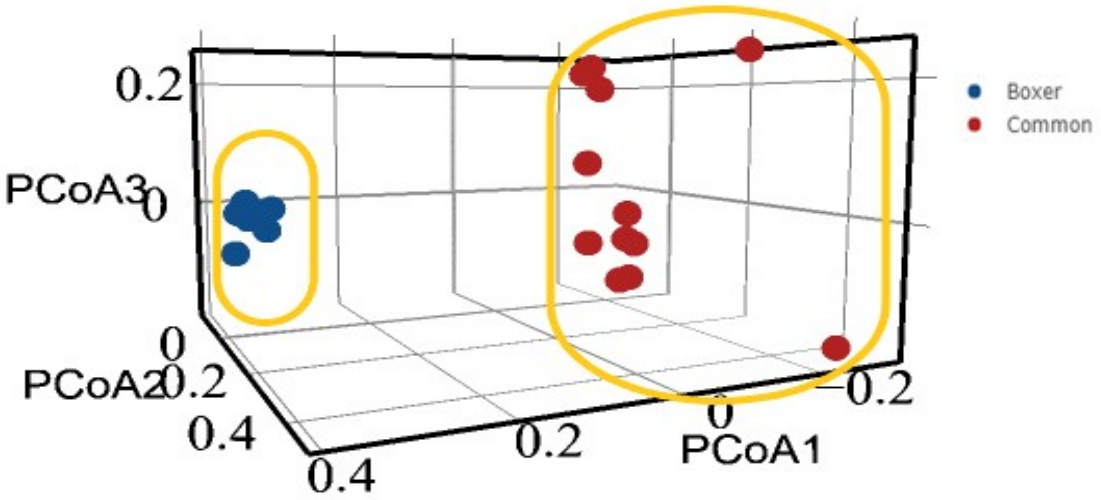



Figure 8.12 -ITS-PCoA graphic

The Shannon index showed greater fungal community diversity in Common dogs, than in Boxers, which is similar to the findings regarding the bacterial microbiome (Fig 8.13).

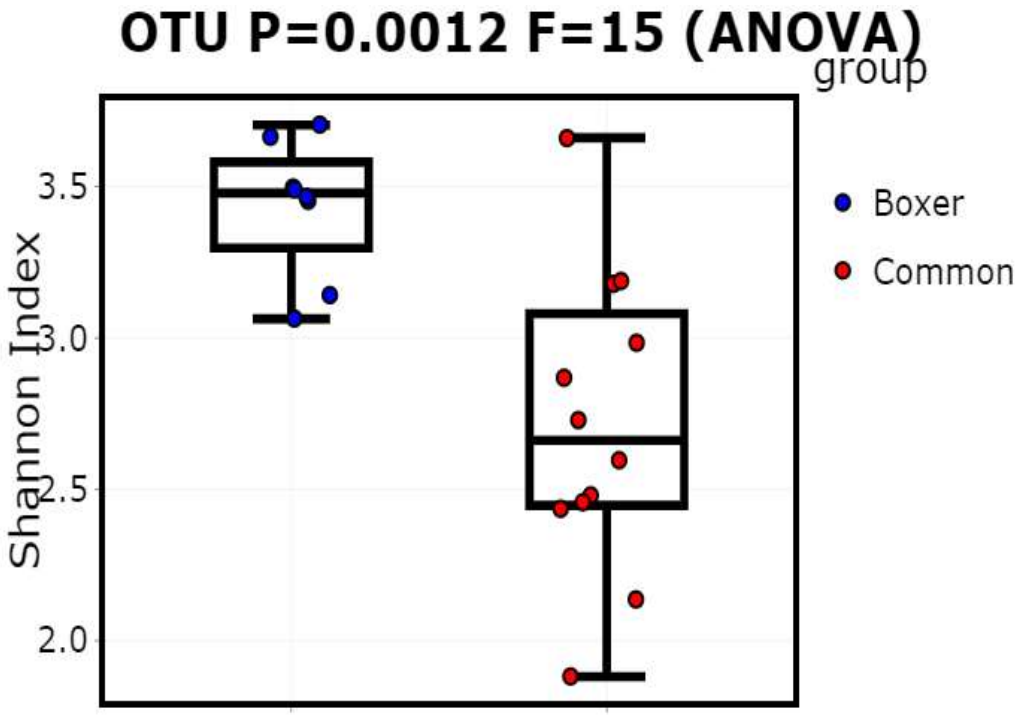



Figure 8.13- ITS- Shannon index

The β diversity weighted UniFrac (quantitative) takes into account the relative abundance of species/taxa shared between samples. We can notice a clear separation of the two groups representing the Boxer and the Common dogs (Fig 8.14).

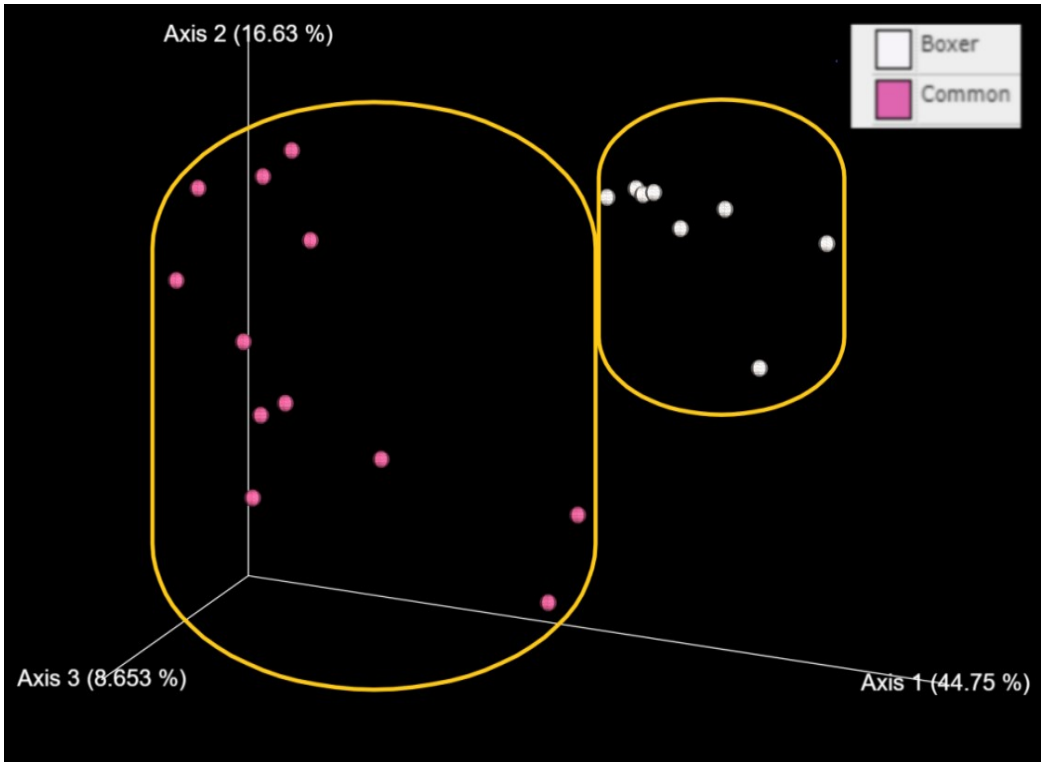



Figure 8.14-ITS-β diversity weighted UniFrac

On the other hand, the β diversity unweighted UniFrac is qualitative analyses, since it only considers the presence or absence of species/taxa shared between samples, without taking into consideration the abundance (http://www.metagenomics.wiki/pdf/definition/alpha-beta-diversity). The similarities appear mainly because the addition of rare taxa rather than abundant taxa (Fig 8.15).We can observe the same clear cluster of the samples into the two studied groups.

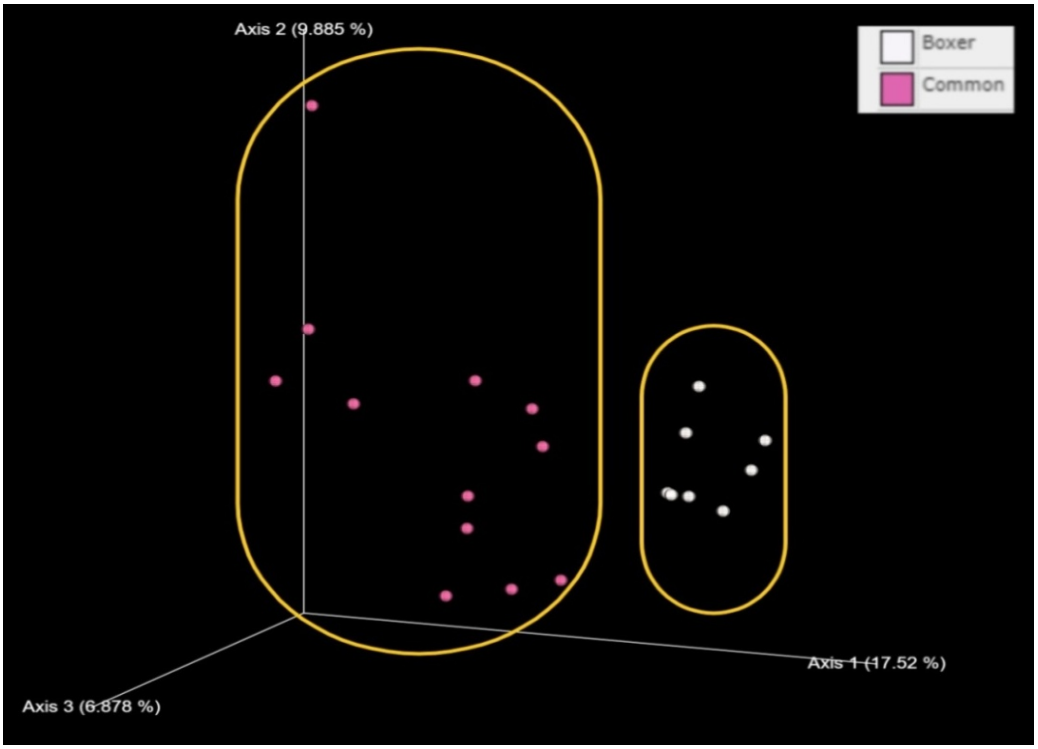



Figure 8.15- ITS-β diversity unweighted UniFrac

### 8.2.2 Comparison of the effect of the treatment

In this chapter we present the changes that appear in the skin microbiome, before and after the treatment with afoxolaner, for the 3 Common breed dogs.

a. Bacterial microbiome

The predominant phyla present in dogs before treatment was Firmicutes (with the genus *Staphylococcus*), Proteobacteria (with the genus *Pseudomonas*), Actinobacteria (with the genus *Nocardioides*), Bacteroidetes (with the genus *Pontibacter, Bacteroides*). After the first treatment with afoxolaner, the main phyla present on their skin were Proteobacteria (with the genus *Pseudomonas*), Actinobacteria (with the genus *Nocardioides*), Bacteroidetes (with the genus *Pontibacter, Bacteroides*) (Fig 8.16).

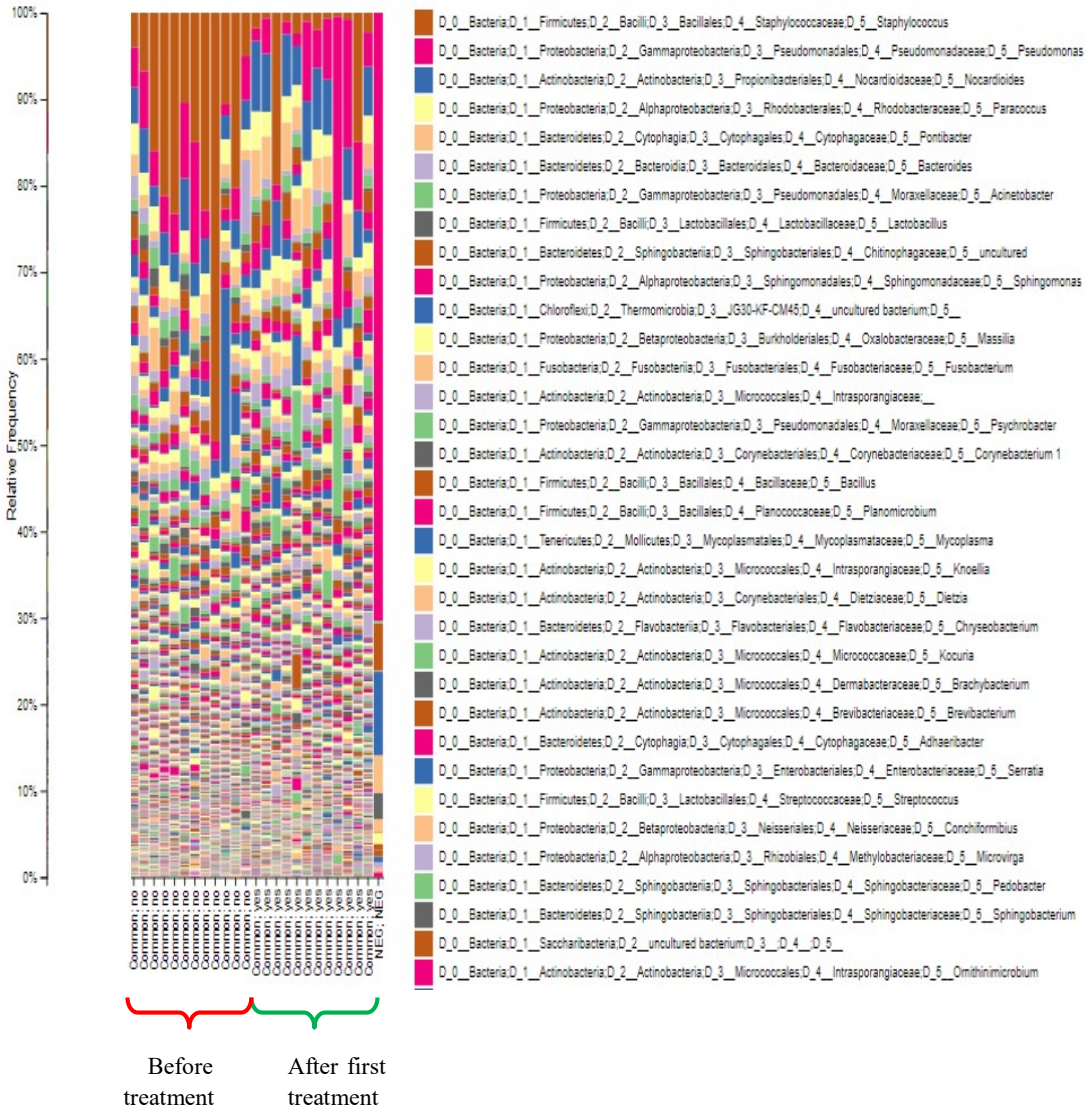



Figure 8.16- General barplot of the main bacteria present before and after first treatment in Common dogs

An interesting observation we could make comparing the before and after treatment groups was that the *Staphylococcus (*mainly *Staphylococcus pseudintermedius)* level decreased significantly after the treatment (Fig 8.17), with the exception of the first two Common dogs, at the leg sampling place, where the level had actually risen after treatment (Fig 8.18).

