DEPARTMENT OF VETERINARY SCIENCES

UNIVERSITY OF TURIN

RESEARCH DOCTORATE IN VETERINARY SCIENCES FOR ANIMAL HEALTH AND FOOD SAFETY

CYCLE XXXIV

PhD CANDIDATE: Barbara Moroni, DVM

PhD TUTOR: Dr. Luca Rossi, DVM, PhD

PhD COORDINATOR: Prof.ssa Maria Teresa Capucchio, DVM, PhD

Disciplinary – Scientific sector: Veterinary Parasitology

Academic Years 2018-2021

PhD Thesis

"*Sarcoptes scabiei* in European wildlife: solving the epidemiological enigma and exploring control options"

INDEX

Abstract

Sarcoptic mange is a contagious parasitic skin disease caused by the borrowing mite *Sarcoptes scabiei* affecting more than 150 mammalian species worldwide. Host range is uniquely broad, and there is an ongoing debate whether *S. scabiei* encompasses (or not) different host-specific variants or subspecies. Since it can result in significant declines in local wildlife populations, sarcoptic mange received particular attention in wildlife conservation and management for decades.

While genetic studies of *Sarcoptes* mites may have a striking role in tracing reservoirs and identifying different circulating strains, further studies are also warranted to explore the feasibility and efficacy of oral acaricide treatment in wildlife. The objective of this project is to investigate the genetic structure of *Sarcoptes* isolates in wildlife and to improve scabies control programmes in free-ranging ungulates. To achieve it, two main research lines have been carried out: i) a molecular study of a large set of *S. scabiei* isolates, aiming to characterize different mite populations using microsatellites as molecular markers, and to reveal possible connections between them; ii) a study on the pharmacological control of scabies by means of oral ivermectin in Iberian ibex model.

Chapter 1:

In Spain, sarcoptic mange was first described in native wildlife in 1987 in Cazorla Natural Park, causing the death of nearly 95% of the local native population of Iberian ibex (*Capra pyrenaica*). Since then, additional outbreaks have been identified in several populations of ibex and other wild ungulate species throughout the country. Although the first epizootic outbreak in wildlife was attributed to the introduction of an infected herd of domestic goats, the origin and the cause of its persistence remain unclear. Ten Sarcoptes microsatellite markers were used to characterize the genetic structure of 266 mites obtained from skin scrapings of 121 mangy wild ruminants between 2011 and 2019 from 11 areas (=populations) in Spain. The results of this study highlight the existence of three genetic strains of *S. scabiei* in the wild ruminant populations investigated. While two genetic clusters of *S. scabiei* were host- and geography-specific, one cluster included multi-host mites deriving from geographically distant populations, suggesting an origin from domestic goats. The molecular epidemiological study of *S. scabiei* in wild ruminants in Spain indicates that the spreading and persistence of the parasite may be conditioned by host species community composition and the permissiveness of each host population to the circulation of individual "strains," among other factors.

Chapter 2:

In 2018, a wild boar (*Sus scrofa*) with lesions compatible with sarcoptic mange was hunted in Ports de Tortosa i Beseit Natural Park (PTB, north-eastern Spain), where an active epizootic outbreak of sarcoptic mange is affecting Iberian ibexes (*Capra pyrenaica*) since 2014.

Mites obtained from the affected wild boar were molecularly genotyped using 10 specific *S. scabiei* microsatellites. For comparison, mites obtained from sympatric Iberian ibexes and allopatric wild boars and Iberian ibexes from southern Spain were also analysed. Three *S. scabiei* genetic clusters were identified: one included mites from southern Iberian ibexes, another included mites from southern wild boars, and a third one distinctively grouped the wild boar from PTB with the sympatric ibexes. This is the first reported case of sarcoptic mange in wild boar in Spain and the first documented case of *S. scabiei* cross-transmission from a wild ruminant host to a wild boar. As for pathology, the wild boar presented an ordinary scabies type reaction, which is typical of the self-limiting infestations reported in other cases of interspecific transmission.

Chapter 3:

The Iberian hare (*Lepus granatensis*) is a popular small game species in the Iberian Peninsula, and it has never been reported to be affected by sarcoptic mange. An adult female Iberian hare with overt skin lesions on forelimbs and ventral thorax, suggestive of sarcoptic mange, was culled in Quart de les Valls municipality in the Valencian Community, Spain, in 2019. Skin scrapings were digested in 10% KOH solutions to confirm the presence of mites. Ten *Sarcoptes* microsatellites markers were used to characterize the genetic structure of mites obtained from the hare, and from sympatric and allopatric wild rabbits (*Oryctolagus cuniculus*) and red foxes (*Vulpes vulpes*). A total of 56 alleles were counted across the 10 microsatellite loci. Six private alleles were found at four loci (Sarms 33, 38, 41, 45). The multivariate analysis characterized three main clusters, corresponding to mites collected on foxes originating from Catalonia, foxes from the Valencian Community and the hare plus sympatric and allopatric wild rabbits. To our knowledge, this is the first reported case of sarcoptic mange in the Iberian hare and one of the very few documented in *Lepus* spp. Based on results, the origin of this case was molecularly traced back to contacts with endemically infected wild rabbits.

Chapter 4:

Domestic and wild felines are considered suitable hosts for the parasitic mite *Sarcoptes scabiei* and sarcoptic mange is reported in several felid species in the scientific literature, although relatively rarely in domestic cat. However, the traditional classification of host-specific varieties of *Sarcoptes* mites does not include *S. scabiei* var. *felis*, and it is not clear whether sarcoptic mange in felines actually implies transmission from canids, other sympatric species or exclusively felines. The aim of this Chapter was to characterize the genetic structure of *S. scabiei* mites from domestic cats (*Felis catus*) and Eurasian lynxes (*Lynx lynx*) comparing them with *Sarcoptes* mites from sympatric domestic and wild carnivores.

Ten *Sarcoptes* microsatellite markers were used to genotype 81 mites obtained from skin scrapings of 36 carnivores: 4 domestic cats, one dog (*Canis lupus familiaris*), 4 lynxes, 23 red foxes (*Vulpes vulpes*) and 4 grey wolves (*Canis lupus lupus*) from Italy, Switzerland and France. Results show the existence of two genetic clusters of *S. scabiei* with a geographical distribution-pattern: mites from Central Italian cats clustered with those from sympatric wolves, while all the other mites from Switzerland, France and Northern Italy clustered together. These results strengthen the previously advanced hypothesis that genetic strains of *S. scabiei* have a predominant geographic-related distribution, with transmission patterns that rely on direct or indirect contacts between different hosts living in the same ecological niche, rather than on species-specific contacts within the same taxon.

Chapter 5:

Zoonotic scabies (ZS), also referred to as "pseudoscabies", is considered a self-limiting disease with a short incubation period and transient clinical skin signs. It is commonly thought that *Sarcoptes scabiei* mites from animals are unable to successfully reproduce and persist on human skin; however, several ZS case reports have mentioned the persistence of symptoms and occasionally mites for weeks. The aim of this Chapter was to collect and organize the sparse literature explicitly referring to *S. scabiei* zoonotic transmission, focusing on the source of the outbreak, the circumstances leading to the transmission of the parasite, the diagnosis including the identification of the *Sarcoptes* "strain" involved, and the applied treatments. A total of 46 articles, one conference abstract and a book were collected describing ZS cases associated with twenty animal hosts in five continents. Dogs were by far the most common source among pet owners, while diverse livestock and wildlife contributed to the caseload as an occupational disease. Genetic epidemiological studies of ZS outbreaks are still limited in number, but tools are available to fill this knowledge gap in the near future. Further research is also needed to understand the apparent heterogeneity in the morbidity, disease severity and timing of the response to treatment among people infected with different animal-derived strains.

Chapter 6:

Sarcoptic mange is considered the main driver of demographic declines occurred in the last decades in Iberian ibex (*Capra pyrenaica*) populations. Mass treatment campaigns by administration of in-feed acaricides are used as a measure to mitigate the impact of mange in the affected populations. However, there are no data on ivermectin pharmacokinetics in this wild caprine, and the treatment through medicated feed is not endorsed by evidence on its effectiveness. The aim of this Chapter was to determine the pharmacokinetic profile of ivermectin in plasma samples of ibexes after the experimental oral administration of ivermectin, using high performance liquid chromatography (HPLC) with automated solid phase extraction and fluorescence detection. A dose of 500 µg of ivermectin per body weight was orally administered in a feed bolus to nine healthy adult ibexes (seven males and

two females). Blood samples were collected by jugular venipuncture into heparin-coated tubes at day 1, 2, 3, 4, 7, 10, 15, and 45 post-administration (dpa). The highest plasma concentration of ivermectin (Cmax = 3.4 ng/ml) was detected 24 hours after the oral administration (T1), followed by a rapid decrease during the first week post-administration. Our results reveal that plasma ivermectin concentration drops drastically within five days of ingestion, questioning the effectiveness of a single in-feed dose of this drug to control sarcoptic mange. This is the first study on plasma availability of ivermectin in ibexes and in any wild ungulate species.

INTRODUCTION

1. *Sarcoptes scabiei*: history and biology

The history of the itch mite *Sarcoptes scabiei* (DeGeer, 1778) is long and intriguing. Scabies, as it is called the skin disease caused by *S. scabiei* in humans, is one of the oldest described in literature, with the first references tracing back to the Bible (1200 b.c) (Roncalli, 1987). The term "scabies" (from Latin, *scabere*: to scratch) seems to be attributed by Celsus, a Roman physician who described papules on his patients (Roncalli, 1987), although the first association between the causative agent and the disease has been made by Bonomo and Cestoni in 1687, marking in the history of medicine a first definitely known cause for any of the diseases of man (Arlian and Morgan, 2017). Linnaeus in 1746 was the first to name the mite *Acarus humanus subcutaneous* in man and *Acarus exulcerans* in animals, although later in 1834, the Corsican medical student Renucci renamed it *Acarus scabiei* after the lesions found on a scabietic patient from Paris.

S. scabiei is a parasitic mite belonging to the superorder Acariformes, order Sarcoptiformes, suborder Oribatida, family Sarcoptidae and the group Astigmata (along with the house dust mites *Dermatophagoides farinae*) (Zhang, 2011). The female is about 400 μm long and the male is approximately half her size. *Sarcoptes* body is characterized by an oval, tortoise-like shape with eight short legs that hardly project beyond the body brim. One of the peculiar characteristics of *S. scabiei* morphology are the numerous ridges and scales on the back, which are not seen on many other mange mites on mammals (Burgess, 1993).

The life cycle of *S. scabiei* includes five developmental stages, typical of any other astigmatid mite: egg, larva, protonymph, tritonymph and adult, but as opposite to other astigmatid free-living mites, the entire life cycle of *S. scabiei* is carried out on the infested host(s). It has been estimated that the duration of the life cycle for *Sarcoptes* mites ranged from 7 to 21 days, with females producing 40-50 eggs over a life span of 26-40 days (Arlian and Morgan, 2017).

Despite *S. scabiei* being an obligate parasite, thus needing a host to maintain and complete its life cycle, this parasite is also able to survive in the environment seeking the host through stimuli such as odor and body temperature. According to experimental studies by Arlian (Arlian *et al.*, 1984), *Sarcoptes* mites can live off- host for 7 days at 15°C with a relative humidity above 75%, although depending on the environmental conditions the time range can largely vary being usually shorter. In general, warmer temperatures and low humidity reduce the survival time, as mites are unable to maintain their water balance and rapidly die of dehydration. While the host-seeking behavior of *Sarcoptes* mites proves the

existence of an environmental transmission through contaminated fomites in both humans and animals, the primary means of transmission is considered to be through direct contact with an infected host.

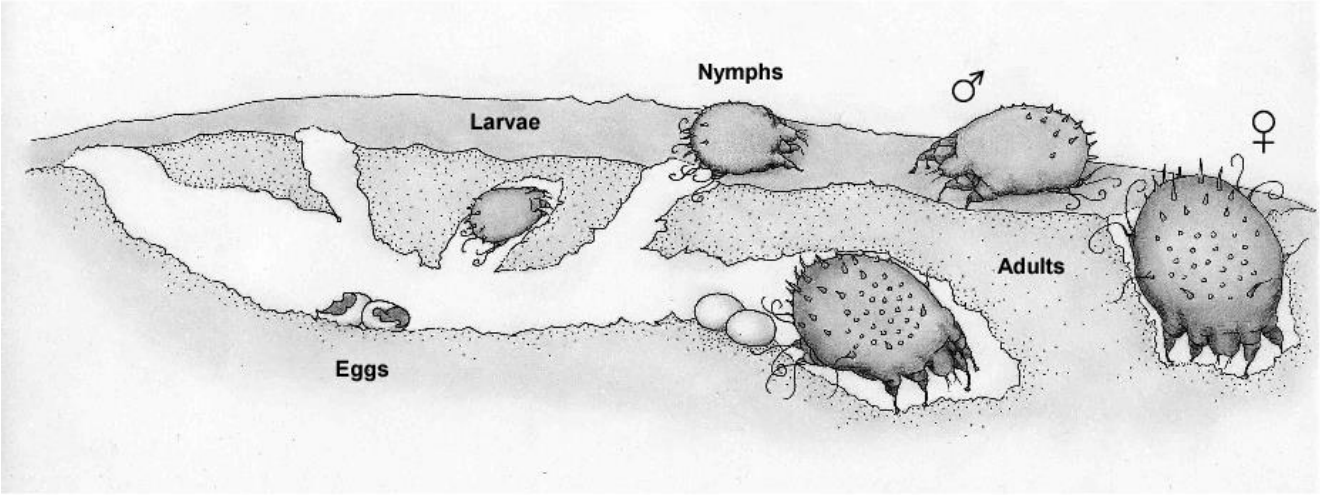


Figure 1. Different developmental stages of the mite *Sarcoptes scabiei* in the epidermis of a host. Photo credit by the doctoral thesis by Molin (Molin, 2009).

2. Genetic Characterization of *Sarcoptes scabiei*

Sarcoptes scabiei is considered a single species with a wide range of host-specific variants or lineages based on the host where they are collected, such as the carnivore or ungulate variants (Zahler *et al.*, 1999; Arlian and Morgan, 2017). However, there is an ongoing debate about the host and geographic specificity and the potential of interspecific transmission of the mite. Understanding the susceptible hosts and transmission pathways for the mite and each one of the variants should allow us to establish the potential host communities for *S. scabiei* and the consequent effects on wildlife conservation, livestock health and zoonotic risk (Escobar *et al.*, 2021). Attempts at clustering *S. scabiei* by host species and geographical localization have ended up with controversial results, at least partly due to the variability of molecular markers used (Berrilli *et al.*, 2002; Rasero *et al.*, 2010; Moroni *et al.*, 2021c).

In the last two decades, genetic molecular tools offered new opportunities to answer old questions on the genetic structure of *S. scabiei*. Amongst these, the main and oldest question is about the nature of *S. scabiei*: a single parasite species structured in host specific "variants" or something else and more complex (e.g., a cocktail of "strains" with different degrees of host specificity, in perennial evolution)? It is now undisputed that, thanks to contribution of molecular epidemiology, the second scenario has become the most accredited.

While morphological studies of *S. scabiei* mites have failed to recognize host-specific differences (Arlian *et al.*, 1984, 1988), growing molecular epidemiological data (Rasero *et al.*, 2010; Gakuya *et al.*, 2011; Matsuyama *et al.*, 2019; Moroni *et al.*, 2021c) have called into question the traditional, still widely accepted, classification of *S. scabiei* into species-specific variants (Arlian and Morgan, 2017).

2.1. Internal transcribed spacer 2

The first contribution on *S. scabiei* genetics dates back to the end of the past century. In a milestone paper, Zahler et al. (1999) analyzed 23 pooled samples of mite isolates from nine host species in four continents, using the second internal transcribed spacer (ITS-2) of ribosomal DNA (rDNA) as genetic marker. Authors concluded that *S. scabiei* consists of a single, heterogeneous species. It was noted by Berrilli et al. (2002) that the "monolithic" results obtained by Zahler et al. (1999) were possibly related to the fact that their study was based on pooled samples and that genetic polymorphism among single individuals may thus have been underestimated. From here on, *S. scabiei* DNA has been obtained by individual mites only. However, the same view as Zahler et al. (1999) was subsequently endorsed by Gu and Yang (2008) who could not differentiate between sympatric and allopatric isolates from pigs and rabbits in China, nor between them and allopatric deposited sequences from humans and a range of other domestic and wild mammals. While ITS-2 has been widely appreciated by molecular taxonomists for distinguishing closely related species and examining phylogenetic relationships within parasitic Arachnida genera (Black and Piesman, 1994; Crampton *et al.*, 1996), further studies clearly showed that ITS-2 is not suitable marker for genetic characterization within a mite species, including *S. scabiei* (Alasaad *et al.*, 2009a) and the related worldwide distributed genera, *Psoroptes* and *Chorioptes* (Essig *et al.*, 1999; Zahler *et al.*, 1999). Accordingly, the use of ITS-2 as the sole marker in the study of *S. scabiei* genetics has been dismissed. Some papers have nevertheless been published in which ITS-2 was used in parallel with markers that were proved to be more informative (Berrilli *et al.*, 2002; Amer *et al.*, 2014; Zhao *et al.*, 2015; Peltier *et al.*, 2017).

2.2. 16S and 12S mitochondrial rRNA genes

Similarly as ITS-2, other genetic markers in large use for the detection of genetic variation among closely related taxa were proved to be suboptimally informative on *S. scabiei* genetics, if not the source of epidemiologically inconsistent results. The so far investigated partial sequences of the 16S and the 12S mitochondrial rRNA genes are undoubtedly amongst them. In a study by Berrilli et al. (2002), the first marker identified: i) a significant amount of genetic differentiation between red fox (*Vulpes vulpes*)-derived mites (originating from two populations in Italy and one in Spain), and ii) a substantial similarity between mites from two geographically isolated but also closely related host taxa, Northern chamois

(*Rupicapra rupicapra*) from the Alps, Italy, and Southern chamois (*R. pyrenaica parva*) from the Cordillera Cantabrica, Spain. However, one of the fox-derived populations unexpectedly clustered with the two chamois-derived mite populations, the first of them sympatric (Alps) but the other one obviously allopatric. Sound epidemiological evidence of similarity or separation between *Sarcoptes* populations, as in Berrilli et al. (2002), are essential prerequisites to infer on the accountability of the genetic markers. In another study on mites obtained from wombats, dogs and humans in Australia (Skerratt *et al.*, 2002), the use of a 326 bp fragment of the mitochondrial 12S rRNA gene did not result in any genetic differentiation between host populations. Authors concluded that this was consistent with the argument that overseas people and/or their dogs introduced to Australia the *S. scabiei* mites that further infected wombats and put a serious threat on their conservation. While the fore mentioned general conclusion was endorsed by other studies using the same and/or other genetic markers (Andriantsoanirina *et al.*, 2015a; Fraser *et al.*, 2017), it is now clear that the polyphyletic associations found in that early study were rather based on short uninformative fragments of the single adopted marker.

2.3. Cytochrome oxydase subunit I gene

The analysis of sequences of the cytochrome oxydase subunit I (COI) gene was first used by Walton et al. (2004), in parallel with microsatellite markers, to investigate the genetic epidemiology of S. scabiei in Northern Australia. According to their study, human derived mites were grouped into three clades, one of them including also animal (mostly dog and wombat) derived mites. While results were encouraging on the potential of this marker, Microsatellite data suggested a different and more coherent genetic structure, in which a wombat, two human, an allopatric dog, and a sympatric "dog plus wallaby" clades could be identified. Interestingly, all subsequent COI-based molecular epidemiological studies where consistent in revealing distinct clades of human derived mites (Amer *et al.*, 2014; Andriantsoanirina *et al.*, 2015b; Zhao *et al.*, 2015), thus calling into question the hypothesis of panmixia for *S. scabiei* in humans. On the other hand, sequences from mites of domestic and wild animal origin rarely clustered according to their host preference, and were most often grouped in a single multi-host clade of uncertain epidemiological meaning (Amer *et al.*, 2014; Makouloutou *et al.*, 2015; Zhao *et al.*, 2015; Peltier *et al.*, 2017; Lastuti *et al.*, 2019; Ueda *et al.*, 2019). Nonetheless, COI has been proved the most informative amongst mitocondrial DNA markers (Fraser *et al.*, 2019). In two recent studies (Fraser *et al.*, 2017, 2019), the first of which also included the phylogenetic analysis of near full-length mitochondrial genomes of mites from Australian wildlife and from humans and dogs from Australia, Asia and Europe (Fraser *et al.*, 2017), analysis of COI gene sequences gave strong support to the hypothesis that Australian native wildlife became exposed to Sarcoptes mites not earlier than a few centuries ago, following multiple Sarcoptes introductions most likely from dogs following colonizers

from across the globe. This hypothesis of multiple introduction events has also been implicated in North American black bears (Peltier *et al.*, 2017) and in wild canids in Japan (Matsuyama *et al.*, 2015). Intriguingly, the phylogenetic analysis of COI gene was used to explore the scientific substantiation of the widely accepted hypothesis (Fain, 1978) that humans were the initial source of the animal - namely dog and possibly wild canid - contamination with Sarcoptes. The results were clearly not consistent with a human origin of S. scabiei mites in dogs and, on the contrary, did not exclude the opposite hypothesis of a spillover from dogs to humans (Andriantsoanirina *et al.*, 2015b).

2.4. Microsatellite markers

Recently, the molecular markers such as microsatellites (known as short tandem repeat -STR- or simple sequence repeat -SSR-) have become the most used and accepted molecular markers for the identification of *Sarcoptes* host-taxon clusters (Walton *et al.*, 2004; Alasaad *et al.*, 2007; Rasero *et al.*, 2010; Moroni *et al.*, 2021c) and in forensic investigations of scabietic traded animals (Alasaad *et al.*, 2012a) to trace the origin of outbreaks and mite genetic population distances.

Thanks to these markers it is possible (at least in wildlife or poorly human-manipulated contexts) to identify species- and origin-related *Sarcoptes* strains and find accurate answer to questions such as: "within a community of *Sarcoptes* sensitive species, how many genetic strains of sarcoptic mange are circulating?" or "may a selected host species be infected by several *Sarcoptes* strains under natural conditions?".

Early microsatellite-based contributions date back to the late 1990s. It was the merit of Walton et al. (1999) to molecularly prove, for the first time, that closely sympatric hosts may harbor *Sarcoptes* mites belonging to different populations. The model, now a renewed one, comprised remote native Australians communities and their dogs, both endemically infected by *S. scabiei*. More than 700 individual mites were characterized by using a panel of only three microsatellites. Results showed that gene flow between mite populations on human and dog hosts was extremely rare if any.

The same results were confirmed in a later study (Walton *et al.*, 2004) by characterizing remarkably less mites albeit with a higher number of microsatellites.

Elsewhere, microsatellite markers were initially used to differentiate between *S. scabiei* populations responsible for outbreaks in free-ranging herbivores and carnivores in Southern Europe (Rasero *et al.*, 2010). The main studied populations were mites from Northern chamois (*R. rupicapra*) and red fox (*Vulpes vulpes*) in Northern Italy, and from a Southern chamois (*R. pyrenaica parva*) population in Northern Spain with no spatial or ecological connection with the former two. No gene flow was found between groups, suggesting the existence of both host- and origin-related lineages.

In a subsequent microsatellite study on a larger number of mites (N=251) obtained from ten wild hosts in three European countries, Rasero et al. (2010) showed that mite populations were clustered into

three main groups: herbivore-, omnivore- and carnivore-derived populations, which for the purposes of the study should be intended as synonyms of ruminants, pigs (wild boars) and carnivores (canids, felids and mustelids), respectively. The separation between these groups was better supported than the geographical separations; nevertheless, a sub-clustering was detected within each of these three groups that separated mite populations according to the geographical origin. These findings demonstrated that *Sarcoptes* is not a single panmictic population, not even within each geographical location.

"Host-taxon law" was the term suggested to identify this major transmission pattern. In a natural multi-host system in Spain, Alasaad et al. (2011) provided evidence of the temporal stability of the genetic structure of *S. scabiei* under the host-taxon law. A subsequent study in Masai Mara, Kenya, revealed a second transmission pattern eventually associated to ecosystems including top predators such as lions (*Panthera leo*) and cheetahs (*Acinonyx jubatus*) and their respective favorite preys, all enzootically infected by *S. scabiei* (Gakuya *et al.*, 2011).

Microsatellite genetic typing showed the following: (i) absence of gene flow between the herbivore (Thomson's gazelle and wildebeest, *Connochaetes taurinus*)-derived and the two carnivore (lion and cheetah)-derived mite populations; (ii) similarity between lion- and wildebeest-derived mite populations, suggesting *S. scabiei* cross-infection from wildebeests (the favourite preys of lions); and (iii) greater complexity of cheetah-derived *Sarcoptes* population which included three different subpopulations. One of these was cheetah-private, one was similar to the wildebeest- and lion-derived mite population, and a third was similar to the mite population derived from Thomson's gazelles, the favourite preys of cheetahs in Masai Mara. This new and complementary pathway was denominated "prey-to-predator".

Another multi-host model in Spain included two herbivores (Southern chamois and red deer), wolves (*Canis lupus*), an efficient predator of both chamois and deers, and red fox, a mesocarnivore with a not specialized opportunistic diet (Oleaga *et al.*, 2013). The study highlighted a greater genetic diversity in wolf-derived mite population, the only one structured in two subpopulations, namely one similar to the single fox-derived mite population and a second one similar to the single herbivore-derived population. This study confirmed that potential prey (herbivore)-top predator (wolf) mite cross-infection may have taken place between investigated host taxa and, in general, that "prey-to-predator transfer may modify the host-taxa relationship" (Arlian and Morgan, 2017).

More recently, a microsatellite study pointed towards a possible further transmission pattern of *S. scabiei* between herbivorous Japanese serows (*Capricornis crispus*) and omnivorous Caniformia mammals in Japan, though under very weak predator-prey relationships (Matsuyama *et al.*, 2019). In more detail, while sympatric and not sympatric Caniformia- and wild boar derived mite populations appeared well differentiated according to the "host-taxon law", a close genetic relationship was found between Caniformia- and serow-derived mite communities at several distant locations. Authors

hypothesized that *S. scabiei*-naive serows initially became infected through direct (eg, with a moribund sick individual) or indirect (environmental) contact with Caniformia during peak years of epizootic mange waves in the latter. Authors used the terms "cryptic" or "hidden" to stress how unexpected genetic relationships of *S. scabiei* populations, mirroring transmission webs locally deviating from previously reported ones, may exist among multi-host systems worldwide.

Overall, the identification of at least three different *Sarcoptes* transmission patterns by means of microsatellite markers provided a great incentive for epidemiologists, specialists in conservation medicine and informed wildlife managers, to insist in molecularly characterizing all possible mite populations within unexplored multi-host mange outbreak areas. Ideally, outcomes of genetic structure studies should drive the debate on possible control options.

Microsatellite typing has also been used to track the origin of *Sarcoptes* infection and/or its main local reservoirs in case of: i) multiple host infections, including humans (Pisano *et al.*, 2019); ii) legal disputes on traded wildlife, if infected before their export or after import (Alasaad *et al.*, 2012a); and iii) emerging infections in alien species (Rentería-Solís *et al.*, 2014).

In Australia, the use of microsatellite markers allowed to explore the biology of scabies recurrence in patients under ivermectin treatment showing that reinfection was a far more common event than recrudescence after unsuccessful treatment (Walton *et al.*, 1999).

Microsatellite typing also accredited the hypothesis that an initial sarcoptic mange outbreak in the endangered San Joaquin kit fox (*Vulpes macrotis mutica*) derived from spillover of mites from the common endemically infected red fox (though not from infected coyotes and domestic dogs), whereas a second (apparently) separate outbreak was primarily maintained by fox-to-kit mite transmission (Rudd *et al.*, 2020), suggesting the need for adjustment of the control strategy.

A similar hypothesis was advanced in Japan, where microsatellite analysis of 27 *S. scabiei* derived from domestic and wild canids (namely dogs (n=5) and raccoon dogs (n=22)) supported the possible transmission of sarcoptic mange between wild canids and domestic dogs, and inversely, the risk of spillover from dogs to wild canids, and re-infection from wild canids to dogs (Matsuyama *et al.*, 2015).

Finally, a recent molecular study using the same panel of 9 microsatellites investigated the ongoing outbreak decimating wild camelid populations (namely vicuñas, *Vicugna vicugna*, and guanacos, *Lama guanicoe*) in San Guillermo National Park, Argentina, suggesting that the mange epidemic originated from a single source and presumably from a single introduction event of livestock (del Valle Ferreyra *et al.*, 2022).

Overall, all the above mentioned genetic models and patterns of transmission rely on the assumption that close contacts between different host species are possible within the same habitat and may result into effective multi-host transmission of *S. scabiei*.

3. Sarcoptic mange in wildlife

Sarcoptic mange has been defined as an emerging panzootic in wildlife due to its ongoing global transmission and sustained spread among areas and wildlife species (Escobar *et al.*, 2021). Overall, outbreaks of sarcoptic mange are currently described in wild canids in North and South America, Europe and Australia, wild cats in Europe and Africa, wild ruminants and wild boars in Europe, camelids in South America, wombats and other marsupials in Australia, and great apes and various wild bovids in Africa.

Nonetheless, the potential long-term effects of mange in wildlife (especially in free-ranging and/or endangered species) and the causes leading to the emergence and spreading of new epidemics are still poorly understood (Pence and Ueckermann, 2002; Astorga *et al.*, 2018).

The disease is characterized by intense pruritus associated with skin lesions such as scales, erythema, alopecia in the acute forms, while skin thickening due to hyperkeratosis and/or exudative crusts formation are more frequent in the chronic forms (Rahman *et al.*, 2010).

Despite being primarily a skin disease, animals affected by sarcoptic mange usually die from emaciation or secondary bacterial infections. In particular, the cascading pathogenic effects following mange infection usually result in an increased metabolism demand and an altered thermoregulation due to the extended alopecia, then followed by a rapid depletion of fat stores, which is not replaced despite the increased foraging effort by the animal (Martin *et al.*, 2018). Behavioral signs like isolation from the group (in social species) are also commonly observed.

S. scabiei causes an immunopathological reaction, arising because of the mechanical cell lysis and the secretion of cytolytic components in the saliva of the mite released in the epidermis of the host. The immunopathogenic and hypersensitivity reactions are mainly activated by these cytolytic components and mite derived antigen (Burgess, 1993; Hengge *et al.*, 2006).

The immune response to sarcoptic mange significantly differs among distinct host species, and different outcomes of the disease can also be seen even among conspecifics. Immunocompetent hosts develop strong types I and IV hypersensitivity responses resulting in a marked decrease and eventual loss of mites in the skin. However, drastic structural and functional changes can be observed in the skin, such as extensive thickening and a marked eosinophilia throughout the epidermis and dermis. There is often almost complete alopecia. For instance, red foxes lack of memory T-cells after the initial infection, thus they do not seem to have a strong immune response to mange, which explains their severe hyperkeratotic skin symptoms, as well as the massive mite infestations (Oleaga *et al.*, 2012) (Figure 1).

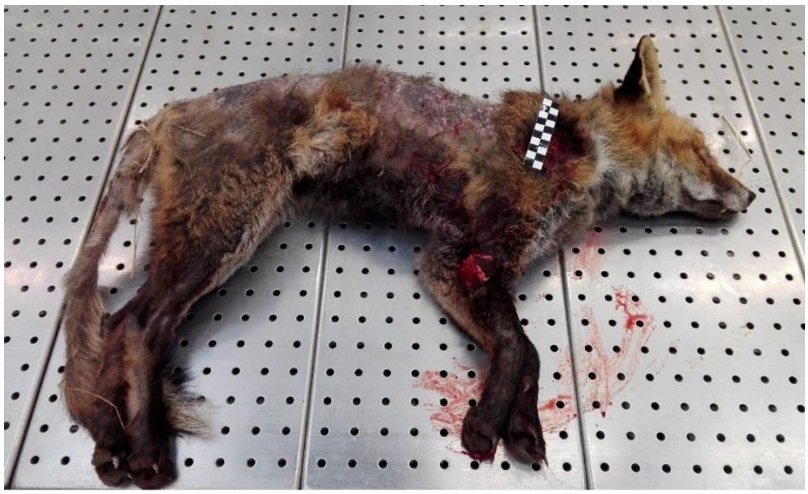


Figure 1. Red fox severely affected by sarcoptic mange, with extended skin lesions and alopecia on the back and on the tail. Photo: Barbara Moroni

Conversely, in grey wolves a prevalence of hypersensitivity response could explain the milder skin symptoms (mainly alopecic), thus their apparent higher ability to control the infection (Oleaga *et al.*, 2011, 2012) (Figure 2). In particular, the neutrophils observed in the inflammatory infiltrate of wolves might be responsible of the oxidation burst favoring the elimination of mites (Oleaga *et al.*, 2012).

Figure 2. European grey wolf affected by sarcoptic mange. Skin lesions included alopecia and skin pigmentation on the proximal part of the tail and on the lombo-sacral region. Photo: Barbara Moroni

In naïve wildlife populations, infection with *S. scabiei* usually results into high morbidity and mortality (Rossi *et al.*, 2019a). This was the case of Iberian ibex in the Cazorla National Park, southern Spain, where the infection spread in few months to over 70% of Iberian ibex, resulting in the death of 95% of the clinically affected individuals (París, 1991). A similarly drastic population decline was observed in

several carnivores in Scandinavia, where S. scabiei was introduced in the late 1970s (Mörner, 1992). Sarcoptic mange can affect the dynamics of exposed populations, by increasing natural mortality rates (Uraguchi *et al.*, 2014), and altering the spatial behavior of animals (Potts *et al.*, 2013).

In Europe, mange seems to have an endemic/persistent cycle in most wildlife species, particularly in free-ranging Caprinae in which naïve populations may be decimated but usually recover and, after the first outbreak, suffer milder consequences on occasion of the following time-spaced epidemic waves.

4. Pharmacological control strategies against sarcoptic mange in free-ranging wildlife

This paragraph has been adapted from: "Comment on: "The treatment of sarcoptic mange in wildlife: a systematic review". Barbara Moroni, Marta Valldeperes, Emmanuel Serrano, Jorge Ramón López-Olvera, Santiago Lavín, Luca Rossi. 13:471. Parasit Vectors (2020)

The pharmacological control of infectious diseases in wildlife has been historically a matter of debate among veterinarians and wildlife specialist (Lyles and Dobson, 1993; Rowe *et al.*, 2019; Moroni *et al.*, 2020). While the zoonotic and sanitary importance of a pathogen as well as the conservation interest of a specific wildlife species have inevitably led to the increasing implementation of human control plans worldwide (e.g for tuberculosis in badgers (Woodroffe *et al.*, 2006), rabies in foxes (Pastoret and Brochier, 1999), or sarcoptic mange in wombats (Fraser *et al.*, 2016)), the spread of a deadly pathogen such as *S. scabiei* in abundant, free-ranging wildlife species of cultural and economic value should also be considered of paramount importance when dealing with local control management solutions.

