Universitat Autònoma de Barcelona

Pathophysiology of sarcoptic mange in Iberian ibex

Arián Ráez Bravo

Universitat Autònoma de Barcelona

Pathophysiology of sarcoptic mange in Iberian ibex

Autora

Arián Ráez Bravo

Director y tutor

Dr. Jorge Ramón López Olvera

Directores

Dr. José Enrique Granados Torres

Dr. Emmanuel Serrano Ferron

Tesis doctoral

Programa de doctorat Medicina i Sanitat Animals

Departament de Medicina i Cirurgia Animals

Facultat de Veterinària

Universitat Autònoma de Barcelona

2019

SEFaS

Wildlife Ecology & Health

The Consejería de Medio Ambiente y Ordenación del Territorio of the Junta de Andalucía supported sample collection and conservation through the projects 676/2006/A/00, 1571/2007/M/00, 173/2009/M/00 and 861/2011/M/00, as did the PAIDI Research Group RNM18 from the Junta de Andalucía. The Ministerio de Economía y Competitividad of the Spanish Government funded the studies of this thesis through the research projects CGL2012-40043-C02-02 and CGL2016-80543-P.

Els doctors **Jorge Ramón López Olvera i Emmanuel Serrano Ferron**, Professor Titular d'Universitat i Investigador Ramón i Cajal, respectivament, del Departament de Medicina i Cirurgia Animals de la Universitat Autònoma de Barcelona i adscrits al *Wildlife Ecopathology and Health Group*; i **José Enrique Granados Torres**, Investigador del Grup d'Investigació *Especies Cinegéticas y Plagas* (RNM-118), adscrit al Parc Nacional de Sierra Nevada,

Informen:

Que la memòria titulada "**Pathophysiology of sarcoptic mange in Iberian ibex**", presentada per **Arián Ráez Bravo** per a la obtenció del grau de Doctora en Medicina i Sanitat Animals per la Universitat Autònoma de Barcelona, s'ha realitzat sota la nostra direcció i, un cop considerada satisfactòriament finalitzada, autoritzem la seva presentació per tal que sigui avaluada per la comissió corresponent.

I perquè així consti als efectes oportuns, signem el present informe a Bellaterra, a 20 de setembre de 2019.

Jorge Ramón López Olvera

Director i tutor

José Enrique Granados Torres

Director

Emmanuel Serrano Ferron

Director

Arián Ráez Bravo

Doctoranda

ACKNOWLEDGEMENTS, AGRADECIMIENTOS

Después de tanto tiempo y situaciones vividas desde que me embarqué en esta aventura, sois muchas las personas con las que he tenido la suerte de compartir muchos momentos y que, de una forma u otra, habéis ayudado a que finalmente pueda cerrar esta etapa de mi vida. No ha sido fácil, pero aquí estamos. Con sólo estas palabras no podré ni acercarme a explicar todo lo que habéis significado, pero espero que sirvan para agradeceros, al menos un poquito, todo vuestro apoyo.

En primer lugar, quisiera dar las gracias a mis tres directores de tesis. Cada uno de vosotros, a vuestra manera, habéis dado lo mejor para que esta tesis pueda haberse realizado. A Jorge, por todo el apoyo que me has dado a lo largo de estos años. Por enseñarme a pensar y a entender qué es la ciencia, y por creer en que podía conseguirlo, incluso cuando yo no lo hacía. He tenido la suerte de poder contar siempre con tu ayuda y consejos en el plano profesional y en el personal. A José Enrique, el artífice de que empezara el doctorado. Sin ti, no estaría hoy aquí. Gracias por haber confiado en mí al inicio de este proyecto, por todos los días de trabajo incansable en campo, y haberme dado la oportunidad de conocer de cerca la gestión de la cabra montés y enamorarme de esta especie y de Sierra Nevada. Y, por último, a Emmanuel. Aunque te uniste al equipo de esta tesis un poco después, he podido contar con tu apoyo y buena energía, sobre todo en el último empujón.