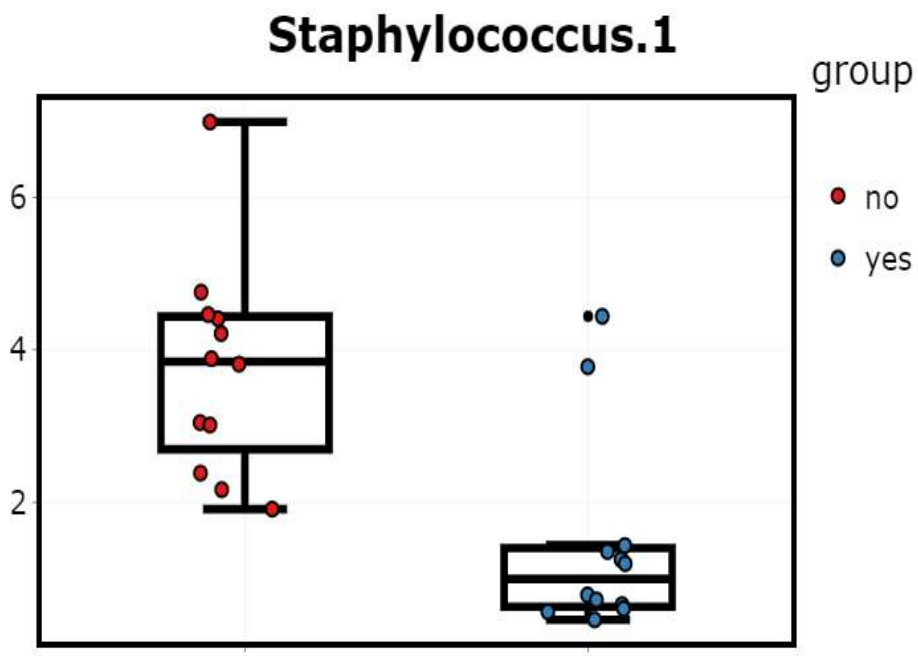
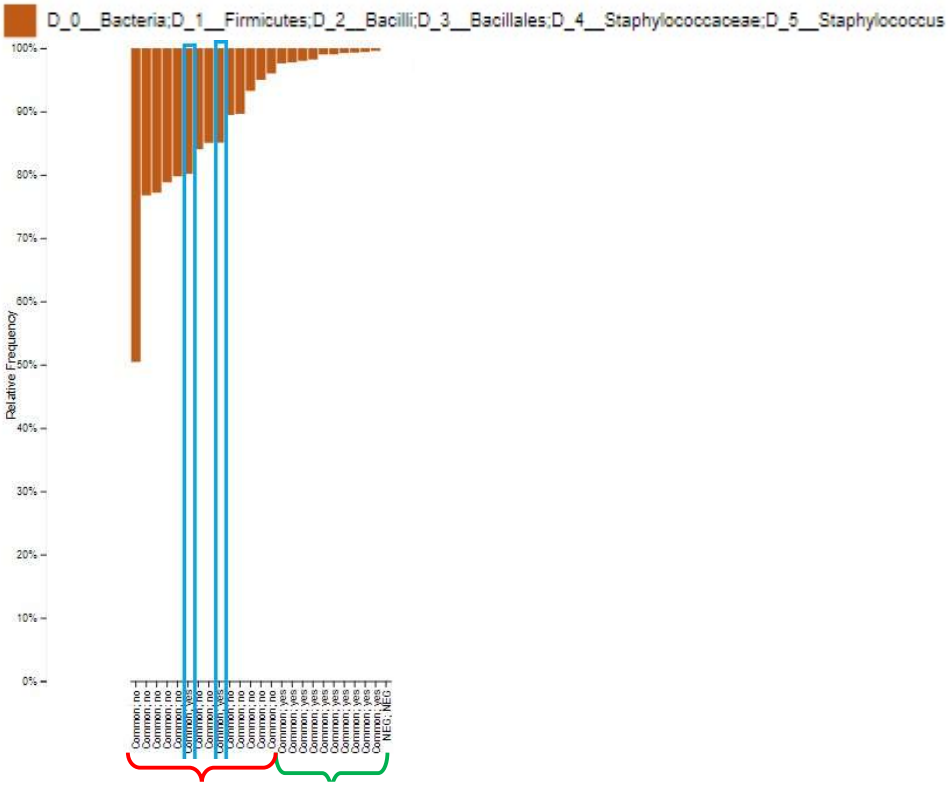



Figure 8.17- Comparison between *Staphylococcus* levels before (no) and after first treatment (yes)




Before treatment (with the exception of the samples in blue)

After treatment

Figure 8.18-Comparative presentation of *Staphylococcus* levels before and after first treatment

The PCoA plot presents individual or group differences and we can notice the clustering of individuals in two groups, one before treatment (with the exception of one leg sample from the first dog) and one after first treatment. This shows the fact that even after first treatment the microbiome has changed (Fig 8.19).

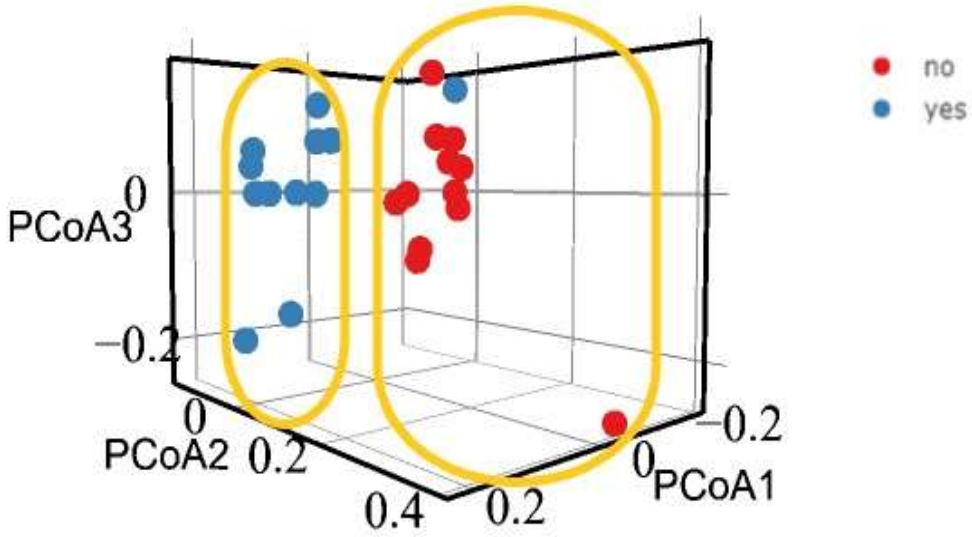



Figure 8.19-16 S- PCoA graphic before (no) and after (yes) first treatment

The Shannon index which assesses the microbial diversity, significantly increased after the first treatment with afoxolaner (Fig 8.20).

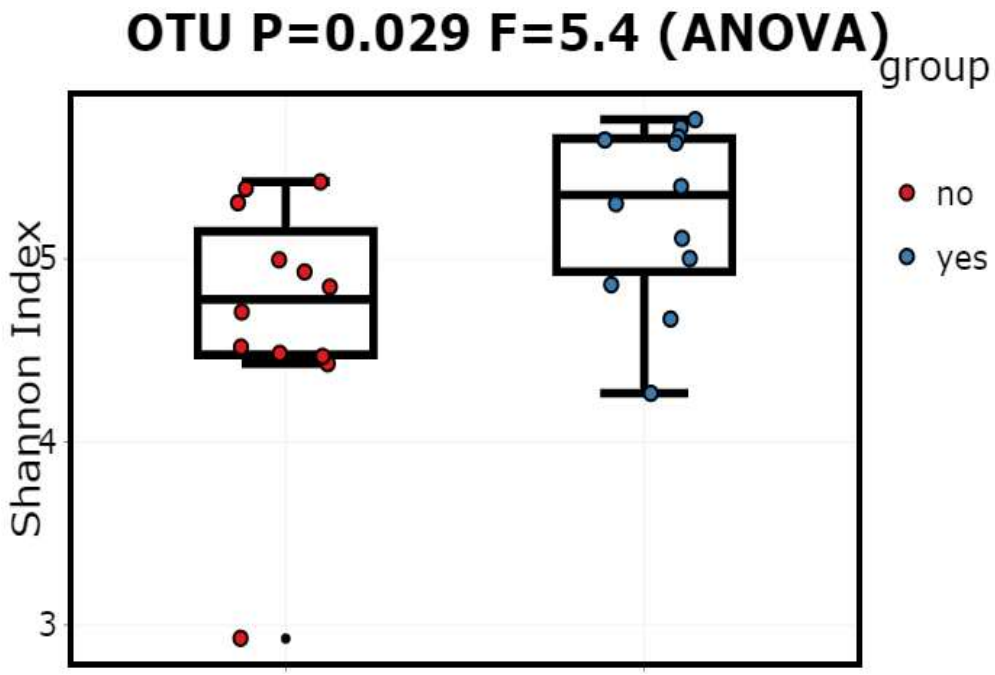



Figure 8.20-16S-Shannon index before (no) and after first treatment (yes)

The β diversity weighted UniFrac did not offer any valuable information, as the samples did not cluster into two groups, but they remained scattered around (Fig 8.21).

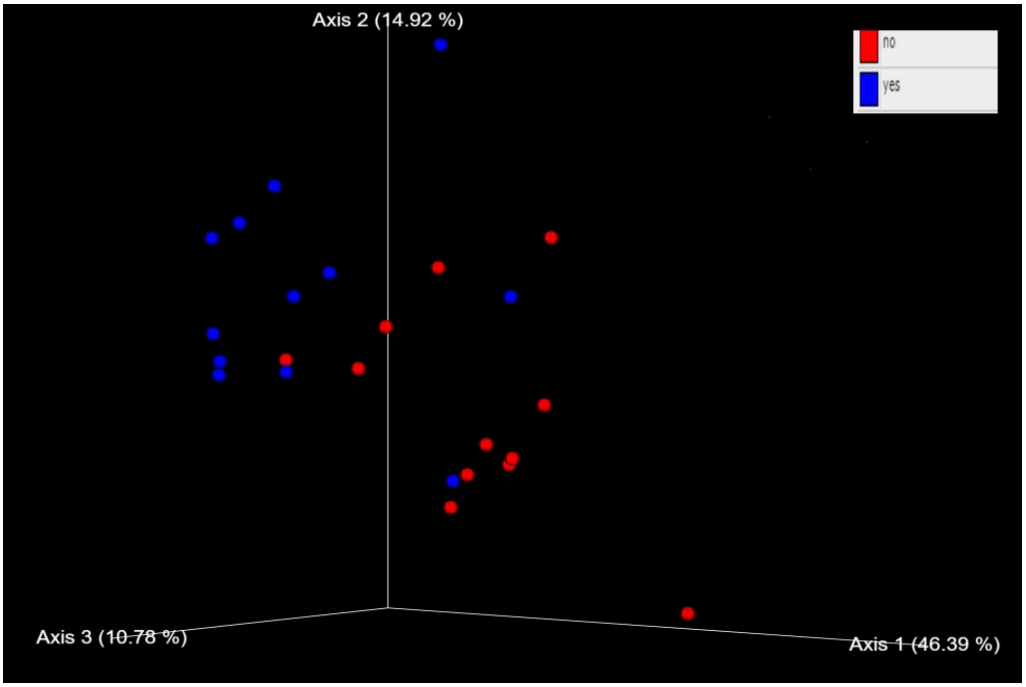



Figure 8.21- 16S-β diversity weighted UniFrac before (no) and after first treatment (yes)

The β diversity unweighted UniFrac shows similarities mainly because the addition of rare taxa rather than abundant taxa. If we consider the influence of the treatment, our samples did not cluster in before and after treatment, but they were rather scattered around (Fig 8.22). The only thing we observed is that if we eliminate the ear site from the graphic, the samples for the leg seem to cluster into a group before treatment and one after treatment, with one sample from after treatment being somehow distant from the group (Fig 8.23). That sample is the same that had the most increased level of *Staphylococcus* after treatment.

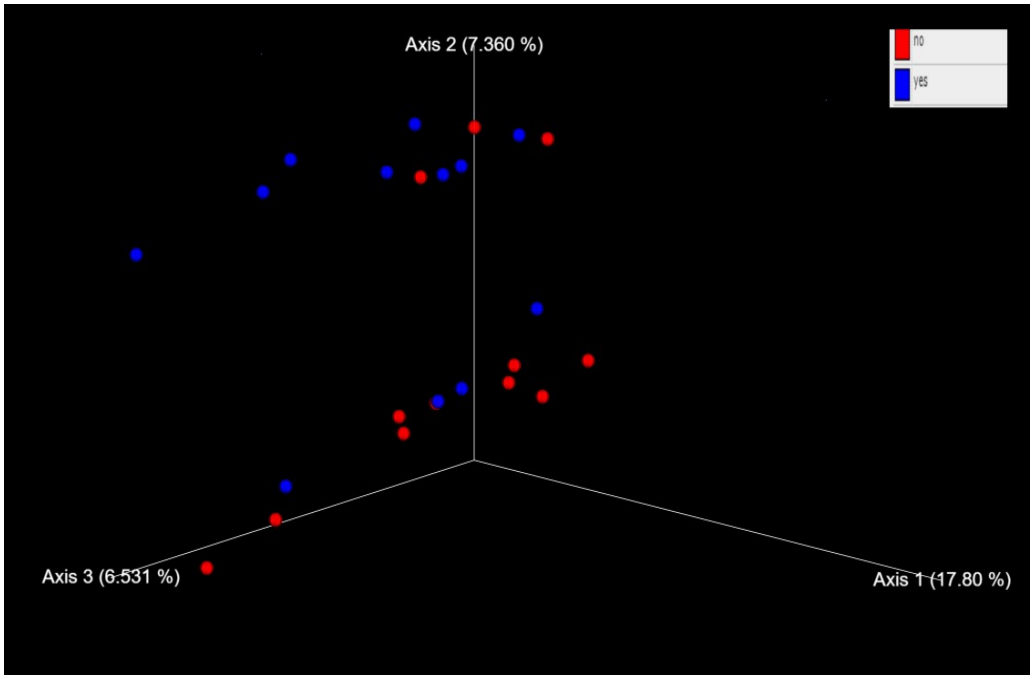



Figure 8.22- 16S-β diversity unweighted UniFrac before (no) and after first treatment (yes)

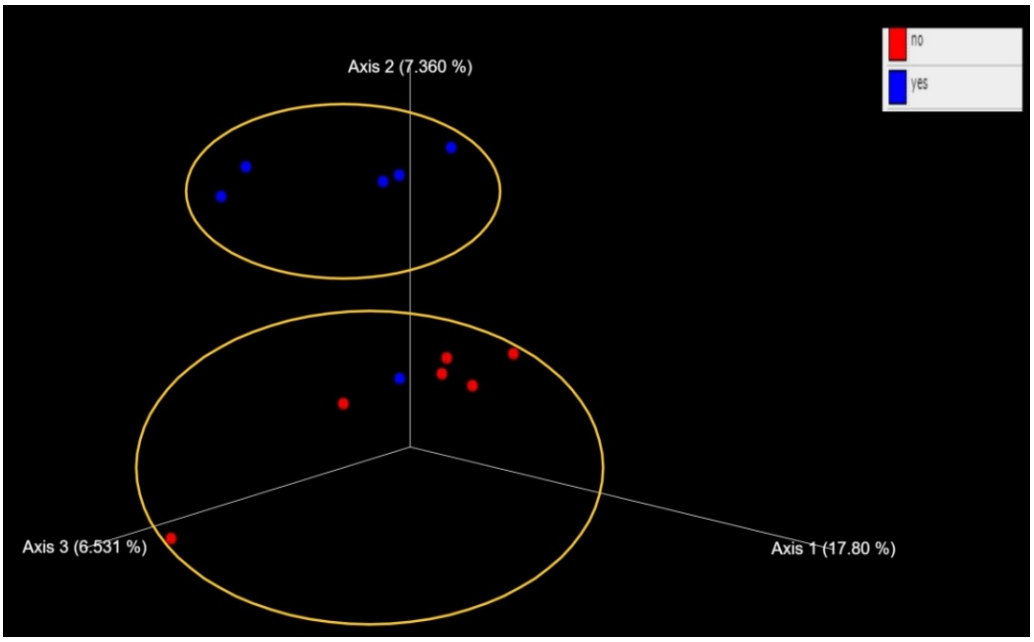



Figure 8.23 16S-β diversity unweighted UniFrac before (no) and after first treatment (yes), without the ear samples

b. Fungal microbiome

The most abundant phylum present in dogs was the same before and after treatment, phylum Ascomycota (with the main species *Cladosporium, Alternaria, Fusarium*) (Fig 8.24).