This is the case of the Iberian ibex in Spain, a highly susceptible wild ungulate, in which the mite has the capacity to spread rapidly, causing mass mortality events, has it happened in the already mentioned severe epidemic observed in Cazorla Natural Park in the late Eighties (León-Vizcaíno *et al.*, 1999).

One strategy that has been commonly applied in epizootic outbreak scenarios in free-ranging wildlife is the selective culling of clinically affected individuals (Alasaad *et al.*, 2013a). However, this population management measure is not free of disadvantages, such as the culling of individuals recovering from scabies (Valldeperes *et al.*, 2019) in detriment of the host population viability, as well as the possible objections from the public opinion in some particular species considered national icon, such as koalas (*Phascolarctos cinereus*) and wombats in Australia.

Another option is the pharmacological treatment of individuals (mostly feasible in captivity or semi-captivity condition), or the mass-treatment of free ranging wildlife.

As a matter of fact, pharmacological treatment of mange in wild animals mostly produces individual healing, but its effects on achieving control or eradication in a population are mostly inconclusive (Martin *et al.*, 2019). Therefore, gathering more information on the population and environmental effects and on the consequences of massive antiparasitic treatments has been recently recommended, approaching the management of sarcoptic mange in wildlife populations from a wider ecological perspective (Espinosa *et al.*, 2020). The success of scabies control in free-ranging wildlife depends on the size of the target population, scabies prevalence, and the feasibility of reaching the required percentage of the population with any specific treatment or measure (Pérez *et al.*, 2021). Individualized pharmacological therapies, however, are desirable for vulnerable or endangered species where the complete recovery of specific individuals is decisive for species recovering (e.g. see an example for the Iberian lynx, *Lynx pardinus* (Oleaga *et al.*, 2019a), or for the black bear, *Ursus americanus* (Wick and Hashem, 2019))

However, in abundant non-threatened and widespread populations with a high prevalence of scabies, it is unlikely that any individual approach would reach the necessary proportion of the population to prevent transmission and reinfection. This is even more evident for ivermectin due to the need for multiple doses to achieve a complete recovery and the total elimination of all mites from the host and the environment, although other long-acting drugs could be a better option. Nevertheless, the environmental and public health concerns of massive antiparasitic drug release in the environment would still persist (Pérez *et al.*, 2021). It is also important to acknowledge the potential for non-target environmental effects of mass administration of ivermectin. Avermectins are excreted during four days post-treatment and can be detected in feces for up to 40 days post-defecation (Pérez *et al.*, 2001) and for more than one year in reindeer pastures (Asbakk *et al.*, 2006). In soil, ivermectin shows a half-life degradation between 7 and 217 days, depending on the solar radiation (Campbell, 1989). Once on the environment, this drug has pre-lethal consequences for dung beetles (Verdú *et al.*, 2018) and for other dung-dwelling invertebrates (Campbell, 1989). If the drug is delivered orally in feeding stuff, as per common practice in intensively managed game populations in Spain, soil contamination and thus the potential effects of ivermectin on other terrestrial fauna, and possibly food chain effects, could be expected not only through fecal contamination but through the drug preparation itself. On the other hand, game treatment would limit venison consumption, as ivermectin withdrawal time in edible tissues may vary from 18 to 48 days depending on the administration route (Slanina *et al.*, 1989; Papich, 2016; Pérez *et al.*, 2021).

Finally, another concern about the use of ivermectin for scabies control in wildlife is the drug resistance phenomenon recently described in human scabies (Currie *et al.*, 2004; Andriantsoanirina *et al.*, 2014) and also suspected in companion animals (Terada *et al.*, 2010).

5. Aims of the PhD project

To address some of the questioned raised in the previous chapter, this PhD research was designed and developed through two main lines:

1. A molecular study on the epidemiology of a large set of *S.scabiei* isolates from European wild and domestic animals, aiming to characterize different mite populations and reveal possible connections between them (developed in Chapters 2,3,4,5);
2. A study on the pharmacological control of scabies by means of oral ivermectin in Iberian ibex model (developed in Chapter 7).

The ultimate purpose of this thesis was to contribute to: a) the understanding, by targeted use of molecular tools, of the epidemiology of *S. scabiei* in a variety of European wildlife species, and b) the current debate on the pros and cons of pharmacological control programmes of scabies in susceptible free-ranging mountain-dwelling ruminants.
A review on the zoonotic potential of scabies was also included (developed in Chapter 6).

6. Research products

The experimental activities carried out during this PhD project granted the publication of 6 original peer reviewed articles in indexed journals, and one manuscript that is currently under review.
For the purpose and homogeneity of this thesis, the published and unpublished articles have been adapted in the following Chapters. For a complete view, please refer to the original articles.
The title and the authors of published manuscripts are listed below (in Chapter order):

Moroni B, Marta Valldeperes, Emmanuel Serrano, Jorge Ramón López-Olvera, Santiago Lavín, Luca Rossi. (2020) Comment on: "The treatment of sarcoptic mange in wildlife: a systematic review". 13:471. Parasit Vectors https://doi.org/10.1186/s13071-020-04347-0

Moroni B, Angelone S, Pérez JM, Molinar AR, Pasquetti M, Tizzani P, Lopez-Olvera JR, Valldeperes M, Granados JE, Lavin S, Mentaberre G, Camacho-Sillero L, Martinez-Carrasco C, Oleaga A, Candela M, Meneguz PG, Rossi L. (2021) Sarcoptic mange in wild ruminants in Spain: solving the epidemiological enigma using microsatellite markers. Parasit Vectors 14:171. https://doi.org/10.1186/s13071-021-04673-x

Valldeperes M, **Moroni B**, Rossi L, Lopez-Olvera JR, Velarde R, Molinar Min AR, Mentaberre G, Serrano

E, Angelone S, Lavin S, Granados JE. (2021) First report of interspecific transmission of sarcoptic mange from Iberian ibex to wild boar. Parasit Vectors 14:481. https://doi.org/10.1186/s13071-021-04979-w

Cardells J, Lizana V, Martí-Marco A, Lavin S, Velarde R, Rossi L, **Moroni B.** (2021) First description of sarcoptic mange in an Iberian hare (*Lepus granatensis*). Curr Res Parasitol Vector-Borne Dis 1:100021. https://doi.org/10.1016/j.crpvbd.2021.100021

Moroni B, Rossi L, Bernigaud C, Guillot J (2022) Zoonotic Episodes of Scabies : A Global Overview. Pathogens 11: 213 https://doi.org/10.3390/ pathogens11020213

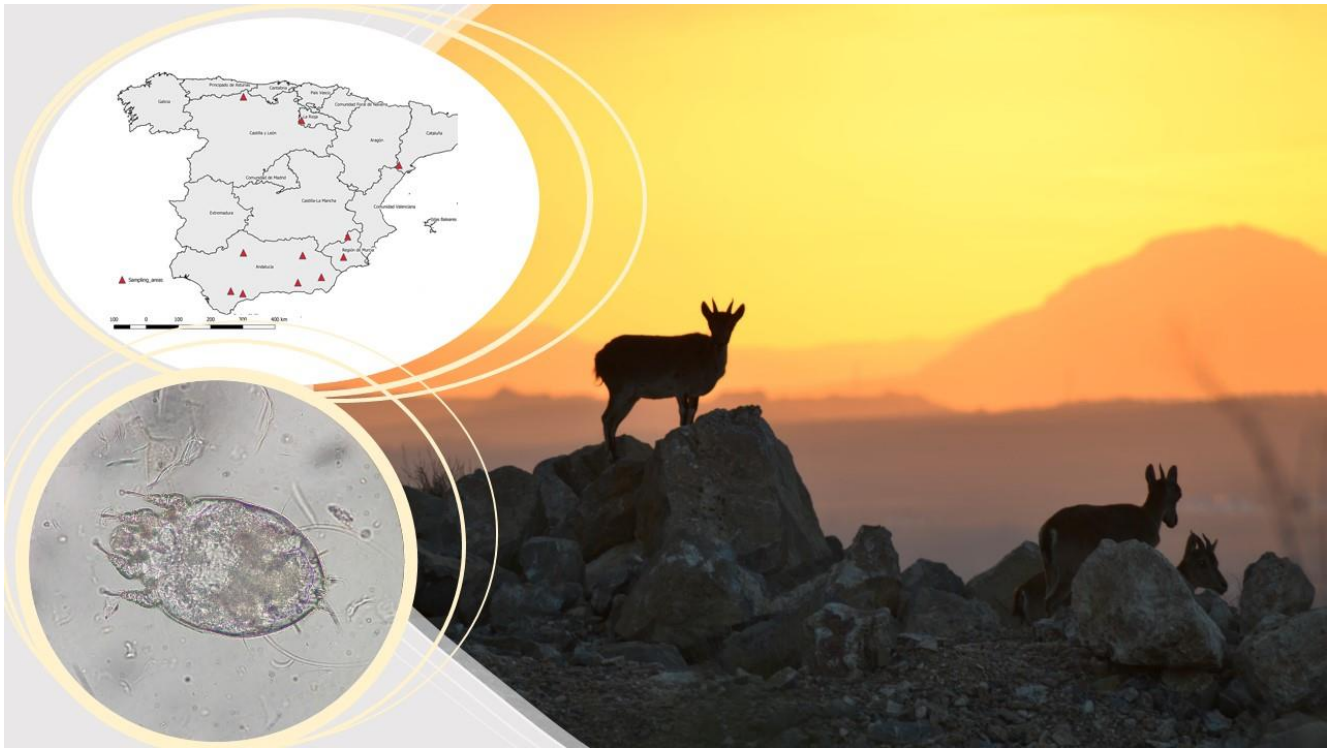
Moroni B, Granados Torres JE, López-Olvera JR, Espinosa Cerrato J, Ráez Bravo A, Mentaberre G, Fandos P, Pazzi M, Romagnoli M, Gardini G, Rossi L, Valldeperes M, Serrano E, Ramos B and Odore R (2022) Ivermectin Plasma Concentration in Iberian Ibex (Capra pyrenaica) Following Oral Administration: A Pilot Study. Front. Vet. Sci. 9:830157. doi: 10.3389/fvets.2022.830157

CHAPTER 1

SARCOPTIC MANGE IN WILD RUMINANTS IN SPAIN

This chapter has been adapted from the published article:

Moroni B, Angelone S, Pérez JM, Molinar AR, Pasquetti M, Tizzani P, Lopez-Olvera JR, Valldeperes M, Granados JE, Lavin S, Mentaberre G, Camacho-Sillero L, Martinez-Carrasco C, Oleaga A, Candela M, Meneguz PG, Rossi L. (2021) Sarcoptic mange in wild ruminants in Spain: solving the epidemiological enigma using microsatellite markers. Parasit Vectors 14:171. https://doi.org/10.1186/s13071-021-04673-x



1. Introduction

In Spain, sarcoptic mange was first described in native free-ranging wild ruminants in late 1987 in the Cazorla Natural Park (Leó N-Vizcaíno *et al.*, 1999)*,* causing a population decline of nearly 95% of the local population of Iberian Ibex (*Capra pyrenaica*) in four years (París, 1991). Since then, additional mange outbreaks were identified in other wild ungulate populations, including red deer (*Cervus elaphus*) , Cantabrian chamois (*Rupicapra pyrenaica parva*) (Fernández-Morán *et al.*, 1997; González-Quirós *et al.*, 2002), fallow deer (*Dama dama*) (León-Vizcaíno, 1992), roe deer (*Capreolus capreolus*)(Oleaga *et al.*, 2008a), European mouflon (*Ovis aries musimon*) (Leó N-Vizcaíno *et al.*, 1999) and the non-native barbary sheep (*Ammotragus lervia*) (González-Candela *et al.*, 2004). Whereas high mortality rates and associated population declines have been recorded in Cantabrian chamois and Iberian ibex (Fernández-Morán *et al.*, 1997; Leó N-Vizcaíno *et al.*, 1999), in the remaining species the infection seems to be less deleterious (Iacopelli *et al.*, 2020).

Since the direct life cycle of *S. scabiei* relies on suitable hosts, a multi-host system can provide the parasite with higher opportunities to persist and spread (Arlian and Morgan, 2017). Wild ruminants in Spain share habitat with different recognized wild hosts for *S. scabiei*, such as red fox and Iberian wolf (*Vulpes vulpes* and *Canis lupus signatus*, respectively) (Oleaga *et al.*, 2012) and wild boar (*Sus scrofa*). While foxes are scavengers and might represent only a marginal and weak transmission pattern of sarcoptic mange for wild cervids and bovids, top predators such as the Iberian wolf, which is currently present in Spain mostly in the Northern region (Asturias, Cantabria), might have prey – predator interactions with red and roe deer and, in some rare occasions, with chamois. Interestingly, sarcoptic mange episodes in wild boar in Spain have never been reported in the scientific literature, although serological positivity to *S. scabiei* has been detected (Haas *et al.*, 2018), and wildlife operators occasionally reported crusted lesions compatible with sarcoptic mange in wild boars.

Although the first epizootic outbreak reported in wildlife in the Iberian Peninsula was attributed to the introduction of an infected herd of domestic goats (Leó N-Vizcaíno *et al.*, 1999), the origin and the cause of the persistence of *S. scabiei* in wild ruminant populations are still unclear. Some decades ago, wild ungulate populations in Spain were mostly evenly distributed in mountain territories, with rare interactions among free-ranging communities located in the different mountain systems. In recent years, wild ungulates have increased in both number and range in Spain, as in the rest of Europe, favored by rural abandonment, reforestations, reintroductions and legislative changes Linell et al., 2020 . This has connected formerly isolated populations through corridors. While the recreation of connection has a major positive effect on biodiversity, on the other hand it can favor the spread and transmission of pathogens such as *S. scabiei*. Whether livestock or human-driven wildlife movement and introduction play a key role in the spread of this parasitosis is still an ongoing and open debate (Lavin *et al.*, 2000).

Using *S. scabiei* mites isolated from eleven populations of six wild ruminant species in Spain, in this chapter I aimed to describe the genetic structure of the circulating *S. scabiei* "strains", namely i) the number of *S. scabiei* strains that can be molecularly identified in wild ruminant populations in Spain, and ii) the epidemiological relationships between *S. scabiei* and the wild ruminant communities within the main outbreak areas countrywide.

2. Material and Methods

Skin samples from one hundred and twenty-one mangy wild ruminants were collected during regular management plan activities or seasonal culling programs between 2011 and 2019 in eleven areas in Spain (Figure 1 and Table 1). The samples belonged to six ungulate species, namely Iberian ibex (83), Cantabrian chamois (16), red deer (18), roe deer (2), aoudad (1) and European mouflon (1) (Table 1).

Geographical origin	**Host species**	**Sampled animals**	***Sarcoptes* isolated[a]**	**Sampling year**	**Index case**
Sierra de Grazalema (Andalucía)	*C. pyrenaica*	4	11	2017	2011
Sierra de las Nieves (Andalucía)	*C. pyrenaica*	6	13	2017	1989
Sierra Nevada (Andalucía)	*C. pyrenaica*	47	100	2017	1992
Tortosa Beceite (Cataluña)	*C. pyrenaica*	10	11	2018	2014
Sierra de Cazorla (Andalucía)	*C. pyrenaica*	9	18	2017	1987
	O. aries musimon	1	5		
	C. elaphus	2	10		
Sierra de los Filabres (Andalucía)	*C. pyrenaica*	3	4	2018	2012

Sierras del Noroeste (Murcia)	*C. pyrenaica*	4	8	2019	1990
Sierra Espuña (Murcia)	*A. lervia*	1	2	2019	1991
Cordillera Cantábrica (Asturias)	*R. pyrenaica*	16	40	2010	1993
	C. capreolus	2	4		
	C. elaphus	9	19		
Sierra Morena (Andalucía)	*C. elaphus*	2	4	2011	NA
Sierra de Demanda (La Rioja)	*C. elaphus*	5	17	2011	2010

Table 1. Geographic origin, host species, sample size, and sampling year of the sarcoptes included in this study. Index case refers to the year of the first mange report in the wild host population of that area.

Skin samples were stored at -20°C or in 70% ethanol tubes until mite isolation. For each skin sample, three mites were isolated and individually stored following the post-frozen isolation method (Alasaad *et al.*, 2009b). All the mites were identified as *S. scabiei* following morphological criteria (Fain, 1971).

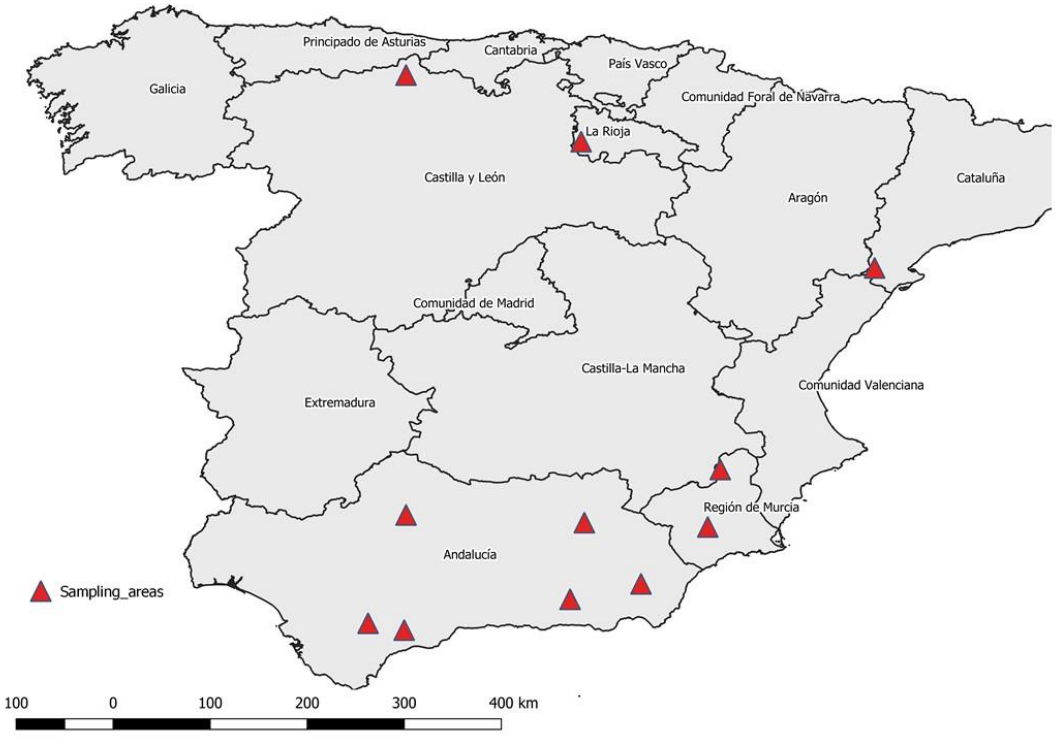


Figure 1. Map showing the 11 sampling sites of ruminants afected by sarcoptic mange in the Iberian Peninsula

DNA was extracted from individual mites following the HotSHOT Plus ThermalSHOCK technique (Alasaad *et al.*, 2008). Then, a 10x multiplex PCR was performed using ten validated primers extracted from the previously published panel (Walton *et al.*, 1997) to target *S. scabiei* mites (Sarms 33, 34, 35, 36, 37, 38, 40, 41, 44, 45) (Soglia *et al.*, 2007). Primers were 5' labelled with 6-FAM, VIC, NED or PET fluorescent dye tag (Applied Biosystem, Foster City, CA, USA). Twelve µl of PCR mixture, consisting in all primer pairs, ranging from 0.04 to 0.01 µM, 10 x PCR buffer (200mMKCl and 100mM Tris–HCl, pH 8.0), 200 µM of each dinucleotide and 0.5 U HotStar Taq polymerase (QIAGEN, Milano, Italy), were admixed with 3 µl of individual mite DNA and submitted to thermal reactions in a 2720 thermal cycler (Applied Biosystems, Foster City, CA, USA), according to the following protocol: 15 minutes at 95°C (initial denaturing), followed by 37 cycles of three steps of 30s at 94°C (denaturation), 45s at 55°C (annealing) and 1.5 minutes at 72°C (extension), before a final elongation of 7 minutes at 72°C. The PCR products (1 µl) were then mixed with 12 µl of formamide with Size Standard 500 Liz (Applied Biosystems, Foster City, CA, USA) in a 96 well plate and heated at 95°C for 5 minutes. Capillary electrophoresis was performed with an ABI PRISM 310 Genetic Analyzer, and the software GeneMapper 4.0 (Applied Biosystems, Foster City, CA, USA) allowed the allele calls and microsatellite visualization. After molecular analysis, only the mites that fulfilled required criteria (eight detectable loci out of the ten analyzed) were included in the molecular analyses.

Three main population genetics analyses were applied to the 266 mite microsatellite outputs: i) Bayesian clustering, ii) genetic distance (to calculate the proportion of shared alleles) and iii) principal

component analysis (PCA). The first one requires Hardy-Weinberg equilibrium (HWE), while no assumptions are required for the second and third analyses.

Descriptive statistics, such as observed and expected heterozygosis (Ho and He, respectively), allelic richness (R) and HWE analysis, were carried out with software R 4.0 using the packages: Adegenet 2.1.3, Pegas 0.3 (Jombart *et al.*, 2008; Paradis, 2010).

P-values for HWE test were based on Monte Carlo permutations of alleles. The Bayesian assignment test was computed with the software STRUCTURE 2.3.4 (Pritchard J.K. *et al.*, 2000). Burn-in and run lengths of Markov chains were 10,000 and 100,000, respectively, and ten independent runs for each K (for K = 1–20) were run. The admixture model was selected as ancestry model. The estimation of clusters was done as previously suggested (Earl and vonHoldt, 2012), using the DK method. Individual mites were then associated to the correspondent inferred cluster.

Genetic distances and multilocus proportion of shared alleles (DPS) among mite populations were computed between all the possible pairs of individuals with microsatellite analyzer (MSA 4.0) and Populations 1.2.32, and then displayed with interactive Tree of Life (iTOL) (Letunic and Bork, 2007) as unrooted dendrogram.

Multivariate analysis (PCA) was performed with R 4.0 without any preliminary assumptions on the origin of the mite samples. The populations of mites in this analysis were labeled as reported in Table 1.

3. Results

Seventy-three different alleles were detected in the 266 mites isolated from the eleven wild ruminant populations using ten microsatellite loci as molecular markers (Additional file 1: Table S1). Depending on the loci, allele count ranged from three (Sarms 37) to 13 (Sarms 45). Thirty-seven private alleles (alleles found only in one population) were detected, ranging from one (Murcia) to 18 (La Rioja), whereas no private alleles were found in the Iberian ibexes from Tortosa Beceite, Sierra de Grazalema, Sierra de los Filabres, and Sierra de las Nieves nor in the red deer from Sierra Morena. Ho and He ranged from 0.03 (Ho) and 0.04 (He) to 0.13 (Ho) and 0.72 (He) in Sarms 44, Sarms 37, and Sarms 34, respectively (Table 2).

	Sarms 33	Sarms 34	Sarms 35	Sarms 36	Sarms 37	Sarms 38	Sarms 40	Sarms 41	Sarms 44	Sarms 45
He	0.72	0.63	0.64	0.57	0.58	0.66	0.73	0.28	0.71	0.71
Ho	0.04	0.13	0.04	0.08	0.03	0.09	0.07	0.05	0.03	0.11
R	0.8	0.61	0.78	0.79	0.94	0.8	0.79	0.68	0.84	0.5

Table 2. Expected (He) and observed (Ho) heterozygosis and allelic richness (R) for each locus corresponding to the microsatellite (Sarms) number.

The mite populations from Sierra Nevada, Asturias, Rioja and Cazorla presented the highest genetic variability, while Sierra de Grazalema, Sierra Morena and Sierra de los Filabres had the mite populations with lowest variability.

Significant deviation from HWE was observed overall (Additional file 2: Table S2). In the Grazalema-, Los Filabres-, and Sierra Morena-derived mite populations, none of the samples supported the HWE ($P < 0.01$). The Bayesian assignment test, according to the DK method of Evanno (K=3), showed three main clusters of ruminant-derived mites (Figure 2).

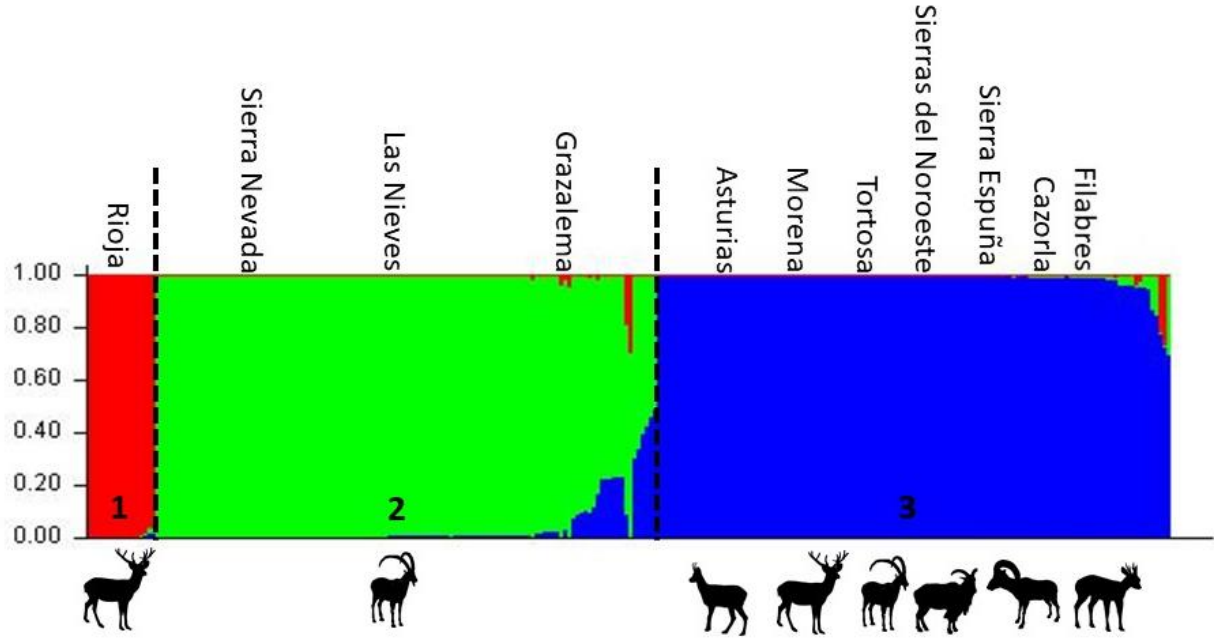


Figure 2. Barplot generated with software Structure 2.3.4 displaying three main clusters (K=3) of Sarcoptes-derived genetic strains

The samples within each individual cluster were consistent with an origin-based classification. The genetic distance among all the mites is displayed in the unrooted dendrogram in Figure 3.

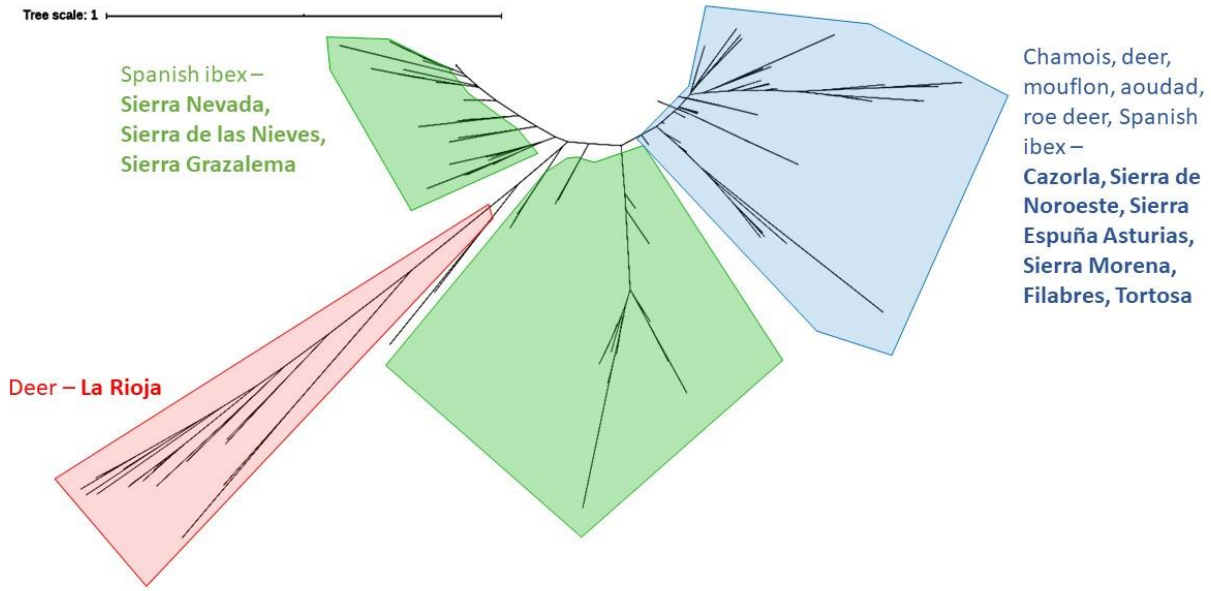


Figure 3. Unrooted distance-based dendrogram constructed with Populations 1.2.32 software and displayed with iTOL 5.5.1 online software representing 266 individual mites from wild herbivores. Main clusters are separated by colors (red, blue, green) with corresponding attributes (host species and origin)

The results of the multivariate analysis with R 4.0 are displayed in Figure 4. The axes one and two accounted for 17.9% of the total variance. The PCA scatter plot revealed three main clusters separated by population origin: the mite population from La Rioja was the most divergent on the first axis. The other two clusters were distributed on the second axis and included the mite populations from Sierra Nevada, Sierra de las Nieves and Sierra de Grazalema, on the one hand (cluster 2) and the mite populations from Cazorla, Asturias, Sierra Morena, Sierra de los Filabres and Tortosa on the other hand (cluster 3).

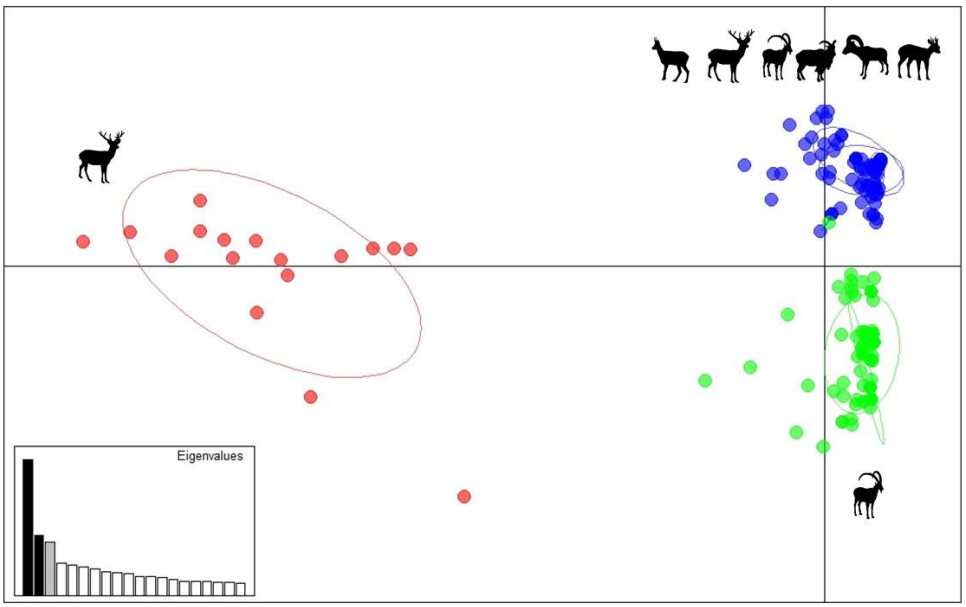


Figure 4. Scatter plot generated with R 4.0 software (implemented by the package adegenet 2.1.3) representing principal component analysis (PCA) of 266 mites from wild herbivores deriving from ten different geographical origins. Variance is explained by 12.4 and 5.5% of components 1 and 2, respectively. The eigenvalues of the two axes are displayed in the bar plot on the left. Label names refer to the origin of host species (see Table 1). Colors (on the red-blue-green scale) and distances display the genetic diversity

Overall, the three different cluster analyses performed, regardless of HWE, agreed in defining three groups of ruminant-derived mites, consistent with the geographical origin, displayed in Figure 5.

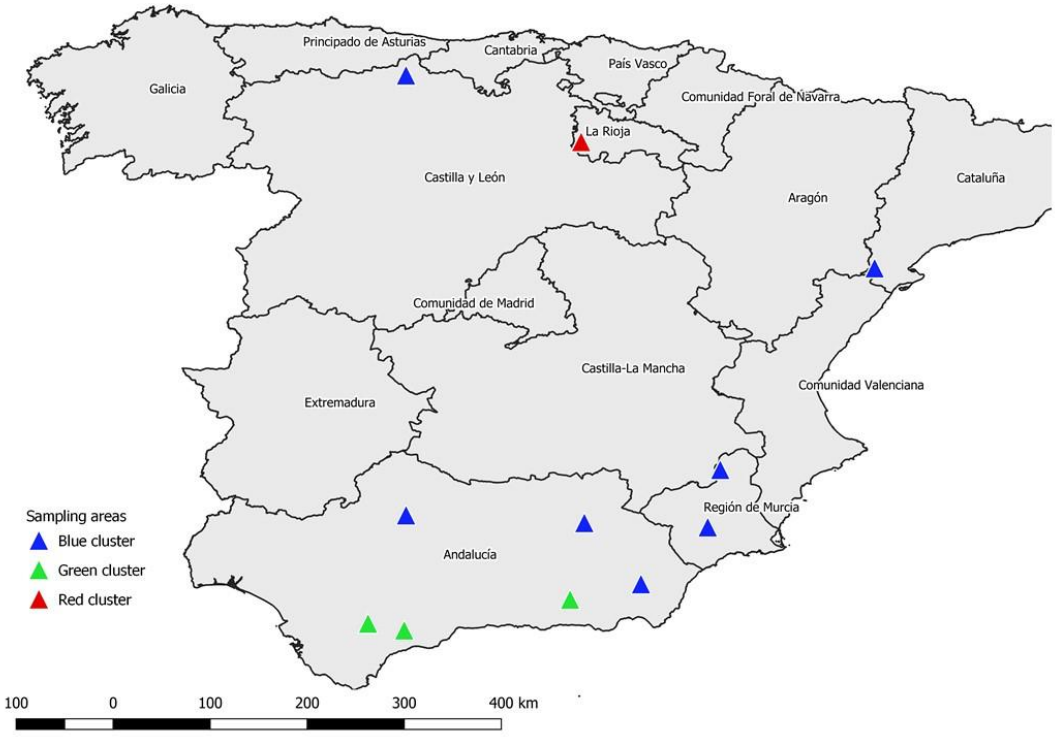


Figure 5. Locations of sampled animals colored accordingly to the genetic cluster analysis

4. Discussion

The cluster analyses performed in our study agreed on identifying three main genetic clusters of *S. scabiei* in the mange-affected wild ruminant populations investigated throughout Spain. The three genetic clusters identified revealed that: i) different wild ruminant species were affected by the same *S. scabiei* strain; ii) circulating *S. scabiei* strains in Spain are both geographically- and host-related, although iii) geographical distance among mange-affected wild ruminant populations is not related to mite strain phylogeny, with distant populations affected by the same *S. scabiei* strain and close populations hosting different strains.

