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.