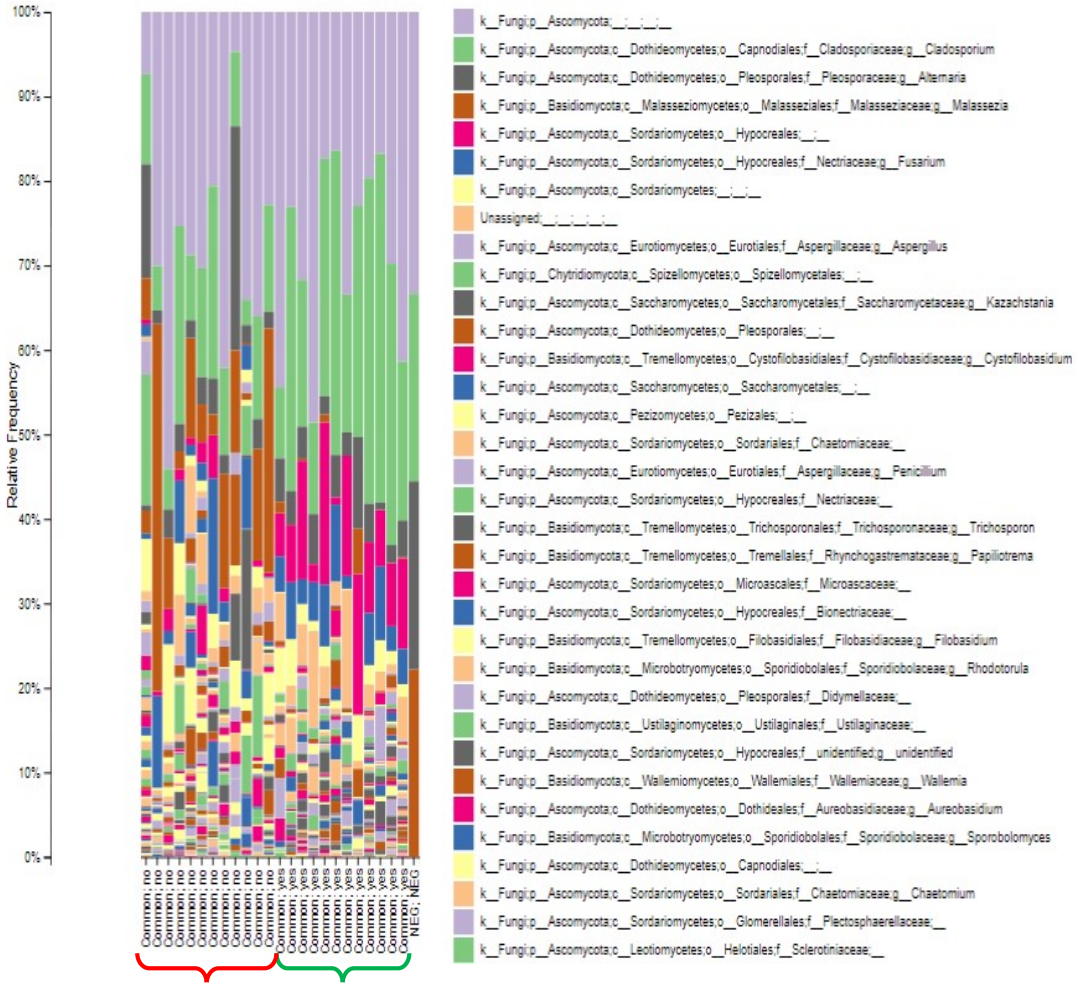



Figure 8.24-ITS-General barplot of the main fungi present before and after first treatment in Common dogs

However, the dogs after the first treatment, presented an important decrese in the relative abundance of the genus *Malassezia* (Fig 8.25-8.26), an increase in the relative abundance of the order Hypocreales, and a decrese in the relative abundance of the genus *Aspergillus* (Fig 8.24).

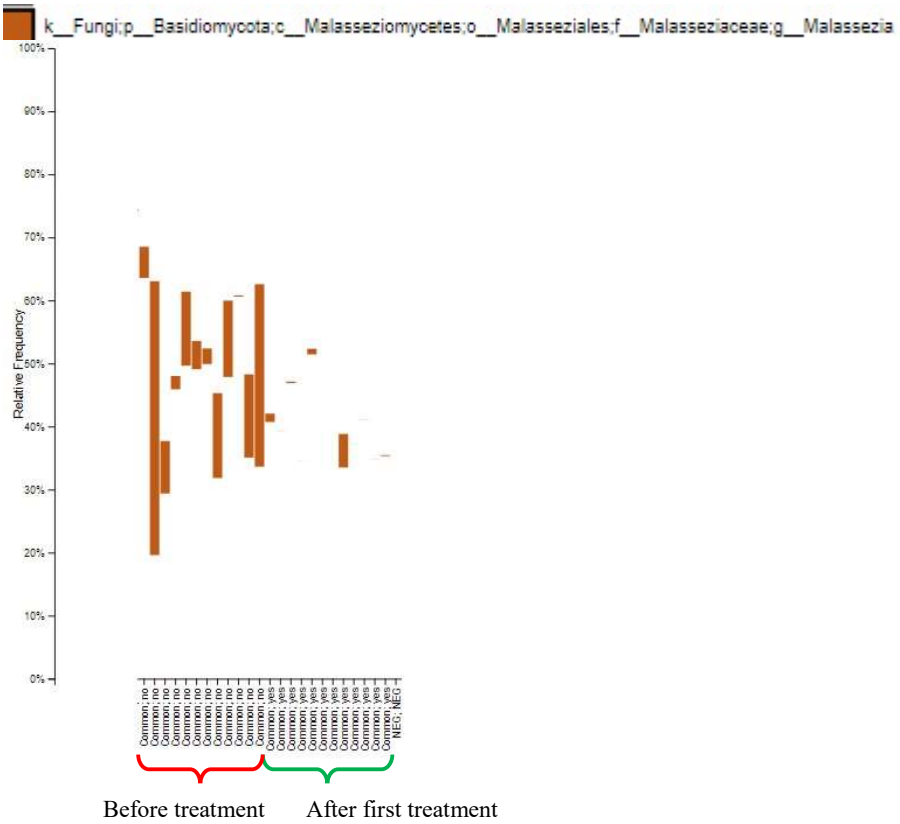



Figure 8.25-ITS-Comparative presentation of *Malassezia* levels before and after first treatment

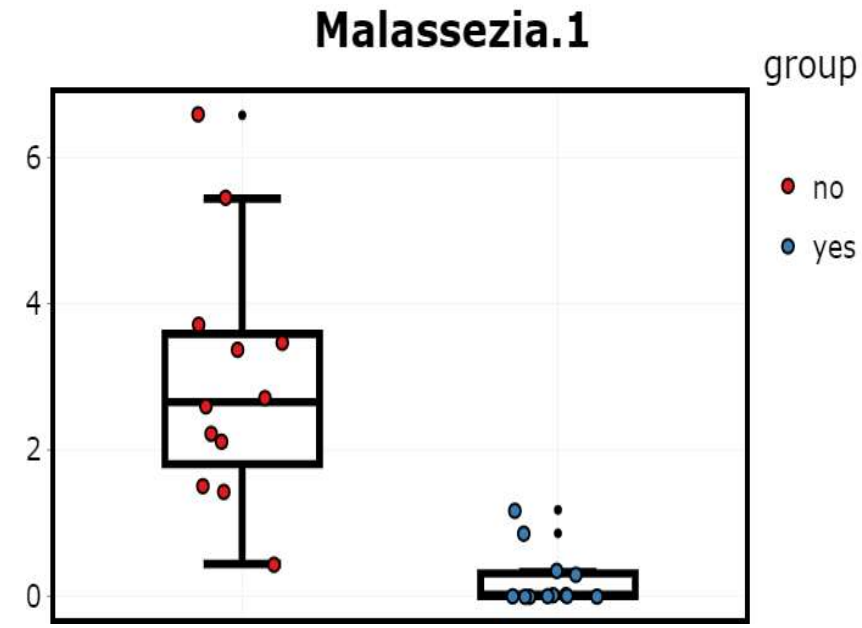



Figure 8.26-ITS Boxplot presentation of *Malassezia* levels before (no) and after first treatment (yes)

In the PCoA plot we can observe the clustering of individuals in two groups, one before treatment (which is more scattered around) and one after first treatment (more homogenous) (Fig 8.27).

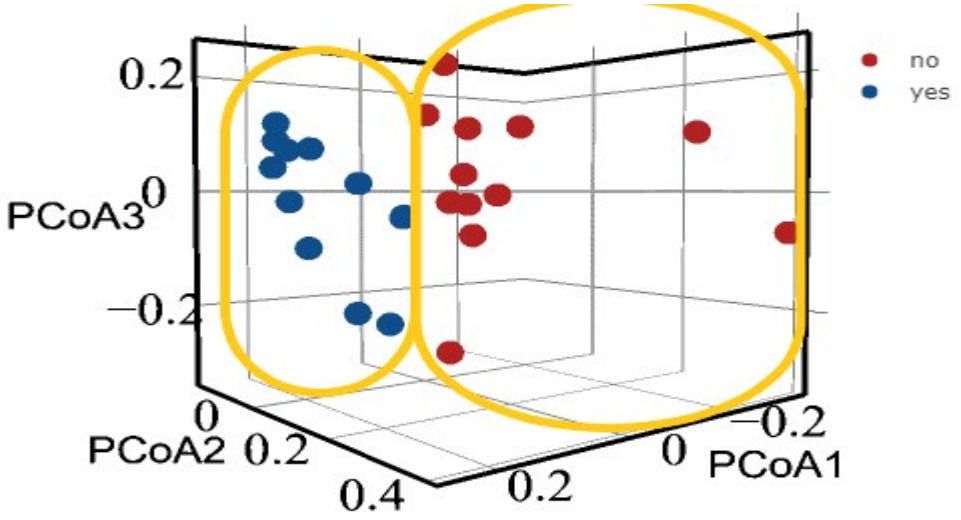



Figure 8.27- ITS- PCoA graphic before (no) and after (yes) first treatment

The Shannon index significantly decreased after the first treatment with afoxolaner, meaning that the treated animals presented a less diverse fungal microbiome (Fig 8.28).

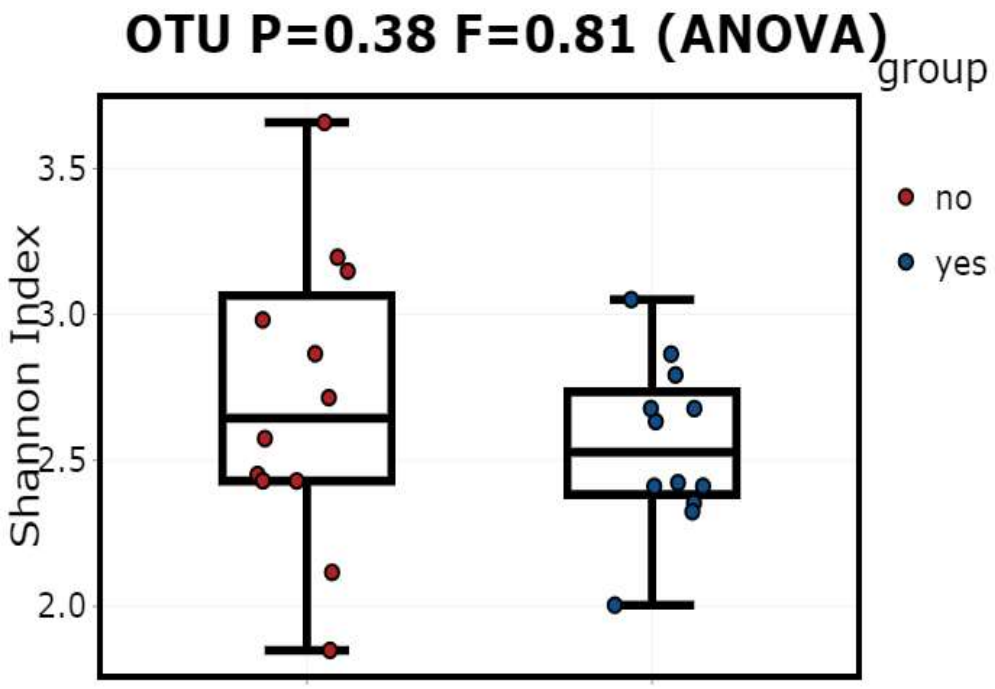



Figure 8.28-ITS- Shannon index before (no) and after first treatment (yes)

The β diversity weighted UniFrac did not show any clustering in the two main groups- the dogs before and the dogs after first treatment (Fig 8.29).

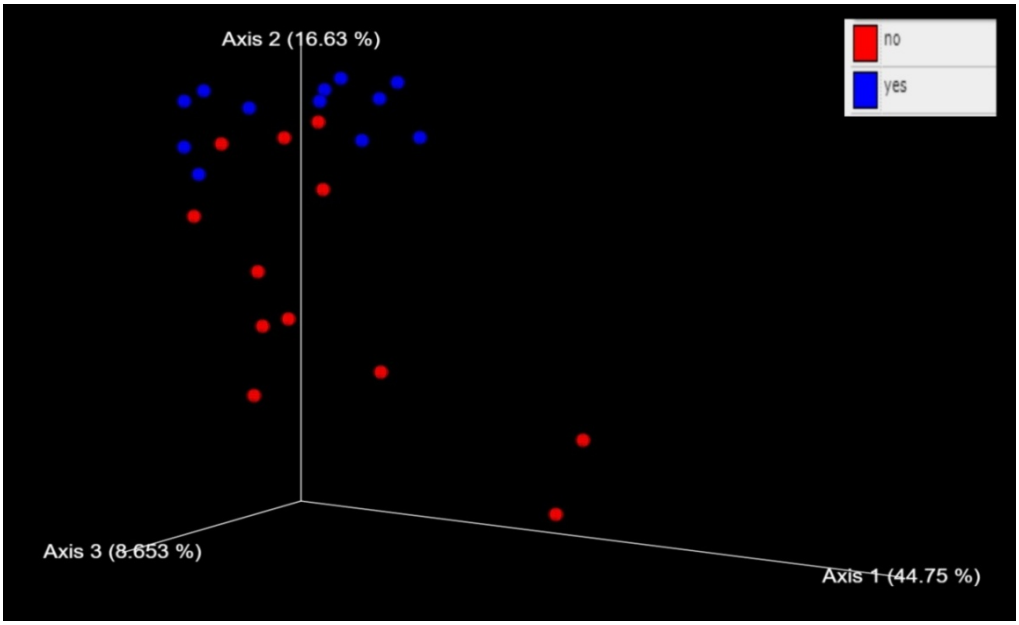



Figure 8.29-ITS-β diversity weighted UniFrac before (no) and after first treatment (yes)

The β diversity unweighted UniFrac shows similarities mainly because the addition of rare taxa rather than abundant taxa. If we consider the influence of the treatment, our samples did somehow group in before treatment and after first treatment (Fig 8.30).