The two clusters related to a single species (La Rioja- and Sierra Nevada-derived, *C. elaphus* and *C. pyrenaica*, respectively) were also geographically limited to a single region or neighboring areas. Conversely, the third cluster (Cazorla/Asturias-derived) encompassed multi-host systems (*C. elaphus*, *C. pyrenaica*, *R. pyrenaica*, *A. lervia*, *C. capreolus* and *O. a. musimon*) and referred to different areas in the Southeast, Northwest and more recently Northeast of the Iberian Peninsula (Figure 5).

Sarcoptes scabiei mites do not have free-living stages, thus main genetic mixing occurs on the same host, and skin-scale patterns of variability have been identified even in the same individual host (Alasaad et al. 2007; Castro et al. 2018). Literature data support the hypothesis that the rare exchange

(and thus possible mating) of mites among different hosts may condition the genetic and epidemiological features of *S. scabiei*, and its spreading patterns in different host communities (Rasero *et al.*, 2010; Gakuya *et al.*, 2011; Matsuyama *et al.*, 2019). In Spain, the spread of each putative "strain" to different sympatric host species (cluster 3) or not (clusters 1 and 2) might be dependent on: i) the host community composition, and ii) the maintenance and transmission capability of each "strain" by the individual host populations and communities. In multi-host scenarios, the more susceptible species would act as reservoir spreading sarcoptic mange to less susceptible host, which would likely not be capable of maintaining the transmission chain in the absence of the source host/s, as suspected in Cantabrian chamois (reservoir host) and red and roe deer (spill-over hosts) in the Cantabrian Mountains (Oleaga *et al.*, 2008a; b, 2012). A similar pattern has been reported in the Alps, where the Northern chamois (*Rupicapra rupicapra*) and the Alpine ibex (*Capra ibex*) play a reservoir role, whereas the red and roe deer and European mouflon are mere spill-over hosts despite their abundance and sympatry with the aforementioned native Caprines (Rossi *et al.*, 2019b).

The imbricated distribution of *S. scabiei* clusters (Figure 5) in scarcely connected wild ruminant populations (in particular, see the distribution of cluster 3), in parallel with the chronology of outbreak eruption which only partially support an "oil spot" spreading pattern of the disease amongst naïve contiguous wild ruminant populations (Palomo *et al.*, 2007), might suggest that *S. scabiei* was likely introduced by infected livestock. The high number of private alleles, particularly in La Rioja population, might indicate low gene flow and high genetic separation among mite populations from the rest of the Iberian Peninsula. Thus, the cluster represented by deer-related *Sarcoptes* from La Rioja (Sierra de la Demanda) might implicate the existence of a new *Sarcoptes*-strain that started to spread after the index case was recorded in the local ungulate population of La Rioja (see Table 1), with unknown origin. Domestic goats and sheep are well-known suitable hosts for *S. scabiei*, and cross-transmission with wild Caprines has been experimentally demonstrated (Lavin *et al.*, 2000). Transmission of *S. scabiei* at the wild-domestic interface has been also reported under natural conditions (León-Vizcaíno *et al.*, 1999; Lavin *et al.*, 2000; Menzano *et al.*, 2007; Rossi *et al.*, 2019b). The presence or introduction of sympatric herds of domestic goats infected with *S. scabiei* have been proposed as the origin of the first epizootic outbreak reported in the Iberian Peninsula and affecting the Spanish ibex (León-Vizcaíno *et al.*, 1999), the subsequent outbreak described in Cantabrian chamois (Oleaga *et al.*, 2008b), and also the most recent sarcoptic mange outbreak affecting the ibex population in Tortosa mountains (Mentaberre). However, mite isolation from goats and molecular confirmation of these suspicions were not feasible since herds had already been treated or were not present in the area at the moment of investigation. In accordance with previous studies (Rasero *et al.*, 2010; Gakuya *et al.*, 2011; Matsuyama *et al.*, 2019), the analyzed samples significantly deviated from HWE, supporting the idea that these assumptions might be inapplicable in most natural populations (Jombart *et al.*, 2008). Moreover, He and allelic

richness were low throughout all loci, implying low gene diversity. Deviations from HWE and from random mating of mite populations might be explained by the nonrandom colonization dynamics of *S. scabiei* at the individual-host level (Alasaad *et al.*, 2007) and at the subpopulation-host level (Wahlund effect).

Not all the ungulate populations or subpopulations in our sample were geographically connected, implying that gene flow between *Sarcoptes* mites was low, and in some cases, absent. We hypothesize that, after the introduction of the mite into naïve wild ruminant populations, the parasite develops a distinctive epidemiological pattern, depending on host species composition, animal density, size and relative abundance, the sensitivity of each species, the specialization of the mite strain, and environmental and social factors, among others (Iacopelli *et al.*, 2020). The role of human-driven introduction or trade of wild and domestic animals should be considered as a viable explanation for sarcoptic mange spread in different areas of the Iberian Peninsula. Given the dramatic consequences of an easier-to-manage disease in domestic livestock, such as sarcoptic mange, when introduction into naïve wild ruminant populations occurs, its importance should not be further neglected by those responsible for livestock health care and treatment (Moroni *et al.*, 2020). This is especially relevant in those scenarios with wildlife-livestock interface, where the jump of shared pathogens may occur among susceptible and phylogenetically related host species.

CHAPTER 2

SARCOPTIC MANGE IN WILDBOAR

This chapter has been adapted from the published article:

Valldeperes M, **Moroni B**, Rossi L, Lopez-Olvera JR, Velarde R, Molinar Min AR, Mentaberre G, Serrano E, Angelone S, Lavin S, Granados JE. (2021) First report of interspecific transmission of sarcoptic mange from Iberian ibex to wild boar. Parasit Vectors 14:481. https://doi.org/10.1186/s13071-021-04979-w

1. Introduction

Sarcoptic mange, though deeply investigated in domestic pig due to its negative impact on productivity and as a model for human scabies (Arends *et al.*, 1990; Davis and Moon, 1990; Davies, 1995; Gutiérrez *et al.*, 1996; Alonso De Vega *et al.*, 1998; Zimmerman *et al.*, 2019), has been poorly studied in wild boar (*Sus scrofa*), where this skin disease is probably underreported (Rasero *et al.*, 2010; Alasaad *et al.*, 2013b; Haas *et al.*, 2018). Sarcoptic mange is present in swine production of Spain (Gutiérrez *et al.*, 1996; Alonso De Vega *et al.*, 1998), and wild boar populations are increasing in Europe, including urban, peri-urban and humanised areas (Licoppe *et al.*, 2013; Massei *et al.*, 2015; Castillo-Contreras *et al.*, 2018; MINUARTIA, 2018). However, in spite of external inspection of all the wild boars hunted and the existence of a national wildlife health surveillance programme, no macroscopic clinical cases of sarcoptic mange have been reported to date in wild boar in Spain. Nevertheless, the detection of a low seroprevalence against *S. scabiei* amongst wild boars in Spain (1.2%) (Haas *et al.*, 2018) suggests that the mite has come in contact with or even circulated in local wild boar populations. Apparently, such contact has not led to widespread clinical disease or epidemic outbreaks, which probably would have been detected through the aforementioned wildlife health surveillance, although some subclinical or clinical cases may have occurred unnoticed. Conversely, sarcoptic mange outbreaks have been widely studied in the Iberian ibex (*Capra pyrenaica*) populations from the Iberian Peninsula since 1987 due to the impact on host demography and the related economy (París, 1991; León-Vizcaíno *et al.*, 1999). The latest of these outbreaks is affecting the Iberian ibex population of Ports de Tortosa i Beseit Natural Park (PTB) in north-eastern Spain since December 2014 (Valldeperes *et al.*, 2018). Wild boars and Iberian ibexes coexist in natural scenarios of the Iberian Peninsula but have different habitat preferences, and pathogen cross-transmission between these two ungulate species is rare, even in areas of high pathogen prevalence (Mentaberre *et al.*, 2010). These factors pose a challenge for sarcoptic mange transmission between both species, particularly considering the relatively short survival of *S. scabiei* off the host.

This study aims to describe the first clinical cases of sarcoptic mange in wild boars from the Iberian Peninsula and to identify the possible source of the infection by comparing mites from different wild boar and sympatric Iberian ibex populations in Spain using microsatellites as molecular markers.

2. Material and Methods

On 29 October 2018, an 8-month-old female wild boar (wildb1) was hunted in a private hunting area (40°52'44.7" N, 0°17'23.3" E) in Arnes (Tarragona, north-eastern Spain), within the PTB. The wild boar had moderate skin lesions consistent with sarcoptic mange (Haas *et al.*, 2018) (Fig. 1) and was

submitted to the Servei d'Ecopatologia de la Fauna Salvatge (SEFaS) of the Universitat Autònoma de Barcelona (UAB) for post-mortem examination as well as histopathology, immunohistochemistry, inflammatory cell and molecular analyses.

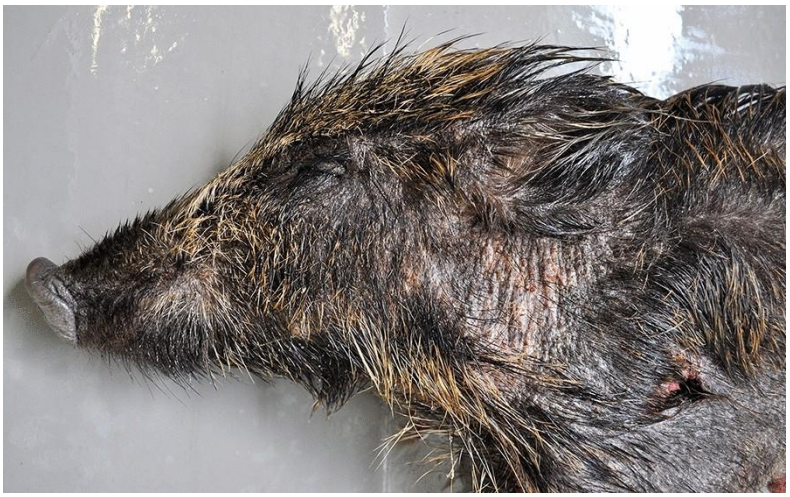


Figure 1. Macroscopic lesions of sarcoptic mange of the wild boar study case (wildb1), with alopecia on the head and neck, mild to moderate skin thickening, papules and crusting

Two additional wild boars, also with moderate skin lesions compatible with sarcoptic mange from southern Spain, were hunted in 2014 in the Lanteira (Granada), within the regular management plan of the species in the National Park of Sierra Nevada (wildb2), and found alive as an orphan piglet in 2017 in Sierra Bermeja (Málaga, wildb3), respectively. The mites obtained from these two wild boars and from 23 sympatric Iberian ibexes were used for molecular analysis to establish phylogenetic relationships (Fig. 2). Shapefiles of Iberian ibex distribution obtained from the Red List of Threatened Species of the International Union for Conservation of Nature (https://www.iucnredlist.org) were used to construct a map using QGIS software 3.2.0 "Bonn".

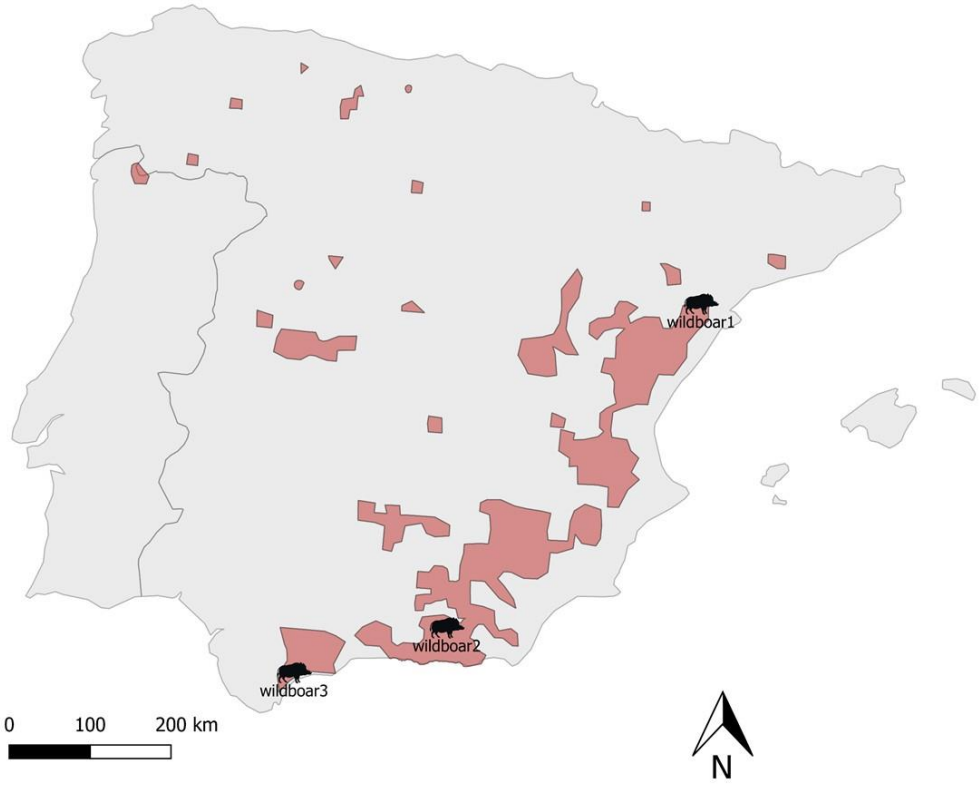


Figure 2. Map of the Iberian Peninsula showing the distribution of *Capra pyrenaica* (light red) and the origin of the three scabietic wild boars included in this study. The shapefiles of Iberian ibex distribution were obtained from the Red List of Threatened Species of the International Union for Conservation of Nature (https://www.iucnredlist.org), and the map was prepared using QGIS software 3.2.0 "Bonn" (http://qgis.osgeo.org)

A complete systematic necropsy following a standardized protocol was performed on wildb1, recording macroscopic lesions. Deep skin scrapings from the area of transition between healthy and affected skin were obtained using a sterile scalpel and evaluated under an optic stereomicroscope (Leica EZ4) at ×35. Three additional wildb1 skin samples of 2.5 × 2.5 cm were collected from the margins of the lesions containing healthy and damaged skin, and processed in a 5% potassium hydroxide (KOH) solution overnight at 43 °C. The digestion products of the three wild boars affected by sarcoptic mange (wildb1, wildb2 and wildb3) and the control wild boar were inspected using an optic stereomicroscope (Leica EZ4) at ×35 (Cook, 1954; Mumcuoglu, 1990) in order to identify *S. scabiei* mites following morphological criteria (Fain, 1968).

Three wild boar mites (one from wildb1, one from wildb2 and one from wildb3) and 40 mites from 23 Iberian ibexes sympatric to these three wild boars from three different geographic areas were used for the genetic analysis. The mites were individually isolated from frozen skin samples using the postponed isolation method (Alasaad *et al.*, 2009b), and DNA was extracted following the HotSHOT plus ThermalSHOCK technique (Alasaad *et al.*, 2008). DNA was amplified through a multiplex 10× polymerase chain reaction (PCR), and multilocus genotyping using 10 specific *Sarcoptes* mite

microsatellites as molecular markers was applied to determine the origin of the parasitic transmission (Soglia *et al.*, 2007). The microsatellites were selected from the panel proposed by Walton et al. (Walton *et al.*, 2004), namely selective androgen receptor modulators (SARMS) 33, 34, 35, 36, 37, 38, 40, 41, 44 and 45. A Bayesian assignment test was carried out to determine the most likely number of genetic clusters through the software STRUCTURE (v. 2.3.4) (Pritchard J.K. *et al.*, 2000). The lengths of burn-in period and number of Markov chain repetitions were 10,000 and 100,000, respectively. Twenty independent runs were performed for each K (for $K = 1–10$), using the admixture option as ancestry model. Selection of K was determined using the DK Evanno method (Earl and vonHoldt, 2012). Descriptive statistics, including multilocus proportion of shared alleles, was carried out with Microsatellite Analyser (MSA 4.1) (Dieringer and Schlötterer, 2003), ignoring any previous clustering information. A consensus dendrogram was obtained using the neighbour-joining algorithm performing 1000 bootstraps in Population 1.2.32 software (https://bioinformatics.org/populations/) and then displayed by MEGA 4.1 (http://www.megasoftware.net).

3. Results

Wildb1 had poor body condition and bilateral hair loss around the eyes, base of the ears, neck, scapular, humeral, axillar, inguinal and anterolateral femoral and tibia regions. On close examination, the skin was moderately thickened with small 1–2-mm orange pustules and crusts, especially on the neck (Fig. 1). Penetrating and exit traumatic injuries consistent with the gunshot were found in the abdominal and left scapular regions, respectively (Fig. 1).

The main internal findings were a general moderate enlargement of the peripheral lymph nodes and areas of reddening, consolidation and septal oedema in the right apical, middle and cranial aspect of the caudal (15%) lung lobes, consistent with a subacute to chronic cranioventral bronchopneumonia.

Both the skin scrapings and the KOH digestions allowed identification of mites that were morphologically consistent with *S. scabiei*.

Forty alleles were obtained from 10 microsatellite loci, ranging from three (SARMS 33, 34, 37, 40, 41, 44) to six (SARMS 36) for each locus. Twenty-four private alleles were detected overall, ranging from nine (wildb3) to one (Ibexto), and no private alleles were detected in the wild boar from PTB and the ibexes from Málaga. The Bayesian assignment test for multilocus genotyping identified three main mite clusters (Fig. 3) obtained through the DK method ($K = 3$).

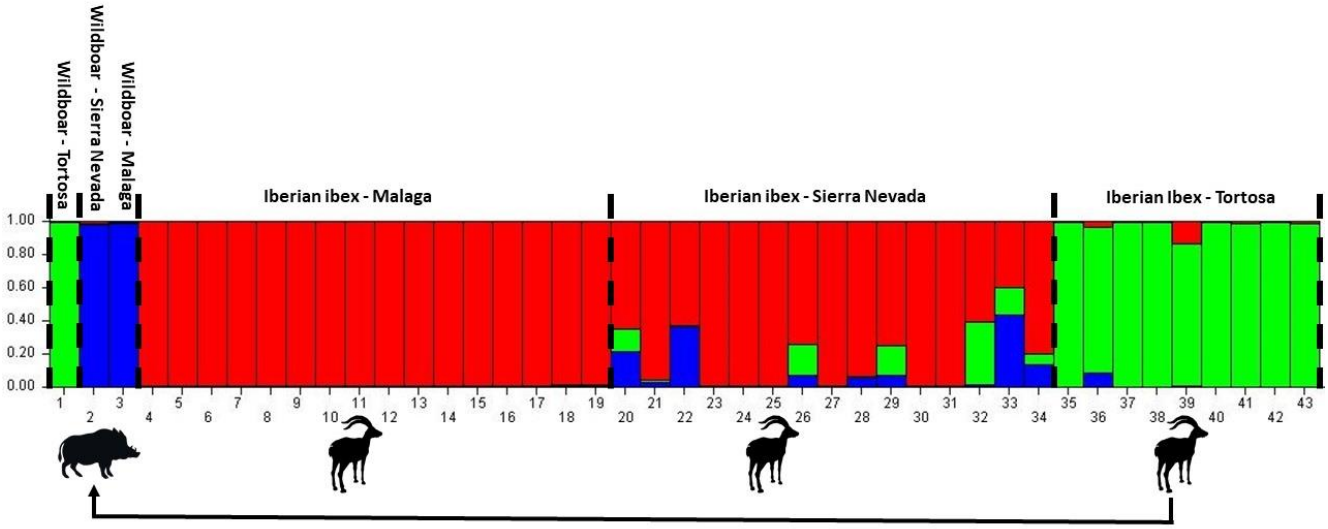


Figure 3. Bar plot generated with STRUCTURE 2.3.4 showing the three genetic mite clusters identified in the wild boars and Iberian ibex sampled. Each bar represents a *Sarcoptes* mite sample, and the height of each coloured segment is proportional to the membership fraction in each cluster.

The mite from wildb1 from Tortosa grouped with the mites from sympatric ibexes with 99% probability of belonging to that ancestry-inferred cluster, while wildb2 and wildb3 mites had 98 and 99% probability of belonging to the same cluster, respectively. Although assigned to the southern ibex cluster, the mites from ibexsn3 and ibexsn14 had 37% and 44% probability, respectively, of belonging to the wildb2 and wildb3 cluster. A consensus dendrogram tree showing the genetic distances between all the individual samples is shown in Fig. 4.

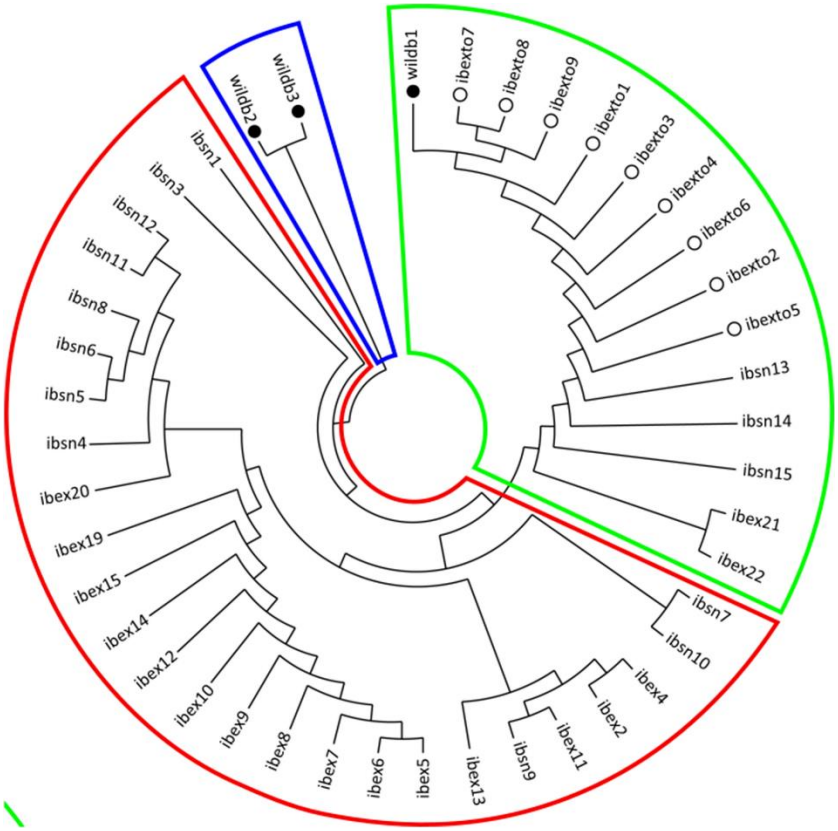


Figure 4. Neighbour-joining phylogenetic tree constructed by using distance matrices with Populations 1.2.32 and displayed with MEGA 4.1. The names of the samples are explained in Additional file 2: Table S2. Full circles represent wild boar-derived mites, while empty circles represent mites from ibexes from Tortosa. Colours are related to the membership clusters explained in Fig. 3

4. Discussion

This study reports the first documented clinical cases of sarcoptic mange in wild boars from the Iberian Peninsula and, most importantly, the first interspecific transmission of *S. scabiei* from a wild ruminant host to wild boar. Cross-transmission of sarcoptic mange between different host species has been widely studied in experimental trials and spontaneous cases. Although sarcoptic mange infection is widespread in the Iberian ibex populations (Pérez, 1997; Pérez *et al.*, 2002, 2021; Acevedo and Cassinello, 2009), to date no cross-transmission of *S. scabiei* with wild boar has been reported. In addition, literature of sarcoptic mange in wild boars is scarce, contrasting with extensive descriptions in domestic pigs (Berrilli *et al.*, 2002; Haas *et al.*, 2018; Escobar *et al.*, 2021).

Sarcoptes scabiei is attributed a certain host specificity (Zahler *et al.*, 1999; Alasaad *et al.*, 2011; Arlian and Morgan, 2017). However, cross-transmissions between phylogenetically related species have been reported both experimentally, for example from domestic goats (*Capra aegagrus hircus*) to Cantabrian chamois (*Rupicapra pyrenaica parva*) (Lavin *et al.*, 2000), and spontaneously, for example from

Cantabrian chamois to red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*) (Oleaga *et al.*, 2008a; b) or from Iberian ibex to red deer (Iacopelli *et al.*, 2020). The success of *S. scabiei* cross-transmission events between phylogenetically distant species, for example between domestic pigs and ruminants, depends on the ecological fitting between the minimal resources required by the mite to survive and reproduce and those that the mite can find in the naïve host species (Araujo *et al.*, 2015). Such phylogenetically distant cross-transmission usually course as self-limiting infestations that heal when the mites supplied by the reservoir host species disappear because they cannot meet the aforementioned minimal requirements in the new host (Alasaad *et al.*, 2012b; Araujo *et al.*, 2015). Nevertheless, cross-transmission of sarcoptic mange between genetically distant species may occur in prey–predator relationships, such as lion (*Panthera leo*) and wildebeest (*Connochaetes* sp.); cheetah (*Acinonyx jubatus*) and Thompson's gazelle (*Eudorcas thomsonii*) (Gakuya *et al.*, 2011); and wolf (*Canis lupus*) and its prey herbivores (Oleaga *et al.*, 2013). In the present case, wildb1 could have acquired the mites from infected sympatric ibexes by scavenging the carcass of a recently dead mangy individual. Alternatively, wildb1 could have been exposed to *S. scabiei* through direct or indirect contact with a dying mangy ibex. The mites from wildb2 and wildb3 clustered together and separately from those isolated in their sympatric Iberian ibexes, suggesting they were infected by a wild boar variant (Escobar *et al.*, 2021). However, a greater number of mites from a range of all sympatric putative sources (e.g., foxes or wild lagomorphs) should have been analysed to support robustly any hypothesis on the origin of mites, including a potential mite exchange between Iberian ibexes, where sarcoptic mange is endemic (Pérez, 1997; Pérez *et al.*, 2021), and wild boars.

Gene flow is commonly used as a proxy for population connectivity, and thus it can be considered a good indicator of *Sarcoptes* mite transmission between different host individuals, as it has been previously described in other wildlife species to understand the source of sarcoptic mange infection (Alasaad *et al.*, 2011; Arlian and Morgan, 2017). The clustering of the mites from the two southern ibex populations separately from the mites from the northern ibex population agrees with a recent molecular epidemiological study (Moroni *et al.*, 2021c). The clustering and occurrence of multiple private alleles in the *Sarcoptes* mites from the southern wild boars (seven for wildb2 and nine in wildb3) suggests that they could be affected by specific omnivorous *S. scabiei* (Rasero *et al.*, 2010; Alasaad *et al.*, 2011) with little gene flow with other populations. However, since the numbers of wild boars studied and mites retrieved and analysed are low, further analyses of *S. scabiei* mites from wild boars in Spain are required to clarify the potential gene flow between mites affecting wild boars and the wild ruminant populations endemically affected by sarcoptic mange countrywide.

The combination of chronic skin lesions and bacterial bronchopneumonia observed in wildb1 has previously been reported in Mediterranean wild boars (Mignone *et al.*, 2014).

The clinical outcome of sarcoptic mange after interspecific cross-transmission does not depend only on the phylogenetic relationship between the host species, but also on the host immunity and status. This

is shown by the different clinical course of each one of the three naïve roe deer that incidentally developed sarcoptic mange after being housed with a mangy chamois (Menzano *et al.*, 2008). Therefore, wildb1 might have developed clinical mange partly because its own immune status or previous conditions, apart from exposure to the mite. Consequently, other wild boars could be carrying *S. scabiei* mites and spreading them in PTB while having just a mild or subclinical infection. Consequently, the surveillance and management of sarcoptic mange outbreaks should not only focus in the more severely affected species, but also cover other sympatric ungulates, in order to detect other complementary reservoir hosts.

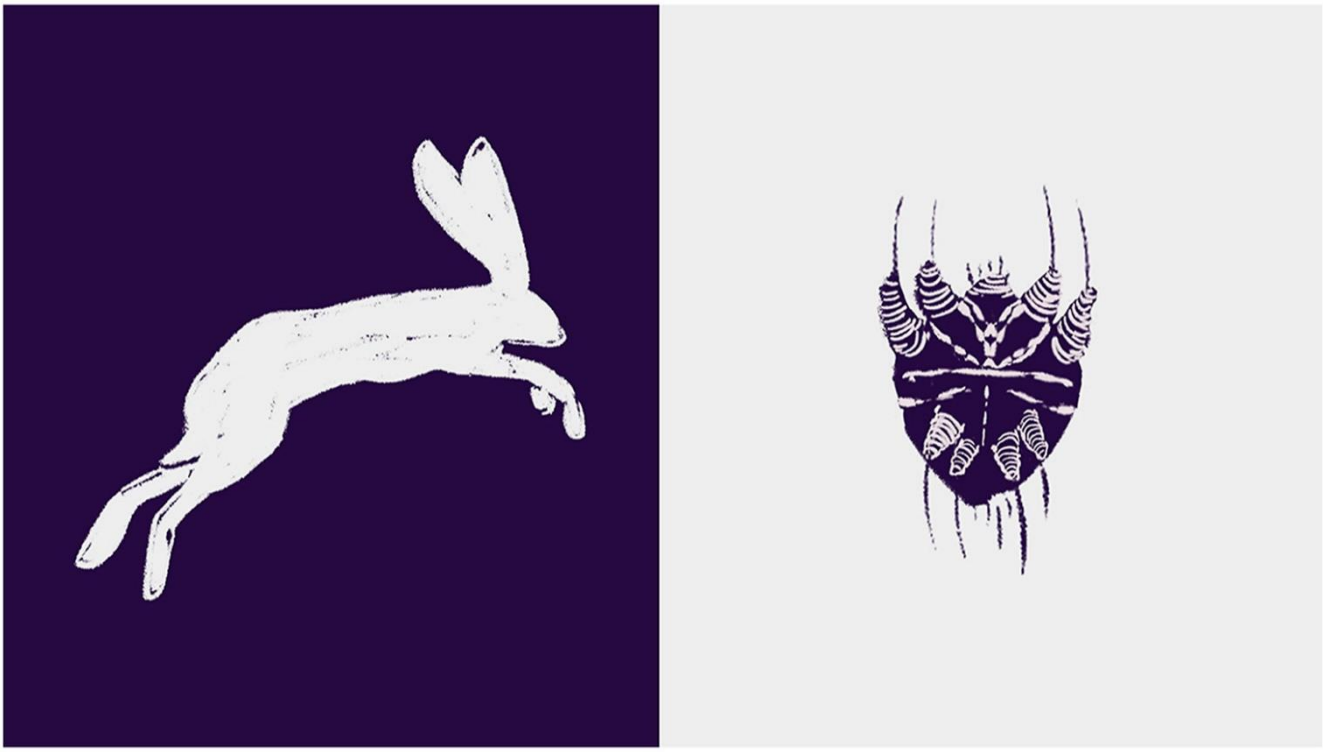
Considering the lesions, the genetics and the ecological circumstances, this case of interspecific cross-transmission of sarcoptic mange could be attributed to: (1) the pressure of infection in the area; (2) the consumption of or contact with carcasses of severely infested Iberian ibex; (3) a state of immunosuppression of the wild boar, which would also fit the opportunistic *E. coli* growth in the lungs and the proliferation of *Demodex* sp.; and (4) a combination of the aforementioned factors.

CHAPTER 3

SARCOPTIC MANGE IN THE IBERIAN HARE

This chapter has been adapted from the published article:

Cardells J, Lizana V, Martí-Marco A, Lavin S, Velarde R, Rossi L, **Moroni B.** (2021) First description of sarcoptic mange in an Iberian hare (*Lepus granatensis*). Curr Res Parasitol Vector-Borne Dis 1:100021. https://doi.org/10.1016/j.crpvbd.2021.100021



1. Introduction

The Iberian hare (*Lepus granatensis*) is native to the Iberian Peninsula. The species is widely distributed in agricultural areas and open fields at altitudes ranging between sea level and 1,200 m above the sea level (Duarte Duarte, 2000). In northern Spain it may be sympatric with the European brown hare (*Lepus europaeus*) and the vulnerable Broom hare (*Lepus castroviejoi*) (Purroy and Salvador Milla, 2017). The Iberian hare is a popular small game species, with more than one million individuals hunted every year (Vargas, 2002). *Lepus granatensis* is apparently resistant to the European brown hare syndrome, a deadly viral disease in European brown hares and Mountain hares (*Lepus timidus*) (Gavier-Widén and Mörner, 1991; Lopes *et al.*, 2014). However, a new strain of Myxoma virus, previously restricted to rabbits, is causing high mortality in Iberian hares since summer 2018 (Águeda-Pinto *et al.*, 2019; Dalton *et al.*, 2019; García-Bocanegra *et al.*, 2019). The immunosuppression associated to Myxoma virus infection (Jeklova *et al.*, 2008) could represent an open gap to other previously undetected diseases. In this chapter, I aimed to describe the molecular profile of the *Sarcoptes* mites collected on the Iberian hare, describing also the first case of sarcoptic mange in this wild lagomorph.

2. Material and Methods

During a surveillance campaign for the detection of myxomatosis in hares in the Valencia Community, Spain, an adult female Iberian hare was found dead with overt skin lesions compatible with sarcoptic mange in the Quart de les Valls municipality (39°44'27.96"N, 0°16'17.04"W), Valencian Community, Spain.

The necropsy was carried out at the Veterinary Faculty of the CEU Cardenal Herrera University. Skin samples from forelimbs and ventral thorax were taken and digested in a 10% KOH solution at 37 °C overnight for the specimen isolation. Samples were then centrifuged at 3,500 rpm for 5 min, and the supernatant was removed and floated with a saturated sucrose solution for 5 min (Bornstein et al., 2001). To collect individual specimens for molecular studies, crusts were scratched under a stereomicroscope (Leica DM750) in an aqueous medium (Soglia *et al.*, 2009) and delivered to the Veterinary Faculty of Turin (University of Turin, Italy).

DNA extraction was performed from individual mites following the HotSHOT Plus ThermalSHOCK technique (Alasaad et al., 2008). Then, a 10× multiplex PCR was carried out using 10 validated primers to target *S. scabiei* mites (Sarms 33, 34, 35, 36, 37, 38, 40, 41, 44, 45) (Soglia *et al.*, 2007; Alasaad *et al.*, 2009b). Capillary electrophoresis was performed with an ABI PRISM 310 Genetic Analyzer, and the software GeneMapper 4.0 (Applied Biosystems, Foster City, CA, USA) was applied to visualize the microsatellite peaks.

The genetic profile of mites isolated from the hare of this study was compared to those of mites previously collected from wild rabbits and red foxes in Spain.