A todas las personas que forman parte del SEFaS. En primer lugar, quiero dar las gracias a Santiago, por haber creado este grupo, del cual he tenido suerte de formar parte. A Gregorio, gracias por hacerme sentir siempre como en casa. A Roser, por ser como eres, por entenderme y los consejos que me has dado para ayudarme en algunas situaciones. Me hubiese gustado tener más tiempo para poder aprender contigo. A Oscar, por tu humor y por siempre tener unas palabras de ánimo.

A todos los amigos y compañeros de doctorado, que habéis hecho todo mucho más sencillo. A Raquel. Me alegro mucho de haber podido llegar a este momento contigo, no habría mejor compañera. Gracias por ayudarme siempre, por las risas, por las charlas de parking, de ferrocarril y de despacho. Sin ti, el tiempo en el SEFaS hubiese sido mucho más gris. A Johan, por tus ánimos y por tu apoyo. Gracias por siempre estar dispuesto a pasar un buen rato entre amigos y juegos delante de una cerveza. A Marta. Lo mejor de esta segunda etapa en el SEFaS ha sido conocerte. Gracias por ser como eres y por siempre estar dispuesta a echar una mano, incluso con la portada de esta tesis. A Carles (y a Karma), vecino de mesa, por tu alegría y los

ánimos que siempre das, y a Carlos, por las risas y las charlas compartidas durante todo este tiempo. Finalmente, a Xavi, Diana y Andreu. Gracias por los buenos ratos vividos. Me hubiese gustado poder estar en las lecturas de vuestras tesis.

A todas las personas que han pasado por el despacho de "Sefitos" a lo largo de este tiempo. A Lucía, Federica, María, Andrea, Irene, Josep, Carme, Mattia, Eugenia, Wendy, Sara, Cristina, Antonio, Elena, Sara y Marta, por todo lo vivido con cada uno de vosotros. Y a Genís, Claudia, Omar, Olivia y Charlotte, los últimos en llegar, por tantos buenos ratos.

Al equipo de Sierra Nevada, Isidro, Pepe, Elías, Antonio, Paco y Manuela. Gracias por vuestra ayuda y los buenos momentos que hemos pasado. Sin vosotros, esto no hubiera sido posible. También a Manolo, por cuidar de las cabras.

A José, compañero de batallas. Gracias por haber sufrido conmigo lo bueno y lo no tan bueno de la infestación.

A Javier, Ramón, Paulino y Jesús. Gracias por haber compartido vuestra experiencia conmigo, por vuestra ayuda y vuestro apoyo.

A los miembros de LETI, en especial a Rubén. Gracias por tu ayuda para desarrollar el ELISA.

A Lara y Magda por vuestra paciencia para ayudarme a entender el análisis genómico.

A Andreea, por todos los buenos ratos que hemos pasado y siempre estar cuando se te necesita. La vida en Sant Quirze, sin duda, no hubiese sido igual.

A mis amigos, en especial a Cris, Belén, Alba, Pablo, Tere, Adri, Manuel y Vero. Aunque durante estos últimos años no nos hayamos visto ni hablado todo lo que me hubiese gustado, sé que siempre estáis ahí.

A mis abuelos, por todo lo han significado en mi vida, y que finalmente no han podido verme terminar esta tesis.

A mi familia. Mamá, Papá y Clara, por estar ahí siempre. Gracias por soportarme en los peores momentos y por ayudarme cada vez que lo he necesitado. Soy quien soy gracias a vosotros.

Y, por último, a Apolo. Gracias por haber creído siempre en mí. Por todo el tiempo y esfuerzo que has invertido en ayudarme, desde tu trabajo en el campo, en la infestación, por las llamadas mientras conducía cuando era ya tarde y por las conversaciones eternas simplemente escuchándome. Parte de esta tesis también es tuya.