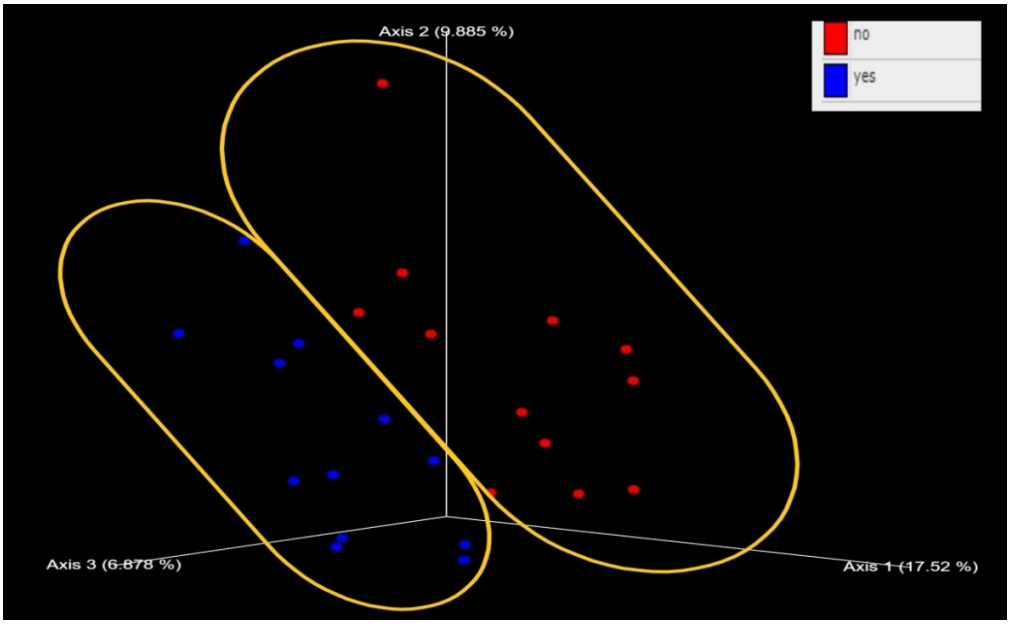



Figure 8.30-ITS-β diversity unweighted UniFrac before (no) and after first treatment (yes)

## 8.2 Discussion

The study that we have conducted regarding the modification of the skin microbiome in dogs affected by sarcoptic mange, is original through the fact that we have described what happens with both bacterial and fungal microbiome during infection with *S. scabiei* and after the first treatment with afoxolaner in dogs.

This is rather new, since all studies regarding the microbiome of dogs investigated healthy or allergic/atopic dogs, in different environmental conditions. There is one study regarding the modifications occurring in the infection with *S. scabiei,* conducted in pigs, by Australian researchers (Swe *et al.*, 2014). A more recent study, by DeCandia *et al.* (2019), observed the alteration of the skin microbiome consecutive to infection with *S. scabiei* in wild canids from North America. However, the use of afoxolaner and its effects on the modification of the skin microbiome of dogs with sarcoptic mange is a new aspect that we studied.

The fungal microbiome of the dog skin has also been studied in a paper which included 10 healthy dogs and 8 allergic dogs, sampled across various sites of the skin (Meason-Smith *et al.*, 2015). The results were that the fungal microbiome on canine skin is highly diverse, and as in bacterial microbiome, more diverse than in humans.

Regarding the number of dogs included in our study, there were a total number of 5 for the breed comparison before any treatment and 3 for the comparison before and after first treatment with afoxolaner. The sampling sites were scabies specific, including the leg and the ear with a clinically affected scabies site and its control in healthy skin, meaning a total of 4 sampling sites per individual.

Rodrigues Hoffman *et al.* (2014) included 12 healthy dogs and 6 allergic dogs, and the sampling sites were 12 for the healthy dogs and 4 for the allergic dogs, including haired skin and mucosal surfaces.

Bradley *et al.* (2016) performed a longitudinal study with 14 dogs diagnosed with atopic dermatitis and 16 healthy dogs, sampled from 4 different skin sites.

Cusco *et al.* (2017 a; 2017 b) included bigger cohorts of dogs, one with 35 healthy dogs from the same environment, with 8 sampling sites and the other one with 9 healthy dogs with sampling from 8 sites.

DeCandia *et al.* (2019), investigated the skin microbiome of 12 coyotes, 11 red foxes and 2 gray foxes, both infected with sarcoptic mange and healthy animals. The number of skin sites sampled was 5 (ear, axilla, groin, dorsal flank, outer back leg).

The study of Swe *et al.* (2014) in pigs with experimental sarcoptic mange, included 15 animals, divided into 3 groups: 5 healthy in the control group, 5 infected with *S. scabiei*, and 5 infected with *S. scabiei* and treated with dexamethasone. The sampling place was the inner sebaceous surface of the pinnae, and the samples were taken before infection, during the infection and after the treatment.

Comparing with the above studies, the number of dogs and skin sites sampled during our thesis seems low, however it still draws some important conclusions regarding the alteration of skin microbiome in sarcoptic mange. The main limitation in our research was the high price of the metagenomics analyses, which resulted in including a smaller number of dogs. Nevertheless, we did include a study of both bacterial and fungal communities present on the skin, and we did include an affected site and a corresponding healthy site from the ear and the leg.

As far as the sampling method is concerned, most of the mentioned studies used sampling by swabbing in dogs, including the study of DeCandia *et al.* (2019) in wild canids. The only exception is the study of Swe *et al.* (2014), which used scraping of the skin in pigs. However, we performed both swabbing and scraping of the skin.

An interesting aspect to mention is that we used an original (detailed per sign) clinical score to evaluate the degree of infection with *S. scabiei*, at D0 for 5 dogs and at D28 for the 3 dogs. DeCandia *et al.*(2019), evaluated also the extent of mange on the body of the animals showing visible characteristic lesions, such as alopecia, crusts, lesions, in a more general way, including animals in mange classes (0- no signs to 3- more than 50% of the body affected).

Regarding the results of our study, we divided them into two main chapters- the influence of breed on the skin microbiome of dogs with sarcoptic mange and the effect of treatment on the skin microbiome of dogs infected with *S. scabiei.*

The breeds we included were Common breed (3 cohabiting dogs) and Boxer (2 cohabiting dogs-mother and puppy). The dogs were sampled before any treatment at D0 and had different clinical scores.

Our results for the bacterial microbiome suggested that the main force driving the skin microbiome is the breed, rather than the individual or the skin site. The PCoA graphic, the β diversity weighted UniFrac and the β diversity unweighted UniFrac all illustrated two clusters of samples, representing the two breeds- Boxer and Common breed. The Shannon index which shows species abundance and evenness of OTUs, showed a marked difference between the Common dogsthat had a more diverse bacterial microbiome than that of the Boxers. Also, the general barplot showed consistent differences between the 2 groups, the Boxers showing greater abundance of: *Bacteroides, Psychrobacter, Corynebacterium, Lactobacillus, Conchiformibius, Brachybacterium, Brevibacterium, and Streptococcus.* Furthermore, the Boxers had a lower number of bacteria than the common dogs, from the following genera: *Pseudomonas, Nocardioides, Paracoccus, Pontibacter, Chitinophagaceae, Planomicrobium, Mycoplasma, Knoellia.* However, the main genus that inhabits the skin of the Boxers infected with mange, is *Staphylococcus*, which is present in a similar abundance in the Common dogs.

Rodrigues-Hoffmann*et al.*(2014) observed that the variability between samples is given by the individual, followed by the skin site (higher number of species on the haired skin

vs. the poorly haired skin, mucous surfaces).Other studies regarding the skin microbiome in healthy dogs (Cusco *et al.*, 2017a, 2017b) researched the influence of individual, skin site, breed and environment. Their results showed that the main force driving the skin microbiome is the individual, followed by the skin site sampled and the breed and the environment in lesser measure.

This aspect did not apply for our study, but we must consider the fact that the number of the dogs was rather small, the number of breeds represented was only 2 and that the Boxers were closely related and probably shared the same microbiome because they were in very close touch, as a mother and a recently weaned puppy. In addition, we compared our results with the results from healthy dogs, which can also be a problem to be interpreted. However, the differences in the skin microbiome in the two groups can easily be observed, even from the general barplot.

As far as the fungal microbiome is concerned, the main phylum present is similar between the 2 groups, with a higher relative abundance of Ascomycota, followed by Basidiomycota. Also, one of the most abundant genus is *Cladosporium*, which has similar abundance within the two mentioned groups.

However, the difference between the two breeds consists in the fact that the Common dogs present a greater abundance of the following genera: *Alternaria, Malassezia, Fusarium*, genera represented in minor abundance in Boxers. On the other hand, the Boxers have a greater abundance of fungi from the order Hypocrales, the genus *Aspergillus*, genus *Kazachstania*, genus *Cystofilobasidium,*order Saccharomycetales, genus *Papilliotrema*, family Microascaceae, and genus *Wallemia*.

The results are the same as in the case of the bacterial microbiome, with the clustering of the samples in the two breeds, for the PCoA, the β diversity weighted UniFrac and the β diversity unweighted UniFrac. Also, the Shannon index showed an important difference between the Common dogs that had a more diverse fungal microbiome than the Boxers.

The study of Meason-Smith *et al.* (2015) on the fungal microbiome of healthy and allergic dogs, revealed a predominance of phyla Ascomycota and Basidiomycota*,* which is similar to our findings. They considered the influence of the body site and the individual in the study. The conclusions were that the individual was again the main force driving the fungal microbiome, rather than the skin site. The variable breed was not analyzed.

The second part of the study, analyzed the differences in the skin microbiome of the 3 cohabiting, Common breed dogs, before any treatment at D0 and after the first treatment with afoxolaner at D28.

Firstly, the bacterial microbiome showed that the predominant phyla present in dogs at D0 were Firmicutes (with the genus *Staphylococcus*), Proteobacteria (with the genus *Pseudomonas*), Actinobacteria (with the genus *Nocardioides*), Bacteroidetes (with the genus *Pontibacter, Bacteroides*). At D28, the main phyla present on their skin were Proteobacteria

(with the genus *Pseudomonas*), Actinobacteria (with the genus *Nocardioides*), Bacteroidetes (with the genus *Pontibacter, Bacteroides*).

An important aspect that we noticed was that the *Staphylococcus (*mainly *Staphylococcus pseudintermedius)*, which represented the most abundant genus at D0, decreased its level significantly after the treatment, which can mean that *S. scabiei* promotes the growth of opportunistic bacteria that are present normally on the skin surface. This is consistent with other studies which investigated the differences between healthy and allergic/atopic dogs' skin, and found out that the levels of *Staphylococcus* were higher in dogs presenting skin problems (Bradley *et al.*, 2016; Pierezan *et al.*, 2016). Furthermore, Swe's study (2014) showed that in the infected groups, there was also a predominance of *Staphylococcus,* which decreased after the treatment with doramectin. The same result was reported in the wild canids infected with mange (DeCandia *et al.*, 2019).

The Shannon index showed an important change in the diversity of skin bacteria, with an increase after the first treatment with afoxolaner. These results correspond with the other studies which showed that allergic/atopic skin of dogs has a less diverse microbial community than the one of healthy dogs (Rodrigues-Hoffmann *et al.*, 2014; Bradley *et al.*, 2016). The same observation has been made in the two studies regarding the modification of skin microbiome in pigs or canids affected by sarcoptic mange (Swe *et al.* 2014; DeCandia *et al.* 2019).

The PCoA plot presented two well defined groups, one before treatment and one after treatment. However, this was not the case for the the β diversity weighted UniFrac and the β diversity unweighted UniFrac, which did not offer any information, since the samples were rather scattered around.

Secondly, the fungal microbiome showed that the most abundant phylum before and even after treatment was Ascomycota (with the main species *Cladosporium, Alternaria, Fusarium*), which is similar to the study of Meason-Smith *et al.* (2015) on fungal microbiome in healthy dogs. An interesting aspect is the particular case of the genus *Malassezia,* which may usually be associated with skin disease in dogs. We observed that its level significantly decreases after the treatment with afoxolaner, this representing a sign of the healing and recovery of the skin barrier.

The Shannon index showed a decrease after the first treatment with afoxolaner, meaning that the treated animals presented a less diverse fungal microbiome. However, this result contradicted those from Measons-Smith *et al.* (2015), where allergic dogs had a lower fungal diversity than the healthy ones.

In the PCoA plot we can observe the clustering of individuals in two groups, one before treatment (which is more scattered around) and one after first treatment (more homogenous). The same can be observed in the β diversity unweighted UniFrac, where the samples seem to cluster in two groups. However, this is not the case for the β diversity weighted UniFrac.

In conclusion, our study confirmed part of the observations from the published papers on the skin microbiome of dogs, the skin microbiome of pigs with sarcoptic mange and the skin microbiome of wild canids infected with *S. scabiei*. The main drawbacks of our study were the little number of dogs analyzed and the few sampling sites, which might explain some of the differences from the other studies. However, we did not have an exact model to compare to, as some of the aspects of our study were done for the first time.

The preliminary results of this chapter were presented in the 27th Conference of the World Association for the Advancement of Veterinary Parasitology (WAAVP), which took place from the 7$^{th}$ to the 11$^{th}$of July 2019 in Madison, WI, USA. The title of the oral presentation was *Clinical Efficacy of Afoxolaner in Dogs Naturally Infected with Sarcoptes Scabiei and Concomitant Modifications of the Skin Microbiota,* and the authors were Raluca MÎNDRU, Andrei Cristian LUPU, Constantin ROMAN, Charlotte BERNIGAUD, Amaury BRIAND, Frédéric BEUGNET, Katja FISCHER, Liviu MIRON, Jacques GUILLOT.

# 9. CONCLUSIONS

Sarcoptic mange represents a problem for all animals and humans, since ancient times. The full mechanisms of action of the *S. scabiei* mites, as well as their genetic variability and the damages that they produce to the skin, remain only partially known.

Furthermore, the drug resistance cases to classic acaricid treatments (such as ivermectin in both humans and animals) (Currie *et al.*, 2004; Mounsey *et al.*, 2009; Terada *et al.*, 2010) trigger an alarm on the necessity of finding new efficient molecules for the treatment of sarcoptic mange in both animals and humans.

The present thesis tried to reveal some new aspects regarding the response of dogs with sarcoptic mange to the treatment with an isoxazoline molecule, afoxolaner, as well as to describe what happens to both bacterial and fungal microbiome in dogs with mange, and how this microbiome changes after the administration of treatment.

The general conclusions of our work are divided according to the 3 studies that we had performed:

- the epidemiological study,
- the study of the efficacy of afoxolaner ,
- the study of the skin microbiome in dogs with sarcoptic mange.

From the epidemiological study we concluded that mange seems to affect mostly young dogs, under one year (n=58.3%).

It has no gender distribution, as the number of males and females analyzed was almost equal (males 48% and females 52%).

We could not interpret the breed predisposition, since most of our dogs were crossbreed or common, and very few pure breed.

Regarding the source of contamination, even though for the majority of the studied dogs it remained unknown, we had a case, in the Boxer household, where the source of contamination came from a fox observed to have severe skin problems.

The clinical score that we used to assess the intensity of mange was an original one, adapted for dogs, but initially designed for pigs infected with *S. scabiei* (Bernigaud *et al.*, 2016). The most scored signs were the extent of skin affected by sarcoptic mange followed closely by alopecia and then crusts and scales and the most affected body site was the head, mainly the ears, which is a characteristic site for the development of *S. scabiei* mites.

The study regarding the clinical efficacy of afoxolaner, in dogs with sarcoptic mange led to the following conclusions.

The clinical efficacy of the treatment at D28 (calculated as [(clinical score at D0 – clinical score at D28)/clinical score at D0] * 100, from Beugnet *et al.*, 2016), varied between 47% and 90%, which means that the clinical signs had visibly improved in all animals.

Even though our study did not include a mite count, which was a primary criteria for evaluating the success of the treatment in the other afoxolaner studies (Beugnet *et al.*, 2016; Hampel *et al.*, 2018), we did resample the dogs at D28 and did not find zero mites present. There were no visible signs of drug intolerance or drug resistance.

We did the clinical evaluation of the dogs at D0 and D28, after the first treatment with afoxolaner, and the results were already positive, showing the high efficacy of the isoxazoline molecule. At the moment of our study, the product was not officially approved for the treatment of sarcoptic mange in dogs.

Regarding the serum biochemical analyses performed in 10 of the 16 dogs before the administration of treatment, the results marked an increase in the urea nitrogen in half of the dogs, but without any correlation with the creatinine level, showing no important renal dysfunctions. We also observed a rise in the aspartate aminotransferase (AST).

From the study of the microbiome of dogs infected with *S. scabiei* mites we have also concluded some interesting aspects.

For the comparison of the breed, where we have included 3 Common breed dogs and 2 Boxers, we found out that, for both bacterial and fungal microbiome analyzed, the main force driving the skin microbiome is the breed, rather than the individual or the skin site.

The PCoA graphic, the β diversity weighted and unweighted UniFrac all illustrated two groups of samples, representing the two breeds- Boxer and Common breed.