Population genetics analyses were carried out using Bayesian clustering approach with the software STRUCTURE 2.3.4 (Pritchard J.K. *et al.*, 2000), while a multivariate principal components analysis (PCA) with microsatellite markers data as variables was performed with R 4.0 using the package *ade 4.0* .

Assessments of observed (Ho) and expected heterozygosity (He), allelic richness (R) and the Hardy-Weinberg Equilibrium (HWE) analysis, were carried out with software R 4.0 using the package *Adegenet 2.1.3* (Jombart *et al.*, 2008).

For the Bayesian analysis, admixture model was selected, "burn-in" and run lengths of Markov chains were 10,000 and 100,000, respectively, and 10 independent runs for each K (for $K = 1–20$) were run. The estimation of clusters was performed using the DK method of Evanno. Individual mites were then associated to the correspondent inferred cluster (Earl and vonHoldt, 2012).

3. Results

At the gross examination of the carcass, extended hyperkeratotic skin lesions were present on the forelimbs and the ventral thorax, with thick crusts and scales (Fig. 1). Overall, the animal was in a good nutritional status and it was in lactation. The post-mortem examination did not show lesions compatible with myxomatosis or European brown hare syndrome.

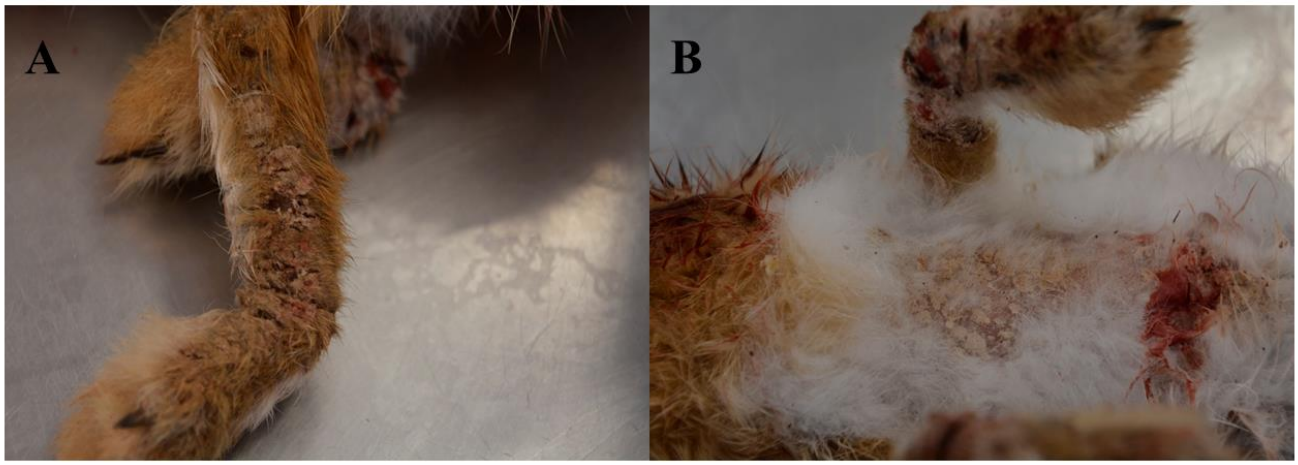


Figure 1. A Skin lesions present on the forelimb of the Iberian hare. **B** Skin lesions present on the ventral thorax of the Iberian hare

Skin samples were collected, and numerous mites of all developmental stages were microscopically observed. They were identified as *S. scabiei* (Fig. 2) based on morphological criteria (Mathison and Pritt, 2014). No other ectoparasites were observed on the animal.

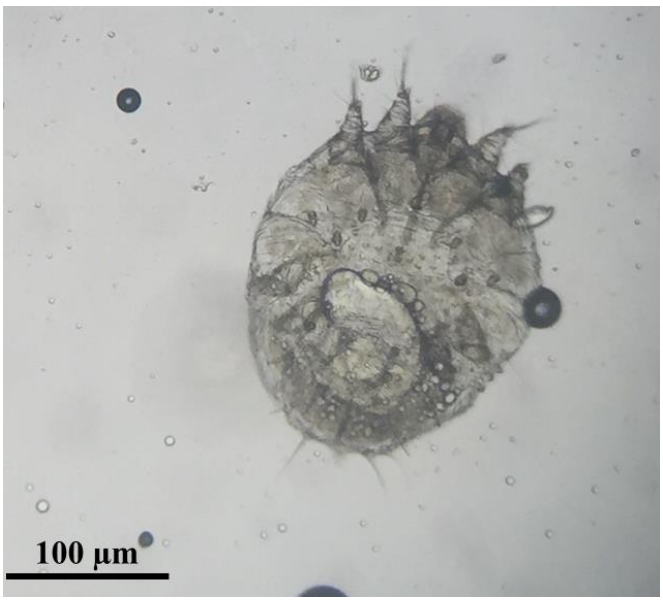


Figure 2. *Sarcoptes scabiei* mite collected from skin scrapings of the Iberian hare and identified under light microscope at a magnification of 100×

Four mites from the hare were successfully isolated and processed for molecular analyses, whereas *S. scabies* mites previously obtained from other species and different populations were used as control groups (Table 1).

Sampling site	**Host species**	***N***	***n***	**Year**
Valencia	*Lepus granatensis*	1	4	2019
Valencia	*Oryctolagus cuniculus*	1	3	2020
Catalonia (Tarragona)	*Oryctolagus cuniculus*	1	4	2010
Mallorca	*Oryctolagus cuniculus*	6	14	2010
Catalonia	*Vulpes vulpes*	8	20	2014
Valencia	*Vulpes vulpes*	2	3	2020

Table 1. Origin, sampling year and sample size of the animals affected by sarcoptic mange included in this study

A total of 56 alleles were detected. Allele count ranged from 3 (Sarms 34) to 10 (Sarms 33). Six private alleles were found across the 10 microsatellite loci, distributed among four loci (Sarms 33, 38, 41, 45). A significant deviation from HWE was detected throughout all loci, except for Sarms 34, 35, 37 ($P < 0.05$). Observed heterozygosity and allelic richness ranged between 0.07 (Sarms 37) and 0.25 (Sarms 35) and between 0.61 (Sarms 33) and 0.88 (Sarms 41), respectively (Table 2).

Mst locus	He	Ho	R
Sarms 33	0.72	0.21	0.61
Sarms 34	0.63	0.09	0.82
Sarms 35	0.64	0.25	0.77
Sarms 36	0.57	0.07	0.81
Sarms 37	0.58	0.15	0.82
Sarms 38	0.66	0.20	0.70
Sarms 40	0.73	0.14	0.69
Sarms 41	0.28	0.11	0.88
Sarms 44	0.71	0.09	0.74
Sarms 45	0.71	0.13	0.67

Table 2. Descriptive statistics of the *Sarcoptes* populations arranged by Sarms locus. He, expected heterozygosity; Ho: observed heterozygosity; R: allelic richness

According to the DK method of Evanno ($K = 2$), the Bayesian assignment test revealed the presence of two geographically separated *Sarcoptes*-derived clusters (Fig. 3), consisting of mites from the hare, wild rabbits and foxes from Valencia (green cluster), and mites from foxes originating from Catalonia (red cluster).

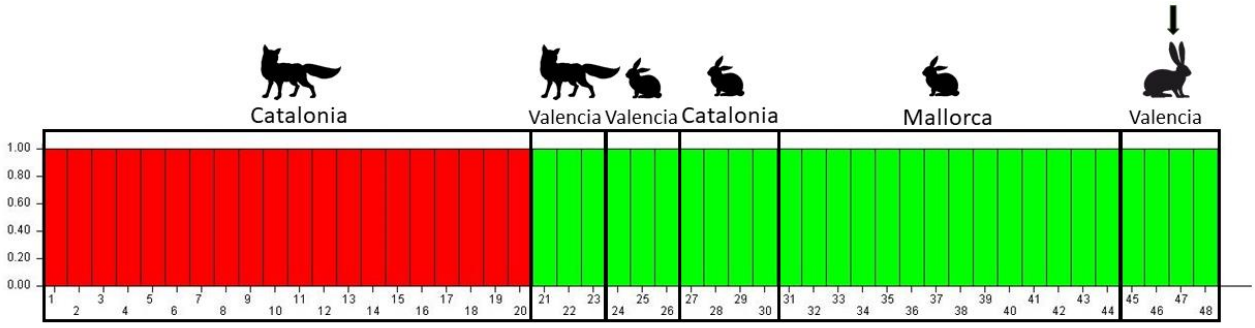


Figure 3. Bar chart of *Sarcoptes*-derived genetic cluster generated with the software Structure 2.3.4 with maximum likelihood $K = 2$. Each mite is represented by a single bar, and the height of each coloured segment is proportional to the membership fraction in each cluster. The arrow indicates the four mite samples from the Iberian hare of this study

The results of the PCA are displayed in Fig. 4. The multivariate analysis revealed three main clusters, prevalently scattered along the first axis: (i) mites collected on foxes from Catalunya; (ii) mites collected on foxes from Valencia; and (iii) mites collected on the hare from Valencia and wild rabbits from Catalonia, Valencia and Mallorca.

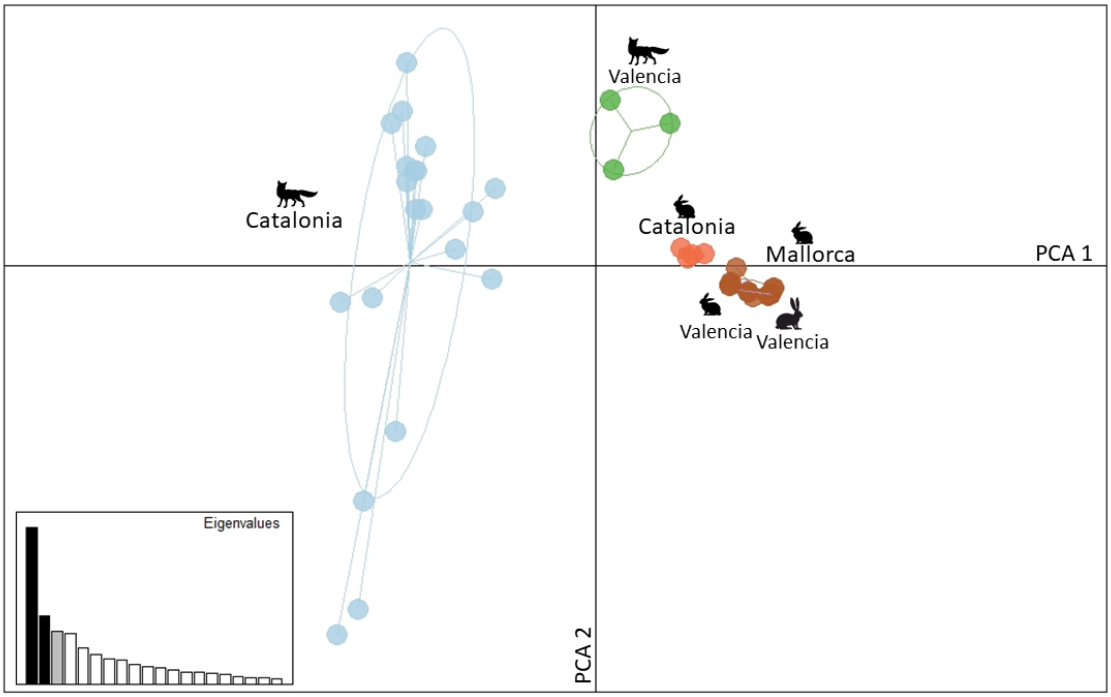


Figure 4. Principal components analysis (PCA) of Mst loci representing hare-, rabbit- and fox-derived mite populations in Spain. Each population is labelled with the host species and the geographical origin. Components 1 and 2 explained 14% and 6.1% of the variance, respectively (black bars of the eigenvalues). The eigenvalues of the two axes are displayed in the barplot on the left.

4. Discussion

In the past decade, microsatellite (Mst) markers have been widely used in *S. scabiei* epidemiological and population genetic investigations (Rasero *et al.*, 2010; Gakuya *et al.*, 2011; Matsuyama *et al.*, 2019; Rudd *et al.*, 2020; Moroni *et al.*, 2021c) and in forensic analyses (Alasaad *et al.*, 2012a). In the present study, ten Msts were used to molecularly type *Sarcoptes* mites derived from a free-ranging Iberian hare and trace the possible source of the infection.

According to the Bayesian assignment test (Fig. 3), the clustering of mites suggests that rabbits or sympatric foxes might be the actual source of infection for this hare, while the PCA analysis highlights three clusters, unambiguously showing that the hare-derived mites clustered with those from wild rabbits. This might be explained by the variance along the first axis of the PCA analysis (Fig. 4), contributing to a clear separation of fox-derived mites from Catalonia and the remaining mite samples, while the second axis separates the fox-derived mites from Valencia and the hare/rabbit-derived mites.

Descriptive genetic analysis revealed an evident deficiency in the observed heterozygosity and allelic richness, confirming the results of previous molecular investigations on *Sarcoptes* mite populations (Rasero *et al.*, 2010; Gakuya *et al.*, 2011; Matsuyama *et al.*, 2019; Rudd *et al.*, 2020). Moreover, the low number of private alleles indicates a reduced genetic divergence and high gene flow between mite populations analyzed in this study.

Sarcoptic mange outbreaks were first reported in wild rabbits at the beginning of the 21st century, namely on Mallorca Island (Millán, 2010) and mainland southern Catalonia (Navarro-Gonzalez *et al.*, 2010). However, results of a large-scale serosurvey showed that *S. scabiei* infection was endemic in wild European rabbits throughout the Iberian Peninsula since at least the 1990s of the previous century (Millán, 2010). Game restocking in the absence of effective sanitary control has been identified as a major risk factor for the spreading of sarcoptic mange amongst resident rabbits (Navarro-Gonzalez et al., 2010). On the other hand, the widespread exposure to *S. scabiei* infection in wild rabbits in Spain and the sympatry between wild rabbits and Iberian hares over large part of the respective distribution areas (Alves and Hackländer, 2008) suggest that *L. granatensis*, described here as a new host for *S. scabiei*, seems to be quite resistant to *S. scabiei* at both, individual and population level. This may be also true for other members of *Lepus* spp., in which sarcoptic mange has never been reported, to the best of our knowledge.

Skin lesions reported in this study resemble those observed in wild rabbits (Millán, 2010), suggesting a similar clinical and pathological course of infection in susceptible individuals.

The clustering of mites collected on a fox from the Valencia Community with mites from wild rabbits and the Iberian hare (Fig. 3) is not surprising, since prey-to-predator transmission of *S. scabiei* has been already documented with molecular tools (Gakuya *et al.*, 2011). However, the overall genetic results of this study (Bayesian assignment test and PCA analysis) confirm that gene flow between mites from similar host taxa occurs more frequently than between those from different sympatric host taxa, and thus, transmission of *S. scabiei* amongst individuals belonging to the same species or closely related ones, according to the "host-taxon law" (Rasero *et al.*, 2010), is prevalent over other reported cross-transmission patterns. Moreover, it is worth noting that rabbit-derived *Sarcoptes* from Mallorca Island are in the same cluster of those originating from mainland southern Catalonia, despite the evident geographical limits (Figs. 3 and 4).

The present report in a previously undetected host of *S. scabiei*, the Iberian hare, should raise awareness in wildlife operators and veterinary authorities, and stimulate monitoring programmes in areas where this endemic game animal shares range with endemically infected wild rabbits. The role of restocking as a risk factor for the spread and persistence of sarcoptic mange in wild rabbits and sympatric lagomorphs should also be elucidated in the future.

CHAPTER 4

SARCOPTIC MANGE IN FELINES

This chapter has been adapted from the manuscript in preparation:

Moroni B, Albanese F, Molinar Min AR, Pasquetti M, Guillot J, Rolando Pisano SR, Ryser-Degiorgis MP, Gauthier D, Cano-Terriza D, Rossi L, Peano A. Sarcoptic mange in felines: does *Sarcoptes scabiei* var. *felis* exist? A preliminary molecular study.

1. Introduction

In domestic cats, sarcoptic mange is considered a rare skin disease. However, several cases including an outbreak affecting 25 felines (Bornstein *et al.*, 2004), have been reported in the scientific literature throughout the last decade (Malik *et al.*, 2006; Hardy *et al.*, 2013; Huang and Lien, 2013; Sivajothi and Reddy, 2015; Singh *et al.*, 2019; Iqomah *et al.*, 2020) (Table 1). The origin of these unusual episodes is empirically attributed to contacts with affected dogs living in the same household (Malik *et al.*, 2006; Sivajothi and Reddy, 2015), or more rarely, with foxes used to visit neighbouring gardens (Malik *et al.*, 2006; Hardy *et al.*, 2013).

Scabies is also known in wild felids, such as Iberian lynx (*Lynx pardinus*) (Oleaga *et al.*, 2019b), Eurasian lynx (*Lynx lynx*) (Ryser-Degiorgis *et al.*, 2002), the Himalayan lynx (*Lynx lynx isabellinus*) (Hameed *et al.*, 2016), lion (*Panthera leo*), cheetah (*Acinonyx jubatus*) (Gakuya *et al.*, 2011), and the European wild cat (*Felis silvestris silvestris*) (Najera *et al.*, 2021)(Table1).

In these species, the source of the transmission has been empirically associated with other affected wildlife species encountered in the same ecological niche, as may occur within carnivore communities (eg Eurasian lynx and red fox in continental Europe (Cardells *et al.*, 2021)) or between preys and predators (eg between gazelle and cheetahs in Eastern Africa, Kenya (Gakuya *et al.*, 2011)).

Sarcoptes scabiei has been traditionally classified into host-specific varieties. Still, growing molecular evidence shows that the mere taxon-oriented approach is not sufficient to embrace the complexity of the issue (Escobar *et al.*, 2021). In this regard, various molecular tools have become available to deepen our understanding of the genetic differences between *Sarcoptes* strains affecting different host species, and track transmission pathway more efficiently and objectively. Amongst these tools, microsatellite markers have been proved more performant than other markers to characterize the genetic strains of *S. scabiei* affecting wildlife populations in Europe (Rasero *et al.,* 2010; Moroni *et al.,* 2021; Cardells *et al.*, 2021; Valldeperes *et al.*, 2021), Africa (Gakuya *et al.,* 2011), Asia (Matsuyama *et al.,* 2019) and North America (Rudd *et al.,* 2020), sometimes revealing unexpected or unproven spreading patterns.

In light of the rare occurrence of scabies in felines, molecular epidemiology of *Sarcoptes* isolates from cats has never been investigated before, living open speculations on the possible transmission models and pathogenic course of sarcoptic mange in these species.

The aim of this study is therefore to investigate the molecular profile of *Sarcoptes* mites from domestic and wild felines from different European countries, and to compare them with *Sarcoptes* mites from sympatric wild species, using microsatellite markers.

Country	Host species	Number of individuals	Suspected origin	Reference
UK	*Felis catus*	1	fox	Hardy et al., 2013
Indonesia	*Felis catus*	9*	NA	Iqomah et al., 2020
Sweden	*Felis catus*	25	dog	Bornstein et al., 2004
Taiwan	*Felis catus*	5*	NA	Huang et al., 2013
Australia	*Felis catus*	4*	Dog, fox, wombat	Malik et al., 2006
India	*Felis catus*	1	dog	Sivajothi et al., 2015
India	*Felis catus*	1	NA	Singh et al., 2019
Switzerland	*Lynx lynx*	2*	Fox/lynx	Degiorgis et al., 2002
Spain	*Lynx pardinus*	1	Fox	Oleaga et al., 2019
Pakistan	*Lynx isabellinus*	1	Fox/ domestic livestock	Hameed et al., 2016
Spain	*Felis silvestris silvestris*	1	Fox/cat/dog/rabbit	Najera et al., 2021
Kenya	*Acinonyx jubatus*	3	Thomson's gazelle	Gakuya et al., 2011
Kenya	*Panthera leo*	3	Wildebeest	Gakuya et al., 2011

Table 1. Felid cases of sarcoptic mange previously reported in the scientific literature. *Multiple cases reported in the same article

2. Material and Methods

Skin scrapings from four cats and one dog were collected in Italy, France and Switzerland during clinical dermatological visit for severe itch and crusted skin lesions, while skin samples from wildlife species were obtained from carcasses found dead individuals submitted to identify the cause of death (Table 2).

Sampling site	**Host species**	***N***	***n***
France	*Felis catus*	1	3
Central Italy	*Felis catus*	2	8
Switzerland	*Felis catus*	1	6
Switzerland	*Lynx lynx*	4	8
France	*Lynx lynx*	1	5
Switzerland	*Vulpes vulpes*	11	11
North Italy	*Vulpes vulpes*	12	28
North Italy	*Canis lupus familiaris*	1	3
France	*Canis lupus*	2	5
Central Italy	*Canis lupus*	2	4

Table 2. Origin and sample size of the animals affected by sarcoptic mange included in this study. N: number of sampled animals; n, number of mites used for Mst analysis

All samples were stored at -20°C in 70% ethanol tubes until mite isolation and later shipped to the Department of Veterinary Sciences of Turin, Italy. Morphological criteria were applied for a preliminary identification of collected mites (Fain *et al.,* 1968). For each skin sample, one to six mites were isolated and individually stored in 70% ethanol (Alasaad et al., 2009).

DNA was extracted from individual mites following the HotSHOT Plus ThermalSHOCK technique (Alasaad *et al.,* 2008). Then, a 10x multiplex PCR was performed using ten validated primers extracted from the previously published panel (Walton *et al.,* 1997) to target *S. scabiei* mites (Sarms 33, 34, 35, 36, 37, 38, 40, 41, 44, 45) following the PCR protocol of Soglia et al. (Soglia *et al*., 2007). Capillary electrophoresis was performed with a Applied Biosystems SeqStudio™, and the software GeneMapper 4.0 (Applied Biosystems, Foster City, CA, USA) allowed the allele calls and microsatellite visualization. After molecular analysis, mites that did not fulfil the required criteria (eight detectable loci out of the ten analyzed) were excluded in the genetic analysis. Two population genetics analyses were applied to the 81 mite microsatellite outputs: i) Bayesian clustering, and ii) principal component analysis (PCA). The first one requires Hardy-Weinberg equilibrium (HWE), while no assumptions are required for the PCA. Descriptive statistics, such as observed and expected heterozygosis (Ho and He, respectively), allelic richness (R) and HWE analysis, were carried out with software R 4.0 using the packages: Adegenet 2.1.3 (Jombart *et al.,* 2008).

P-values for HWE test were based on Monte Carlo permutations of alleles. The Bayesian assignment test was computed with the software STRUCTURE 2.3.4. Burn-in and run lengths of Markov chains were 10,000 and 100,000, respectively, and five independent runs for each K (for K = 1–20) were run. The ancestry model selected was the admixture type. Clusters were estimated as suggested by Evanno (Earl *et al.,* 2012), using the DK method.

3. Results

A total of 53 alleles were detected. Allele count ranged from 2 (Sarms 34) to 11 (Sarms 45). Eleven private alleles were found across the 10 microsatellite loci, distributed among six loci (Sarms 33, 34, 38, 41, 44, 45). Deviation from HWE was detected only in Sarms 34 (Supplementary material). Observed heterozygosity ranged between 0.07 (Sarms 37) and 0.25 (Sarms 35) and between 0.61 (Sarms 33) and 0.88 (Sarms 41), respectively (Table 3).

According to the DK method of Evanno (K = 2), the Bayesian assignment test revealed the presence of two geographically separated *Sarcoptes*-derived clusters (Fig. 1). One included mites from the cats and wolves from Central Italy (green cluster), the other mites from foxes, lynx, wolves, dog and cats originating from France, Switzerland, Northern Italy (red cluster).

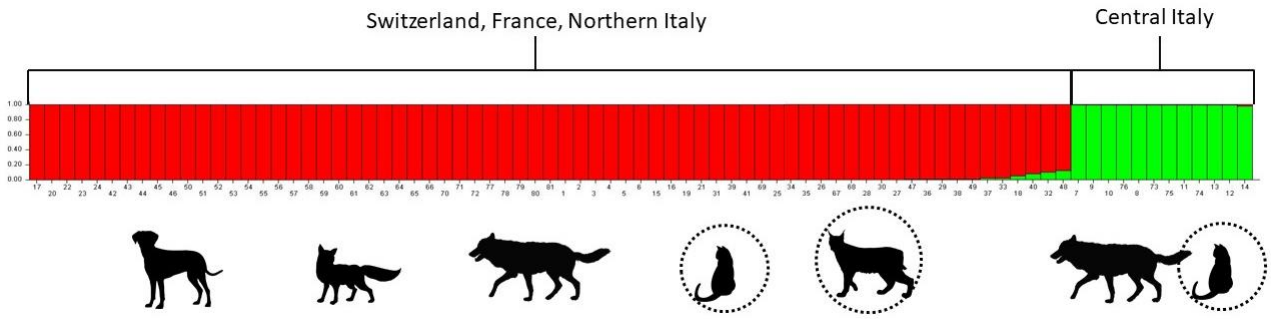


Figure 1. Barplot of *Sarcoptes*-derived genetic cluster generated with the software Structure 2.3.4 with maximum likelihood $K = 2$. Each mite is represented by a single bar, and the height of each coloured segment is proportional to the membership fraction in each cluster.

The results of the PCA are displayed in Fig. 2. The multivariate analysis revealed two main clusters, mainly scattered along the first axis: (i) mites collected on cats and wolves from Central Italy; (ii) mites collected on cats, dog, wolves, foxes and lynx from France, Switzerland, Northern Italy (see also Table 2).

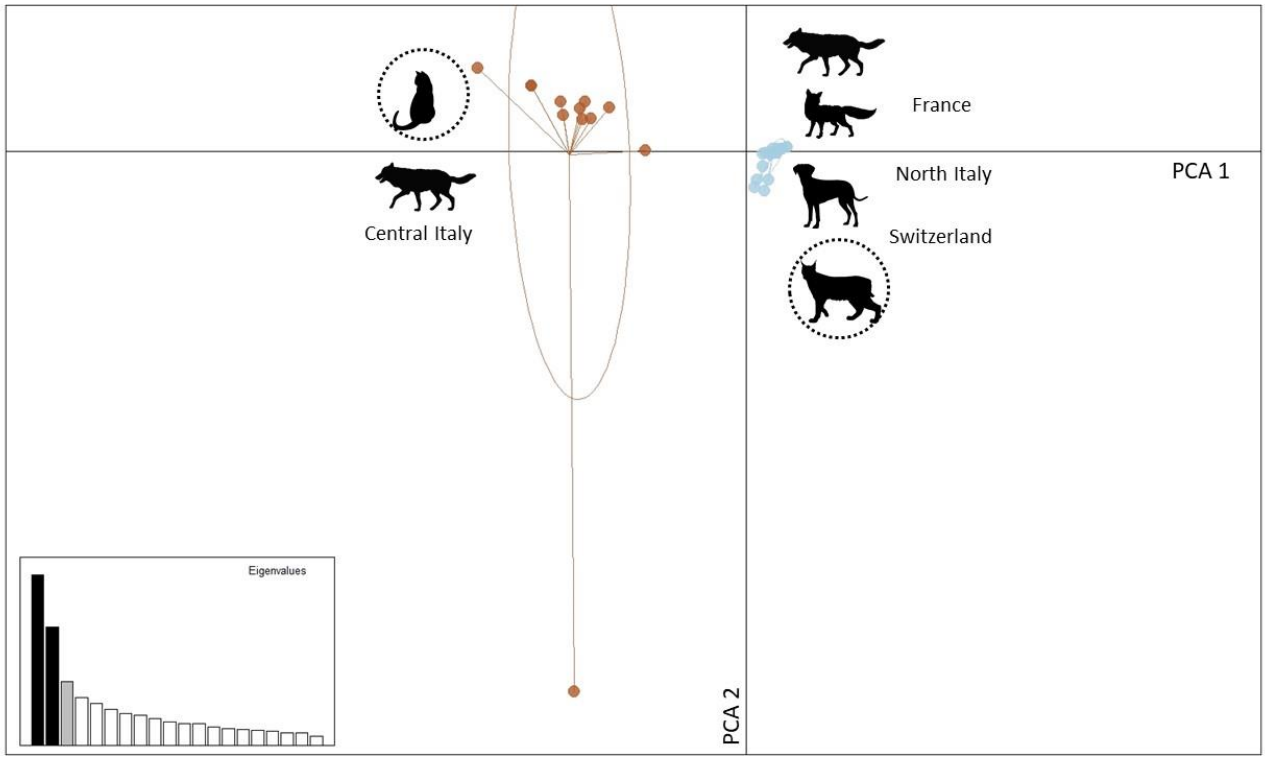


Figure 2. Principal components analysis (PCA) of Mst loci representing cat-, dog-, lynx-, wolf- and fox-derived mite populations in Spain. Each population is labelled with the host species and the geographical origin. Components 1 and 2 explained 11.3% and 7.9 % of the variance, respectively (black bars of the eigenvalues). The eigenvalues of the two axes are displayed in the barplot on the left.

4. Discussion

The main finding of this study is the identification of two genetic clusters of European *S. scabiei* with a geographical distribution pattern: cat-derived mites from Central Italy clustered with those obtained from sympatric wolves. In contrast, all the other mites from Switzerland, France and Northern Italy clustered together. These results are in line with previous evidence, though referred to different models, that genetic lineages of *S. scabiei* have a geographic-related rather than a host-related distribution. The transmission within broad taxa would thus rely on direct or indirect interactions between different hosts sharing the same ecological niche (Rasero *et al.,* 2010; Matsuyama *et al.,* 2020; Moroni *et al.,* 2021). This is a substantial deviation from the classical view that *S.scabiei* exclusively embraces strictly host-specific varieties, and instead supports the view that, in the same environment, mammal species with different social behavior and habits (eg predation, scavenging, allogrooming, territorial fights, denning, mating, etc.) may have from occasional to frequent opportunities of direct or indirect contact, with consequent sharing of pathogens (Rossi *et al.,* 2019; Escobar *et al.,* 2021).

While numerous species-specific varieties of *S. scabiei* have been traditionally associated to a range of different hosts, a feline-specific variety (eg *S. scabiei* var. "*felis*") has never been described, possibly because of the infrequent occurrence of sarcoptic mange in domestic cats, in which notoedric mange by a closely related burrowing mite, *Notoedres cati,* is predominant. Nonetheless, in the last few decades, an increasing number of sarcoptic mange cases in both domestic and wild felines have been reported in the scientific literature (Malik *et al.,* 2006; Gakuya *et al*., 2011; Degiorgis *et al.,* Hardy *et al.,* 2012; Iqomah *et al.,* 2020), referred to different epidemiological scenarios in which scabietic domestic dogs, foxes or natural preys of wild felines, were pinpointed as the likely source of infection (Table 1).

Recently, nuclear molecular markers such as microsatellites were proved valuable tools to investigate population genetic differences and putative transmission pathways within *S. scabiei* (Rasero *et al.,* 2010; Rudd *et al.,* 2020; Matsuyama *et al.,* 2020; Moroni *et al.,* 2021). The present study represents the first application of such tools on multiple domestic/wild felid-derived mites.

Interestingly, Bornstein et al., 2004 mentioned a genetic characterization (although not specifying the molecular markers employed) of *Sarcoptes* mites from six out of 25 mangy cats involved in a single outbreak in Sweden. These authors pointed out that "the mites had DNA sequences identical to *S. scabiei* from naturally infected dogs and Swedish wildlife". These results agree with our findings, regarding the molecular profiles of sympatric wild carnivores that similar to those from domestic cats (Figure 1, 2). These results compel evidence that *S. scabiei* taxonomy cannot be simplified in clear-cut host-specific varieties or subspecies, as already outlined (Escobar *et al.,* 2021; Valldeperes *et al.,* 2021).

According to empirical information in previous studies (Table 1), dogs and foxes are the most reported source of spillover infection for domestic and wild felids (n=8), suggesting that these canids play a key

role to understand and predict the transmission of *S. scabiei* to domestic and wild felids in a range of scenarios, from urban to remote natural zones. Instead, unlike what was observed in two large wild felines in Eastern Africa (Table 1), no prey-to-predator pattern of *S. scabiei* transmission has been identified in this study.

It should however be noted that, in Switzerland and the Western Alps in general (see Table 1 for the origin of lynx samples), scabies is unknown pathology in the lynx main preys, namely in Roe deer (*Capreolus capreolus*) and the Northern chamois (*Rupicapra rupicapra*), as well as in minor preys except for the red fox.

The use of canid-derived mites in our study (Table 2), allowed for the first time to compare *Sarcoptes* genetic profiles from dogs and wolves, with those of sympatric foxes and felids. Our results, far from suggesting that domestic cats and wolves from Central Italy were sharing the pathogen by direct contact (which is ecologically unreasonable), show that the same *Sarcoptes* strain circulates in different carnivore hosts living in the same geographical area. It seems reasonable to assume that scabietic dogs or red foxes represent the missing link between wolves and cats in Central Italy (see Figure 1 and 2).

Interestingly, the owners of the cats from this study area reported in the anamnestic interview that both had free access to the green area near the house, and that foxes were often seen roaming in the same place.

While sarcoptic mange may not always represent a threat for domestic animals, in which the veterinarian can easily perform diagnosis and treatment, numerous cases resulted in zoonotic transmission to the owners or members of the family who had contact with the infected cat (Moroni *et al.*, 2022). Moreover, infection by *Sarcoptes* mites circulating in carnivore communities in Europe, can put additional demographic pressure on species of conservation concern, such as the Eurasian lynx. In this wild feline, and other European wild felids not included in this study, the recognition of canids (and most likely the red fox) as the expected source of infection may have practical consequences in the planning and running of delicate conservation and management interventions, such as restocking and reintroductions.

In conclusion, our results suggest that domestic and wild felines in Italy and neighboring countries are affected by the same *Sarcoptes* strains affecting sympatric canids. A prevalent geographical pattern may influence the transmission pathways of *Sarcoptes* mites in the investigated carnivore hosts, rather than a "species-specific only" pattern. The collection of samples from different domestic and wild host species originating from different countries in Europe required the coordination of several scientific organisms such as Universities, wildlife authorities and veterinary clinicians, and may represent a successful example of collaboration between international *Sarcoptes* specialists.