TABLE OF CONTENTS

1. Summary of the thesis

1.1. Summary

Sarcoptic mange is a parasitic skin disease caused by the burrowing mite *Sarcoptes scabiei*. It affects mammals worldwide, including humans. Sarcoptic mange in wildlife is considered an emerging disease, and can cause severe population declines. Iberian ibex (*Capra pyrenaica*) is a medium-sized mountain ungulate endemic to the Iberian Peninsula. Since the end of the '80s, the Iberian Ibex populations of Southern and Eastern Spain have been affected by mange, suffering variables mortalities reported to reach up to 90%. Most of the studies on sarcoptic mange in Iberian ibex have focused on the epidemiology and the population consequences of the diseases, thus existing a lack of knowledge about the pathophysiology and pathogenesis of this disease in this species.

The two first studies of this thesis analysed the acute phase proteins (APP) (**Study I**) and validated a test for the detection of immunoglobulins G (IgG) against *S. scabiei* (**Study II**) in free-ranging Iberian ibexes, both healthy and affected by sarcoptic mange. In the **Study I**, an increase of serum amyloid protein type A (SAA) and in lower magnitude of alpha-1 acid glicoprotein (AGP) concentrations was observed, in correlation with the extent of the skin lesions caused by sarcoptic mange. Conversely, haptoglobin (Hp) concentration was not different between the healthy and infested ibexes. Since there is not an effective laboratory diagnostic method, in the **Study II** three enzyme-linked immunosorbent assays (ELISA) were evaluated for IgG detection against *S. scabiei* in Iberian ibex, and one of the three showed high specificity and sensitivity by using the avidin-biotin system, which allowed it to be validated.

The **Studies III and IV** were carried out on Iberian ibexes with different alleles of the DRB1 gen of the major histocompatibility complex (MHC) class II, experimentally infested with *S. scabiei*. Although all the infested ibexes developed lesions compatible with sarcoptic mange, the clinical evolution varied from extensive lesions affecting most of the body surface to mild lesions and clinical recovering of the disease (**Study III**). However, such clinical differences seemed unrelated to MHC differences. The severely affected ibexes showed anaemia, possibly related to the inflammation caused by the mite, as well as neutrophilia and lymphopenia, probably due to secondary infections favoured by sarcoptic mange. Immunoglobulin G concentration also increased in agreement with the severity of the lesions. Finally, the **Study IV** addressed the genomic response of Iberian ibexes to the experimental infestation with *S. scabiei*. The severely affected Iberian ibexes showed an increase in the gene expression of

pathways related to immunity and inflammation, agreeing with the exacerbated and non-effective generalized immune response induced by the mite and the response to secondary infections. Moreover, the Iberian ibexes that recovered showed an increase in the local skin expression of genes related with antigen presentation and T-lymphocytes activation.

To summarize, sarcoptic mange induces both systemic and local changes in the Iberian Ibex, causing an increase in APP and antibodies, as well as haematological and local and systemic gene expression disorders. Although the causes of the differences found in the clinical evolution have not been completely elucidated, local skin cellular immunity may be key in controlling the infestation. Immunoglobulin G detection by ELISA can be a useful and effective diagnostic tool for sarcoptic mange in Iberian Ibex, while APP are a prognostic indicator.

1.2. Resumen

La sarna sarcóptica es una enfermedad parasitaria causada por el ácaro *Sarcoptes scabiei*. Afecta a mamíferos de todo el mundo, incluyendo al ser humano. En la fauna salvaje se considera una enfermedad emergente, pudiendo causar graves consecuencias poblacionales. La cabra montés (*Capra pyrenaica*) es un ungulado de montaña endémico de la Península Ibérica. Desde finales de los años 80, las poblaciones de cabra montés del sur y del este peninsular se han visto afectadas por esta parasitosis, con mortalidades variables que han llegado a superar el 90%. La mayoría de los estudios sobre la sarna en la cabra montés se han centrado en la epidemiología y los efectos poblacionales, por lo que no se conoce totalmente la fisiopatología y la patogenia de esta enfermedad en esta especie.