The Shannon index showed a marked difference between the Common dogs that had a more diverse bacterial and fungal microbiome than the one of the Boxers.

Concerning the bacterial microbiome of the Boxers, they had greater abundance of: *Bacteroides, Psychrobacter, Corynebacterium, Lactobacillus, Conchiformibius, Brachybacterium, Brevibacterium, and Streptococcus* and a lower number of bacteria than the Common dogs, from the following genera: *Pseudomonas, Nocardioides, Paracoccus, Pontibacter, Chitinophagaceae, Planomicrobium, Mycoplasma, Knoellia.*

However, the main genus present on the skin of both Boxers and Common dogs, in similar abundance, was represented by *Staphylococcus.*

As far as the fungal microbiome is concerned, the main phylum present is similar between the 2 groups, with a higher relative abundance of Ascomycota, followed by

Basidiomycota. Also, one of the most abundant genus is *Cladosporium*, which has similar abundance within the two mentioned groups.

However, the Common dogs present a greater abundance of the following genera: *Alternaria, Malassezia, Fusarium*, genera represented in minor abundance in Boxers.

On the other hand, the Boxers have a greater abundance of fungi from the order Hypocrales, the genus *Aspergillus*, genus *Kazachstania*, genus *Cystofilobasidium,* order Saccharomycetales, genus *Papilliotrema*, family Microascaceae, and genus *Wallemia*.

For the comparison of the effect of the first treatment with afoxolaner, for the bacterial microbiome, we noticed that the predominant phyla present in dogs at D0 were Firmicutes (with the genus *Staphylococcus*), Proteobacteria (with the genus *Pseudomonas*), Actinobacteria (with the genus *Nocardioides*), Bacteroidetes (with the genus *Pontibacter, Bacteroides*).

At D28, the main phyla present on their skin were Proteobacteria (with the genus *Pseudomonas*), Actinobacteria (with the genus *Nocardioides*), Bacteroidetes (with the genus *Pontibacter, Bacteroides*).

An interesting aspect that we observed, was that the *Staphylococcus (*mainly *Staphylococcus pseudintermedius)*, which represented the most abundant genus at D0, decreased its level significantly after the treatment, which can mean that *S. scabiei* promotes the growth of opportunistic bacteria that are present normally on the skin surface.

The Shannon index showed an important shift in the diversity of skin bacterial microbiome, with an important increase after the first treatment with afoxolaner.

The PCoA plot presented two well defined groups, one before treatment and one after treatment, but this aspect was not observed in the β diversity weighted and unweighted UniFrac graphics.

For the fungal microbiome, the most abundant phylum before and even after treatment was Ascomycota (with the main species *Cladosporium, Alternaria, Fusarium*), but we could notice, for the dogs after the first treatment, an important decrese in the relative abundance of the genus *Malassezia*, an increase in the relative abundance of the order Hypocreales, and a decrese in the relative abundance of the genus *Aspergillus.* Since *Malassezia* may be associated with skin disease in dog, the significant decrease after the treatment can be a sign of the process of skin recovery.

The Shannon index showed a decrease after the first treatment with afoxolaner, meaning that the treated animals presented a less diverse fungal microbiome, which is rather contradictory, since the healing of the skin usually means an enrichment of the fungal microbiome.

The clustering of individuals in two groups, before treatment and after treatment, can be observed in the PCoA and β diversity unweighted UniFrac graphic.

To sum up, we must, of course, be aware of the fact that our results cannot be generalized, as the number of individuals included in the study was rather low. However, the results of our work on the skin microbiome of dogs with sarcoptic mange are interesting and as far as we know, some of the analyses, such as the fungal microbiome, have been done for the first time in dogs with sarcoptic mange, as well as the comparison of the evolution of both bacterial and fungal microbiome before and after the first treatment with afoxolaner.

# BIBLIOGRAPHY


1. Abdel-Ghaffar F., Al-Quraishy S., Sobhy H., Semmler M., 2008, *Neem seed extract shampoo, Wash Away Louse®, an effective plant agent against Sarcoptes scabiei mites infesting dogs in Egypt.* Parasitol. Res. 104, 145–148
2. Alasaad S., Soglia D., Maione S., Sartore S., Soriguer R.C., Pérez J.M.., Rasero R., Rossi L., 2009, *Effectiveness of postponed isolation (post-frozen isolation) method for PCR-quality Sarcoptes mite gDNA*, Exp Appl Acarol, 47, p. 173–178
3. Andriantsoanirina V., Ariey F., Izri A., Bernigaud C., Fang F., Charrel R., Foulet F., Botterel F., Guillot J., Chosidow O., Durand R., 2015a, *Sarcoptes scabiei mites in humans are distributed into three genetically distinct clades.* Clin. Microbiol. Infect. 21, 1107–1114
4. Andriantsoanirina V., Ariey F., Izri A., Bernigaud C., Fang F., Guillot J., Chosidow O., Durand R., 2015b, *Wombats acquired scabies from humans and/or dogs from outside Australia.* Parasitol. Res.114(6):2079-83
5. Angelone-Alasaad S., Molinar Min A., Pasquetti M., Alagaili A.N., D'Amelio S., Berrilli F., Obanda V., Gebely M. A., Soriguer R.,Rossi L., 2015, *Universal conventional and real-time PCR diagnosis tools for Sarcoptes scabiei,* Parasites and Vectors, 8:587
6. Arlian L.G., Runyan R.A., Achar S., Estes S.A., 1984, *Survival and infectivity of Sarcoptes scabiei var. canis and var. hominis*. J. Am. Acad. Dermatol. 11, 210–215
7. Arlian LG, Vyszenski-Moher DL, 1988, *Life cycle of Sarcoptes scabiei var. canis.* J Parasitol 74:427
8. Arlian L.G., Estes S.A., Vyszenski-Moher D.L., 1988a, *Prevalence of Sarcoptes scabiei in the homes and nursing homes of scabietic patients* 19, 806–811
9. Arlian L.G., Moher D.V., Cordova D., 1988b, *Host specificity of S. scabiei var. canis (Acari: Sarcoptidae) and the role of host odor.* J. Med. Entomol. 25, 52–56.
10. Arlian L.G., Vyszenski-Moher D.L., Pole M.J., 1989a, *Survival of adults and developmental stages of Sarcoptes scabiei var. canis when off the host.* Exp. Appl. Acarol. 6
11. Arlian L.G., 1989b, *Biology, host relations, and epidemiology of Sarcoptes scabiei.* Annu. Rev. Entomol. 34, 139–161
12. Arlian L.G., Morgan M.S., Rapp C.M., Vyszenski-Moher D.L., 1995, *Some effects ofsarcoptic mange on dogs*. J. Parisitol. 81, 698–702
13. Arlian L.G., Morgan M.S., Neal J.S., 2003, *Modulation of cytokine expression in humankeratinocytes and fibroblasts by extracts of scabies mites.* Am J Trop MedHyg.;69(6):652–6
14. Arlian LG, Morgan MS, 2017, *A review of Sarcoptes scabiei: past, present and future.* Parasit Vectors 10:297. Review
15. Averof M., Akam M., 1995, *Insect-crustacean relationships: insights from comparative developmental and molecular studies*. Philos. Trans. R. Soc. B Biol. Sci. 347, 293–303

16. Aydıngöz İ.E., Mansur A.T., 2011, *Canine scabies in humans: a case report and review of the literature*, Dermatology 223, 104–106
17. Barnard E., Huiying L., 2017, *Shaping of cutaneous function by encounters with commensals,* J Physiol 595.2, pp 437-450
18. Beck A.L., 1965, *Animal scabies affecting man*, Arch. Dermatol. 91, 54–55
19. Becskei C., De Bock F., Illambas J., Cherni J.A., Fourie J., Lane M., Mahabir S.P., Six R.H., 2016, *Efficacy and safety of a novel oral isoxazoline, sarolaner (SimparicaTM), for the treatment of sarcoptic mange in dogs*. Veterinary Parasitology, 222, 56–61
20. Beech R., Levitt N., Cambos M., Zhou S., Forrester S.G., 2010, *Association of ion-channel genotype and macrocyclic lactone sensitivity traits in Haemonchus contortus.* Mol. Biochem. Parasitol. 171, 74–80
21. Beigh S.A., Soodan J.S., Bhat A.M., 2016, *Sarcoptic mange in dogs: Its effect on liver, oxidative stress, trace minerals and vitamins,* Veterinary Parasitology 227 30–34
22. Bergvall K.E., 1998, *Clinical efficacy of milbemycin oxime in the treatment of canine scabies. a study of 56 cases,* ,Veterinary Dermatology; **9**: 231–3
23. Bernigaud C., Fang F., Fischer K., Lespine A., Aho L.S., Dreau D., *et al*., 2016, *Preclinical Study of Single-Dose Moxidectin, a New Oral Treatment for Scabies: Efficacy, Safety, and Pharmacokinetics Compared to Two-Dose Ivermectin in a Porcine Model*. PLoS Negl Trop Dis 10(10): e0005030
24. Beugnet F., deVos C., Liebenberg J., Halos L., Fourie J., 2014a, *Afoxolaner against fleas: immediate efficacy and resultant mortality after short exposure on dogs.* Parasite 21:42
25. Beugnet F., Halos L., Larsen D., Labuschagné M., Erasmus H., Fourie J., 2014b, *The ability of an oral formulation of afoxolaner to block the transmission of Babesia canis by Dermacentor reticulatus ticks to dogs.* Parasit Vectors 7, 283
26. Beugnet F., de Vos C., Liebenberg J., Halos L., Larsen D., Fourie J., 2016, *Efficacy of afoxolaner in a clinical field study in dogs naturally infested with Sarcoptes scabiei.* Parasite, 23, 26
27. Beugnet F., Lenaig H., Guillot J., 2018, *Textbook of Clinical Parasitology in dogs and cats*, Ed. Grupo Asis Biomedia
28. Bhat A.S., Mounsey K.E., Liu X., Walton S.*F.,*2017*, Host immune responses to the itch mite, Sarcoptes scabiei, in humans,* Parasites & Vectors, 10:385
29. Bloomquist J.R., 2003, *Chloride channels as tools for developing selective insecticides*. Arch. Insect Biochem. Physiol. 54, 145–156
30. Bochkov A.V., 2010, *A review of mammal-associated Psoroptidia (Acariformes: Astigmata). Acarina.*;18:99–260
31. Borchard P., Eldridge D.J.,Wright I.A., 2012, *Sarcoptes mange (Sarcoptes scabiei) increases diurnal activity of bare-nosed wombats (Vombatus ursinus) in an agricultural riparian environment*. *Mamm. Biol.* 77, 244–248
32. Bordeau W., Hubert B., 2000, *Treatment of 36 cases of canine Sarcoptes using a 0.25 % fipronil solution.*Veterinary Dermatology; **11** (Suppl. 1): 27

33. Bornestein S., 1991, *Experimental infection of dogs with Sarcoptes scabiei derived from naturally infected wild red foxes (Vulpes vulpes): clinical observations.* Vet. Dermatol. 2, 151–159
34. Bornstein S. Thebo P., Zakrisson G., 1996, *Evaluation of an enzyme-linked immunosorbent assay (ELISA) for the serological diagnosis of canine sarcoptic mange*. VetDermatol 7:21
35. Brown D.D.R., Siddiqui S.Z., Kaji M.D., Forrester S.G., 2012, *Pharmacological characterization of the Haemonchus contortus GABA-gated chloride channel, Hco-UNC-49: Modulation by macrocyclic lactone anthelmintics and a receptor for piperazine*. Vet. Parasitol. 185, 201–209
36. Buffet M., Dupin N., 2003, *Current treatments for scabies*. Fundam. Clin. Pharmacol. 17, 217–225
37. Burkhart C.G., Burkhart C.N., Burkhart K.M., 2000, *An epidemiologic and therapeutic reassessment of scabies.* CUTIS-N. Y.- 65, 233–242
38. Campbell W.C., 1989, *Ivermectin and Abamectin*. Springer-Verlag. New York.
39. Cangelosi G.A., Meschke J.S., 2014, *Dead or alive: molecular assessment of microbial viability*. Appl Environ Microbiol80:5,884–5,891
40. Chandramathi S., Suresh K., Anita Z.B., Kuppusamy U.R., 2009, *Elevated levels of urinary hydrogen peroxide, advanced oxidative protein prod-uct (AOPP) and malonaldialdehyde in humans infected with intestinal parasites.* Parasitology 136, 359–363
41. Charlesworth E.N., Johnson J.L., 1974, *An epidemic of canine scabies in man*, Arch. Dermatol. 110, 572–574
42. Chen Y.Z., Liu G.H., Song H.Q., Lin R.Q., Weng, Y.B., Zhu, X.Q. ,2014, *Prevalence ofSarcoptes scabiei infection in pet dogs in southernChina*. The Scientific World Journal, 718590
43. Chosidow, O., 2000. *Scabies and pediculosis.*The Lancet 355, 818
44. Chosidow, O., 2006, *Scabies*. N. Engl. J. Med. 354, 1718–1727
45. Chosidow O, Giraudeau B , 2012, *Topical ivermectin -- a step toward making head lice dead lice?* N Engl J Med 367:1750-1752
46. Cosoroabă I., 2014, *Tratat de Medicină Veterinară, Volumul VI,Secțiunea a XII-a: Parazitologie Veterinară*, Râile (Scabiile), p.973-1026, Ed. Risoprint, Cluj-Napoca
47. Costello E.K., Lauber C.L., Hamady M. *et al*., 2009, *Bacterial community variation in human body habitats across space and time*. Science; 326: 1,694–1,697
48. Cross P.C.Almberg E.S., Haase C.G., Hudson P.J., Maloney S.K., Metz M.C., Munn A.J., Nugent P., Putzeys O., Stahler D.R., Stewart A.C., Smith D.W.,2016, *Energetic costs of mange in wolves estimated from infrared thermography.Ecology* 97, 1938–1948
49. Currie B.J., Harumal P., McKinnon M., Walton S.F., 2004, *First documentation of in vivo and in vitro ivermectin resistance in Sarcoptes scabiei.* Clin. Infect. Dis. 39, e8–e12
50. Currier R.W., Walton S.F., Currie B.J., 2011, *Scabies in animals and humans: history, evolutionary perspectives, and modern clinical management*, Ann. N. Y. Acad. Sci. 1230, E50–E60