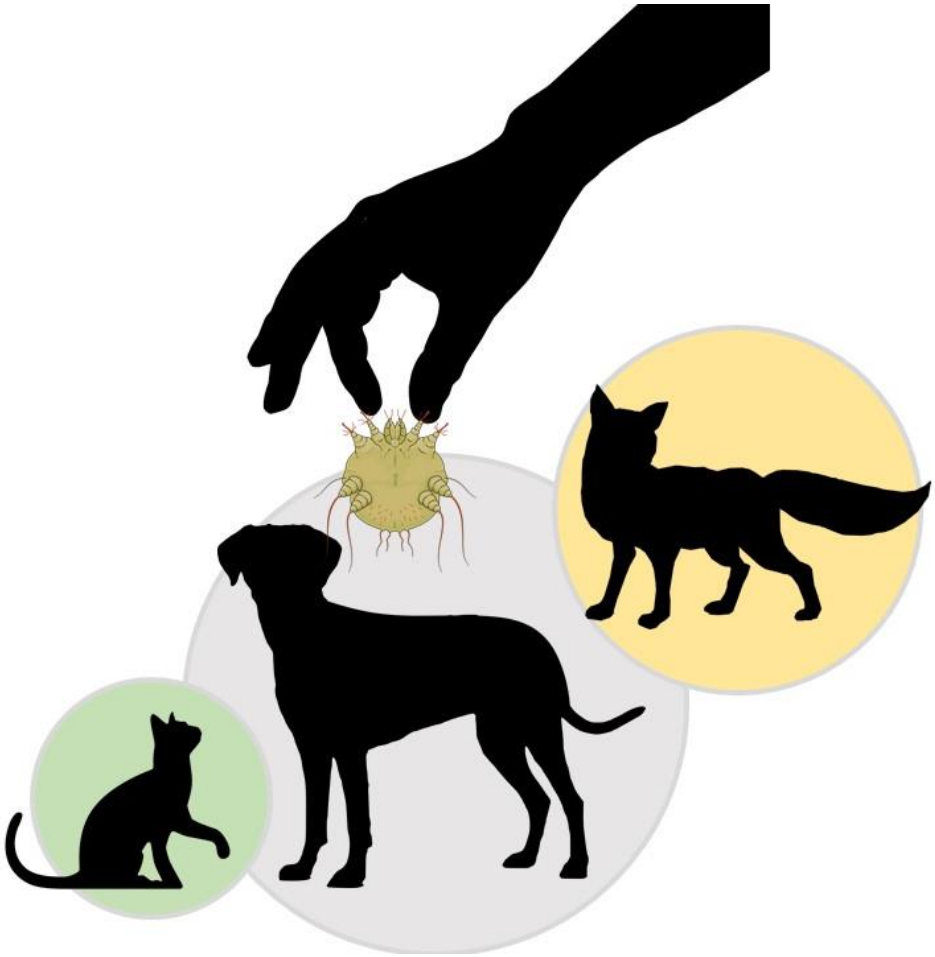
A geographical implementation of the *Sarcoptes* mite population from felids would be a desirable future step for further genetic analysis.

CHAPTER 5

ZOONOTIC SCABIES: A GLOBAL OVERVIEW

This chapter has been adapted from the published article:

Moroni B, Rossi L, Bernigaud C, Guillot J (2022) Zoonotic Episodes of Scabies : A Global Overview. Pathogens 11: 213
https://doi.org/10.3390/ pathogens11020213



1. Introduction

Scabies, also named sarcoptic mange when referred to animals, is a contagious parasitic skin disease caused by the burrowing mite, *Sarcoptes scabiei*, affecting more than 150 mammal species worldwide. It was stated that "no other permanent parasitic mite has such a large variety of hosts as does *S. scabiei"* (Fain, 1978). Scabies was listed by the World Health Organization (WHO) among neglected tropical diseases in 2017, acknowledging the need for greater awareness on the part of practitioners and health organizations, and for a global consensus on control guidelines and strategies. According to WHO, more than 200 million people suffer from scabies globally, but the real extent of the disease is likely grossly underestimated (Karimkhani *et al.*, 2015; Engelman *et al.*, 2019). The source of human scabies is commonly attributed to direct or less frequently indirect (via fomites) contacts with affected people. More rarely, the origin of scabies episodes is traced back to contacts with affected animals. Zoonotic scabies (from here on, ZS), also referred to as "pseudoscabies", is considered a self-limiting disease, with a short incubation period and transient skin clinical signs. Usually, ZS is limited to some topographic regions of the body (*ie* chest, abdomen, hands), resolving with avoidance and/or treatment of the animal source (Rehmus and Prendiville, 2019). It is commonly thought that animal "strains" of *S. scabiei* are unable to successfully reproduce and persist on human skin (Burgess, 1993). However, human experimental infections with scabies mites of dog origin resulted, in four of six volunteers, in a successful replication with hatching and development of mites (Delafond, 1862; Estes *et al.*, 1983), and several spontaneous ZS case reports mentioned the persistence of symptoms for weeks until an effective acaricide treatment was applied (Rabinowitz and Gordon, 2004; Aydingöz and Mansur, 2011; Gallegos *et al.*, 2014). Those reports suggest that scabies mite needs may be fulfilled in human skin, too. Anthropozoonotic transmission (*ie* transmission from humans to animals) has been also documented in mountain gorillas (Graczyk *et al.*, 2001).

Online bibliographic enquiries using terms such as "pseudoscabies" or "ZS" point out that literature on ZS is not as abundant as expected, and is partially outdated and difficult to retrieve. In addition, reviews on ZS episodes are lacking, except for sporadic contributions focusing on zoonotic canine scabies (Roncalli, 1987; Burroughs and Elston, 2003; Aydingöz and Mansur, 2011). Because of all this, flaws exist on multiple aspects of ZS global comprehension, ranging from the proper use of terms (*eg* pseudoscabies, see below) to the epidemiology (*eg* diversity and relative importance of animal sources, or mite "strains" involved), the diagnosis and the treatment to recommend. This review is conceived as a collection and critical analysis of the available literature on *S. scabiei* zoonotic episodes, focusing on the source of the outbreaks, and the circumstances leading to the transmission of the parasite. We searched and selected relevant papers through three electronic databases *via* Web and interlibrary services (Scopus, Web of Science, and Google Scholar) until September 2021 with no time and language limits. The search strategy included different combinations of two or more of the following

key words: "*Sarcoptes scabiei*", "scabies", "human", "animals", "sarcoptic mange", "pseudoscabies", "zoonosis", "zoonotic disease". Initially, titles and abstracts were screened, identifying articles for their relevance to the present topic. Finally, 47 relevant full papers were analyzed. Complementary information was obtained from the online version of an ancient monography by Delafond and Bourguignon (Delafond, 1862).

2. Overview of zoonotic scabies episodes

At a global scale, ZS episodes are associated to six continents (Africa, North and South America, Europe, Asia and Oceania) (Figure 1), with the majority of reports originating from North America and Asia (Table 1).

	Country	**Epidemic aspect (number of people infected)**	**Diagnosis in humans**	**References**
Contact with dogs (*Canis lupus familiaris*)				
	Turkey	no	Dermoscopy	(Aydingöz and Mansur, 2011)
	USA	yes (n=9)	Clinical	(Beck, 1965)
	USA	yes (n=15)	Skin scraping	(Charlesworth and Johnson, 1974)
	South Korea	no	Skin scraping	(Chun *et al.*, 2009)
	USA	yes (n=10)	Clinical	(EMDE, 1961)
	Chile	yes (n=7)	Skin scraping	(Gallegos *et al.*, 2014)
	South Korea	No	Skin scraping	(Kang *et al.*, 1988)
	UK	No	Clinical	(Hewitt *et al.*, 1971)

	Brazil	yes (n=58*)	Skin scraping/clinical	(Larsson, 1978)
	USA	-	-	(JF, 1965)
	Egypt	-	-	(Morsy *et al.*, 1994)
	USA	yes (n=4)	Skin scraping	(Newton and Gerrie, 1966)
	USA	-	Skin scraping	(Norins, 1969)
	Mexico	No	Skin scraping	(Ruiz-Maldonado *et al.*, 1977)
	USA	yes (n=22*)	Clinical/ex-juvantibus	(Smith and Claypoole, 1967)
	India	No	-	(Reddy and Kumari, 2013)
	USA	yes (n=7)	Skin scraping	(Tannenbaum, 1965)
	UK	No	Clinical	(Thomsett, 1968)
	USA	yes (n=67*)	-	(Warner, 1984)
Contact with red foxes (*Vulpes vulpes*)				
	Germany	No	Clinical/ex-juvantibus	(Birk *et al.*, 1999)
	Switzerland	yes (n=4)	Dermoscopy	(Pisano *et al.*, 2019)
	USA	No	Clinical/ex-juvantibus	(Rabinowitz and Gordon, 2004)

	Italy	No	Dermoscopy	(Moroni *et al.*, 2021a)
	Sweden	-	-	(Mörner, 1992)
Contact with Bovidae				
Cow (*Bos taurus*)	-	No	Skin scraping	(Mumcuoglu and Rufli, 1979)
Water buffalo (*Bubalus bubalis*)	India	yes (n=35)	Skin scraping and clinical	(Chakrabarti *et al.*, 1981a)
Goat (*Capra hircus*)	India	yes	-	(Chakrabarti *et al.*, 1981b)
	India	yes (n=13)	Skin scraping	(Chakravorty *et al.*, 1953)
	India	No	Skin scraping	(Mahendra *et al.*, 2006)
	India	yes	-	(Mitra *et al.*, 1995)
Chamois (*Rupicapra rupicapra*)	Italy	yes (n=7)	Clinical	(Menzano *et al.*, 2004)
Goitrerd gazelle (*Gazella subgutturosa)*	Iran	yes (n=6)	Skin scraping	(Bazargani *et al.*, 2007)
Contact with horses (*Equus caballus*)				
	UK	No	Clinical	(Sleutjens, 2015)
	UK	No	Clinical	(Littlewood, 2011)
Contact with wombats (*Vombatus ursinus*)				
	Australia	yes (n=3)	Clinical	(Skerratt and

				Beveridge, 1999)
Contact with Camelidae				
Alpaca (*Vicugna pacos*)	UK	No	Clinical/ex-juvantibus	(Twomey *et al.*, 2009)
Llama, alpaca (*Lama glama, Vicugna pacos*)	Germany	No	Clinical	(Beck, 2020)
Contact with rabbits (*Oryctolagus cuniculus*)				
	South Korea	No	Ex-juvantibus	(Choe *et al.*, 2020)
	China	-	-	(Li *et al.*, 1999)
	China	-	-	(Duan *et al.*, 2000)
Contact with primates				
Gibbon (*Hylobates leuciscos*)	UK	yes (n=10)	Dermoscopy	(Goldman and Feldman, 1949)
Contact with pigs (*Sus scrofa*)				
	Switzerland	No	Clinical	(Grahofer *et al.*, 2018)
	India	yes (n=30)	Skin scraping/clinical	(Chakrabarti, 1990)
Contact with cats (*Felis catus*)				
	UK	No	Clinical	(Hardy *et al.*, 2013)
	Indonesia	No	Clinical	(Iqomah *et al.*, 2020)
	Taiwan	yes (n=5)	Clinical	(Huang and Lien, 2013)
	Australia	No	Clinical	(Malik *et al.*, 2006)

*more than a single epidemic event

Table 1. Reported episodes of zoonotic scabies. Relevant information on the source of the transmission, country of the episode, epidemic aspect (when more than 2 people were infected), and type of diagnosis were collected. ZS cases observed by Delafond and Bourguignon (Delafond, 1862) were not included in this table.

According to data showed in Figure 2, both domestic and wildlife animals are involved in the transmission pathways (Figure 2). The temporal range of collected articles is seventy years wide (1949–2020) with no clustering pattern along years, highlighting a low, though stable global reporting rate of ZS. As opposite to human-derived scabies, ZS seems to follow a scattered pattern of spreading associated to high-risk circumstances, rather than a cyclical pattern.

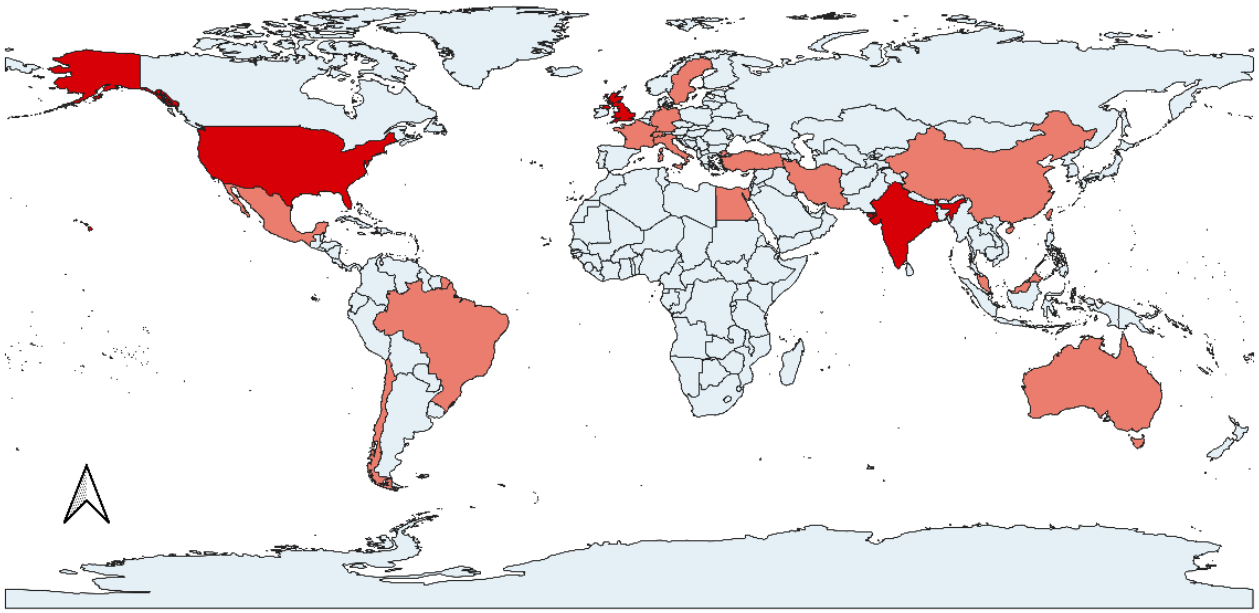


Figure 1. World map showing countries with episodes of zoonotic scabies reported in the scientific literature (dark red: 4 or more articles associated to this country; light red: less than 4 articles).

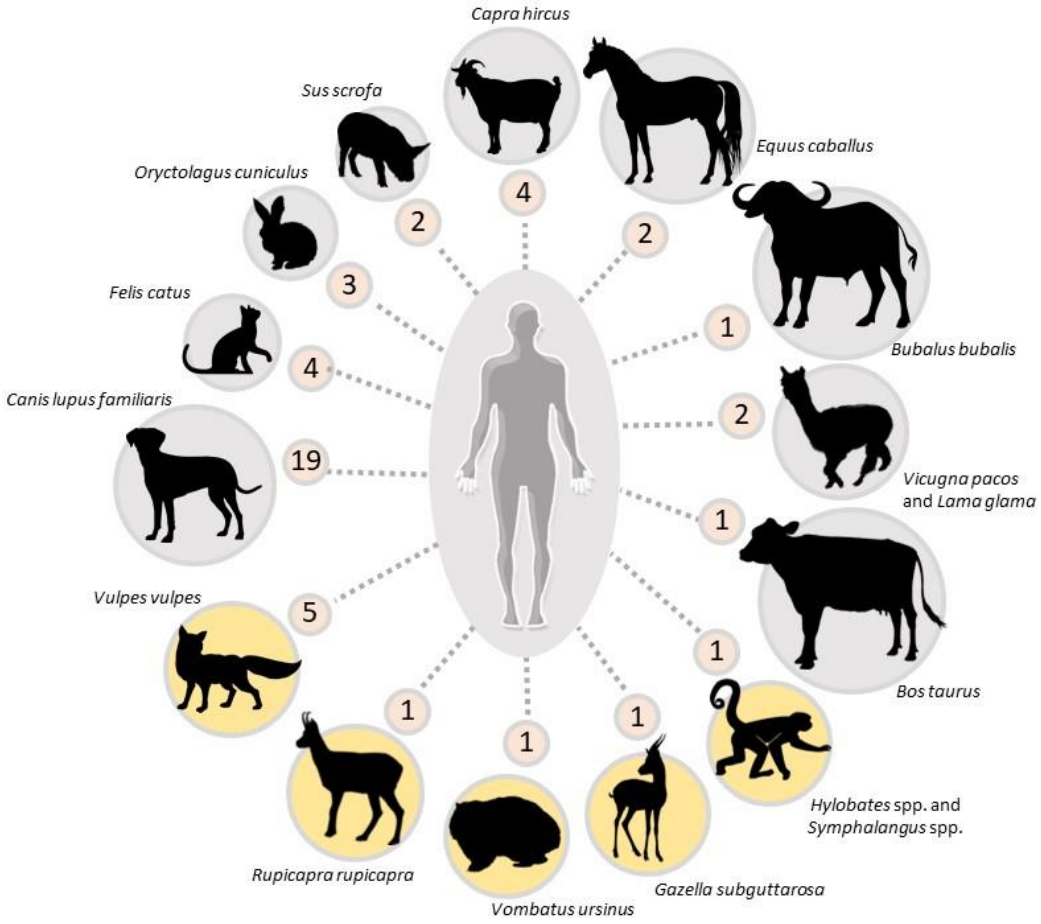


Figure 2. Animal species origin of zoonotic episodes of scabies represented by number of articles/animal species collected in this review. Wildlife species are highlighted in yellow. The ZS episodes described by Delafond and Bourguignon (Delafond, 1862) were not included in this figure but are mentioned in the main text.

Pet owners are an important ZS target. So far, five pet species were identified as source for their owners, namely dog, cat, miniature pig, horse and rabbit (Figure 2). Dog is by far the most reported source in ZS episodes worldwide (Figure 2). It is not clear whether this is a bias due to the fact that dog is the most popular pet in several countries, or because direct contacts are particularly frequent and intimate in the case of dog ownership, or a combination of both. As a matter of fact, while sarcoptic mange is commonly diagnosed in stray, low care or sheltered dogs (Chee *et al.*, 2008), the condition is relatively rare amongst cats, another popular pet worldwide (Malik *et al.*, 2006). However, compared with other carnivore hosts of *S. scabiei* (*eg* red foxes, *Vulpes vulpes*), dogs are less prone to develop the crusted "Norwegian" form of the disease, which is associated with high mite densities and a subsequent greater infectious potential than other sarcoptic mange presentations (Rentería-Solís *et al.*, 2014). Worth of note, in five studies, the canine origin of the ZS outbreak was associated to the recent adoption of a puppy with crusty lesions and/or harboring abundant mites (Charlesworth and Johnson,

1974; Kang *et al.*, 1988; Chun *et al.*, 2009; Aydingöz and Mansur, 2011; Gallegos *et al.*, 2014). The authentic zoonotic nature remains debatable in the case of a 14-year-old girl with an eight-year history of disseminated pruritic crusted lesions, in a household where scabietic dogs were also present (Ruiz-Maldonado *et al.*, 1977).

ZS originating from livestock is also a well-known condition, earning appellations such as "goat handler's itch", "dairyman's itch", "buffaloman's itch", "pig handler's itch", "cavalryman's itch" (Chakrabarti *et al.*, 1981b; a; Chakrabarti, 1990; Burgess, 1993). Reports involve different livestock species, namely goats, sheep, pigs, cows, alpacas, llamas and water buffaloes (Figure 2), (Mumcuoglu and Rufli, 1979; Chakrabarti *et al.*, 1981b; a; Chakrabarti, 1990; Twomey *et al.*, 2009; Beck, 2020). These ZS cases mainly include operators who were in intimate contact with infested livestock because of their daily work (*eg* milkmen, breeders, animal attendants). On a global scale, it is reasonable to assume that ZS episodes linked with livestock animals are underreported compared to ZS episodes related to pets. Nonetheless, compared with literature analysed in this review, the rich and varied caseload in Delafond and Bourguignon (Delafond, 1862) suggest that ZS of livestock origin is now uncommon in the Western world compared to the 19th century, likely due to the different attitude and management of animals and the availability of efficient and user-friendly acaricides. This is particularly true for ZS of horse origin, that collapsed in Europe in parallel with the contraction of the use of horses as military and non-military work animals. Sarcoptic mange has turned into a rare condition in equines throughout developed countries, and recent cases reported in Europe have been rather traced back to spillover contact with mangy foxes (De Pennington and Colles, 2011; Littlewood, 2011; Sleutjens, 2015; Pisano *et al.*, 2019).

Wildlife does not appear to be a common source of ZS, as only nine species in different parts of the globe have been found to be responsible for mite transmission to humans (Figure 2), although this might be another bias due to the infrequent skin-to-skin contacts between humans and wild animals. Red foxes were associated with human scabies in five cases (Mörner, 1992; Birk *et al.*, 1999; Rabinowitz and Gordon, 2004; Pisano *et al.*, 2019; Moroni *et al.*, 2021a), in both, urban and rural contexts. Worthy of note is an outbreak involving four people in a farm where a moribund fox with generalized lesions had sought shelter (Pisano *et al.*, 2019). In this episode, people became infested through direct contacts with different species of domestic animals (pig, goat, dog, horse, oxen) which, in turn, had previous contacts with the scabietic fox. However, in other wildlife-derived ZS episodes, the affected individuals were involved in a wildlife-related occupation as keepers, veterinarians, or specialized operators, or were private citizens who found themselves rescuing a scabietic animal or handling a fresh carcass (Goldman and Feldman, 1949; Boch and Schneidawind, 1988; Mörner, 1992; Skerratt and Beveridge, 1999; Menzano *et al.*, 2004; Rabinowitz and Gordon, 2004; Bazargani *et al.*, 2007). Reportedly, wearing gloves did not guarantee protection in all cases (Skerratt and Beveridge, 1999; Bazargani *et al.*, 2007), possibly due to the enormous number of mites crawling on the skin surface of source individuals

affected by generalized crusted scabies. Beyond literature, authors have personal experience that ZS is not uncommon among hunters and gamekeepers in areas of Central and Southern Europe where mountain-dwelling ruminants are endemically infected with sarcoptic mange (Skerratt and Beveridge, 1999; Bazargani *et al.*, 2007). However, to the authors' knowledge, no ZS cases have been reported in wild boar hunters, although the disease is observed in this game throughout Europe (Haas *et al.*, 2018; Sannö *et al.*, 2021; Valldeperes *et al.*, 2021). Delafond and Bourguignon (Delafond, 1862) mention a single ZS episode following contact with the skin of a scabietic wild boar, in Germany. In the same monography, a ZS outbreak was reported in zookeepers after contacts with affected lions (*Panthera leo*), hyena (*Hyaena hyaena*) and bear (*Ursus arctos*) within weeks from importation in France.

On occasion, people were infected by other people who had acquired scabies from a wild or domestic animal source (EMDE, 1961; Skerratt and Beveridge, 1999; Menzano *et al.*, 2004; Bazargani *et al.*, 2007), implying the possibility of a person-to-person transmission of animal-derived mites. Symptoms in these people were apparently milder than in source contacts and resolved without any treatment within two weeks after exposure.

ZS episodes in this review either involved single patients (n=21/47 citations) or occurred in form of small outbreaks, with clinical signs appearing in more members of the same family (n=20/47 citations) (Tannenbaum, 1965; Newton and Gerrie, 1966; Gallegos *et al.*, 2014), or in professionals who handled the same infested animal/s in the same working environment (Chakravorty *et al.*, 1953; Chakrabarti, 1990; Menzano *et al.*, 2004)(Table 1).

3. Characterization of *S. scabiei* mites in zoonotic scabies episodes

It is accepted that the study of morphology is of limited, if any, value in characterizing the different host- or host-group-adapted variants ("varietates") recognized so far within the globally distributed *S. scabiei*. Consequently, the mite variants involved in ZS episodes have generally been identified on the basis of patient history and epidemiological background. While this is still a primary approach, sharing of similar mite variants can now be demonstrated on the basis of robust molecular evidence. Amongst several genetic markers available, microsatellites were shown to be the most informative and accurate for the purpose of characterizing interspecific cross-transmission of *Sarcoptes* mites (Rentería-Solís *et al.*, 2014; Rudd *et al.*, 2020; Cardells *et al.*, 2021; Moroni *et al.*, 2021c). In a promising way, last generation techniques, such as mitochondrial metagenome sequencing (Mofiz *et al.*, 2016a) or whole mite genome sequencing (Mofiz *et al.*, 2016b) can now be used to further clarify the genetic diversity within mite species.

Walton et al. (Walton *et al.*, 1999) were the first to show that randomly sampled Australian aborigine patients were occasionally infested by *Sarcoptes* mites genetically clustering with those collected on sympatric community dogs. More recently, in the context of the fore mentioned ZS episode that

occurred in a farm in Switzerland (Pisano *et al.*, 2019), mites collected from different and zoologically distant domestic hosts were found to be genetically similar to those isolated from the scabietic fox that likely prompted the outbreak. In Italy, Moroni et al. (Moroni *et al.*, 2021a) provided unambiguous evidence that two patients and the scabietic fox they incautiously rescued, harbored the same vulpine variant of the pathogen. The authors are not aware of any studies based on other genetic markers (namely mitochondrial and ribosomal), in which mites collected on occasion of spontaneous ZS episodes have been characterized. However, the study of polymorphisms in the cytochrome c oxidase subunit 1 gene (Andriantsoanirina *et al.*, 2015a) identified remarkable differences between sympatric and allopatric mites of human origin, and showed that some human-derived isolates clustered with mites of animal origin, eg, dog, pig and raccoon dog (*Nyctereutes procyonoides*), suggesting that *Sarcoptes* is anything but a panmictic taxon and that *Sarcoptes* taxonomy is not as simplified as the "traditional" denomination according to clear host-based variants would suggest.

Despite recent genetic advances, *Sarcoptes* molecular typing remains challenging for several factors, including the difficulty of isolating individual mites from skin scrapings or biopsies, and the low success rate of DNA extraction and PCR amplification of mite DNA (Alasaad *et al.*, 2009b; Moroni *et al.*, 2021c).

4. Diagnosis of zoonotic scabies

ZS diagnosis is often based on compatible signs coupled with consistent historical features, as the visualization of animal-derived mites on a patient can be challenging, but still described in literature (Norins, 1969; Burroughs and Elston, 2003; Menzano *et al.*, 2004; Aydingöz and Mansur, 2011).

ZS usually manifests as an intensely pruritic papulovesicular rash, affecting areas such as the trunk, abdomen, forearms, thighs and legs, while sparing those parts of the body (e.g., hand and foot palms and genitalia) that are frequently involved in human scabies by *S. scabiei* var. *hominis* (Burroughs and Elston, 2003; Chosidow, 2006; Engelman *et al.*, 2020) (Figure 3). However, some exceptions are known in children exposed to scabietic pets, where the distribution pattern of skin lesions, involving the palms, web of fingers, head and neck, may resemble human scabies (Smith and Claypoole, 1967; Norins, 1969; Estes *et al.*, 1983). Body areas in contact with the animal source are the first affected, but other areas may be involved later (Skerratt and Beveridge, 1999; Menzano *et al.*, 2004). The different distribution of skin lesions between human and ZS should be further investigated through systematic clinical and dermatological examinations to draw a valid conclusion on the different immuno-pathological processes behind. In a more recent paper (Aydingöz and Mansur, 2011) that used dermoscopy, emphasis was put on the diagnostic significance of papules showing a curvilinear crust over a yellow background, possibly corresponding to the remnant of a short superficial burrow. Typically, the onset of itching is much more rapid (*eg* hours or few days) than in the case of human

scabies (ie weeks), and a nocturnal exacerbation is often reported (EMDE, 1961; Skerratt and Beveridge, 1999; Menzano *et al.*, 2004; Beck, 2020).

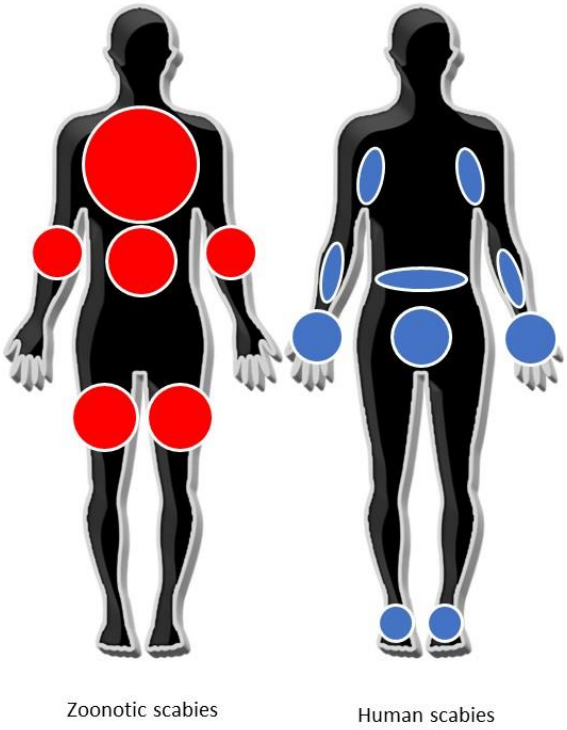


Figure 3. Distribution of typical clinical skin manifestation in zoonotic (in red) and human (in blue) scabies, adapted from Engelman *et al*. (Engelman *et al.*, 2020).

In one of the experimental infections mentioned previously (Delafond, 1862; Estes *et al.*, 1983), the prototypical lesions were erythematous papules and vesicles, and burrows. Pruritus was evident within 24 hours in both volunteers, and histologic biopsy findings were similar to those observed in human scabies.

As it is well known, the search for the parasite on patients may be unsuccessful and the final diagnosis is usually based on clinical signs such as typical pruritic skin lesions (19/47 cases investigated in this review, see Table 1) and/or on the success of clinical response after application of an acaricide treatment (ex-juvantibus). Nonetheless, the presence of burrows with mites and/or eggs has been reported in ZS patients following skin scrapings or dermoscopic examination (Goldman and Feldman, 1949; Smith and Claypoole, 1967; Norins, 1969; Gallegos *et al.*, 2014; Moroni *et al.*, 2021a). Experimentally (Estes *et al.*, 1983), more than half of the adult female mites deposited on the skin of two volunteers managed to survive until the end of the trial (96 hours) and some laid viable eggs.

In two case reports, *S. scabiei* was erroneously diagnosed by the authors as the primary cause of a pruritic rash in a 56-year-old man and in two farmers, respectively. However, pictures in the articles showed a chigger mite (Bandi and Saikumar, 2013) and a *Chorioptes* spp. mite (Ulmer *et al.*, 2007),

suggesting that ZS misdiagnosis may occur even when mites are collected and observed under a microscope, if for some reason physicians are not familiar with zoonotic ectoparasites.

Considering the self-limiting nature of the majority of ZS episodes, more attention should be drawn to prevention and early diagnosis. This is particularly important in vulnerable categories such as the elderly, the young, or immunocompromised patients, in whom early diagnosis would make a difference. Pruritus conditions can be easily misdiagnosed in these categories as psychogenic, degenerative or senile conditions, rather than of parasitic origin. It is therefore essential to include scabies in the differential diagnosis of any persistent non-classic pruritus erythematous rash, especially in settings where the patient may have had contacts with potentially mangy animals.

5. Treatment and control

Because of the origin of ZS and its usually self-limiting nature, resolution of symptoms does not necessarily imply the etiological treatment of the patient, as evidence exists that treating and/or interrupting direct contacts with the animal source(s) are sufficient for the purpose. Nonetheless, some dermatologists testify that patients are rarely willing to wait for the disease to run its course (Burroughs and Elston, 2003).

Regarding the benefits of applying an etiological treatment, Smith and Claypoole (Smith and Claypoole, 1967) reported a shorter resolution time in ZS patients receiving a single 24-hour topical lindane treatment compared with untreated patients, but this was not tested in a randomized trial scheme. A relatively long resolution time (two weeks) was instead observed in an adult patient treated with 10% lindane lotion (Bazargani *et al.*, 2007), and also failure to clear the rash was reported after massive exposure to an infested fox (Rabinowitz and Gordon, 2004). Application of 5% permethrin cream represents another recommended treatment option (Aydingöz and Mansur, 2011; Gallegos *et al.*, 2014; Sleutjens, 2015), but the resolution times are unfortunately not available, with the exception of a single report that mentions clinical resolution in approximately one week after treatment application, which is less than the traditionally reported 2-3 weeks characterizing the spontaneous course of the disease (Menzano *et al.*, 2004). From all the above, it is clear that additional evidence-based information on the efficacy of current etiological treatment protocols is necessary. Symptomatic treatment, involving the administration of sedative antihistamines and/or emollient cream, can help to relieve itching, reduce scratching and preserve sleep time.

The identification and successful etiological treatment of the animal source(s) is crucial not only to establish a correct diagnosis, but also to avoid potentially unsafe over-treatment in patients with recurrent symptoms. Effective treatment protocols against sarcoptic mange are available in several domestic and wildlife species, including the new drug class of isoxazolines (eg. fluranaler, afoxolaner) (Hampel *et al.*, 2018; Chiummo *et al.*, 2020; Wilkinson *et al.*, 2021a), although the treatment may be

challenging in animal patients with generalized crusted mange (Beck, 2020; Deak *et al.*, 2021) and in susceptible free-ranging wildlife (Rowe *et al.*, 2019; Moroni *et al.*, 2020).

6. Knowledge gaps and conclusions

"Pseudoscabies" is a broad definition to describe a "skin eruption caused by mites for which humans are not the normal host" (Cafiero *et al.*, 2008; Rehmus and Prendiville, 2019). As it can be applied to ZS as well as several dermatological conditions that clinically resemble human scabies while having a different etiology (*eg*, those attributable to bird or rodent mites, namely *Dermanyssus gallinae* and *Ornithonyssus sylviarum* (Cafiero *et al.*, 2008; Moroni *et al.*, 2021b), or to environmental and plant mites (*eg*, *Trombicula* spp, *Pyemotes ventricosus* (Rehmus and Prendiville, 2019)), we believe that such definition is ambiguous and adds more confusion to the already complex scenario of scabies sources and epidemiology. We therefore encourage dermatologists and parasitologists to prioritize the term "zoonotic scabies", specifying the animal source of the infection, if available.

Descriptive data on ZS episodes are still limited. The number of events may be greatly underestimated considering that the scientific literature in this review also embraces outdated case reports, anecdotal reports, and grey literature. Moreover, most of the cases were only reported as supplement or added details in the frame of another study, and not as primary description of ZS, which explains why keywords such as "pseudoscabies" or "zoonotic" did not appear in the abstract. This might be also attributed to the fact that ZS originating from numerous sources has already been described, and new reports in the scientific literature would now seem repetitive or obsolete when known animal sources are involved. Yet, ZS remains a neglected zoonosis, with no surveillance nor coordinated reporting system at regional and global-scale, so information on its prevalence and spread can only be extrapolated by the available scientific literature, or by anecdotal reports, with implicit inaccuracy.

With the advent of next-generation sequencing such as whole genome sequencing, new methodologies will be available to explore in detail genomic differences among animal and human *S. scabiei* lineages (Mofiz *et al.*, 2016b). To enhance the new opportunities at the doors, collaboration between scabies researchers all over the world would be essential in order to share samples from different animal species and geographical origins, and to standardize techniques and data interpretation. More first-hand information by entitled dermatologists is also warranted to understand the variability in the morbidity, disease severity and timing of the response to treatment among people infected with different animal-derived strains.

Finally, we believe that key priorities in understanding ZS are represented by an increased surveillance in high-risk occupations and, on occasion of outbreaks, improved communication between animal and human health specialists, in line with the One-Health perspective.