En los dos primeros estudios de esta tesis, se analizaron las proteínas de fase aguda (PFA) (**Estudio I**) y se validó una prueba para la detección de inmunoglobulinas G (IgG) frente *S. scabiei* (**Estudio II**) en cabras monteses en libertad, tanto sanas como afectadas por sarna sarcóptica. En el **Estudio I**, se observó el aumento de las concentraciones de la proteína amiloide sérica tipo A (SAA) y de la alfa-1 glicoproteína ácida (AGP), aunque en menor medida, en función de la extensión de las lesiones causadas por la sarna sarcóptica. Por el contrario, la concentración de haptoglobina (Hp) no fue diferente entre las cabras monteses sanas y las infestadas. Debido a la falta de un método diagnóstico de laboratorio efectivo, en el **Estudio II** se evaluaron tres ensayos por inmunoabsorción ligada a enzimas (ELISA) para detectar IgG frente a *S. scabiei* en cabra montés, validando uno de los tres ELISA que mostró una elevada especificidad y sensibilidad, al emplear el sistema de avidina-biotina.

Los **Estudios III y IV** se llevaron a cabo con cabras monteses que presentaban diferentes alelos del gen DRB1 del complejo mayor de histocompatibilidad (MHC) clase II, infestadas experimentalmente con *S. scabiei*. Aunque todas las cabras monteses infestadas desarrollaron lesiones compatibles con sarna sarcóptica, la evolución clínica varió desde lesiones extensas que afectaron la mayor parte de la superficie corporal hasta lesiones leves y recuperación clínica de la enfermedad (**Estudio III**). Sin embargo, estas diferencias clínicas no parecieron estar relacionadas con diferencias en el MHC. En las cabras monteses que desarrollaron cuadros clínicos graves de sarna se observó anemia, posiblemente relacionada con la inflamación causada por el ácaro, así como neutrofilia y linfopenia, probablemente debidas a las infecciones secundarias facilitadas por la sarna sarcóptica. La concentración de IgG también

aumentó en función de la gravedad de las lesiones. Finalmente, en el **Estudio IV** se estudió la respuesta genómica de las cabras monteses frente a la infestación experimental con *S. scabiei*. En las cabras monteses con cuadros clínicos graves se observó un aumento de la expresión génica de vías relacionadas con la inmunidad y la inflamación, reflejo de la respuesta inmune generalizada, exacerbada e ineficaz inducida por el ácaro y de la respuesta frente las infecciones secundarias. Asimismo, las cabras monteses que se recuperaron mostraron un aumento de la expresión de genes relacionados con la presentación de antígeno y de activación de linfocitos T en la piel.

Como resumen, la sarna sarcóptica produce cambios tanto sistémicos como locales, causando un aumento de PFA y anticuerpos, así como alteraciones hematológicas y en la expresión génica local y sistémica. Aunque las causas de las diferencias encontradas en la evolución clínica no han podido ser completamente dilucidadas, la inmunidad celular local cutánea puede ser clave en el control de la infestación. La detección de IgG mediante ELISA puede ser útil como método diagnóstico efectivo de la sarna sarcóptica en cabra montés, mientras que las PFA son un indicador pronóstico.

2. Introduction

2.1. Iberian ibex

2.1.1. Taxonomy and distribution

Iberian ibex (*Capra pyrenaica*) is a medium size mountain ungulate endemic to the Iberian Peninsula, although it has recently been reintroduced in the northern side of the Pyrenees (Crampe, Sourp, & Cavailhes, 2015). Four subspecies were originally described based on morphological differences (Cabrera, 1911):

- *C. p. hispanica*
- *C. p. lusitanica*
- *C. p. pyrenaica*
- *C. p. victoriae*

Although this classification is still being questioned, it is the currently accepted by the International Union for the Conservation of Nature and Natural resources (Shackleton & IUCN/SSC Caprinae Specialist Group, 1997). However, genetic studies based on mitochondrial DNA analysis of different Iberian ibex populations did not support the recognition of the subspecies *C. p. victoriae* and *C. p. hispanica*, but found differences with *C. p. pyrenaica* (Manceau, Crampe, Boursot, & Taberlet, 1999). Incomplete linage sorting or hybridization between *C. p. victoriae* and *C. p. hispanica* before this study could have blurred the adaptive differences between both subspecies (Putman, Apollonio, & Andersen, 2011).