51. Curtis C.F., 1996, *Use of 0.25 per cent fipronil spray to treat sarcoptic mange in a litter of five-week-old puppies.*Veterinary Record; **139**: 43–4
52. Curtis C., Paradis M., 2003, *Sarcoptic mange, cheyletiellosis and trombiculosis*,Foster AP, Foil CS (eds) BSAVA manual of small animal dermatology. British Small Animal Veterinary Association, Gloucester, pp 146–149
53. Cuscó A., Sánchez A., Altet L., Ferrer L., Francino O., 2017a, *Individual Signatures Define Canine Skin Microbiota Composition and Variability*,Front. Vet. Sci. 4:6.
54. Cuscó A., Belanger J.M., Gershony L., Islas-Trejo A., Levy K., Medrano J.F., Sánchez A., Oberbaue A.M., Francino O., 2017b, *Individual signatures and environmental factors shape skin microbiota in healthy dogs,* Microbiome 5:139
55. De U.K., Dey S., 2010, *Evaluation of organ function and oxidant/antioxidant status in goats with sarcoptic mange,* Trop Anim Health Prod42:1663–1668
56. DeCandia A.L. Leverett K.N., vonHoldt B.M., 2019, *Of microbes and mange: consistent changes in the skin microbiome of three canid species infected with Sarcoptes scabiei mites,* Parasites & Vectors, *12:488*
57. Delafond O, 1862, *Traité pratique d'entomologie et de pathologie comparées de la psore ou gale de l'homme et des animaux domestiques*. Paris
58. Dimri U., Bandyopadhyay S., Singh S.K., Ranjan R.,Mukherjee R.,Yatoo M.I.,Patra P.H., De U.K, Dar A.A.,2014, *Assay of alterations in oxidative stress markers in pigsnaturally infested with Sarcoptes scabiei var. suis,* Veterinary Parasitology 205295–299
59. Dumont P., Blair J., Fourie J.J., Chester T.S., Larsen D.L., 2014, *Evaluation of the efficacy of afoxolaner against two European dog tick species: Dermacentor reticulatus and Ixodes ricinus.* Vet. Parasitol., Special Issue: NEXGARD®. Afoxolaner, a new oral insecticide-acaricide to control fleas and ticks in dogs 201, 216–219
60. Emde R.N., 1961, *Sarcoptic mange in the human: a report of an epidemic of 10 cases of infection by Sarcoptesscabiei, variety canis.* Arch. Dermatol. 84, 633–636
61. Fain A., 1968, *Etude de la variabilite de Sarcoptes scabiei avec une revision des Sarcoptidae.*Acta Zool Pathol Antverp 47, 1–196
62. Fain A., 1978, *Epidemiological problems of scabies.* Int. J. Dermatol. 17, 20–30
63. Fang F., Candy K.,Melloul E., Bernigaud C., Chai L., Darmon C., Durand R., Botterel F., Chosidow O.,Izri A., Huang W.,Guillot J., 2016, *In vitro activity of ten essential oils againstSarcoptes scabiei,* Parasites & Vectors 9:594
64. Faust E.C., Beaver P.C., Jung R.C., 1962, *Animal Agents and Vectors of Human Disease*, Acad. Med. 37, 804
65. Feather L., Gough K, Flynn R.J., Elsheikha H.M., 2010, *A retrospective investigation into risk factors of sarcoptic mange in dogs,* Parasitol Res, 107:279–283
66. Findley K., Oh J., Yang J., Conlan S., Deming C., Meyer J.A., Schoenfeld D., Nomicos E., Park M., Becker J *et al*, 2013, *Topographic diversity of fungal and bacterial communities in human skin,* Nature 498:367-70
67. Folz S.D., Kratzer D.D., Kakuk T.J. , Rector D.L., 1984, *Evaluation of a sponge-on therapy for canine scabies.*Journal of Veterinary, Pharmacology and Therapeutics, p. 29–34

68. Fraser A.T., Holme R., Martin A., Whiteley P., Montarello M., Raw C., Carver S.,Polkinghorne A., 2019, *Expanded Molecular Typing of Sarcoptes scabiei Provides Further Evidence of Disease Spillover Events in the* Epidemiology *of Sarcoptic Mange in Australian Marsupials,* Journal of Wildlife Diseases 55(1), 231-237
69. Fukuto T.R., 1990, *Mechanism of action of organophosphorus and carbamate insecticides.* Environ. Health Perspect. 87, 245–254
70. Gao Z., Tseng C.H., Pei Z., Blaser M.J., 2007, *Molecular analysis of human forearm superficial skin bacterial biota.* Proc Natl Acad Sci USA 104, 2927-2932
71. García-Reynaga P., Zhao C., Sarpong R., Casida J.E., 2013, *New GABA/Glutamate Receptor Target for [3H]Isoxazoline Insecticide.* Chem. Res. Toxicol. 26, 514–516
72. Gassel M., Wolf C., Noack S., Williams H., Ilg T., 2014, *The novel isoxazoline ectoparasiticide fluralaner: Selective inhibition of arthropod γ-aminobutyric acid- and l-glutamate-gated chloride channels and insecticidal/acaricidal activity.* Insect Biochem. Mol. Biol. 45, 111–124
73. Grice E.A., Kong H.H., Renaud G. *et al*., 2008, *A diversity profile of the human skin microbiota*. Genome Res; 18: 1,043–1,050
74. Grice E.A., Kong H.H., Conlan S. *et al*., 2009, *Topographical and temporal diversity of the human skin microbiome*. Science; 324:1,190–1,192
75. Hamady M., Knight R., 2009, *Microbial community profiling for human microbiome projects: tools, techniques, and challenges.* Genome Res 2009; 19:1, 141-1,152
76. Hampel V., Knaus M., Schäfer J., Beugnet F., Rehbein S., 2018, *Treatment of canine sarcoptic mange with afoxolaner (NexGard®) and afoxolaner plus milbemycin oxime (NexGard Spectra®) chewable tablets: efficacy under field conditions in Portugal and Germany*. Parasite 25, 63
77. Han S.H., Cheon H.I., Hur M.S., Kim M.J., JungW.H., Lee Y.W., Choe Y.B., Ahn K.J, 2018, *Analysis of the skin mycobiome in adult patiens with atopic dermatitis,* Exp Dermatol. 00:1-8
78. Hay R.J., Steer A.C., Engelman D., Walton S., 2012, *Scabies in the developing world–-its prevalence, complications, and management*. Clin Microbiol Infect;18(4):313–23
79. Hebra F.,1868*, On disease of the skin including the exanthemata*, vol. 2, p.164–252. The New Sydenham Society, London, United Kingdom. (Translated by C. H. Fagge and P. H. Pye-Smit.)
80. Heilesen B., 1946, *Studies on Acarus scabiei and scabies*
81. Hengge U.R., Currie B.J., Jäger G., *et al*, 2006, *Scabies: a ubiquitous neglected skin disease.* Lancet Infect Dis 6:769–779
82. Heukelbach, J., Feldmeier, H., 2004, *Ectoparasites—the underestimated realm.* The Lancet 363, 889–891
83. Hollingworth R.M., 1976, *Chemistry, biological activity, and uses of formamidine pesticides.* Environ. Health Perspect. 14, 57–69
84. Jannic A., Bernigaud C., Brenaut E., Chosidow O., 2018, *Scabies Itch*, Dermatol Clin., Jul;36(3):301-308
85. Jasmine P., 2011, *Clinical handbook on clinical dermatology*, 3rd edition,175 p

86. Jo J.H., Kennedy E.A., Kong H.H., 2017, *Topographical and physiological differences of the skin mycobiome in health and disease,* Virulence, 8:3, 324-333
87. Kennis R., 2004, *Arthropod parasite*, Fathman L, Gower J (eds) Small animal dermatology secrets. Mosby, St. Louis, Mo, London, pp 130–131
88. Kido N., Kamegaya C., Omiya T., Wada Y., Takahashi M., Yamamoto Y., 2011, *Hematology and serum biochemistry in debilitated, free-ranging raccoon dogs (Nyctereutes procyonoides) infested with sarcoptic mange,* Parasitol Int.Dec;60(4):425-8
89. Kiki-Mvouaka S., Menez C., Borin C., Lyazrhi F., Foucaud-Vignault M., Dupuy J., Collet X., Alvinerie M., Lespine A., 2010, *Role of P-Glycoprotein in the Disposition of Macrocyclic Lactones: A Comparison between Ivermectin, Eprinomectin, and Moxidectin in Mice.* Drug Metab. Dispos. 38, 573–580
90. Kilp S., Ramirez D., Allan M.J., Roepke R.K., Nuernberger M.C., 2014, *Pharmacokinetics of fluralaner in dogs following a single oral or intravenous administration*.Parasit Vectors 7, 85
91. Kim K.W., Oh Y.J., Cho B.K., Houh W., Kim J.A., Lee Y.S., 1990, *Norwegian scabies - dissemination of mites by medical intruments.* Ann. Dermatol. 2, 50–54
92. Lebon W., Beccati M., Bourdeau P., Brement T., Bruet V., Cekiera A.,Crosaz O.,Darmon C., Guillot J., Mosca M., Pin D., Popiel J., Handwerker D.P., Larsen D., Tielemans E., Beugnet F., Halos L.,2018, *Efficacy of two formulations of afoxolaner (NexGard® and NexGard Spectra®) for the treatment of generalised demodicosis in dogs, in veterinary dermatology referral centers in Europe,* Parasites & Vectors
93. Lespine A., Alvinerie M., Sutra J.F., *et al*, 2005, *Influence of the route of administration on efficacy and tissue distribution of ivermectin in goat*. Vet Parasitol 128:251–260
94. Letendre L., Huang R., Kvaternick V., Harriman J., Drag M., Soll M., 2014, *The intravenous and oral pharmacokinetics of afoxolaner used as a monthly chewable antiparasitic for dogs.* Vet. Parasitol. 201, 190–197
95. Leung M.H., Chan K.C., Lee P.K, 2016, *Skin fungal community and its correlation with bacterial community of urban Chinese individuals,* Microbiome 4:46
96. Lifschitz A., Virkel G., Imperiale F., *et al*., 1999, *Moxidectin in cattle: correlation between plasma and target tissues disposition.*J Vet Pharmacol Ther 22:266–273
97. Lower K.S., Medleau L.M., Hnilica K., Bigler B.,2001, *Evaluation of an enzyme-linked immunosorbant assay (ELISA) for the serological diagnosis of sarcoptic mange in dogs*. Vet Dermatol 12:315.
98. Lozupone C., Knight R., 2005, *UniFrac: a new phylogenetic method for comparing microbial communities.* Appl Environ Microbiol;71(12):8228–8235
99. Lozupone C., Knight R., 2008, *Species divergence and the measurement of microbial diversity*. FEMS Microbiol Rev; 32: 557–558
100. Mägi E., Järvis T., Miller I., 2006, *Effects of Different Plant Products against Pig Mange Mites,* Acta Vet. Brno, 75: 283-287
101. Mangowi A.L., 2014, *Effect of Agriculture on Abundance and Diversity of Arthropods with Chewing Mouth Parts at Sokoine University of Agriculture Main Campus.* J. Nat. Sci. Res. 4, 55–62

102. Marchesi J.R., Ravel J., 2015, *The vocabulary of microbiome research: a proposal.* Microbiomr 2015; 3:31
103. Martin A.M., Fraser T.A., Lesku J.A., Simpson K., Roberts G.L., Garvey J., Polkinghorne A., Burridge C.P., Carver S., 2018, *The cascadingpathogenic consequences of Sarcoptes scabiei infection that manifest in host disease*. *R. Soc. open sci.* 5: 180018
104. McKellar Q.A., Benchaoui H.A., 1996, *Avermectins and milbemycins*. J. Vet. Pharmacol. Ther. 19, 331–351
105. Meason-Smith C., Diesel A., Patterson A.P. , Older C.E., Mansell J.M., Suchodolski J.S., Rodrigues Hoffman A., 2015, *What is living on your dog's skin? Characterization of the canine cutaneous mycobiota and fungal dysbiosis in canine allergic dermatitis*,FEMS Microbiol Ecol 2015; 91: fiv139
106. Ménez C., Sutra J.F., Prichard R., Lespine A., 2012, *Relative Neurotoxicity of Ivermectin and Moxidectin in Mdr1ab (−/−) Mice and Effects on Mammalian GABA(A) Channel Activity.* PLoS Negl Trop Dis 6:e1883
107. Mika A., Reynolds S.L., Pickering D., McMillan D., Sriprakash K.S., Kemp D.J., Fischer K., 2012, *Complement inhibitors from scabies mites promote streptococcal growth – a novel mechanism in infected epidermis?* PLoS Negl Trop Dis ; 6(7):e1563
108. Miller W.H., Griffin C.E., Campbell K.L.., 2012, *Muller & Kirk's Small Animal Dermatology* 7th Edition, Ed. Elsevier, 938 p
109. Miller W.H. Jr., de Jaham C. Scott D.W. *et al*, 1996, *Treatment of canine scabies with milbemycin oxime*. CanadianVeterinary Journal; **37**: 219–21
110. Mofiz E., Seemann T., Bahlo M., Holt D., Currie B.J., Fischer K., Papenfuss A.T., 2016, *Mitochondrial Genome Sequence of the Scabies Mite Provides Insight into the Genetic Diversity of Individual Scabies Infections.* PLoS Negl. Trop. Dis. 10, e0004384
111. Mok W.Y., Barretto da Silva M.S., 1984, *Mycoflora of the human dermal surfaces,* Can J Microbiol, 30:1205-9
112. Morsy T.A., Bakr M.E., Ahmed M.M., *et al*, 1994*, Human scabies acquired from a pet puppy.* J Egypt Soc Parasitol 24:305–308
113. Mounsey K.E., Holt D.C., McCarthy J.S., Currie B.J., Walton S.F., 2009, *Longitudinal evidence of increasing in vitro tolerance of scabies mites to ivermectin in scabies-endemic communities.* Arch. Dermatol. 145, 840–841
114. Mounsey K.E., Pasay C.J., Arlia, L.G., Morgan M.S., Holt D.C., Currie B.J., Walton S.F., McCarthy J.S., 2010, *Increased transcription of Glutathione S-transferases in acaricide exposed scabies mites*
115. Mueller R.S., Bettena, S.V., Shipstone M., 2001, *Value of the pinnal-pedal reflex in the diagnosis of canine scabies*. Vet. Rec. 148, 621–623
116. Narahashi T., Zhao X., Ikeda T., Salgado V.L., Yeh J.Z., 2010, *Glutamate-activated chloride channels: Unique fipronil targets present in insects but not in mammals*. Pestic. Biochem. Physiol. 97, 149–152
117. Nathanson, J.A., 1985, *Characterization of octopamine-sensitive adenylate cyclase: elucidation of a class of potent and selective octopamine-2 receptor agonists with toxic effects in insects.* Proc. Natl. Acad. Sci. 82, 599–603

118. Neveu-Lemaire M., 1938, *Traité d'entomologie medicale et vétérinaire*
119. Nuttall T., Harvey R.G., McKeever P.J., 2009, *A Colour Handbook of Skin Diseases of the Dog and Cat,* Second Edition,Ed. Manson Publishing/The Veterinary Press, 336 p
120. Omura S., 2008, I*vermectin: 25 years and still going strong.* Int J Antimicrob Agents 31:91–8
121. Omura S, Crump A, 2014*, Ivermectin: panacea for resource-poor communities?* Trends Parasitol 30:445-455. Review
122. Overskaug K., 1994*, Behavioural changes in free-ranging red foxes (Vulpes vulpes) due to sarcoptic mange.Acta Vet. Scand.* 35, 457–459
123. Paradis M., 1998, *Ivermectin in small animal dermatology. Part II. Extralabel applications*, Compendium of Continuing Education for the Practicing Veterinarian ; **20**:459–69
124. Pasay C., Arlian L., Morgan M., Gunning R., Rossiter L., Holt D., Walton S., Beckham S., McCarthy J., 2009, *The Effect of Insecticide Synergists on the Response of Scabies Mites to Pyrethroid Acaricides.* PLoS Negl Trop Dis 3, e354
125. Pasay C., Walton S., Fischer K., Holt D., Mccarthy J., 2006, *Pcr-Based Assay to Survey for Knockdown Resistance to Pyrethroid Acaricides in Human Scabies Mites (Sarcoptes scabiei var hominis).* Am. J. Trop. Med. Hyg. 74, 649–657
126. Pasay C., Arlian L., Morgan M., Vyszenski-Moher D., Rose A., Holt D., Walton S., McCarthy J., 2008, *High-resolution melt analysis for the detection of a mutation associated with permethrin resistance in a population of scabies mites.* Med. Vet. Entomol. 22, 82–88
127. Pierezan F., Olivry T., Paps J., Lawhon S.D., Wu J., Steiner J.M., Suchodolski J.S, Rodrigues Hoffmann A., 2016, *The skin microbiota in an allergen challenged canine model of atopic dermatitis.* Vet Dermatol; 27: 332–e82
128. Ramos-e-Silva M., 1998, *Giovan Cosimo Bonomo (1663–1696): discoverer of the etiology of scabies*. Int. J. Dermatol. 37:625–630
129. Rapp C.M., Morgan M.S., Arlian L.G., 2006, *Presence of host immunoglobulin in the gut of Sarcoptes scabiei (Acari: Sarcoptidae).* J Med Entomol 43:539-42
130. Rodrigues Hoffmann A., 2017, *The cutaneous ecosystem: the roles of the skin microbiome in health and its association with inflammatory skin conditions in humans and animals,* Vet Dermatol 28; 60-e15
131. Rodrigues Hoffmann A., Patterson A.P., Diesel A., Lawhon S.D., Ly H.J., Stephenson C.E., Mansell J.,Steiner J.M., Dowd S.E.,Olivry T., Suchodolski J.S., 2014, *The Skin Microbiome in Healthy and Allergic Dogs*. PLoS ONE 9(1):e83197
132. Romero C.,Heredia R., Pineda J., Serrano J.A., Mendoza G.D., Trapala P., Cordero A. M., 2016, *Efficacy of fluralaner in 17 dogs with sarcoptic mange,* Vet Dermatology; 27: 353–e88
133. Roncalli R.A., 1987, *The history of scabies in veterinary and human medicine frombiblical to modern times*. Vet Parasitol. 1987;25(2):193–8
134. Rosumeck S., Nast A., Dressler C., 2018, *Ivermectin and permethrin for treating scabies*.Cochrane Database Syst Rev. 4:CD012994. Review