CHAPTER 7

CONTROL STRATEGIES FOR SARCOPTIC MANGE IN IBERIAN IBEX

This chapter has been adapted from the published manuscript:

Moroni B, Granados Torres JE, López-Olvera JR, Espinosa Cerrato J, Ráez Bravo A, Mentaberre G, Fandos P, Pazzi M, Romagnoli M, Gardini G, Rossi L, Valldeperes M, Serrano E, Ramos B and Odore R (2022) Ivermectin Plasma Concentration in Iberian Ibex (Capra pyrenaica) Following Oral Administration: A Pilot Study. Front. Vet. Sci. 9:830157. doi: 10.3389/fvets.2022.830157

1. Introduction

Iberian ibex (*Capra pyrenaica*) is a wild ungulate native to the mountain ranges of the Iberian Peninsula and has recently been reintroduced in the French side of the Pyrenees. This caprine currently enjoys a favorable conservation status ("least concern" according to the IUCN), which allows sustainable hunting as a valuable game species. Besides its biological and ecological value, ibex trophy hunting and the associated related tourism represent a significant source of revenue for remote and disadvantaged rural communities in Spain (Pérez *et al.*, 2002). As for the majority of large herbivores in Europe, the hunting quotas are established by public conservation agencies, based on the estimated population size, although hunting is banned in specific protected areas such as National Parks (Foose and Ballou; Granados *et al.*, 2001).

Since the late eighties of the 20th century, free-ranging Iberian ibex populations suffered from epidemic waves of sarcoptic mange, a contagious skin disease caused by the burrowing mite *Sarcoptes scabiei*. After the epidemics, mange has remained endemic in all the populations affected. Devastating demographic effects have been recorded in naïve herds, including mortality rates over 90% (París, 1991; León-Vizcaíno *et al.*, 1999). Sarcoptic mange is now considered the main driver of short to medium term population declines in this wild ruminant (Espinosa *et al.*, 2020).

Control of sarcoptic mange epizootics in wildlife is challenging. Amongst other measures like non–intervention (*laissez-faire*), massive lethal control, and selective culling of clinically-affected animals (Wobeser, 2002), individual and mass treatments with acaricides have been proposed and empirically implemented at local scale with unknown success (Rowe *et al.*, 2019). However, beyond ethical considerations, treating free-ranging wildlife has disadvantages, such as the impracticability of drug administration on a large population scale, and the potential environmental contamination with drug residues (Artois *et al.*, 2011; Moroni *et al.*, 2020). In Spain, the lack of shared protocols among regions to tackle the population decline caused by sarcoptic mange in exposed wild ruminants has promoted the empirical use of acaricides massively administered through medicated mixture feed (Espinosa *et al.*, 2020). Nevertheless, no studies support the actual effectiveness of this common practice. In fact, essential gaps in knowledge need to be filled before considering oral mass treatments as a possible management control measure to control sarcoptic mange in free-ranging Iberian ibex and other susceptible wild ruminants (Valldeperes *et al.*, 2021; Moroni *et al.*, 2021c). Among the knowledge gaps to fill before implementing any in-field treatment, the pharmacokinetics of orally-administered candidate acaricides in the target hosts are crucial. Pharmacokinetic is the keystone to establish effective drugs, dosages, vehicles, frequency of administration, number of feeding points according to surface area and population, and uptake rates of medicated feed that should be reached in order to

obtain a mass effect limiting the impact of the disease under field conditions. Difficulties in recruiting, maintaining and repeatedly handling Iberian ibexes have, so far, understandably limited the necessary trials.

In captive wild ruminants, the macrocyclic lactone ivermectin (IVM) has been used against *S. scabiei* infection at dose rates of 200-400 µg/kg, and repeated subcutaneous administrations were necessary to eliminate severe clinical signs (Rowe *et al.*, 2019). Oral administration has also been described in wild free-ranging animals (Rajković-Janje *et al.*, 2004; Rowe *et al.*, 2019; Espinosa *et al.*, 2020); nevertheless, the plasma concentration in treated individuals, and the drug plasma therapeutic concentrations against sarcoptic mange were unknown, as well as the proportion of individuals having access to medicated feed. In domestic ruminants, interspecies and intra-species variability in IVM pharmacokinetics has been observed. In domestic goat, a greater plasma clearance of IVM leads to lower plasma concentrations than in cattle and sheep, regardless of the route of drug administration (González Canga *et al.*, 2009). After oral administration, IVM bioavailability is about four times greater and plasma concentrations can be detected longer in sheep than in goats (Cerkvenik *et al.*, 2002). Moreover, IVM pharmacokinetics may depend on sex and age, and the presence of rumen digesta significantly influences the systemic availability of the drug (Steel, 1993).

The main findings related to domestic ruminants and the paucity of data concerning wild species recommend caution when extrapolating treatment protocols (e.g. dose and formulation) from one species to another and, as a consequence, extended pharmacokinetic data in target animal species are much-needed to avoid drug misuse. Therefore, the aim of the present experimental study is to begin filling the existing knowledge gaps by exploring, for the first time, the pharmacokinetic profile of a single oral dose of IVM in Iberian ibexes.

2. Material and Methods

Nine adult healthy Iberian ibexes (seven males and two females, age ranging one to five years) were captured in Sierra Nevada Natural Space as part of the regular management of the species and transferred to the "Iberian ibex stock reservoir El Toril", in Dílar, Granada (37°02'-37°03'N, 3°22'-3°33'W), southern Spain (Espinosa *et al.*, 2017). The ibexes were kept isolated from the rest of the reservoir ibex population during all the study period. Average weight was 19 kg for females and 26 kg for males. The study was approved by the Ethics on Animal Welfare Committee of the University of Jaén and authorized by the Dirección General de Producción Agrícola y Ganadera of the Consejería de Agricultura, Pesca y Medio Ambiente of the Junta de Andalucía (Ref: SA/SIS/MD/ps/October 25, 2012). The Sierra Nevada National and Natural Park Administration also approved this study.

After an acclimation period of at least one month in the experimental facilities, an *ad hoc* formulated bolus containing 0.5 mg of IVM per kg of body weight was orally administered to each ibex. For this, specialized wildlife operators physically immobilized, blindfolded, and administered the boluses to the ibexes using commercial applicators. Blood samples (20 ml) were collected from the jugular vein into heparin-coated vacutainers just prior to drug administration (time 0) and 1 (T1), 2 (T2), 3 (T3), 4 (T4), 7 (T5), 10 (T6), 15 (T7), and 45 (T8) days after the bolus administration (dpa). Plasma was obtained immediately after the blood collection by centrifugation at 2000 g for 20 minutes and stored in 2 ml containers at -80°C until analyzed.

IVM was analyzed in the plasma samples by high performance liquid chromatography (HPLC) with automated solid phase extraction and fluorescence detection following a validated method previously described (Alvinerie *et al.*, 1993). Briefly, 1 ml plasma aliquot was mixed with 5 ng internal standard abamectin and vortexed. The solution was mixed with 1 ml acetonitrile/water solution (4:1) for 30 minutes and then centrifuged at 17000 g at room temperature for 5 minutes. The resultant supernatant was applied at 10 mg/ml to a cartridge (Strata-X 33 µm Polymeric Reversed Phase, Phenomenex, Torrance, CA, US), previously activated with 1 ml methanol and 1 ml of water. Elution was performed with 1.3 ml methanol. The dried residue was dissolved in 100 µl N-methylmidazole solution in acetonitrile (1:2) and derivatized with 150 µl trifluoroacetic anhydride solution in acetonitrile (1:3).

A 100-µl aliquot of the derivatized samples was injected into the chromatograph. The mobile phase consisted of acetic acid 0.2% in water/methanol/acetonitrile (10:40:50) pumped at a flow rate of 1.4 ml/min through a column (Gemini C18, 150 x 4.6 mm, Phenomenex) with fluorescence detection. Fluorescence detection (RF 2000 Fluorescence Detector, Dionex) was performed at 365 nm excitation and 475 nm emission wavelength. Recovery rate was calculated for each sample through internal standard recovery correction.

A Linear Mixed Model (LMM with a normal error distribution and identity link function) was fitted to explore the pharmacokinetics of plasma IVM concentrations (ng/ml, log-transformed) in the study ibexes. Time since drug administration was included as fixed factors whereas ibex identity as a random intercept term in the LMM (19). For the mixed models we used the library lme4 1.1-15 version (Bates *et al.*), whereas the library MuMIn 1.43.6 version (Barton, 2009) was employed to assess the marginal and conditional contribution of the fixed and random terms (Nakagawa and Schielzeth, 2013). All the statistical analysis were performed with R Statistical Software 4.1.0 version (Team and others, 2013).

3. Results

The limit of quantification was 0.2 ng/ml. Although ivermectin was detected in plasma from dpa 1 to dpa 45, mean plasma ivermectin concentration dropped from the initial 3.40 ng/ml in dpa 1 to 0.63 ng/ml in dpa 4 (Figure 1A), and stabilised thereafter around 0.25 ng/ml (min = 0.07, max= 0.56).

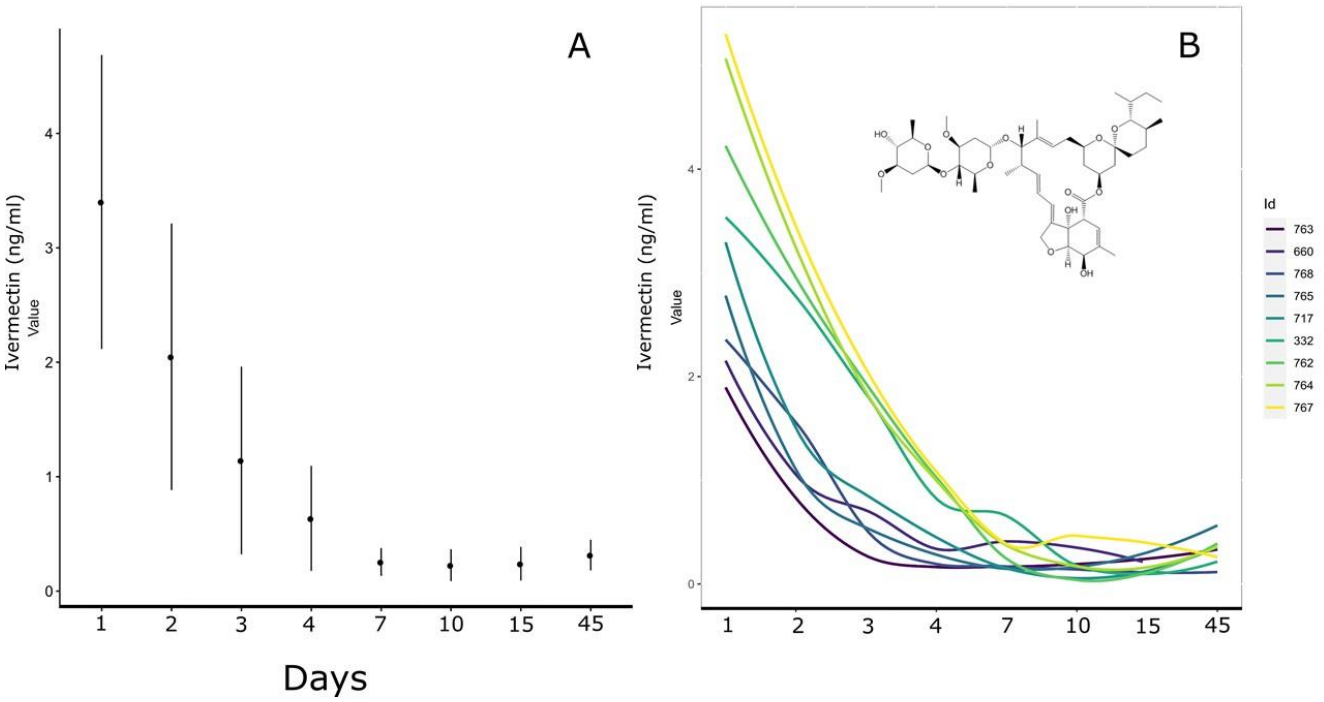


Figure 2. A: Time trend of the mean plasma ivermectin concentration (ng/ml) in the nine Iberian ibexes orally administered with ivermectin from 1 (T1) to 45 (T8) days post-administration; B: differences among individual ibexes in the decrease of plasma ivermectin concentration throughout the study period.

This temporal decrease (β =- 0.4, SE = 0.04, t = -11.11, p-value < 0.01) explained 61% of the plasma ivermectin concentration variability in the ibexes of the study. Moreover, the individual contribution to the observed patterns was 8% (Figure 1B).

The pharmacokinetic values of orally-administered ivermectin in the Iberian ibexes are reported in Table 1. No adverse response was observed throughout the study.

Cmax (ng/ml)	**Tmax (days)**	**AUC (days · ng/ml)**	**Tlast (day)**	**MRT (days)**
3.40 ±1.2	1	21.27	45	30.80

Cmax = maximum concentration; Tmax = time for maximum concentration; AUC = Area Under Curve; Tlast = time of last detection of ivermectin; MRT = mean residence time

Table 1. Ivermectin pharmacokinetic values in plasma of the experimental Iberian ibexes after the oral administration of a 0.5 mg/kg dose.

4. Discussion

To the best of our knowledge, this is the first study investigating the pharmacokinetic profile of orally administered IVM in Iberian ibex and in any wild ruminant species. The controlled conditions of the experiment will allow the use of the results as reference for future experimental scenarios and/or field studies. Sarcoptic mange has been pharmacologically treated in captive wildlife, and some experiences in free-ranging wildlife populations have also been carried out, mostly empirically (Rowe *et al.*, 2019; Rossi *et al.*, 2019b; Moroni *et al.*, 2020).

However, the complete eradication of mange from a population in endemic areas through pharmacological control strategies has been never achieved and so far deemed as unrealistic or inconclusive (Moroni *et al.*, 2020).

Literature is poorly informative on the efficacy of different doses of orally administered macrocyclic lactones for the treatment of sarcoptic mange in wild ruminants (Rowe *et al.*, 2019). Hence, the IVM dose in this study (0.5 mg/kg) must be intended as an empiric trade-off between a limited number of relevant sources. Amongst them, Yeruham and colleagues (Yeruham *et al.*, 1996) successfully treated five ruminant species affected by sarcoptic mange in zoo gardens, including the Nubian ibex (*Capra nubiana*), a close relative of the Iberian ibex. Their protocol provided for the administration of an oral dose of 0.2 mg/kg of IVM for three consecutive days repeated three times at two week intervals. This protocol is clearly unfit for mass use under free-ranging conditions, since regular access of target wildlife to medicated feed is far from being guaranteed; nevertheless, the results are encouraging with respect to possible oral treatment options in *Sarcoptes*-exposed wild ruminants. Moreover, Leon-Vizcaino and colleagues (León-Vizcaíno *et al.*, 2001) showed that 0.4 mg/kg of subcutaneously administered IVM were preferable to 0.2 mg/kg for the treatment of spontaneously infested Iberian ibex, and Foreyt (Foreyt, 1993) reported the successful in-feed use of IVM for 7 consecutive days at the dose of 1 mg/kg for the treatment of Rocky Mountain bighorns (*Ovis canadensis*) experimentally infested with *Psoroptes* mites. The 0.5 mg/kg IVM dose in our study took into account the above findings, the lower antiparasitic efficacy of oral IVM when administered at the same dosage recommended via subcutaneous inoculation (Oksanen *et al.*, 1993) and, prospectively, the individual variability in medicated food intake by free-ranging wild ruminants.

This study revealed that IVM plasma concentrations in orally-treated Iberian ibexes drop drastically within four dpa. Not surprisingly, the oral route leads to lower IVM plasma concentrations compared to those reached after a subcutaneous administration (Oksanen *et al.*, 2014), likely due to the binding to the particulate phase of digesta (Mestorino *et al.*, 2003; González Canga *et al.*, 2009). Moreover, the

binding of IVM to plasma albumin and lipoproteins should be taken into account especially in animals with chronic stages of sarcoptic mange usually showing emaciation and poor body condition, in which a decrease in plasma proteins (thus a higher free fraction of IVM in the plasma), might be more likely associated to an unsuccessful treatment (González Canga *et al.*, 2009).

The maximum drug concentration and bioavailability found in Iberian ibex (3.40 ± ng/ml) were lower than those described in goats (6.03 ±0.95 ng/ml; 15.85 ±5.29 ng/ml, respectively) after the oral administration of an IVM dose of 0.2 mg/kg (Scott *et al.*, 1990; Lespine *et al.*, 2005). By contrast, the time to maximum concentration (1 day) was consistent with previous reports of oral administration in other medium-sized ruminants, sheep (1.7 day), goat (2.8 days), and reindeer (2 days for oral mixture, 1 day for oral paste) (Alvinerie *et al.*, 1993; Cerkvenik *et al.*, 2002; Oksanen *et al.*, 2014). By comparing two different oral formulations in reindeer, Oksanen et al. (Oksanen *et al.*, 2014) found a lower relative plasma availability for a paste than for an oral liquid drench formulation, which could also partially explain the lower values observed in this study. The solid bolus formulation used in this study does not appear in previous similar trials, but we deemed it: i) better representative, than a liquid drench, of the medicated pellet administration that is already empirically used in Iberian ibex (43); ii) better suited to provide an accurate and precise IVM dosing than medicated pellets.

Several factors known to influence drug absorption and bioavailability may explain the interindividual variability in IVM plasma concentrations, observed in particular at T1-T4 (Hoener and Benet, 2002). Nevertheless, as anticipated, the variability could be even greaterunder field conditions (e.g. mass-delivery treatment schemes with delivery of medicated feed), the variability in the IVM plasma concentrations reached should likely be greater due to inter-individual variability in medicated food intake, with unpredictable consequences on IVM efficacy. The antiparasitic action of macrocyclic lactones is related to their plasma concentrations, and more specifically to the bioavailability (C. Campbell, 2012). On the other hand, due to its high lipophilic nature, IVM is widely distributed within the body in all species, with highest concentrations achieved in liver and adipose tissue, where it tends to accumulate (González Canga *et al.*, 2009). This might explain the rapid drop of IVM observed in the plasma of Iberian ibexes in this study, as well as the long MRT (30.8), pointing to a possible quick deposit in other tissues. Interestingly, it has been experimentally observed that IVM is detectable in the skin of scabietic pigs for at least 10 days after a single oral administration of 0.2 mg/kg (Bernigaud *et al.*, 2016).

Therapeutic or effective threshold for IVM plasma concentrations against sarcoptic mange has not been defined in any ruminant model. In cattle, the minimum IVM blood concentration for anthelmintic activity against nematodes ranges from 0.5 to 1 ng/ml (Lifschitz *et al.*, 2000). By contrast, a higher therapeutic threshold level (8 ng/ml) has been suggested after a subcutaneous injection of 0.6 mg/kg long-acting IVM against common bovine ticks (*Boophilus spp*) (Davey *et al.*, 2010), showing that therapeutic blood levels may depend, among others, on the animal species and related metabolism, as well as the target

parasite. Moreover, the therapeutic efficacy and the associated threshold of IVM plasma concentrations may vary depending on the severity of sarcoptic mange, with the most affected individuals being less responsive to the therapeutic effect, thus requiring a higher dosage to eliminate the mite and heal (31), as shown in other animal species (32).

The efficacy of IVM plasma concentrations against *S. scabiei* in Iberian ibex and other wild ungulate species should be further investigated, also considering that underdosing is a recognized driver for the development of resistance to endo and ectoparasiticides (Jokelainen *et al.*, 2019a; b; Rowe *et al.*, 2019; Moroni *et al.*, 2020). At present, resistance of *S. scabiei* against IVM has been described both, *in vitro* and *in vivo,* limited to human model (Currie *et al.*, 2004; Mounsey *et al.*, 2009).In the particular case of sustained oral administration of IVM to free-ranging Iberian ibexes, resistance to the drug could be also developed by generalist nematodes of the digestive tract, eg the blood-feeding *Haemonchus contortus*, that may be cross-transmitted with sympatric sheep and goats (Lavin *et al.*, 1997; Cardoso *et al.*, 2021). This could result in negative herd health management issues at the wildlife/livestock interface (Rossi *et al.*, 2019b).

Some limitations of the present study include the relatively low number of dosed individuals and the absence of time points within the first 24 h post treatment, which would have provided more precise information concerning IVM absorption rate and plasma maximum concentrations.

The pharmacological treatment of sarcoptic mange in wildlife is a complex and controversial management measure, with absence or paucity of protocols regarding dosage, administration times, density of medicated baits per surface or animal population unit, and a lack of knowledge on the pharmacokinetics of macrocyclic lactones in most wildlife species, on the chances for resistance to appear due to underdosage and on the potential environmental consequences of the massive release of antiparasitic drugs in the environment (Iglesias *et al.*, 2006; Römbke *et al.*, 2010; Rowe *et al.*, 2019; Vokřál *et al.*, 2019; Moroni *et al.*, 2020). In Iberian ibex, traditional management has mostly relied in decreasing host density and/or the selective culling of affected individuals (Wobeser, 2002; Valldeperes *et al.*, 2019). However, short-term strategies including the non-selective mass administration of medicated feed to affected ibex populations has also been tried, with little or no effect on the disease prevalence and demographic impact (Sánchez-Isarria *et al.*, 2007; Espinosa *et al.*, 2020; Pérez *et al.*, 2021). The rapid drop of plasma IVM concentration in Iberian ibex after a single oral dose found in this study, and the lack of published results on therapeutic trials with oral IVM in scabietic free-ranging individuals suggest caution in promoting the mass delivery of IVM medicated pellets as a measure to efficiently control sarcoptic mange at the individual and population level in this wild caprine. The epidemiological effects of partially ineffective treatment strategies could even be opposite, by lengthening the infective stage of the individuals affected by sarcoptic mange and therefore increasing the dissemination and spread of the disease (León-Vizcaíno *et al.*, 2001), potentially to other mammal species too (Valldeperes *et al.*, 2021; Moroni *et al.*, 2022). Moreover, aspects such as the proportion of

the target population actually receiving the treatment, the access to the drug by non-target species, and the environmental effects of the massive release of IVM in the environment, are still in need to be thoroughly investigated and controlled (Rowe *et al.*, 2019; Moroni *et al.*, 2020). A major concern is that IVM and its metabolites are mainly eliminated in the feces of treated individuals(Mancini *et al.*, 2020), with potential impact of the residues on the biology and reproduction of dung invertebrate fauna (Iglesias *et al.*, 2006; Mancini *et al.*, 2020).

In vitro and *in vivo* studies carried out with *Sarcoptes* mites var. *suis* showed that moxidectin concentration required to kill 50% of mites was lower than that of IVM, and that a single dose of moxidectin or fluralaner were more effective than two consecutive of IVM, suggesting that IVM should be soon replaced in the treatment approach for sarcoptic mange (Bernigaud *et al.*, 2016, 2018a; Mounsey *et al.*, 2017). New long-acting oral and topic isoxazolines are emerging as more efficient therapeutic options against sarcoptic mange, as recently suggested by recent experimental studies in both domestic animals and captive wildlife (Bernigaud *et al.*, 2018b; Smith *et al.*, 2020; Wilkinson *et al.*, 2021b).

Nonetheless, and similarly to IVM, numerous gaps in knowledge must be addressed before the in-field use of these alternative drugs may be considered a valid option (Moroni *et al.*, 2020). Recently, Mounsey et al. (Mounsey *et al.*, 2022) highlighted the precarious balance between dosage, route of administration, ecotoxicity, drug resistance and efficacy of macrocyclic lactones in the Australian wombats (*Vombatus ursinus* and *Lasiorhinus latifrons*), two highly susceptible wildlife species to sarcoptic mange, suggesting that a better cooperation and a continuous debate between stakeholders, including veterinarians, wildlife carers and researchers, should be encouraged to achieve best treatment options for wildlife.

There is therefore a need to further investigate the pharmacokinetics, pharmacodynamics, population target and environmental consequences of the administration of available macrocyclic lactones, isoxazolines and other candidate molecules, including long-acting formulations, before their in-field delivery become a management option to control sarcoptic mange in free-ranging wild ruminants.

CONCLUSIONS

1. Concluding remarks

The research in this PhD project illustrates how entangled and fascinating are the linkages between human-wildlife-domestic animal scabies in a complex epidemiological scenario in continuous evolution. Overall, the obtained results contribute in establishing the distribution of *S. scabiei* genetic clusters in different host species in Europe, and give some evidences on the low efficacy of the current pharmacological control measures empirically applied in scabies sensitive wild ruminants.

The results of the molecular study in **Chapter 1** supported the hypothesis that livestock likely contributed to the onset of epidemic sarcoptic mange in previously mange-free wild ruminants in Spain. Naïve populations of wild ruminants still exist in the country and they may be exposed to infected domestic ruminants in the future. Therefore, further genetic investigations, mainly targeted on livestock derived mites, are required to better understand the current epidemiological role of domestic ungulates in the spread of sarcoptic mange at the wildlife-livestock interface in the Iberian Peninsula, and optimize the resources for prevention of this deadly disease in valuable naïve game.

In **Chapter 2**, evidence on the cross-transmission of sarcoptic mange between Iberian ibex and wild boar in Spain were provided, suggesting again how hidden and unpredictable may be the spreading dynamics of this disease in wildlife. However, it must be also acknowledged that in the described case, the potentially compromised immune state of the infected wild boar might have influenced the apparent persistence and pathogenicity of *Sarcoptes* mites of ruminant origin in a new unexpected hosts. The immunosuppressive role of bacterial and other infectious agents should not be neglected in ecopathological studies; from this perspective, sarcoptic mange in wildlife seems to represent an interesting and accessible model to refer to.

In Chapter 3, the host range of *S. scabiei* was expanded with a new species, the locally vulnerable Iberian hare, and the origin of infection was molecularly traced back to sympatric wild rabbits, a very popular small game in Spain and the object of diffuse restocking throughout the Country, to meet hunters' demand and compensate for the losses due to endemic Myxomatosis and Rabbit Hemorrhagic Disease under natural conditions. From the conservation perspective, one suggestion deriving from the results of this study, is that control (and possibly eradication) of sarcoptic mange should be prioritized at the wild rabbit farms level. On a more general level, the spreading of scabies in new free-ranging wildlife hosts may be accounted as sentinel event for the possible interaction with domestic species, in which effective control measures of pathogens are easier to implement. An additional point of interest

of this case report is that mites from a single sympatric fox clustered with mites from wild rabbits and the Iberian hare, suggesting a possible prey-to-predator transmission pathway of *S. scabiei*, as already documented in other models involving large carnivores and their main preys (Gakuya et al., 2011; Oleaga et al., 2013).

In **Chapter 4**, molecular analysis of *Sarcoptes* mites in carnivores suggested that different strains of sarcoptic mange may circulate in wild and domestic felines, and that canids are a major source of infection for them, thus rejecting the theory for which only host-specific *Sarcoptes* varieties are responsible for the transmission of the disease under natural conditions. These results strengthen the previously advanced hypothesis that genetic strains of *S. scabiei* have a predominant geographic-related distribution, with somehow "cryptic" transmission patterns relying on the possible direct or indirect interactions between different hosts living in the same shared ecological niche, rather than a simple species-specific infection between hosts belonging to the same taxa.

Taken together, the results of the first studies (**Chapter 1 to 4**) provided further evidence of the usefulness of microsatellite markers applied to the genetic epidemiology of *S. scabiei,* in particular to unveil the origin of scabies outbreaks, as well as the transmission patterns of the disease at the wildlife/domestic animal interface, with potentially important implications for the design of wildlife conservation and management policies wherever applicable. Beyond all doubt, well designed broad-based studies are needed on livestock and companion animals to drive control strategies, thus limiting animal welfare and economy issues, lowering the risk of zoonotic *Sarcoptes* infection in humans, and preventing the undesirable spillover of permissive *S. scabiei* strains towards wildlife.

At the same time, results of this PhD project suggest that the "traditional" classification of *S. scabiei* into host-related variants (*varietates*), still widely reported in books and several papers, is definitely insufficient to represent the complexity that a growing mass of molecular epidemiological studies referred to natural scenarios are revealing.

The role played by different host species in zoonotic scabies has been elucidated in **Chapter 5**, with clear evidence on the more frequent occurrence of transmission by contact with domestic animals, rather than wildlife. Dogs were by far the most common source among pet owners, while diverse livestock and wildlife contributed to the caseload as an occupational disease deserving greater attention in future. Genetic epidemiological studies of zoonotic scabies outbreaks are still limited in number, but molecular tools are available to fill this knowledge gap in the near future. Further research is needed to understand the apparent heterogeneity in the morbidity, disease severity and timing of the response to treatment among people infected with different animal-derived strains.

Finally, in **Chapter 6** the pharmacokinetic profile of orally administered ivermectin in plasma samples of captive Iberian ibexes was examined using high performance liquid chromatography (HPLC) was examined. The obtained results revealed that plasma ivermectin concentration drops drastically within five days of ingestion, questioning the effectiveness of in-feed administration of this drug to control sarcoptic mange in field conditions. While this is the first study on plasma availability of ivermectin in any wild ruminant species, will all related difficulties, the results obtained contribute to a better understanding of the pharmacokinetics and safety of ivermectin in a host which, differently from other Caprines in other parts of Europe and Asia, is frequently subjected to mass treatments with this or related acaricide drugs in the empiric attempts to mitigate the effects of sarcoptic mange at the individual and population level. This study may serve as baseline data in view of monitoring more objectively future in-field trials, and somehow responds to the increasing demand by wildlife specialists and authorities in Spain to improve new mass delivery methods using effective acaricides with sufficient host retention and little environmental impact.

2. Future directions

Within the international frame of this PhD project, a multi-disciplinary approach has been adopted, highlighting the importance of collaboration between institutions and specialists from different countries. Nonetheless, despite the enormous efforts in coordinating and collecting *Sarcoptes* mites from different parts of the Europe, samples from human and domestic animals are still difficult to gather. This is certainly due to the fact that wildlife species are usually sampled by skin samples from fresh carcasses, so usually a big number of mites can be easily collected per animal, thus in terms of time and economic resources it is easier to process material from dead wildlife. On the other hand, *Sarcoptes* mites from pets or human beings are difficult to collect for several reasons: i) it is usual for dermatologists to deal with patients (e.g. dogs or humans) with mild forms and few mites; ii) patients are often treated without direct visualization of mites (detriments, eggs or mite feces are also considered diagnostic); iii) obtaining mites with *in vivo* invasive methods which may cause discomfort and/or pain in patients raise obvious ethical concerns. While these difficulties are somehow insurmountable, the tighter and more frequent cooperation between parasitologists, wildlife specialist and human/animal physicians might simplify the chain of work, possibly leading to further promising results in the near future.

Sarcoptes genetic analysis should be implemented in the future, widening the formerly known models of transmission in wildlife, and revealing possible new epidemiological scenarios in human-derived *Sarcoptes* mites. In particular, next generation sequencing such as the whole genome sequencing and advanced phylogenetic modelling will be soon applied to a variety of host species to improve the robustness and repeatability of *S. scabiei* genetic studies in animals and humans.

Considering the tighter contacts between animals and people, and bearing in mind that scabies is listed among neglected tropical disease by the WHO, zoonotic episodes of scabies might increase in the future, and the establishment of spill-over origin and transmission patterns should be better investigated, especially when dealing with more fragile categories such as elderlies and immunocompromised people.

The pharmacological control of scabies in wildlife is controversial and still debated in most parts of the world. The results of this thesis advocate for careful consideration on the potential limitations of it, especially in free-ranging wildlife, taking into account several factors including feasibility and efficacy, ecological impact, drug resistance, drug residues in meat (for animal and human consumption) and economics, among others. Balancing the relative merits of traditional ecological population-based management approaches to handle scabies outbreaks independent of drug-based treatments may be warranted in many free-ranging wildlife contexts. Similarly, a pragmatic assessment of whether the pharmacological control can be achieved, and such intervention therefore justified, should be at least considered on the animal welfare ground.

Bibliography

Acevedo, P. and Cassinello, J. (2009). Biology, ecology and status of Iberian ibex Capra pyrenaica: A critical review and research prospectus. *Mammal Review* **39**, 17–32. doi: 10.1111/j.1365-2907.2008.00138.x.

Águeda-Pinto, A., de Matos, A. L., Abrantes, M., Kraberger, S., Risalde, M. A., Gortázar, C., McFadden, G., Varsani, A. and Esteves, P. J. (2019). Genetic characterization of a recombinant myxoma virus leap into the Iberian hare (Lepus granatensis). *bioRxiv* 1–16. doi: 10.1101/624338.

Alasaad, S., Soglia, D., Sarasa, M., Soriguer, R. C., Granados, J. E., Rasero, R., Zhu, X. Q. and Rossi, L. (2007). Skin-scale genetic structure of Sarcoptes scabiei populations from individual hosts : empirical evidence from Iberian ibex-derived mites.

Alasaad, S., Rossi, L., Maione, S., Sartore, S., Soriguer, R. C., Pérez, J. M., Rasero, R., Zhu, X. Q. and Soglia, D. (2008). HotSHOT Plus ThermalSHOCK, a new and efficient technique for preparation of PCR-quality mite genomic DNA. *Parasitology Research* **103**, 1455–1457. doi: 10.1007/s00436-008-1127-9.

Alasaad, S., Soglia, D., Spalenza, V., Maione, S., Soriguer, R. C., Pérez, J. M., Rasero, R., Degiorgis, M. P. R., Nimmervoll, H., Zhu, X. Q. and Rossi, L. (2009a). Is ITS-2 rDNA suitable marker for genetic characterization of Sarcoptes mites from different wild animals in different geographic areas? *Veterinary Parasitology* **159**, 181–185. doi: 10.1016/j.vetpar.2008.10.001.

Alasaad, S., Soglia, D., Maione, S., Sartore, S., Soriguer, R. C., Pérez, J. M., Rasero, R. and Rossi, L. (2009b). Effectiveness of the postponed isolation (post-frozen isolation) method for PCR-quality Sarcoptes mite gDNA. *Experimental and Applied Acarology* **47**, 173–178. doi: 10.1007/s10493-008-9196-0.

Alasaad, S., Oleaga, Á., Casais, R., Rossi, L., Min, A., Soriguer, R. C. and Gortázar, C. (2011). Temporal stability in the genetic structure of Sarcoptes scabiei under the host-taxon law: Empirical evidences from wildlife-derived Sarcoptes mite in Asturias, Spain. *Parasites and Vectors* **4**, 1–7. doi: 10.1186/1756-3305-4-151.

Alasaad, S., Schuster, R. K., Gakuya, F., Theneyan, M., Jowers, M. J., Maione, S., Min, A. M., Soriguer, R. C. and Rossi, L. (2012a). Applicability of molecular markers to determine parasitic infection origins in the animal trade: A case study from Sarcoptes mites in wildebeest. *Forensic Science, Medicine, and Pathology* **8**, 280–284. doi: 10.1007/s12024-011-9268-z.

Alasaad, S., Fickel, J., Rossi, L., Sarasa, M., Benítez-Camacho, B., Granados, J. E. and Soriguer, R. C. (2012b). Applicability of major histocompatibility complex DRB1 alleles as markers to detect vertebrate hybridization: A case study from Iberian ibex × domestic goat in southern Spain. *Acta Veterinaria Scandinavica* **54**, 1–6. doi: 10.1186/1751-0147-54-56.