135. Roth W.I., 1991, *Scabies resistant to lindane 1% lotion and crotamiton 10% cream.* J. Am. Acad. Dermatol. 24, 502–503
136. Ruiz-Maldonado R., Tamayo L., Domiňguez J., 1977, Norwegian scabies due to *Sarcoptes scabiei* var *canis*. Arch. Dermatol. 113, 1733–1733
137. Samuel W.M., 1981, *Attempted experimental transfer of sarcoptic mange (Sarcoptes scabiei, Acarina: Sarcoptidae) among red fox, coyote, wolf and dog*. J. Wildl. Dis. 17, 343–347
138. Samuel W.M., Kocan A.A., Pybus M.J., Davis J.W., 2001, *Parasitic diseases of wild mammals*. Iowa State University Press, Ames
139. Schumann R.J., Morgan M.S., Glass R., Arlian L.G.,2002, *Characterization of house dust mite and scabies allergens by the use of canine serum antibodies*, Am J Vet Res 62:1344–1348
140. Senthilkumaran B., 2015, *Pesticide- and sex steroid analogue-induced endocrine disruption differentially targets hypothalamo–hypophyseal–gonadal system during gametogenesis in teleosts – A review.* Gen. Comp. Endocrinol. 219, 136–142
141. Shoop W.L., Mrozik H., Fisher M.H., 1995, *Structure and activity of avermectins and milbemycins in animal health.* Vet. Parasitol. 59, 139–156.
142. Shoop W.L., Hartline E.J., Gould B.R., Waddell M.E., McDowell R.G., Kinney J.B., Lahm G.P., Long J.K., Xu M., Wagerle T., Jones G.S., Dietrich R.F., Cordova D., Schroeder M.E., Rhoades D.F., Benner E.A., Confalone P.N., 2014*, Discovery and mode of action of afoxolaner, a new isoxazoline parasiticide for dogs.* Vet. Parasitol. 201, 179–189
143. Singh S.K., Dimri U., Sharma M.C., Swarup, Sharma B., 2011, *Determination of oxidative status and apoptosis in peripheral blood of dogs with sarcoptic mange,* Veterinary Parasitology 178330–338
144. Sitterle E., Rodriguez C., Mounier R., Calderaro J., Foulet F., Develoux M., Pawlotsky J., Botterel F., 2017, *Contribution of Ultra Deep Sequencing in the Clinical Diagnosis of a New Fungal Pathogen Species: Basidiobolus meristosporus*. Frontiers in microbiology.8:334. PubMed PMID: 28326064. Pubmed Central PMCID: 5339647. Epub 2017/03/23. eng
145. Six R.H., Clemence R.G., Thomas C.A. *et al*., 2000, *Efficacy and safety of selamectin against Sarcoptes scabiei on dogs and Otodectes cynotis on dogs and cats presented as veterinary patients.*Veterinary Parasitology ; **91**:291–309
146. Smith E.B., Claypoole T.F., 1967, *Canine scabies in dogs and in humans.* Jama 199, 59–64
147. Soderlund D.M., Bloomquist J.R., 1989*, Neurotoxic actions of pyrethroid insecticides*. Annu. Rev. Entomol. 34, 77–96
148. Soderlund D.M., Clark J.M., Sheets L.P., Mullin L.S., Piccirillo V.J., Sargent D., Stevens, J.T., Weiner M.L., 2002, *Mechanisms of pyrethroid neurotoxicity: implications for cumulative risk assessment*. Toxicology 171, 3–59
149. Solcan Gh., 2011, *Medicina internă a animalelor, vol 2,* Cap.Dermatitele purulente ale carnivorelor de companie (Piodermite),p.450-463, Ed. Eurostampa, Timișoara

150. Song S.J., Lauber C., Costello E.K. *et al*., 2013, *Cohabiting family members share microbiota with one another and with their dogs*. Elife ; 2: e00458
151. Soulsbury C.D., Iossa G., Baker P.J., Cole N.C., Funk S.M., Harris S., 2007, *The impact of sarcoptic mange Sarcoptes scabiei on the British fox Vulpes vulpes population.* Mammal Rev. 37, 278–296
152. Swe P.M., Zakrzewski M., Kelly A., Krause L., Fischer K., 2014, *Scabies Mites Alter the Skin Microbiome and Promote Growth of Opportunistic Pathogens in a Porcine Model*. PLoS Negl Trop Dis 8(5): e2897
153. Swe P.M., Reynolds S.L., Fischer K., 2014a, *Parasitic scabies mites and associated bacteria joining forces against host complement defence*. Parasite Immunol.; 36((1)1):585–93
154. Taenzler J., Liebenberg J., Roepke R.K.A., Frénais R. , Heckeroth A.J.,2016, *Efficacy of fluralaner administered either orally or topically for the treatment of naturally acquired Sarcoptes scabiei var. canis infestation in dogs,* Parasites and Vectors, 9:392
155. Tannenbau, M.H., 1965, *Canine scabies in man: A report of human mange*, JAMA 193, 321–322
156. Taylor M.A., Coop R.L., Wall R.L., 2016, *Veterinary Parasitology*, 4th Edition.ed
157. Terada Y., Murayama N., Ikemura H., Morita T., Nagata M., 2010,*Sarcoptes scabiei var. canis refractory to ivermectin treatment in two dogs: Ivermectin-refractory canine scabies*. Vet. Dermatol. 21, 608–612
158. Thomas J., Peterson G.M., Walton S.F., Carson C.F., Naunton M., Baby K.E., 2015, *Scabies: an ancient global disease with a need for new therapies*. BMC Infect. Dis. 15
159. Tizard I.R., Jones S.W., 2018, *The microbiota regulates immunity and immunologic diseases in dogs and* cats*,* Vet Clin Small Anim 48, 307-322
160. Ursell L.K., Clemente J.C., Rideout J.R. *et al*., 2012, *The interpersonal and intrapersonal diversity of human-associated microbiota in key body sites*. J Allergy Clin Immunol; 129: 1,204–1,208
161. Wagner R., Wendlberger U., 2000, *Field efficacy of moxidectin in dogs and rabbits naturally infested with Sarcoptes spp., Demodex spp. and Psoroptes spp. mites.*Veterinary Parasitology; **93**: 149–58
162. Walton S.F., Currie B.J., Kemp D.J., 1997, *A DNA fingerprinting system for the ectoparasite Sarcoptes scabiei.* Mol. Biochem. Parasitol. 85, 187–196
163. Walton S.F., Choy J.L., Bonson A., Valle A., McBroom J., Taplin D., Arlian L., Mathews J.D., Currie B., Kemp D.J., 1999a, *Genetically distinct dog-derived and human-derived Sarcoptes scabiei in scabies-endemic communities in northern Australia.* Am. J. Trop. Med. Hyg. 61, 542–547
164. Walton S.F., McBroom J., Mathews J .D., Kemp D.J., Currie B.J., 1999b, *Crusted scabies: a molecular analysis of Sarcoptes scabiei variety hominis populations from patients with repeated infestations.* Clin. Infect. Dis. 29, 1226–1230.
165. Walton S.F., Holt D.C., Currie B.J., Kemp D.J., 2004, *Scabies: New Future for a Neglected Disease, in: Advances in Parasitology.* Elsevier, pp. 309–376

166. Weber T., Selzer P.M., 2016, Isoxazolines: *A Novel Chemotype Highly Effective on Ectoparasites.* ChemMedChem n/a–n/a

167. Wolstenholme, A.J., Rogers, A.T., 2005, *Glutamate-gated chloride channels and the mode of action of the avermectin/milbemycin anthelmintics.* Parasitology 131, S85–S95

168. Wolstenholme A.J., 2012, *Glutamate-gated Chloride Channels.* J. Biol. Chem. 287, 40232–40238

169. Zakrzewski M., Proietti C., Ellis J., Hasan S., Brion M.J., Berger B., Krause L., 2016, *Calypso: A User-Friendly Web-Server for Mining and Visualizing Microbiome-Environment Interactions*. Bioinformatics

170. Zeeuwen P.L., Boekhorst J., van den Bogaard E.H., de Koning H.D., van de Kerkhof P.M., Saulnier D.M., van Swam I.I., van Hijum S.A., Kleerebezem M., Schalkwijk J., Timmerman H.M, 2012, *Microbiome dynamics of human epidermis following skin barrier disruption.*Genome Biol 13(11):R101

171. Zhang Z.Q., 2011, *Animal biodiversity: an outline of higher-level classification andsurvey of taxonomic richness*. Zootaxa.; 3148:237

172. Zhao C., Casida J.E., 2014, *Insect γ-Aminobutyric Acid Receptors and Isoxazoline Insecticides: Toxicological Profiles Relative to the Binding Sites of [ $^{3}$ H]Fluralaner, [ $^{3}$ H]-4'-Ethynyl-4- n -propylbicycloorthobenzoate, and [ $^{3}$ H]Avermectin.* J. Agric. Food Chem. 62, 1019–1024

173. Zhao Y., Cao Z., Cheng J., Hu L., Ma J., Yang Y., Wang X., Zeng J., Wang T., 2015, *Population identification of Sarcoptes hominis and Sarcoptes canis in China using DNA sequences.* Parasitol. Res. 114, 1001–1010.

***https://www.pathologyoutlines.com/topic/skinnontumorscabies.html, Accessed June 12th, 2019.

*** http://cgenome.net/calypso/, Accesed July 1st, 2019

***Illumina "16S Metagenomic Sequencing Library Preparation ", Document #15044223 Rev.B of 27 November 2013, Accesed September 2nd, 2019

https://support.illumina.com/downloads/16s_metagenomic_sequencing_library_preparation.html, Accesed September 28th , 2019

***https://rdrr.io/bioc/phyloseq/man/UniFrac-methods.html, Accesed October 5th, 2019

***http://www.metagenomics.wiki/pdf/definition/alpha-beta-diversity, Accesed October 7th, 2019

***https://www.researchgate.net/publication/319663057_The_Role_of_Cutaneous_Microbiota_Harmony_in_Maintaining_a_Functional_Skin_Barrier/fulltext/59f529c5458515547c21cfc9/The-Role-of-Cutaneous-Microbiota-Harmony-in-Maintaining-a-Functional-Skin-Barrier.pdf, Accesed February 2020

***Merck Veterinary Manual, https://www.merckvetmanual.com/special-subjects/reference-guides/serum-biochemical-reference-ranges, Accesed January 21st, 2020

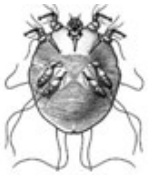
*** CDC, 2011. *CDC Health Information for International Travel 2012 : The Yellow Book: The Yellow Book.* Oxford University Press

*** GBD 2015 Disease and Injury Incidence and Prevalence Collaborators, 2016, *Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015*. Lancet 388:1545-1602.Erratum in: Lancet (2017) 389:el.

*** ESCCAP Guideline 03 Sixth Edition, March 2018

## Annex 1- Dog questionnaire 1st visit

**Questionnaire for dogs**
**1st visit**



Date: ____/____/____

Number (*e.g.* DOG001) of the dog: _____

**DOG:** Age or year of birth: _____ Gender: ☐ M ☐ F

**Dog info**: _____

**Habitat:**

- Other animal(s) in house: ☐ No ☐ Yes / Type: _____
- Number of animals in the house: _____

**Predisposing factors for scabies and/or associated pathology:**

**History of Dermatological disease**: ☐ No ☐ Yes

☐ Ectoparasites ☐ Allergic skin disease ☐ Other: _____

- Duration of sarcoptic mange signs: _____

**Treatment(s) taken the last month:**

☐ Antiparasitic treatment : _____

☐ Antibiotic: _____

☐ Topical steroid ☐ Systemic steroids: dose and how long? _____

☐ Antifungal therapy: name, when, how long? _____

☐ Other treatment: name, when, how long? _____

## Clinical exam

**Diagnosis:** ☐ Common mange ☐ Crusted mange

**Pruritus:** ☐ Yes ☐ No / Intensity: **+** **++** **+++**

**Sarcoptic mange lesions:**

Body distribution and type of lesions:

**Severity of the lesions:** ☐ Mild (≤ 10 lesions) ☐ Moderate (11–49 lesions) ☐ Severe (≥ 50)

**Associated pyoderma**: ☐ Yes ☐ No /

**Severity of pyoderma:**

☐ Very Mild (≤ 5 lesions) ☐ Mild (6–10 lesions) ☐ Moderate (11–49 lesions) ☐ Severe (≥ 50)

**Other skin lesions?** ☐ yes ☐ no

Details _____

## Additional Information

_____

_____

_____

_____

**Microbiome STUDY**

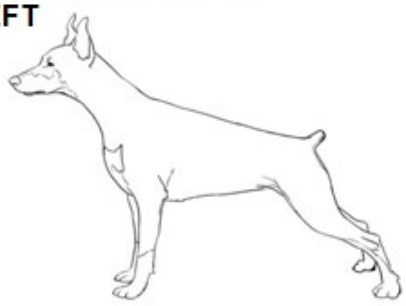
*Sample 3 infected sites (pinna, leg, groin) + the same equivalent sites in healthy skin as a control (6 samples in total per dog). Identify the sampled sites by surrounding the site on the figure bellow and give a number.*

*Write **S** for infected site and **C** for control, healthy site above the surrounding area; S**1** and C**1** for the site 1 (pinna), S**2** and C**2** for the site 2 (leg) and S**3** and C for the site 3 (groin).*

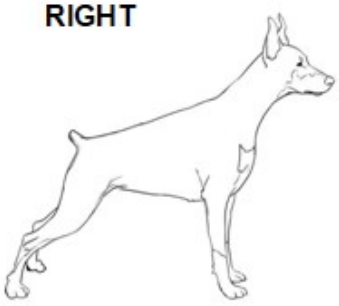
*Write the date on the tubes.*

- **Localizations of the sampling:**

**LEFT**



**RIGHT**



## Annex 2- Dog questionnaire 2nd visit

### Questionnaire for dogs
### 2nd visit

Date | _____/_____/_____

Number (*e.g.* DOG001) of the dog _____

**Treatment used after the 1st visit**

Molecule(s) used and modality (dose, delay between the doses) for sarcoptic mange:
* Preferred treatments for the study

☐ ivermectin* _____

☐ afoxolaner* _____

☐ Other:

_____

**Other treatment used**:

☐ antibiotic / precise : _____

Other: _____

**Clinical exam**

**Final Diagnosis:** ☐ Common mange ☐ Crusted mange

**Persistence of pruritus:** ☐ yes ☐ no / Intensity: **+** **++** **+++**

**Are cutaneous lesions still observed?** ☐ yes ☐ no

If yes, body distribution and type of lesions: _____

_____

**Severity of the persistent lesions:**

☐ Mild (≤ 10 lesions) ☐ Moderate (11–49 lesions) ☐ Severe (≥ 50)

**Associated pyoderma**: ☐ Yes ☐ No

**Severity of pyoderma:**

☐ Very Mild (≤ 5 lesions) ☐ Mild (6–10 lesions) ☐ Moderate (11–49 lesions) ☐ Severe (≥ 50)