Alasaad, S., Granados, J. E., Fandos, P. and Soriguer, R. C. (2013a). The use of radio-collars for monitoring wildlife diseases : a case study from Iberian ibex affected by Sarcoptes scabiei in Sierra Nevada , Spain. 2–6. doi: 10.1186/1756-3305-6-242.

Alasaad, S., Rossi, L., Heukelbach, J., Pérez, J. M., Hamarsheh, O., Otiende, M. and Zhu, X. Q. (2013b). The neglected navigating web of the incomprehensibly emerging and re-emerging Sarcoptes mite. *Infection, Genetics and Evolution* **17**, 253–259. doi: 10.1016/j.meegid.2013.04.018.

Alonso De Vega, F., Mendez De Vigo, J., Ortiz Sanchez, J., Martinez-Carrasco Pleite, C., Albaladejo Serrano, A. and Ruiz De Ybañez Carnero, M. R. (1998). Evaluation of the prevalence of sarcoptic mange in slaughtered fattening pigs in southeastern Spain. *Veterinary Parasitology* **76**, 203–209. doi: 10.1016/S0304-4017(97)00212-4.

Alves, P. C. and Hackländer, K. (2008). Lagomorph species: geographical distribution and conservation status. *Lagomorph biology: evolution, ecology, and conservation. Berlin (Germany): Springer-Verlag* 395–405.

Alvinerie, M., Sutra, J., Galtier, P., Alvinerie, M., Sutra, J. and Ivermectin, P. G. (1993). Ivermectin in goat plasma and milk after subcutaneous injection To cite this version : HAL Id : hal-00902156 Ivermectin in goat plasma and milk.

Amer, S., El Wahab, T. A., El Naby Metwaly, A., Ye, J., Roellig, D., Feng, Y. and Xiao, L. (2014). Preliminary molecular characterizations of sarcoptes scaibiei (Acari: Sarcoptidae) from farm animals in Egypt. *PLoS ONE* **9**, 1–6. doi: 10.1371/journal.pone.0094705.

Andriantsoanirina, V., Izri, A., Botterel, F., Foulet, F., Chosidow, O. and Durand, R. (2014). Molecular survey of knockdown resistance to pyrethroids in human scabies mites. *Clinical Microbiology and Infection* **20**, 2013–2015. doi: 10.1111/1469-0691.12334.

Andriantsoanirina, V., Ariey, F., Izri, A., Bernigaud, C., Fang, F., Charrel, R., Foulet, F., Botterel, F., Guillot, J., Chosidow, O. and Durand, R. (2015a). Sarcoptes scabiei mites in humans are distributed into three genetically distinct clades. *Clinical Microbiology and Infection* **21**, 1107–1114. doi: 10.1016/j.cmi.2015.08.002.

Andriantsoanirina, V., Ariey, F., Izri, A., Bernigaud, C., Fang, F., Charrel, R., Foulet, F., Botterel, F. and Guillot, J. (2015b). Sarcoptes scabiei mites in humans are distributed into three genetically distinct clades. *Clinical Microbiology and Infection* **21**, 1107–1114. doi: 10.1016/j.cmi.2015.08.002.

Araujo, S. B. L., Braga, M. P., Brooks, D. R., Agosta, S. J., Hoberg, E. P., Von Hartenthal, F. W. and Boeger, W. A. (2015). Undestanding host-switching by ecological fitting. *PLoS ONE* **10**, 1–17. doi: 10.1371/journal.pone.0139225.

Arends, J. J., Stanislaw, C. M. and Gerdon, D. (1990). Effects of sarcoptic mange on lactating swine and growing pigs. *Journal of animal science* **68**, 1495–1499. doi: 10.2527/1990.6861495x.

Arlian, L. G. and Morgan, M. S. (2017). A review of Sarcoptes scabiei : past , present and future. 1–22. doi: 10.1186/s13071-017-2234-1.

Arlian, L. G., Runyan, R. A., Achar, S. and Estes, S. A. (1984). Survival and infestivity of Sarcoptes scabiei var. canis and var. hominis. *Journal of the American Academy of Dermatology* **11**, 210–215. doi: 10.1016/S0190-9622(84)70151-4.

Arlian, L. G., Vyszenski-Moher, D. L. and Cordova, D. (1988). Host specificity of S. scabiei var. canis (Acari: Sarcoptidae) and the role of host odor. *Journal of medical entomology* **25**, 52–56. doi: 10.1093/jmedent/25.1.52.

Artois, M., Blancou, J., Dupeyroux, O. and Gilot-Fromont, E. (2011). Sustainable control of zoonotic pathogens in wildlife: How to be fair to wild animals? *OIE Revue Scientifique et Technique* **30**, 733–743. doi: 10.20506/rst.30.3.2069.

Asbakk, K., Hrabok, J. T., Oksanen, A., Nieminen, M. and Waller, P. J. (2006). Prolonged persistence of fecally excreted ivermectin from reindeer in a sub-arctic environment. *Journal of agricultural and food chemistry* **54**, 9112—9118. doi: 10.1021/jf061633l.

Astorga, F., Carver, S., Almberg, E. S., Sousa, G. R., Wingfield, K., Niedringhaus, K. D., Wick, P. Van, Rossi, L., Xie, Y., Cross, P., Angelone, S., Gortázar, C. and Escobar, L. E. (2018). International meeting on sarcoptic mange in wildlife , June 2018 , Blacksburg , Virginia , USA. 1–10.

Aydingöz, I. E. and Mansur, A. T. (2011). Canine scabies in humans: A case report and review of the literature. *Dermatology* **223**, 104–106. doi: 10.1159/000327378.

Bandi, K. M. and Saikumar, C. (2013). Sarcoptic mange: A zoonotic ectoparasitic skin disease. *Journal of Clinical and Diagnostic Research* **7**, 156–157. doi: 10.7860/JCDR/2012/4839.2694.

Barton, K. (2009). MuMIn multi-model inference, R package version 0.12. 0. *httpr-forge. r-project. orgprojectsmumin*.

Bates, D., Mächler, M., Bolker, B. M. and Walker, S. C. Fitting linear mixed-effects models using lme4.

Bazargani, T. T., Hallan, J. A., Nabian, S. and Rahbari, S. (2007). Sarcoptic mange of gazelle (Gazella subguttarosa) and its medical importance in Iran. *Parasitology Research* **101**, 1517–1520. doi: 10.1007/s00436-007-0669-6.

Beck, A. L. (1965). Animal Scabies Affecting Man. *Archives of Dermatology* **91**, 54–55. doi: 10.1001/archderm.1965.01600070060007.

Beck, W. (2020). Treatment of sarcoptic mange in llamas (Lama glama) and alpacas (Vicugna pacos) with repeated subcutaneous moxidectin injections. *Veterinary Parasitology* **283**, 109190. doi: 10.1016/j.vetpar.2020.109190.

Bernigaud, C., Fang, F., Fischer, K., Lespine, A., Aho, S., Dreau, D., Kelly, A., Moreau, F., Lilin, T. and Guillot, J. (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. 1–18. doi: 10.1371/journal.pntd.0005030.

Bernigaud, C., Fang, F., Fischer, K., Lespine, A., Aho, L. S., Mullins, A. J., Tecle, B., Kelly, A., Sutra, J. F., Moreau, F., Lilin, T., Beugnet, F., Botterel, F., Chosidow, O. and Guillot, J. (2018a). Efficacy and pharmacokinetics evaluation of a single oral dose of afoxolaner against sarcoptes scabiei in the porcine scabies model for human infestation. *Antimicrobial Agents and Chemotherapy* **62**, 1–12. doi: 10.1128/AAC.02334-17.

Bernigaud, C., Fang, F., Fischer, K., Lespine, A., Aho, L. S., Mullins, A. J., Tecle, B., Kelly, A., Moreau, F., Lilin, T., Chosidow, O. and Guillot, J. (2018b). crossm Efficacy and Pharmacokinetics Evaluation of a Single Oral Dose of Afoxolaner against Sarcoptes scabiei in the Porcine Scabies Model for Human Infestation. 1–12.

Berrilli, F., D'Amelio, S. and Rossi, L. (2002). Ribosomal and mitochondrial DNA sequence variation in Sarcoptes mites from different hosts and geographical regions. *Parasitology Research* **88**, 772–777. doi: 10.1007/s00436-002-0655-y.

Birk, R. W., Tebbe, B., Schein, E., Zouboulis, C. C. and Orfanos, C. E. (1999). Pseudoskabies durch rotfuchs ubertragen. *Hautarzt* **50**, 127–130. doi: 10.1007/s001050050876.

Black, W. C. and Piesman, J. (1994). Phylogeny of hard-and soft-tick taxa (Acari: Ixodida) based on mitochondrial 16S rDNA sequences. *Proceedings of the National Academy of Sciences* **91**, 10034–10038.

Boch, J. and Schneidawind, H. (1988). *Krankheiten des jagdbaren Wildes: mit 19 Tabellen*. Parey.

Bornstein, S., Gidlund, K., Karlstam, E., Bergstrom, K., Zakrisson, G., Nikkila, T., Bergvall, K., Renstrom, L. and Mattsson, J. G. (2004). Sarcoptic mange epidemic in a cat population. *Veterinary Dermatology* **15**, 34.

Burgess, I. (1993). Sarcoptes scabiei and scabies. *Advances in parasitology* **33**, 235.

Burroughs, R. F. and Elston, D. M. (2003). What's Eating You? Canine Scabies. *Cutis* **72**, 21–23.

C. Campbell, W. (2012). History of Avermectin and Ivermectin, with Notes on the History of Other Macrocyclic Lactone Antiparasitic Agents. *Current Pharmaceutical Biotechnology* **13**, 853–865. doi: 10.2174/138920112800399095.

Cafiero, M. A., Camarda, A., Circella, E., Santagata, G., Schino, G. and Lomuto, M. (2008). Pseudoscabies caused by Dermanyssus gallinae in Italian city dwellers: a new setting for an old dermatitis. *Journal of the European Academy of Dermatology and Venereology* **22**, 1380–1382. doi: 10.1111/j.1468-3083.2008.02644.x.

Campbell, W. C. (1989). *Ivermectin and abamectin*, 1st Edn. ed. Campbell, W. C. Springer-Verlag, New Yotk.

Cardells, J., Lizana, V., Martí-Marco, A., Lavín, S., Velarde, R., Rossi, L. and Moroni, B. (2021). First description of sarcoptic mange in an Iberian hare (Lepus granatensis). *Current Research in*

Parasitology & Vector-Borne Diseases **1**, 100021. doi: 10.1016/j.crpvbd.2021.100021.

Cardoso, B., Pessoa, B., Figueiredo, P., Rinaldi, L., Cringoli, G., D\'\iaz, A., Gomes, L., Santos, N. and de Carvalho, L. M. (2021). Comparative survey of gastrointestinal parasites in sympatric Iberian ibex (Capra pyrenaica) and domestic goats using molecular host specific identification. *Parasitology Research* **120**, 2291–2296.

Castillo-Contreras, R., Carvalho, J., Serrano, E., Mentaberre, G., Fernández-Aguilar, X., Colom, A., González-Crespo, C., Lavín, S. and López-Olvera, J. R. (2018). Urban wild boars prefer fragmented areas with food resources near natural corridors. *Science of the Total Environment* **615**, 282–288. doi: 10.1016/j.scitotenv.2017.09.277.

Castro, I., Espinosa, J., Granados, J. E., Cano-Manuel, F. J., Fandos, P., Ráez-Bravo, A., López-Olvera, J. R., Soriguer, R. C. and Pérez, J. M. (2018). Characterizing the growth of Sarcoptes scabiei infrapopulations. *Experimental and Applied Acarology* **76**, 41–52. doi: 10.1007/s10493-018-0287-2.

Cerkvenik, V., Grabnar, I., Skubic, V., Doganoc, D. Z., Beek, W. M. J., Keukens, H. J., Drobniˇ, M. and Pogaˇ, M. (2002). Ivermectin pharmacokinetics in lactating sheep. **104**, 175–185.

Chakrabarti, A. (1990). Pig Handler's Itch. **29**, 205–207.

Chakrabarti, A., Chatterjee, A., Chakrabarti, K. and Sengupta, D. N. (1981a). Human scabies from contact with water buffaloes infested with Sarcoptes scabiei var. bubalis. *Annals of Tropical Medicine |& Parasitology* **75**, 353–357.

Chakrabarti, A., Dutta, A. K., Mandal, S. B., Chakraborty, A. K., Sengupta, D. N. and others (1981b). Transmission of scabies from goats to human subjects. *Indian journal of dermatology* **26**, 7.

Chakravorty, M. A. N., Ghosh, S. and Banerjee, A. K. (1953). Case notes of scabies in a family transmitted from goats. *The Indian medical gazette* **88**, 153.

Charlesworth, E. N. and Johnson, J. L. (1974). An Epidemic of Canine Scabies in Man. *Archives of Dermatology* **110**, 572–574. doi: 10.1001/archderm.1974.01630100032008.

Chee, J., Kwon, J., Cho, H., Cho, K., Lee, Y. and El-aty, A. M. A. B. D. (2008). A Survey of Ectoparasite Infestations in Stray Dogs of Gwang-ju City , Republic of Korea. **46**, 23–27. doi: 10.3347/kjp.2008.46.1.23.

Chiummo, R., Petersen, I., Plehn, C., Zschiesche, E., Roepke, R. and Thomas, E. (2020). Efficacy of orally and topically administered fluralaner (Bravecto®) for treatment of client-owned dogs with sarcoptic mange under field conditions. *Parasites and Vectors* **13**, 1–7. doi: 10.1186/s13071-020-04395-6.

Choe, S., Kim, S., Na, K. J., Nath, T. C., Ndosi, B. A., Kang, Y., Bia, M. M., Lee, D., Park, H., Eamudomkarn, C., Jeon, H. K. and Eom, K. S. (2020). First infestation case of sarcoptic mange

from a pet rabbit oryctolagus cuniculus in republic of korea. *Korean Journal of Parasitology* **58**, 315–319. doi: 10.3347/kjp.2020.58.3.315.

Chosidow, O. (2006). Clinical practices. *Scabies N Engl J Med* **354**, 1718–1727.

Chun, B. M., Park, J. H., Her, Y., Kim, C. W. and Kim, S. S. (2009). A case of human infestation of canine scabies. *Korean Journal of Dermatology* **47**, 104–107.

Cook, E. F. (1954). A modification of Hopkin's technique for collecting ectoparasites from mammalian skins. *Entomol. News* **65**, 35–37.

Crampton, A., McKay, I. and Barker, S. C. (1996). Phylogeny of ticks (Ixodida) inferred from nuclear ribosomal DNA. *International journal for parasitology* **26**, 511–517.

Currie, B. J., Harumal, P., McKinnon, M. and Walton, S. F. (2004). First documentation of in vivo and in vitro ivermectin resistance in Sarcoptes scabiei. *Clinical Infectious Diseases* **39**, e8–e12. doi: 10.1086/421776.

Dalton, K. P., Martín, J. M., Nicieza, I., Podadera, A., de Llano, D., Casais, R., Gimenez, S., Badiola, I., Agüero, M., Duran, M., Buitrago, D., Romero, L. J., García, E. and Parra, F. (2019). Myxoma virus jumps species to the Iberian hare. *Transboundary and Emerging Diseases* **66**, 2218–2226. doi: 10.1111/tbed.13296.

Davey, R. B., Pound, J. M., Miller, J. A. and Klavons, J. A. (2010). Therapeutic and persistent efficacy of a long-acting (LA) formulation of ivermectin against Rhipicephalus (Boophilus) microplus (Acari: Ixodidae) and sera concentration through time in treated cattle. *Veterinary Parasitology* **169**, 149–156. doi: 10.1016/j.vetpar.2009.12.040.

Davies, P. R. (1995). Sarcoptic mange and production performance of swine: A review of the literature and studies of associations between mite infestation, growth rate and measures of mange severity in growing pigs. *Veterinary Parasitology* **60**, 249–264. doi: 10.1016/0304-4017(95)00795-3.

Davis, D. P. and Moon, R. D. (1990). Dynamics of swine mange: a critical review of the literature. *Journal of medical entomology* **27**, 727–737. doi: 10.1093/jmedent/27.5.727.

De Pennington, N. and Colles, K. M. (2011). Sarcoptes scabiei infestation of a donkey in the UK. *Equine Veterinary Education* **23**, 19–23. doi: 10.1111/j.2042-3292.2010.00114.x.

Deak, G., Moroni, B., Boncea, A. M., Rambozzi, L., Rossi, L. and Mihalca, A. D. (2021). Case Report: Successful Treatment of Sarcoptic Mange in European Camelids. *Frontiers in Veterinary Science* **8**, 6–11. doi: 10.3389/fvets.2021.742543.

del Valle Ferreyra, H., Rudd, J., Foley, J., Vanstreels, R. E. T., Martín, A. M., Donadio, E. and Uhart, M. M. (2022). Sarcoptic mange outbreak decimates South American wild camelid populations in San Guillermo National Park, Argentina. *PLoS ONE* **17**, 1–23. doi: 10.1371/journal.pone.0256616.

Delafond, H. M. O. (1862). *Traité pratique d'entomologie et de pathologie comparées de la psore: ou, Gale de l'homme et des animaux domestiques*. Impr. impériale.

Dieringer, D. and Schlötterer, C. (2003). Microsatellite analyser (MSA): a platform independent analysis tool for large microsatellite data sets. *Molecular ecology notes* **3**, 167–169.

Duan, H. F., Wang, L. and Wang, J. (2000). A investigation report of an outbreak of human scabies caused by Sarcoptes scabies from rabbit factory in Harbin. *Harbin Med J* **20**, 36–37.

Duarte Duarte, J. (2000). Liebre Ibérica (Lepus granatensis Rosenhauer, 1856). *Galemys: Boletín informativo de la Sociedad Española para la conservación y estudio de los mamíferos* **12**, 3–14.

Earl, D. A. and vonHoldt, B. M. (2012). STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* **4**, 359–361. doi: 10.1007/s12686-011-9548-7.

EMDE, R. N. (1961). Sarcoptic Mange in the Human: A Report of an Epidemic of 10 Cases of Infection by Sarcoptes Scabiei, Variety Canis. *Archives of dermatology* **84**, 633–636.

Engelman, D., Cantey, P. T., Marks, M., Solomon, A. W., Chang, A. Y., Chosidow, O., Enbiale, W., Engels, D., Hay, R. J., Hendrickx, D., Hotez, P. J., Kaldor, J. M., Kama, M., Mackenzie, C. D., McCarthy, J. S., Martin, D. L., Mengistu, B., Maurer, T., Negussu, N., Romani, L., Sokana, O., Whitfeld, M. J., Fuller, L. C. and Steer, A. C. (2019). The public health control of scabies: priorities for research and action. *The Lancet* **394**, 81–92. doi: 10.1016/S0140-6736(19)31136-5.

Engelman, D., Yoshizumi, J., Hay, R. J., Osti, M., Micali, G., Norton, S., Walton, S., Boralevi, F., Bernigaud, C., Bowen, A. C., Chang, A. Y., Chosidow, O., Estrada-Chavez, G., Feldmeier, H., Ishii, N., Lacarrubba, F., Mahé, A., Maurer, T., Mahdi, M. M. A., Murdoch, M. E., Pariser, D., Nair, P. A., Rehmus, W., Romani, L., Tilakaratne, D., Tuicakau, M., Walker, S. L., Wanat, K. A., Whitfeld, M. J., Yotsu, R. R., Steer, A. C. and Fuller, L. C. (2020). The 2020 International Alliance for the Control of Scabies Consensus Criteria for the Diagnosis of Scabies. *British Journal of Dermatology* **183**, 808–820. doi: 10.1111/bjd.18943.

Escobar, L. E., Carver, S., Cross, P. C., Rossi, L., Almberg, E. S., Yabsley, M. J., Niedringhaus, K. D., Van Wick, P., Dominguez-Villegas, E., Gakuya, F., Xie, Y., Angelone, S., Gortázar, C. and Astorga, F. (2021). Sarcoptic mange: An emerging panzootic in wildlife. *Transboundary and Emerging Diseases* 1–16. doi: 10.1111/tbed.14082.

Espinosa, J., López-olvera, J. R., Cano-manuel, F. J., Fandos, P., Pérez, J. M., López-graells, C., Ráez-bravo, A., Mentaberre, G., Romero, D., Soriguer, R. C. and Granados, J. E. (2017). Guidelines for managing captive Iberian ibex herds for conservation purposes. *Journal for Nature Conservation* **40**, 24–32. doi: 10.1016/j.jnc.2017.09.002.

Espinosa, J., Pérez, J. M., Raéz–bravo, A., Fandos, P., Cano–manuel, F. J., Soriguer, R. C., López–olvera, J. R. and Granados, J. E. (2020). Recommendations for the management of sarcoptic mange in free–ranging iberian ibex populations. *Animal Biodiversity and Conservation* **43**, 137–149. doi: 10.32800/abc.2020.43.0137.

Essig, A., Rinder, H., Gothe, R. and Zahler, M. (1999). Genetic differentiation of mites of the genus Chorioptes (Acari: Psoroptidae). *Experimental |& applied acarology* **23**, 309–318.

Estes, S. A., Kummel, B. and Arlian, L. (1983). Experimental canine scabies in humans. *Journal of the American Academy of Dermatology* **9**, 397–401. doi: 10.1016/S0190-9622(83)70148-9.

Fain, A. (1968). Etude de la variabilite de Sarcoptes scabiei avec une revision des Sarcoptidae. *Acta zool pathol Antverp* **47**, 1–196.

Fain, A. (1971). EPIDEMIOLOGICAL PROBLEMS OF SCABIES Caused by Mites.

Fain, A. (1978). Epidemiological problems of scabies. *Int J Dermatol* **17**, 20–30.

Fernández-Morán, J., Gómez, S., Ballesteros, F., Quirós, P. Q., Benito, J. L., Feliu, C. and Nieto, J. M. (1997). Epizootiology of sarcoptic mange in a population of cantabrian chamois (Rupicapra pyrenaica parva) in Northwestern Spain. *Veterinary Parasitology* **73**, 163–171. doi: 10.1016/S0304-4017(97)00061-7.

Foose, T. J. and Ballou, J. D. Population management : theory and practice Management of small populations.

Foreyt, W. J. (1993). Efficacy of in-feed formulation ivermectin against Psoroptes sp. in bighorn sheep. *Journal of Wildlife Diseases* **29**, 85–89.

Fraser, T. A., Charleston, M., Martin, A., Polkinghorne, A. and Carver, S. (2016). The emergence of sarcoptic mange in Australian wildlife : an unresolved debate. *Parasites & Vectors* 1–11. doi: 10.1186/s13071-016-1578-2.

Fraser, T. A., Shao, R., Fountain-Jones, N. M., Charleston, M., Martin, A., Whiteley, P., Holme, R., Carver, S. and Polkinghorne, A. (2017). Mitochondrial genome sequencing reveals potential origins of the scabies mite Sarcoptes scabiei infesting two iconic Australian marsupials. *BMC Evolutionary Biology* **17**,. doi: 10.1186/s12862-017-1086-9.

Fraser, T. A., Holme, R., Martin, A., Whiteley, P., Montarello, M., Raw, C., Carver, S. and 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**, 231–237.

Gakuya, F., Rossi, L., Ombui, J., Maingi, N., Muchemi, G., Ogara, W., Soriguer, R. C. and Alasaad, S. (2011). The curse of the prey: Sarcoptes mite molecular analysis reveals potential prey-to-predator parasitic infestation in wild animals from Masai Mara, Kenya. *Parasites and Vectors* **4**, 1–7. doi: 10.1186/1756-3305-4-193.

Gallegos, J. L., Budnik, I., Peña, A., Canales, M., Concha, M. and López, J. (2014). Sarna sarcóptica: Comunicación de un brote en un grupo familiar y su mascota. *Revista Chilena de Infectologia* **31**, 47–52. doi: 10.4067/S0716-10182014000100007.

García-Bocanegra, I., Camacho-Sillero, L., Risalde, M. A., Dalton, K. P., Caballero-Gómez, J., Agüero, M., Zorrilla, I. and Gómez-Guillamón, F. (2019). First outbreak of myxomatosis

in Iberian hares (Lepus granatensis). *Transboundary and Emerging Diseases* **66**, 2204–2208. doi: 10.1111/tbed.13289.

Gavier-Widén, D. and Mörner, T. (1991). Epidemiology and diagnosis of the European brown hare syndrome in Scandinavian countries: a review. *Revue scientifique et technique (International Office of Epizootics)* **10**, 453–458. doi: 10.20506/rst.10.2.555.

Goldman, L. and Feldman, M. D. (1949). Human infestation with scabies of monkeys. *Archives of Dermatology and Syphilology* **59**, 175–178. doi: 10.1001/archderm.1949.01520270051005.

González-Candela, M., Leónzcaíno, L. and Cubero-Pablo, M. J. (2004). Population effects of sarcoptic mange in Barbary sheep (Ammotragus lervia) from Sierra Espuña Regional Park, Spain. *Journal of Wildlife Diseases* **40**, 456–465. doi: 10.7589/0090-3558-40.3.456.

González-Quirós, P., Manzano, P. S. and Rodr\'\iguez, S. S. (2002). Population evolution of Cantabrian chamois (Rupicapra pyrenaica parva) with sarcoptic mange (Sarcoptes scabiei) in centre-eastern Asturias (Northwest Spain). *Pirineos* **157**, 201–210.

González Canga, A., Sahagún Prieto, A. M., José Diez Liébana, M., Martínez, N. F., Vega, M. S. and Vieitez, J. J. G. (2009). The pharmacokinetics and metabolism of ivermectin in domestic animal species. *The Veterinary Journal* **179**, 25–37. doi: 10.1016/j.tvjl.2007.07.011.

Graczyk, T. K., Mudakikwa, A. B., Cranfield, M. R. and Eilenberger, U. (2001). Hyperkeratotic mange caused by Sarcoptes scabiei (Acariformes: Sarcoptidae) in juvenile human-habituated mountain gorillas (Gorilla gorilla beringei). *Parasitology Research* **87**, 1024–1028. doi: 10.1007/s004360100489.

Grahofer, A., Bannoehr, J., Nathues, H. and Roosje, P. (2018). Sarcoptes infestation in two miniature pigs with zoonotic transmission - a case report. *BMC Veterinary Research* **14**, 1–5. doi: 10.1186/s12917-018-1420-5.

Granados, J. E., Pérez, J. M., Marquez, F. J., Serrano, E., Soriguer, R. C. and Fandos, Y. P. (2001). La cabra montés (Capra pyrenaica, SCHINZ 1838). 3–37.

Gutiérrez, J. F., De Vigo, J. M., Castellá, J., Muñoz, E. and Ferrer, D. (1996). Prevalence of sarcoptic mange in fattening pigs sacrifice in a slaughterhouse of northeastern Spain. *Veterinary Parasitology* **61**, 145–149. doi: 10.1016/0304-4017(95)00814-4.

Haas, C., Origgi, F. C., Rossi, S., López-Olvera, J. R., Rossi, L., Castillo-Contreras, R., Malmsten, A., Dalin, A. M., Orusa, R., Robetto, S., Pignata, L., Lavín, S. and Ryser-Degiorgis, M.-P. (2018). Serological survey in wild boar (Sus scrofa) in Switzerland and other European countries: Sarcoptes scabiei may be more widely distributed than previously thought. *BMC Veterinary Research* **14**, 1–10. doi: 10.1186/s12917-018-1430-3.

Hameed, K., Angelone-alasaad, S., Din, J. U., Nawaz, M. A. and Rossi, L. (2016). The threatening but unpredictable Sarcoptes scabiei : first deadly outbreak in the Himalayan lynx , Lynx lynx isabellinus , from Pakistan. *Parasites & Vectors* 1–3. doi: 10.1186/s13071-016-1685-0.

Hampel, V., Knaus, M., Schäfer, J., Beugnet, F. and 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**, 1–9. doi: 10.1051/parasite/2018064.

Hardy, J. I., Fox, M. T., Loeffler, A. and Sinclair, G. (2013). Feline sarcoptic mange in the UK. *Veterinary Record Case Reports* **1**,. doi: 10.1136/vr.101001.

Hengge, U. R., Currie, B. J., Jäger, G., Lupi, O. and Schwartz, R. A. (2006). Scabies : a ubiquitous neglected skin disease.

Hewitt, M., Walton, G. S. and Waterhouse, M. (1971). Pet animal infestations and human skin lesions. *British journal of Dermatology* **85**, 215–225.

Hoener, B. and Benet, L. Z. (2002). Factors influencing drug absorption and drug availability. In *Modern Pharmaceutics*, pp. 166–207. CRC Press.

Huang, H. P. and Lien, Y. H. (2013). Feline sarcoptic mange in Taiwan: A case series of five cats. *Veterinary Dermatology* **24**,. doi: 10.1111/vde.12049.

Iacopelli, F., Fanelli, A., Tizzani, P., Berriatua, E., Prieto, P., Martínez-Carrasco, C., León, L., Rossi, L. and Candela, M. G. (2020). Spatio-temporal patterns of sarcoptic mange in red deer and Iberian ibex in a multi-host natural park. *Research in Veterinary Science* **128**, 224–229. doi: 10.1016/j.rvsc.2019.11.014.

Iglesias, L. E., Saumell, C. A., Fernández, A. S., Fusé, L. A., Lifschitz, A. L., Rodríguez, E. M., Steffan, P. E. and Fiel, C. A. (2006). Environmental impact of ivermectin excreted by cattle treated in autumn on dung fauna and degradation of faeces on pasture. *Parasitology Research* **100**, 93–102. doi: 10.1007/s00436-006-0240-x.

Iqomah, M., Suwarno, N. and Yuliani, P. (2020). Cat Scabies at The Animal Health Clinic of Salatiga Agriculture Service on August to November 2020. *Journal of Parasite Science* **4**, 45. doi: 10.20473/jops.v4i2.29152.

Jeklova, E., Leva, L., Matiasovic, J., Kovarcik, K., Kudlackova, H., Nevorankova, Z., Psikal, I. and Faldyna, M. (2008). Characterisation of immunosuppression in rabbits after infection with myxoma virus. *Veterinary Microbiology* **129**, 117–130. doi: 10.1016/j.vetmic.2007.11.039.

JF, M. (1965). Human infestation with canine scabies. *The Journal of the Maine Medical Association* **56**, 131.

Jokelainen, P., Moroni, B., Hoberg, E., Oksanen, A. and Laaksonen, S. (2019a). Gastrointestinal parasites in reindeer (Rangifer tarandus tarandus) calves from Fennoscandia: An epidemiological study. *Veterinary Parasitology: Regional Studies and Reports* **16**,. doi: 10.1016/j.vprsr.2019.100277.

Jokelainen, P., Moroni, B., Hoberg, E., Oksanen, A. and Laaksonen, S. (2019b). Gastrointestinal parasites in reindeer (Rangifer tarandus tarandus): A review focusing on Fennoscandia. *Veterinary*

Parasitology: Regional Studies and Reports **17**,. doi: 10.1016/j.vprsr.2019.100317.

Jombart, T., Devillard, S., Dufour, A. B. and Pontier, D. (2008). Revealing cryptic spatial patterns in genetic variability by a new multivariate method. *Heredity* **101**, 92–103. doi: 10.1038/hdy.2008.34.

Kang, S. B., Lee, J. Y., Cho, B. K. and Houh, W. (1988). A case of human infestation of canine scabies. *Korean Journal of Dermatology* **26**, 570–574.

Karimkhani, C., Colombara, D. V, Drucker, A. M., Norton, S. A., Hay, R., Engelman, D., Steer, A., Whitfeld, M., Bill, F. and Foundation, M. G. (2015). Article The global burden of scabies : a cross-sectional analysis from the Global Burden of Disease Study 2015. *The Lancet Infectious Diseases* **17**, 1247–1254. doi: 10.1016/S1473-3099(17)30483-8.

Larsson, M. H. M. A. (1978). Evidências epidemiológicas da ocorrência de escabiose, em humanos, causada pelo Sarcoptes scabiei (DeGeer, 1778) var. canis (Bourguignon, 1853). *Revista de Saúde Pública* **12**, 333–339. doi: 10.1590/s0034-89101978000300007.

Lastuti, N. D. R., Ma'ruf, A. and Yuniarti, W. M. (2019). Characterization of mitochondrial COX-1 gene of Sarcoptes scabiei from rabbits in East Java, Indonesia. *Journal of Advanced Veterinary and Animal Research* **6**, 445–450. doi: 10.5455/javar.2019.f366.

Lavin, S., Marco, I., Rossi, L., Meneguz, P. G. and Viñas, L. (1997). Haemonchosis in Spanish ibex. *Journal of Wildlife Diseases* **33**, 656–659.

Lavin, S., Ruiz-Bascaran, M., Marco, I., Fondevila, M. D. and Ramis, A. J. (2000). Experimental infection of chamois (Rupicapra pyrenaica parva) with Sarcoptes scabiei derived from naturally infected goats. *Journal of Veterinary Medicine, Series B* **47**, 693–699. doi: 10.1046/j.1439-0450.2000.00406.x.

Leó N-Vizcaíno, L., Ruíz De Ybáñ Ez, M. R., Cubero, M. J., Ortíz, J. M., Espinosa, J., Pé, L., Simó, M. A. and Alonso, F. (1999). *Sarcoptic mange in Spanish ibex from Spain*.

León-Vizcaíno, L. (1992). No Title. In *Epidemiología de la sarna sarcóptica en el Parque Natural de las Sierras de Cazorla, Segura y Las Villas.*, p. Sevilla.

León-Vizcaíno, L., Ruíz de Ybáñez, M. R., Cubero, M. J., Ortíz, J. M., Espinosa, J., Pérez, L., Simón, M. A. and Alonso, F. (1999). Sarcoptic mange in Spanish ibex from Spain. *Journal of Wildlife Diseases* **35**, 647–659. doi: 10.7589/0090-3558-35.4.647.

León-Vizcaíno, L., Cubero, M. J., González-Capitel, E., Simón, M. A., Pérez, L., Ruiz De Ybáñez, M. R., Ortíz, J. M., González Candela, M. and Alonso, F. (2001). Experimental ivermectin treatment of sarcoptic mange and establishment of a mange-free population of Spanish ibex. *Journal of Wildlife Diseases* **37**, 775–785. doi: 10.7589/0090-3558-37.4.775.

Lespine, A., Alvinerie, M., Pors, I. and Chartier, C. (2005). Influence of the route of administration on efficacy and tissue distribution of ivermectin in goat. **128**, 251–260. doi: 10.1016/j.vetpar.2004.11.028.

Letunic, I. and Bork, P. (2007). Interactive Tree Of Life (iTOL): an online tool for phylogenetic tree display and annotation. *Bioinformatics* **23**, 127–128.

Li, J., Yu, Y. and Zhang, W. (1999). Diagnosis and treatment for the cross infection of scabies mites between rabbit and human. *Chin J Rabbit Farm* **04:38**,.