**Other skin lesions?** ☐ yes ☐ no

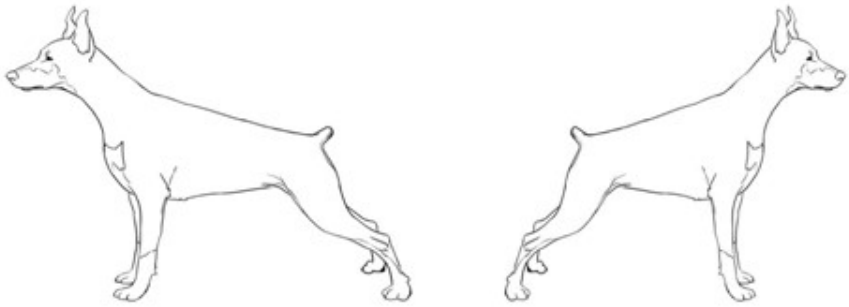
Details _____

**Microbiome Study**

***You need to sample (scrape and swab) <u>exactly the same areas as VISIT 1</u>*** *(the 3 infected sites + the 3 controls in adjacent healthy skin), even if there are no more skin lesions.*
*Identify the sampled sites by surrounding the site on the figure bellow and give a number.*
*Write **S** for scabies-infected site and **C** for control, healthy site above the surrounding area; S**1** and C**1** for the site 1 (pinna), S**2** and C**2** for the site 2 (leg) and S**3** and C for the site 3 (groin).*
*Write the date on the tubes.*

- **<u>Localizations of the samplings</u>:**

**LEFT** **RIGHT**



## Annex 3- Abreviation list

°C- degree Celsius

AchE-acetyl cholinesterase

ALT-Alanine amino transferase

AST- Aspartate amino transferase

bp- base pairs

CD 81 T cells- Clustr of differentiation 81 T cells

cm- centimeters

D- day

DNA-deoxy ribonucleic acid

ELISA- Enzyme linked immunosorbent assay

GABA- gamma aminobutyric acid

GAS- Group A *Streptococcus*

GluCls- glutamate gated chloride channels

HDM- house dust mite

h-hour

Ig E-immunoglobuline E

Ig G- immunoglobuline G

ITS-internal transcribed spacer

kdr- knockdown resistance

kg- kilograms

mg – milligrams

Min-minut

ML- macrocyclic lactones

ml- milliliter

Mm- millimeter

mM- millimolar

mt DNA cox 1- mithocondrial cytochrome C oxidase subunit

mt DNA-mithocondrial DNA

NGS- Next generation sequencing

nM-nanomolar

OTU- operational taxonomic unit

PCoA-Principal coordinates analysis

PCR- polymerase chain reaction

pM-picomolar

rRNA-ribosomal ribonucleic acid

sc- subcutaneous

SMPP- proteolytically inactive serine protease paralogs secreted by *Sarcoptes scabiei*

SMS- scabies mite serpins

spp-species

s-second

Th cell- T helper cell

TSS-total sum normalization

α-alpha

β-beta

µl- microlitre

## Annex 4- Figures list

## Annex 5- Tables list

## Annex 6- List of published papers

### In ISI proceedings journals (first author/coauthor)

PhD field (2)

1. **MÎNDRU Raluca**, ROMAN Constantin, LUPU C. Andrei, Gabriela MARTINESCU Larisa, IVĂNESCU, ACATRINEI M. Dumitru, GUILLOT Jacques, MIRON Liviu, 2019 - *Efficacy Assessment of Afoxolaner (Nexgard®) in Dogs Naturally Infected with Sarcoptes scabiei*. ISI Proceedings of the Int.Sci.Congress "Life sciences, a challenge for the future" p.426-431. ISBN: 978-88-85813-63-2
2. IVĂNESCU Maria Larisa , GRECU (MĂTIUȚ) Doina-Simona , MARTINESCU Gabriela , **MÎNDRU Raluca** , MIRON Liviu, 2019- *Case Report: Demodex Cornei of Dog Can Also Affect Humans*. ISI Proceedings of the Int.Sci.Congress "Life sciences, a challenge for the future" p.401-405. ISBN: 978-88-85813-63-2

### In BDI journals (first author/coauthor)

PhD field (2)

1. **Raluca MÎNDRU**, Constantin ROMAN, Andrei C. LUPU, Gabriela V. MARTINESCU, Larisa M. IVĂNESCU, Dumitru M. ACATRINEI, Ilie BODALE, Jacques GUILLOT, Liviu D. MIRON, 2020, *Epidemiology and clinical presentation of dogs infected with sarcoptic mange*, Lucr. ȘT. USAMV Iași, seria Medicină Veterinară, Ed. "Ion Ionescu de la Brad" Iași, vol. 62 partea a4a/2019, ISSN 2393 – 4603, p. 407-414
2. **Raluca MÎNDRU**, Constantin ROMAN, Liviu D. MIRON, Ștefan IRIMICIUC, Vlad GHIZDOVĂȚ, 2019*, Case study of a severely infected dog with Sarcoptes scabiei mites and the mathematical study of the interactions between mites and host,* BULETINUL INSTITUTULUI POLITEHNIC DIN IAŞI, Publicat de Universitatea Tehnică „Gheorghe Asachi" din Iaşi, Volumul 65 (69), Nr. 4, 2019, Secţia MATEMATICĂ. MECANICĂ TEORETICĂ. FIZICĂ, p.57-67

Other publications (4)

1. Andrei-Cristian LUPU, Alin BARBACARIU, Constantin ROMAN, **Raluca MÎNDRU**, GabrielaVictoria MARTINESCU, Andrei-Alexandru CÎMPAN, Liviu Dan MIRON, 2018, *In vivo study of conjugated diminazene aceturate for ichthyophthiriosis of farmed carp*, Lucr. ȘT. USAMV Iași, seria Medicină Veterinară, Ed. "Ion Ionescu de la Brad" Iași, vol. 61/2018, ISSN 2393-4603, p. 65-75

2. Andrei-Cristian LUPU, Alin BARBACARIU, Constantin ROMAN, Andrei-Alexandru CÎMPAN, **Raluca MÎNDRU**, Gabriela-Victoria MARTINESCU, Liviu Dan MIRON, 2018, *In vitro study of diminazene aceturate complex with β-cyclodextrin for Ichthyophthirius multifiliis*, Lucr. ȘT. USAMV Iași, seria Medicină Veterinară, Ed. "Ion Ionescu de la Brad" Iași, vol. 61/2018, ISSN 2393-4603, p. 58-64
3. Constantin ROMAN, Lavinia CIUCĂ, **Raluca MARDARE (MÎNDRU)**, Andrei-Cristian LUPU, Andrei-Alexandru CÎMPAN, Maria-Larisa IVĂNESCU, Olimpia IACOB, Dumitru ACATRINEI, Liviu Dan MIRON, 2017, *Observations regarding in vitro hatching of Raillietina spp. (Cestoda: Cyclophyllidea) onchosphere*, Lucr. ȘT: - Medicina Veterinara, Universitatea de Stiinte Agricole si Medicina Veterinara Ion Ionescu de la Brad Iasi, Vol. 60 (4); p. 477-480, ISSN 1454-7406
4. Constantin ROMAN, Lavinia CIUCĂ, **Raluca MARDARE**, Larisa M. IVĂNESCU, Dumitru M. ACATRINEI, Liviu D. MIRON, *Research regarding endoparasitosis in some wild mammals living in zoological parks,* LUCRĂRI ȘTIINȚIFICE MEDICINA VETERINARĂ VOL. L (2), 2017, TIMIȘOARA,**ISSN: -1221-5295**

**In ISI journals (coauthor)**

Other publications(4)

1. Andrei-Cristian LUPU, Mihaela BOMBOȘ, Cristian-Alin BARBACARIU, Constantin ROMAN, **Raluca MÎNDRU**, Gabriela Victoria MARTINESCU, Liviu Dan MIRON, - *Conditioning of praziquantel and florfenicol for some heterologous coinfections of farmed carp*, Revista de Chimie, vol. 70, nr. 7.2019 - IF 1.412 ).
2. Andrei-Cristian LUPU, Andra-Cristina BOSTĂNARU, Mihai MAREȘ, Laura URSU, Constantin ROMAN, **Raluca MÎNDRU**, Liviu Dan MIRON, *Ultrastructural aspects of Yersinia ruckeri cells after treatment with non-thermal plasma-activated water*, Revista de Chimie, ISSN: 0034-7752, vol. 70 (1) nr. 7. pp.118-121, 2019 - IF 1.412 ).
3. Ana Vasić, Nemanja Zdravković, Dragoș Aniță, Jovan Bojkovski, Mihai Marinov, Alexander Mathis, Marius Niculaua, Elena Luanda Oșlobanu, Ivan Pavlović, Dušan Petrić, Valentin Pflüger, Dubravka Pudar, Gheorghe Savuța, Predrag Simeunović, Eva Veronesi, Cornelia Silaghi and the SCOPES AMSAR training group (Adriana Aniță, Ioana Alexandra Anton, Andrei Cîmpan, Lavinia Ciucă, Luciana Crivei, Aleksandar Cojkić, Darko Davitkov, Vladimir Draskovic, Bojan Gajic, Uroš Glavinić, Maria-Larisa Ivănescu, Mihaela Kavran, Andrei-Cristian Lupu, **Raluca Mîndru**, Daniela Porea, Radiša Prodanović, Oliver Radanović, Cristian Raileanu, Stefan Raileanu, Marko Ristanić, Constantin Roman, Ljubodrag Stanišić, Slavica Vaselek, Miloje Đurić.), (2019) - *Species diversity, host preference and arbovirus detection of Culicoides (Diptera: Ceratopogonidae) in South-Eastern Serbia,* Parasites and Vectors, 2019 12:61, (IF 3.62).
4. Ana Vasic, Luanda Elena Oșlobanu, Mihai Marinov, Luciana Alexandra Crivei, Ioana Alexandra Rațoi, Adriana Anița, Dragos, Anița, Alexandru Dorosencu, Vasile

Alexe, Ștefan Raileanu, Predrag Simeunovic, Cristian Răileanu, Elena Falcuta, Florian Liviu Prioteasa, Jovan Bojkovski, Ivan Pavlovic , Alexander Mathis, Birke Andrea Tews, Gheorghe Savuța, Eva Veronesi, Cornelia Silaghi and the SCOPES AMSAR training group (Andrei Cimpan, Lavinia Ciuca, Aleksandar Cojki´c, Vladimir Draškovic, Miloje Djuric, Uroš Glavinic , Maria Larisa Ivanescu, Mihaela Kavran, Andrei Lupu, **Raluca Mindru**, Daniela Porea, Oliver Radanovic, Marko Ristanic , Constantin Roman, Ljubodrag Stanišic, Nemanja Zdravkovic, Slavica Vaselek), (2019)- *Evidence of West Nile Virus (WNV) Circulation in Wild Birds and WNV RNA Negativity in Mosquitoes of the Danube Delta Biosphere Reserve, Romania, 2016*, Tropical Medicine and Infectious Disease, 2019, 4,116

**Communications in international conferences- first author**

1. **Raluca MÎNDRU**, Andrei Cristian LUPU, Constantin ROMAN, Charlotte BERNIGAUD, Amaury BRIAND, Frédéric BEUGNET, Katja FISCHER, Liviu MIRON, Jacques GUILLOT, *Clinical Efficacy of Afoxolaner in Dogs Naturally Infected With Sarcoptes Scabiei and Concomitant Modifications of the Skin Microbiota*, 27th Conference of the World Association for the Advancement of Veterinary Parasitology (WAAVP), 7-11 Iulie, Madison, WI, SUA (Prezentare orală)
2. **Raluca MINDRU**, Charbel AL KHOURY, Fang FANG, Charlotte BERNIGAUD, Jacques GUILLOT, *In Vitro Assays to Evaluate Lethal or Repulsive Activity of Chemical Products Against Sarcoptes Scabiei,* 27th Conference of the World Association for the Advancement of Veterinary Parasitology (WAAVP), 7-11 Iulie, Madison, WI, SUA (Poster)

Books (coauthor)

1. *HANDBOOK of main zoonotic diseases identified in 4 EU countries: Romania, Italy, Croatia and Lithuania* - Miron Liviu (coord.), Dumitru Acatrinei, Olimpia Iacob, Larisa Ivanescu, Lavinia Ciuca,Constantin Roman, **Raluca Mindru**, Andrei Lupu, Andrei Cimpan, Gabriela Martinescu, ElenaVelescu, Mioara Matei, Carmen Manciuc, Alina Manole, Doina Azoicai, Florentina-Manuela Miron, Gianina-Ana Massari, Anca Colibaba, Cintia Colibaba, Stefan Colibaba, Elza Gheorghiu, Andreea Ionel (2016- 2019) - ZOE/Zoonosis Online Education Project 2016-1-RO01-KA203-024732,Editura ”Ion Ionescu de la Brad”, ISBN 978-973-147-334-5.
2. *Dirofilariosis in humans and animals - Dirofilarioza la om și animale* - Miron Liviu (coord.), Dumitru Acatrinei, Olimpia Iacob, Larisa Ivanescu, Lavinia Ciuca, Constantin Roman, **Raluca Mindru**, Andrei Lupu, Andrei Cimpan, Gabriela Martinescu, Elena Velescu, Mioara Matei, Carmen Manciuc, Alina Manole, Doina Azoicai, Florentina-Manuela Miron, Gianina-Ana Massari, Anca Colibaba, Cintia Colibaba, Stefan Colibaba, Elza Gheorghiu, Andreea Ionel (2016- 2019) -

ZOE/Zoonosis Online Education Project 2016-1-RO01-KA203-024732, Editura ”Ion Ionescu de la Brad”, ISBN 978-973-147-313-0.

3. *La dirofilariose chez les humains et les animaux - Dirofilarioza la om și animale* - Miron Liviu (coord.), Dumitru Acatrinei, Olimpia Iacob, Larisa Ivanescu, Lavinia Ciuca, Constantin Roman, **Raluca Mindru**, Andrei Lupu, Andrei Cimpan, Gabriela Martinescu, Elena Velescu, Mioara Matei, Carmen Manciuc, Alina Manole, Doina Azoicai, Florentina-Manuela Miron, Gianina-Ana Massari, Anca Colibaba, Cintia Colibaba, Stefan Colibaba, Elza Gheorghiu, Andreea Ionel (2016- 2019)- ZOE/Zoonosis Online Education Project 2016-1-RO01-KA203-024732, Editura ”Ion Ionescu de la Brad”, ISBN 978-973-147-314-7.
4. *Malaria* - Miron Liviu (coord.), Dumitru Acatrinei, Olimpia Iacob, Larisa Ivanescu, Lavinia Ciuca, Constantin Roman, **Raluca Mindru**, Andrei Lupu, Andrei Cimpan, Gabriela Martinescu, Elena Velescu, Mioara Matei, Carmen Manciuc, Alina Manole, Doina Azoicai, Florentina-Manuela Miron, Gianina-Ana Massari, Anca Colibaba, Cintia Colibaba, Stefan Colibaba, Elza Gheorghiu, Andreea Ionel (2016- 2019) - ZOE/Zoonosis Online Education Project 2016-1-RO01-KA203-024732, Editura ”Ion Ionescu de la Brad”, ISBN 978-973-147-315-4.
5. *Le paludisme - Malaria* - Miron Liviu (coord.), Dumitru Acatrinei, Olimpia Iacob, Larisa Ivanescu, Lavinia Ciuca, Constantin Roman, **Raluca Mindru**, Andrei Lupu, Andrei Cimpan, Gabriela Martinescu, Elena Velescu, Mioara Matei, Carmen Manciuc, Alina Manole, Doina Azoicai, Florentina-Manuela Miron, Gianina-Ana Massari, Anca Colibaba, Cintia Colibaba, Stefan Colibaba, Elza Gheorghiu, Andreea Ionel (2016- 2019) - ZOE/Zoonosis Online Education Project 2016-1- RO01-KA203-024732, Editura ”Ion Ionescu de la Brad”, ISBN 978-973-147-316-1.