Licoppe, A., Prévot, C., Heymans, M., Bovy, C., Casaer, J. and Cahill, S. (2013). Wild boar/ feral pig in (peri-)urban areas. *International survey report as an introduction to the workshop:"Managing wild boar in human-dominated landscapes" International Union of Game Biologists.* **30**, 1200–1211. doi: 10.1111/cobi.12731.

Lifschitz, A., Virkel, G., Sallovitz, J., Sutra, J. F., Galtier, P., Alvinerie, M. and Lanusse, C. (2000). Comparative distribution of ivermectin and doramectin to parasite location tissues in cattle. *Veterinary Parasitology* **87**, 327–338. doi: 10.1016/S0304-4017(99)00175-2.

Littlewood, J. D. (2011). Equine sarcoptic mange: Re-emergence of a previously notifiable disease? *Equine Veterinary Education* **23**, 24–26. doi: 10.1111/j.2042-3292.2010.00169.x.

Lopes, A. M., Marques, S., Silva, E., Magalhães, M. J., Pinheiro, A., Alves, P. C., Le Pendu, J., Esteves, P. J., Thompson, G. and Abrantes, J. (2014). Detection of RHDV strains in the Iberian hare (Lepus granatensis): Earliest evidence of rabbit lagovirus cross-species infection. *Veterinary Research* **45**, 1–7. doi: 10.1186/s13567-014-0094-7.

Lyles, A. M. and Dobson, A. P. (1993). Infectious disease and intensive management: population dynamics, threatened hosts, and their parasites. *Journal of zoo and wildlife medicine* 315–326.

Mahendra, P., Pratibha, D. and others (2006). Human scabies contracted from a goat. *Intas Polivet* **7**, 487–488.

Makouloutou, P., Suzuki, K., Yokoyama, M., Takeuchi, M., Yanagida, T. and Sato, H. (2015). Involvement of two genetic lineages of Sarcoptes scabiei mites in a local mange epizootic of wild mammals in Japan. *Journal of Wildlife Diseases* **51**, 69–78.

Malik, R., McKellar Stewart, K., Sousa, C. A., Krockenberger, M. B., Pope, S., Ihrke, P., Beatty, J., Barrs, V. R. D. and Walton, S. (2006). Crusted scabies (sarcoptic mange) in four cats due to Sarcoptes scabiei infestation. *Journal of Feline Medicine and Surgery* **8**, 327–339. doi: 10.1016/j.jfms.2006.05.005.

Mancini, L., Lacchetti, I., Chiudioni, F., Cristiano, W., Di Domenico, K., Marcheggiani, S., Carere, M., Bindi, L. and Borrello, S. (2020). Need for a sustainable use of medicinal products: the impacts on the environment of ivermectin. *Annali dell'Istituto Superiore di Sanità* **56**, 492–496.

Martin, A. M., Fraser, T. A., Lesku, J. A., Simpson, K., Roberts, G. L., Garvey, J., Polkinghorne, A., Burridge, C. P. and Carver, S. (2018). The cascading pathogenic consequences of sarcoptes scabiei infection that manifest in host disease. *Royal Society Open Science* **5**,. doi: 10.1098/rsos.180018.

Martin, A. M., Richards, S. A., Fraser, T. A., Polkinghorne, A., Burridge, C. P. and Carver, S. (2019). Population-scale treatment informs solutions for control of environmentally transmitted wildlife disease. *Journal of Applied Ecology* **56**, 2363–2375. doi: 10.1111/1365-2664.13467.

Massei, G., Kindberg, J., Licoppe, A., Gačić, D., Šprem, N., Kamler, J., Baubet, E., Hohmann, U., Monaco, A., Ozoliņš, J., Cellina, S., Podgórski, T., Fonseca, C., Markov, N., Pokorny, B., Rosell, C. and Náhlik, A. (2015). Wild boar populations up, numbers of hunters down? A review of trends and implications for Europe. *Pest Management Science* **71**, 492–500. doi: 10.1002/ps.3965.

Mathison, B. A. and Pritt, B. S. (2014). Laboratory identification of arthropod ectoparasites. *Clinical Microbiology Reviews* **27**, 48–67. doi: 10.1128/CMR.00008-13.

Matsuyama, R., Yabusaki, T., Kuninaga, N. and Morimoto, T. (2015). Veterinary Parasitology Coexistence of two different genotypes of Sarcoptes scabiei derived from companion dogs and wild raccoon dogs in Gifu , Japan : The genetic evidence for transmission between domestic and wild canids. *Veterinary Parasitology* **212**, 356–360. doi: 10.1016/j.vetpar.2015.06.023.

Matsuyama, R., Yabusaki, T., Senjyu, N., Okano, T., Baba, M., Tsuji-Matsukane, T., Yokoyama, M., Kido, N., Kadosaka, T., Kato, T., Suzuki, M. and Asano, M. (2019). Possible transmission of Sarcoptes scabiei between herbivorous Japanese serows and omnivorous Caniformia in Japan: A cryptic transmission and persistence? *Parasites and Vectors* **12**,. doi: 10.1186/s13071-019-3630-5.

Mentaberre, G. Nuevo brote de sarna sarcoptic.

Mentaberre, G., Serrano, E., Velarde, R., Marco, I., Lavin, S., Mateos, A., De Juan, L., Domínguez, L., Olivé, X. and Romeva, J. (2010). Absence of TB in Iberian ibex (Capra pyrenaica) in a high-risk area. *Veterinary Record* **166**, 700. doi: 10.1136/vr.c2552.

Menzano, A., Rambozzi, L. and Rossi, L. (2004). Outbreak of scabies in human beings, acquired from chamois (Rupicapra rupicapra). *Veterinary Record* **155**, 568. doi: 10.1136/vr.155.18.568.

Menzano, A., Rambozzi, L. and Rossi, L. (2007). A severe episode of wildlife-derived scabies in domestic goats in Italy. *Small Ruminant Research* **70**, 154–158. doi: 10.1016/j.smallrumres.2006.02.010.

Menzano, A., Rambozzi, L., Molinar Min, A. R., Meneguz, P. G. and Rossi, L. (2008). Description and epidemiological implications of S. scabiei infection in roe deer (Capreolus capreolus) originating from chamois (Rupicapra rupicapra). *European Journal of Wildlife Research* **54**, 757–761. doi: 10.1007/s10344-008-0195-6.

Mestorino, N., Turic, E., Pesoa, J., Echeverr\'\ia, J. and Errecalde, J. O. (2003). Pharmacokinetics in plasma of ivermectin after its oral (solution and tablets) administration to sheep. *Journal of Veterinary Pharmacology and Therapeutics* **26**, 307–309.

Mignone, W., Poggi, M., Pistone, G. C., Caramelli, M., Bollo, E. and Biolatti, B. (2014).

Pathology of Wild boar (Sus scrofa) in Liguria, Italy, between 1989 and 1992. *Journal of Mountain Ecology* **3**,.

Millán, J. (2010). First description of sarcoptic mange in wild European rabbit (Oryctolagus cuniculus). *European Journal of Wildlife Research* **56**, 455–457. doi: 10.1007/s10344-009-0347-3.

MINUARTIA (2018). Programa de Seguiment de les poblacions De Senglar a Catalunya Temporada 2017-2018.

Mitra, M., Mahanta, S. K., Sen, S., Ghosh, C. and Hati, A. K. (1995). Transmission of Sarcoptes scabiei from animal to man and its control. *Journal of the Indian Medical Association* **93**, 142–143.

Mofiz, E., Seemann, T., Bahlo, M., Holt, D., Currie, B. J., Fischer, K. and Papenfuss, A. T. (2016a). Mitochondrial Genome Sequence of the Scabies Mite Provides Insight into the Genetic Diversity of Individual Scabies Infections. *PLoS Neglected Tropical Diseases* **10**, 1–16. doi: 10.1371/journal.pntd.0004384.

Mofiz, E., Holt, D. C., Seemann, T., Currie, B. J., Fischer, K. and Papenfuss, A. T. (2016b). Genomic resources and draft assemblies of the human and porcine varieties of scabies mites, Sarcoptes scabiei var. hominis and var. suis. *GigaScience* **5**, 0–3. doi: 10.1186/s13742-016-0129-2.

Molin, E. U. (2009). In vitro characterization of glutathione transferases from Sarcoptes scabiei.

Mörner, T. (1992). Sarcoptic mange in Swedish wildlife. *Revue Scientifique et Technique-Office International des Epizooties* **11**, 1115.

Moroni, B., Valldeperes, M., Serrano, E., López-Olvera, J. R., Lavín, S. and Rossi, L. (2020). Comment on: "the treatment of sarcoptic mange in wildlife: A systematic review." *Parasites and Vectors* **13**, 1–4. doi: 10.1186/s13071-020-04347-0.

Moroni, B., Dutto, M., Barrasetas, M., Molinar Min, A. and Rossi, L. (2021a). Red foxes as a source of zoonotic scabies in a rural area of Piedmont, Italy. 1.

Moroni, B., Barlaam, A., Misia, A. L., Peano, A., Rossi, L. and Giangaspero, A. (2021b). Dermanyssus gallinae in non-avian hosts: A case report in a dog and review of the literature. *Parasitology International* **84**, 102378. doi: 10.1016/j.parint.2021.102378.

Moroni, B., Angelone, S., Pérez, J. M., Molinar Min, A. R., Pasquetti, M., Tizzani, P., López-Olvera, J. R., Valldeperes, M., Granados, J. E., Lavín, S., Mentaberre, G., Camacho-Sillero, L., Martínez-Carrasco, C., Oleaga, A., Candela, M., Meneguz, P. G. and Rossi, L. (2021c). Sarcoptic mange in wild ruminants in Spain: solving the epidemiological enigma using microsatellite markers. *Parasites & Vectors* **14**, 171. doi: 10.1186/s13071-021-04673-x.

Moroni, B., Rossi, L., Bernigaud, C. and Guillot, J. (2022). Zoonotic Episodes of Scabies : A Global Overview. *Pathogens* **11**,.

Morsy, T. A., Bakr, M. E., Ahmed, M. M. and Kotb, M. M. (1994). Human scabies acquired from a

pet puppy. *Journal of the Egyptian Society of Parasitology* **24**, 305–308.

Mounsey, K. E., Holt, D. C., McCarthy, J. S., Currie, B. J. and Walton, S. F. (2009). Longitudinal evidence of increasing in vitro tolerance of scabies mites to ivermectin in scabies-endemic communities. *Archives of dermatology* **145**, 840–841.

Mounsey, K. E., Walton, S. F., Innes, A., Cash-Deans, S. and McCarthy, J. S. (2017). In vitro efficacy of moxidectin versus ivermectin against Sarcoptes scabiei. *Antimicrobial Agents and Chemotherapy* **61**,. doi: 10.1128/AAC.00381-17.

Mounsey, K., Harvey, R. J., Wilkinson, V., Takano, K., Old, J., Stannard, H., Wicker, L., Phalen, D. and Carver, S. (2022). Drug dose and animal welfare: important considerations in the treatment of wildlife. *Parasitology Research*. doi: 10.1007/s00436-022-07460-4.

Mumcuoglu, K. Y. (1990). A technique for quantitative evaluation of ectoparasitic mites and insects of domestic animals. *Experimental & Applied Acarology* **9**, 97–101. doi: 10.1007/BF01198987.

Mumcuoglu, Y. and Rufli, T. (1979). Human infestation by Sarcoptes scabiei var. bovis (cattle itch mite). *Der Hautarzt; Zeitschrift fur Dermatologie, Venerologie, und verwandte Gebiete* **30**, 423–426.

Najera, F., Crespo, E., Garc, A., Grande-Gomez, R., Herrera-Sanchez, F., Gentil, M., Cort, C., Müller, E., Calero-Bernal, R. and Revuelta, L. (2021). European Wildcat (Felis silvestris silvestris) from Spain. 1–8.

Nakagawa, S. and Schielzeth, H. (2013). A general and simple method for obtaining R 2 from generalized linear mixed-effects models. **2**, 133–142. doi: 10.1111/j.2041-210x.2012.00261.x.

Navarro-Gonzalez, N., Serrano, E., Casas-Díaz, E., Velarde, R., Marco, I., Rossi, L. and Lavín, S. (2010). Game restocking and the introduction of sarcoptic mange in wild rabbit in north-eastern Spain. *Animal Conservation* **13**, 586–591. doi: 10.1111/j.1469-1795.2010.00390.x.

Newton, D. K. and Gerrie, W. (1966). Sarcoptic scabies in a dog (with human involvement). *The Canadian Veterinary Journal. la Revue Veterinaire Canadienne* **7**, 43–43.

Norins, A. L. (1969). Canine Scabies in Children: "Puppy Dog" Dermatitis. *American Journal of Diseases of Children* **117**, 239–242. doi: 10.1001/archpedi.1969.02100030241023.

Oksanen, A., Nieminen, M. and Soveri, T. (1993). A comparison of topical, subcutaneous and oral administrations of ivermectin to reindeer. *The Veterinary Record* **133**, 312–314.

Oksanen, A., Åsbakk, K., Raekallio, M. and Nieminen, M. (2014). The relative plasma availabilities of ivermectin in reindeer (Rangifer tarandus tarandus) following subcutaneous and two different oral formulation applications. *Acta Veterinaria Scandinavica* **56**, 76. doi: 10.1186/s13028-014-0076-9.

Oleaga, A., Balseiro, A. and Gortázar, C. (2008a). Sarcoptic mange in two roe deer (Capreolus capreolus) from northern Spain. *European Journal of Wildlife Research* **54**, 134–137. doi: 10.1007/s10344-007-0105-3.

Oleaga, A., Casais, R., González-Quirós, P., Prieto, M. and Gortázar, C. (2008b). Sarcoptic mange in red deer from Spain: Improved surveillance or disease emergence? *Veterinary Parasitology* **154**, 103–113. doi: 10.1016/j.vetpar.2008.03.002.

Oleaga, Á., Casais, R., Balseiro, A., Espí, A., Llaneza, L., Hartasánchez, A. and Gortázar, C. (2011). Veterinary Parasitology New techniques for an old disease : Sarcoptic mange in the Iberian wolf. **181**, 255–266. doi: 10.1016/j.vetpar.2011.04.036.

Oleaga, A., Casais, R., Prieto, J. M., Gortázar, C. and Balseiro, A. (2012). Comparative pathological and immunohistochemical features of sarcoptic mange in five sympatric wildlife species in Northern Spain. *European Journal of Wildlife Research* **58**, 997–1000. doi: 10.1007/s10344-012-0662-y.

Oleaga, A., Alasaad, S., Rossi, L., Casais, R., Vicente, J., Maione, S., Soriguer, R. C. and Gortázar, C. (2013). Genetic epidemiology of Sarcoptes scabiei in the Iberian wolf in Asturias , Spain. *Veterinary Parasitology* **196**, 453–459. doi: 10.1016/j.vetpar.2013.04.016.

Oleaga, A., García, A., Balseiro, A., Casais, R., Mata, E. and Crespo, E. (2019a). First description of sarcoptic mange in the endangered Iberian lynx (Lynx pardinus): clinical and epidemiological features. *Eur. J. Wildl. Res.* **65**,.

Oleaga, A., García, A., Balseiro, A., Casais, R., Mata, E. and Crespo, E. (2019b). First description of sarcoptic mange in the endangered Iberian lynx (Lynx pardinus): clinical and epidemiological features.

Palomo, L. J., Gisbert, J. and Blanco, J. C. (2007). *Atlas y libro rojo de los mammiferos terrestres de España*. Organismo Autónomo de Parques Nacionales Madrid.

Papich, M. G. (2016). Ivermectin. In *Saunders handbook of veterinary drugs* (ed. Elseiver), pp. 420–423. Saunders Elseiver doi: 10.1016/B978-0-323-24485-5.00323-5.

Paradis, E. (2010). pegas: an {R} package for population genetics with an integrated--modular approach. *Bioinformatics* **26**, 419–420.

París, F. P. (1991). *La cabra montés (Capra pyrenaica) en el Parque Natural de las Sierras de Cazorla, Segura y Las Villas*. Instituto Nacional para la Conservación de la Naturaleza.

Pastoret, P.-P. and Brochier, B. (1999). Epidemiology and control of fox rabies in Europe. *Vaccine* **17**, 1750–1754.

Peltier, S. K., Brown, J. D., Ternent, M., Niedringhaus, K. D., Schuler, K., Bunting, E. M., Kirchgessner, M. and Yabsley, M. J. (2017). Genetic Characterization of Sarcoptes scabiei from Black Bears (Ursus americanus) and Other Hosts in the Eastern United States . *Journal of Parasitology* **103**, 593–597. doi: 10.1645/17-26.

Pence, D. B. and Ueckermann, E. (2002). Sarcoptic mange in wildlife Aetiology. **21**, 385–398.

Pérez, J. (1997). The dynamics of sarcoptic mange in the ibex population of Sierra Nevada in Spain–influence of climatic factors. *Journal of Wildlife Research* **2**, 86–89.

Pérez, R., Cabezas, I., Sutra, J. F., Galtier, P. and Alvinerie, M. (2001). Faecal excretion profile of moxidectin and ivermectin after oral administration in horses. *Veterinary Journal* **161**, 85–92. doi: 10.1053/tvjl.2000.0521.

Pérez, J. M., Granados, J. E., Soriguer, R. C., Fandos, P., Márquez, F. J. and Crampe, J. P. (2002). Distribution, status and conservation problems of the Spanish Ibex, Capra pyrenaica (Mammalia: Artiodactyla). *Mammal Review* **32**, 26–39. doi: 10.1046/j.1365-2907.2002.00097.x.

Pérez, J. M., Giuseppe, P., Dipartimento, M., Torino, U. and Paolo, L. (2021). Biology and management of sarcoptic mange in wild Caprinae populations. **51**, 82–94. doi: 10.1111/mam.12213.

Pisano, S. R. R., Ryser-Degiorgis, M. P., Rossi, L., Peano, A., Keckeis, K. and Roosje, P. (2019). Sarcoptic mange of fox origin in multiple farm animals and scabies in humans, Switzerland, 2018. *Emerging Infectious Diseases* **25**, 1235–1238. doi: 10.3201/eid2506.181891.

Potts, J. R., Harris, S. and Giuggioli, L. (2013). Quantifying behavioral changes in territorial animals caused by sudden population declines. *American Naturalist* **182**,. doi: 10.1086/671260.

Pritchard J.K., Stephens P. and Donnelly P. (2000). Inference of population structure using multilocus genotype data. *Genetics* **155**, 945–959.

Purroy, F. J. and Salvador Milla, A. (2017). Liebre ibérica--Lepus granatensis Rosenhauer, 1856.

Rabinowitz, P. M. and Gordon, Z. (2004). Outfoxing a Rash: Clinical Example of Human–Wildlife Interaction. *EcoHealth* **1**, 404–407. doi: 10.1007/s10393-004-0137-z.

Rahman, M. M., Lecchi, C., Fraquelli, C., Sartorelli, P. and Ceciliani, F. (2010). Acute phase protein response in Alpine ibex with sarcoptic mange. *Veterinary Parasitology* **168**, 293–298. doi: 10.1016/j.vetpar.2009.12.001.

Rajković-Janje, R., Manojlović, L. and Gojmerac, T. (2004). In-feed 0.6% ivermectin formulation for treatment of wild boar in the Moslavina hunting ground in Croatia. *European Journal of Wildlife Research* **50**, 41–43. doi: 10.1007/s10344-003-0033-9.

Rasero, R., Rossi, L., Soglia, D., Maione, S., Sacchi, P., Rambozzi, L., Sartore, S., Soriguer, R. C., Spalenza, V. and Alasaad, S. (2010). Host taxon-derived Sarcoptes mite in European wild animals revealed by microsatellite markers. *Biological Conservation* **143**, 1269–1277. doi: 10.1016/j.biocon.2010.03.001.

Reddy, B. S. and Kumari, K. N. (2013). Canine Scabies-Its Therapeutic Management and Zoonotic importance. *Intas Polivet* **14**, 292–294.

Rehmus, W. E. and Prendiville, J. S. (2019). Scabies and Pseudoscabies. *Harper's Textbook of Pediatric Dermatology* 711–722. doi: 10.1002/9781119142812.ch59.

Rentería-Solís, Z., Min, A. M., Alasaad, S., Müller, K., Michler, F. U., Schmäschke, R., Wittstatt, U., Rossi, L. and Wibbelt, G. (2014). Genetic epidemiology and pathology of raccoon-derived Sarcoptes mites from urban areas of Germany. *Medical and Veterinary*

Entomology **28**, 98–103. doi: 10.1111/mve.12079.

Römbke, J., Krogh, K. A., Moser, T., Scheffczyk, A. and Liebig, M. (2010). Effects of the veterinary pharmaceutical ivermectin on soil invertebrates in laboratory tests. *Archives of Environmental Contamination and Toxicology* **58**, 332–340. doi: 10.1007/s00244-009-9414-8.

Roncalli, R. A. (1987). The history of scabies in veterinary and human medicine from biblical to modern times. *Veterinary Parasitology* **25**, 193–198. doi: 10.1016/0304-4017(87)90104-X.

Rossi, L., Tizzani, P., Rambozzi, L., Moroni, B. and Meneguz, P. G. (2019a). Sanitary emergencies at the wild/domestic caprines interface in Europe. *Animals* **9**, 1–13. doi: 10.3390/ani9110922.

Rossi, L., Tizzani, P., Rambozzi, L., Moroni, B. and Meneguz, P. G. (2019b). Sanitary emergencies at the wild/domestic caprines interface in Europe. *Animals* **9**,. doi: 10.3390/ani9110922.

Rowe, M. L., Whiteley, P. L. and Carver, S. (2019). The treatment of sarcoptic mange in wildlife : a systematic review. *Parasites & Vectors* 1–14. doi: 10.1186/s13071-019-3340-z.

Rudd, J. L., Clifford, D. L., Cypher, B. L., Hull, J. M., Jane Riner, A. and Foley, J. E. (2020). Molecular epidemiology of a fatal sarcoptic mange epidemic in endangered San Joaquin kit foxes (Vulpes macrotis mutica). *Parasites and Vectors* **13**, 1–11. doi: 10.1186/s13071-020-04328-3.

Ruiz-Maldonado, R., Tamayo, L. and Domiňguez, J. (1977). Norwegian scabies due to Sarcoptes scabiei var canis. *Archives of dermatology* **113**, 1733.

Ryser-Degiorgis, M. P., Ryser, A., Bacciarini, L. N., Angst, C., Gottstein, B., Janovsky, M. and Breitenmoser, U. (2002). Notoedric and sarcoptic mange in free-ranging lynx from Switzerland. *Journal of Wildlife Diseases* **38**, 228–232. doi: 10.7589/0090-3558-38.1.228.

Sánchez-Isarria, M. A., Hermoso, J., de la Peña, J., Casanova, G., Burgui, J. M., Sanchis, G., Arévalo, P. R. S. and Sánchez, R. (2007). La ivermectina como tratamiento de la sarna sarcóptica en el medio natural: vias de aplicación. *Tendencias actuales en el Estudio y Conservación de los caprinos europeos* 269–276.

Sannö, A., Ander, M., Ågren, E. and Troell, K. (2021). Sarcoptic mange in the wild boar, Sus scrofa, in Sweden. *Current Research in Parasitology & Vector-Borne Diseases* **1**, 100060. doi: 10.1016/j.crpvbd.2021.100060.

Scott, E. W., Kinabo, L. D. and McKellar, Q. A. (1990). Pharmacokinetics of ivermectin after oral or percutaneous administration to adult milking goats. *Journal of Veterinary Pharmacology and Therapeutics* **13**, 432–435. doi: 10.1111/j.1365-2885.1990.tb00800.x.

Singh, R., Turkar, S., Dua, K. and Khan, I. S. (2019). A rare case of Sarcoptes Scabiei in Persian cat. **39**, 56–58.

Sivajothi, S. and Reddy, B. S. (2015). Cat affected with sarcoptic mange in Y. S. R. District of Andhra Pradesh, India. *Comparative Clinical Pathology* **24**, 1031–1032. doi: 10.1007/s00580-014-2023-6.

Skerratt, L. F. and Beveridge, I. (1999). Human scabies of wombat origin. *Australian Veterinary Journal* **77**, 607. doi: 10.1111/j.1751-0813.1999.tb13202.x.

Skerratt, L., Campbell, N., Murrell, A., Walton, S., Kemp, D. and Barker, S. (2002). The mitochondrial 12S gene is a suitable marker of populations of Sarcoptes scabiei from wombats, dogs and humans in Australia. *Parasitology Research* **88**, 376–379. doi: 10.1007/s00436-001-0556-5.

Slanina, P., Kuivinen, J., Ohlsén, C. and Ekström, L. G. (1989). Ivermectin residues in the edible tissues of swine and cattle: Effect of cooking and toxicological evaluation. *Food Additives and Contaminants* **6**, 475–481. doi: 10.1080/02652038909373807.

Sleutjens, J. (2015). Successful treatment of Sarcoptes scabiei in a 33-year-old pony with underlying pituitary pars intermedia dysfunction. *Equine Veterinary Education* **27**, 22–25. doi: 10.1111/eve.12255.

Smith, E. B. and Claypoole, T. F. (1967). Canine Scabies in Dogs and in Humans. *JAMA: The Journal of the American Medical Association* **199**, 59–64. doi: 10.1001/jama.1967.03120020053008.

Smith, J. S., Berger, D. J., Hoff, S. E., Jesudoss Chelladurai, J. R. J., Martin, K. A. and Brewer, M. T. (2020). Afoxolaner as a Treatment for a Novel Sarcoptes scabiei Infestation in a Juvenile Potbelly Pig. *Frontiers in Veterinary Science* **7**, 1–6. doi: 10.3389/fvets.2020.00473.

Soglia, D., Rasero, R., Rossi, L., Sartore, S., Sacchi, P. and Maione, S. (2007). Microsatellites as markers for comparison among different populations of Sarcoptes scabiei. *Italian Journal of Animal Science* **6**, 214–216. doi: 10.4081/ijas.2007.1s.214.

Soglia, D., Rambozzi, L., Maione, S., Spalenza, V., Sartore, S., Alasaad, S., Sacchi, P. and Rossi, L. (2009). Two simple techniques for the safe Sarcoptes collection and individual mite DNA extraction. 1465–1468. doi: 10.1007/s00436-009-1580-0.

Steel, J. W. (1993). Pharmacokinetics and metabolism of avermectins in livestock. *Veterinary Parasitology* **48**, 45–57. doi: 10.1016/0304-4017(93)90143-B.

Tannenbaum, M. H. (1965). Canine scabies in man: A report of human mange. *JAMA* **193**, 321–322.

Team, R. C. and others (2013). R: A language and environment for statistical computing.

Terada, Y., Murayama, N., Ikemura, H., Morita, T. and Nagata, M. (2010). Sarcoptes scabiei var. canis refractory to ivermectin treatment in two dogs. *Veterinary dermatology* **21**, 608–612.

Thomsett, L. R. (1968). Mite Infestations of Man Contracted from Dogs and Cats. *British Medical Journal* **3**, 93–95. doi: 10.1136/bmj.3.5610.93.

Twomey, D. F., Birch, E. S. and Schock, A. (2009). Outbreak of sarcoptic mange in alpacas (Vicugna pacos) and control with repeated subcutaneous ivermectin injections. *Veterinary Parasitology* **159**, 186–191. doi: 10.1016/j.vetpar.2008.10.023.

Ueda, T., Tarui, H., Kido, N., Imaizumi, K., Hikosaka, K., Abe, T., Minegishi, D., Tada, Y., Nakagawa, M., Tanaka, S., Omiya, T., Morikaku, K., Kawahara, M., Kikuchi-Ueda, T.,

Akuta, T. and Ono, Y. (2019). The complete mitochondrial genome of Sarcoptes scabiei var. nyctereutis from the Japanese raccoon dog: Prediction and detection of two transfer RNAs (tRNA-A and tRNA-Y). *Genomics* **111**, 1183–1191. doi: 10.1016/j.ygeno.2018.09.002.

Ulmer, A., Schanz, S., Röcken, M. and Fierlbeck, G. (2007). A papulovesicular rash in a farmer and his wife. *Clinical Infectious Diseases* **45**, 395–396. doi: 10.1086/519435.

Uraguchi, K., Ueno, M., Iijima, H. and Saitoh, T. (2014). Demographic analyses of a fox population suffering from sarcoptic mange. *Journal of Wildlife Management* **78**, 1356–1371. doi: 10.1002/jwmg.794.

Valldeperes, M., Granados, J. E., Gregorio, M., Prieto, P., Escribano, F., Cardells, J., Foj, R., Puigdemont, A., Fandos, P., Ráez-Bravo, A., Espinosa, J., Pérez, J. M., Lizana, V., Soriguer, R. and López-Olvera, J. R. (2018). Serological survey of sercoptic mange in Mediterranean Iberian ibex (Capra pyrenaica) populations. In *13th EWDA Conference*, p. 18071. Larissa.

Valldeperes, M., Granados, J. E., Pérez, J. M., Castro, I., Bravo, A. R., Fandos, P., Ramón, J., Olvera, L., Serrano, E. and Mentaberre, G. (2019). How sensitive and specific is the visual diagnosis of sarcoptic mange in free - ranging Iberian ibexes? *Parasites & Vectors* 1–7. doi: 10.1186/s13071-019-3665-7.

Valldeperes, M., Moroni, B., Rossi, L., López-Olvera, J. R., Velarde, R., Molinar Min, A. R., Mentaberre, G., Serrano, E., Angelone, S., Lavín, S. and Granados, J. E. (2021). First report of interspecific transmission of sarcoptic mange from Iberian ibex to wild boar. *Parasites & Vectors* **14**, 481. doi: 10.1186/s13071-021-04979-w.

Verdú, J. R., Cortez, V., Martinez-Pinna, J., Ortiz, A. J., Lumaret, J. P., Lobo, J. M., Sánchez-Piñero, F. and Numa, C. (2018). First assessment of the comparative toxicity of ivermectin and moxidectin in adult dung beetles: Sub-lethal symptoms and pre-lethal consequences. *Scientific Reports* **8**, 1–9. doi: 10.1038/s41598-018-33241-0.

Vokřál, I., Michaela, Š., Radka, P., Jiří, L., Lukáš, P., Dominika, S., Kateřina, L., Barbora, S. and Lenka, S. (2019). Ivermectin environmental impact: Excretion profile in sheep and phytotoxic effect in Sinapis alba. *Ecotoxicology and Environmental Safety* **169**, 944–949. doi: 10.1016/j.ecoenv.2018.11.097.

Walton, S. F., Currie, B. J. and Kemp, D. J. (1997). A DNA fingerprinting system for the ectoparasite Sarcoptes scabiei. *Molecular and Biochemical Parasitology* **85**, 187–196. doi: 10.1016/S0166-6851(96)02825-3.

Walton, S. F., Choy, J. L., Bonson, A., Valle, A., McBroom, J., Taplin, D., Arlian, L., Mathews, J. D., Currie, B. and Kemp, D. J. (1999). Genetically distinct dog-derived and human-derived Sarcoptes scabiei in scabies-endemic communities in northern Australia. *American Journal of Tropical Medicine and Hygiene* **61**, 542–547. doi: 10.4269/ajtmh.1999.61.542.

Walton, S. F., Dougall, A., Pizzutto, S., Holt, D., Taplin, D., Arlian, L. G., Morgan, M., Currie, B. J. and Kemp, D. J. (2004). Genetic epidemiology of Sarcoptes scabiei (Acari: Sarcoptidae) in northern Australia. *International Journal for Parasitology* **34**, 839–849. doi: 10.1016/j.ijpara.2004.04.002.

Warner, R. D. (1984). Occurrence and impact of zoonoses in pet dogs and cats at US Air Force bases. *American Journal of Public Health* **74**, 1239–1243. doi: 10.2105/AJPH.74.11.1239.

Wick, M. Van and Hashem, B. (2019). Treatment of Sarcoptic Mange in an American Black Bear (Ursus americanus) with a Single Oral Dose of Fluralaner. **55**, 250–253. doi: 10.7589/2017-12-310.

Wilkinson, V., Tokano, K., Nichols, D., Martin, A., Holme, R., Phalen, D., Mounsey, K., Charleston, M., Kreiss, A., Pye, R., Browne, E., Næsborg-Nielsen, C., Richards, S. A. and Carver, S. (2021a). Fluralaner as a novel treatment for sarcoptic mange in the bare-nosed wombat (Vombatus ursinus): safety, pharmacokinetics, efficacy and practicable use. *Parasites and Vectors* **14**, 1–21. doi: 10.1186/s13071-020-04500-9.

Wilkinson, V., Takano, K., Nichols, D., Martin, A., Holme, R., Phalen, D., Mounsey, K., Charleston, M., Kreiss, A., Pye, R., Browne, E., Næsborg-Nielsen, C., Richards, S. A. and Carver, S. (2021b). Fluralaner as a novel treatment for sarcoptic mange in the bare-nosed wombat (Vombatus ursinus): safety, pharmacokinetics, efficacy and practicable use. *Parasites & Vectors* **14**, 18. doi: 10.1186/s13071-020-04500-9.

Wobeser, G. (2002). Disease management strategies for wildlife. *Revue Scientifique et Technique-Office international des epizooties* **21**, 159–178.

Woodroffe, R., Donnelly, C. A., Jenkins, H. E., Johnston, W. T., Cox, D. R., Bourne, F. J., Cheeseman, C. L., Delahay, R. J., Clifton-Hadley, R. S., Gettinby, G. and others (2006). Culling and cattle controls influence tuberculosis risk for badgers. *Proceedings of the National Academy of Sciences* **103**, 14713–14717.

Yeruham, I., Rosen, S., Hadani, A. and Nyska, A. (1996). Sarcoptic mange in wild ruminants in zoological gardens in Israel. *Journal of Wildlife Diseases* **32**, 57–61.

Zahler, M., Essig, A., Gothe, R. and Rinder, H. (1999). Molecular analyses suggest monospecificity of the genus Sarcoptes (Acari: Sarcoptidae). *International Journal for Parasitology* **29**, 759–766. doi: 10.1016/S0020-7519(99)00034-X.

Zhang, Z.-Q. (2011). *Animal biodiversity: An outline of higher-level classification and survey of taxonomic richness*. Magnolia press.

Zhao, Y. E., Cao, Z. G., Cheng, J., Hu, L., Ma, J. X., Yang, Y. J., Wang, X. P., Zeng, J. H. and Wang, T. P. (2015). Population identification of Sarcoptes hominis and Sarcoptes canis in China using DNA sequences. *Parasitology Research* **114**, 1001–1010. doi: 10.1007/s00436-014-4266-1.

Zimmerman, J. J., Karriker, L. A., Ramirez, A., Schwartz, K. J., Stevenson, G. W. and Zhang,

J. (2019). *Diseases of swine*, 11th Edn. ed. Zimmerman, J. J., Karriker, L. A., Ramirez, A., Schwartz, K. J., Stevenson, G. W., and Zhang, J. Wiley Blackwell, Hoboken, USA.