Currently, two of the four subspecies are extinct: *C. p. lusitanica*, which was located in northern Portugal and southern Galicia, become extinct in 1980; and *C. p. pyrenaica*, which occupied the Pyrenees and died out in 2000, when the last known female was found dead (Alados & Escós, 2012; Pérez et al., 2002). *Capra p. victoriae* occupies mainly the central mountains of the Iberian Peninsula, but also there are some small populations in the Cantabrian Mountains and Galicia, and in the Peneda-Gerês National Park located in Portugal (Moço et al., 2006). This is also the subspecies reintroduced in the French Pyrenees (Crampe et al., 2015). *Capra p. hispani*ca is the most widespread subspecies, distributed throughout the South and East of the Iberian Peninsula (Figure 2.1).

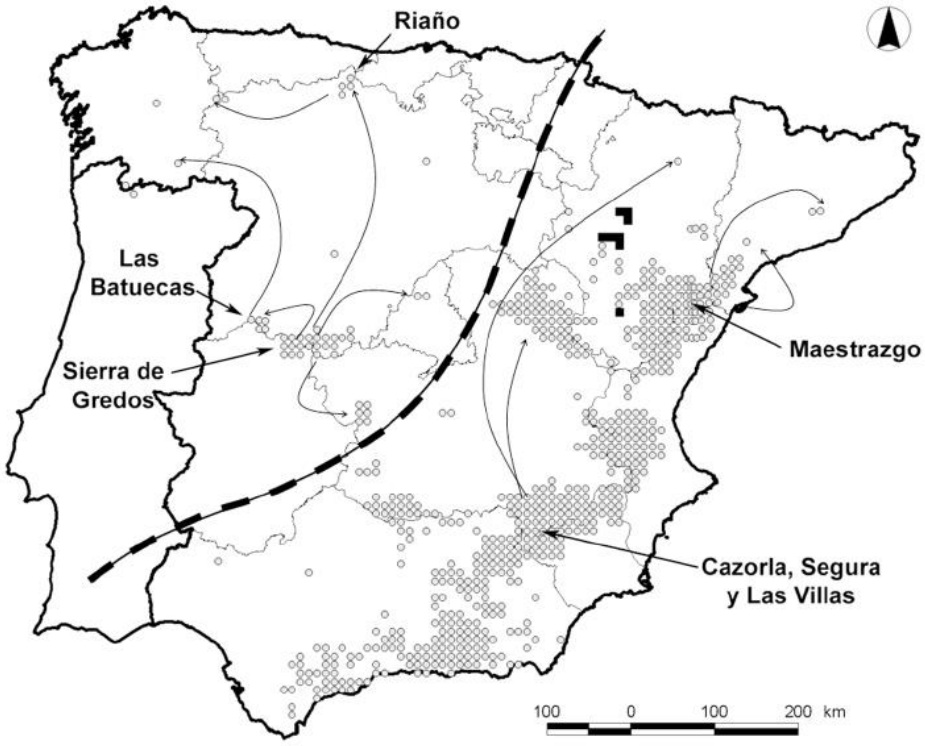

Figure 2.1. Distribution of the two extant subspecies of Iberian ibex: *Capra pyrenaica victoriae* (left to the line) and *Capra pyrenaica hispani*ca (right to the line) (Acevedo & Cassinello, 2009).

2.1.2. Biology

The Iberian Ibex shows a pronounced sexual dimorphism (Figure 2.2). Males present larger horns and greater size and weight than females. Moreover, coat colour varies in adult males, increasing the proportion of black over time from the age of four years (Fandos, 1991). Horn growth continues throughout the whole ibex life. Annual horn growth is influenced by the previous year horn growth, environmental factors, and mainly by age, so it is used for age determination (Fandos, 1991, 1995). Age can also be determined by deciduous dental eruption and growth marks in teeth (Fandos, 1991).

Like other ungulates, particularly those species with pronounced sexual size dimorphism, Iberian ibex males and females are segregated for most of the year, with adult male groups and mixed groups of females and young males, which differ in their habitat use (Acevedo & Cassinello, 2009; Alados & Escós, 1996; Granados, Pérez, et al., 2001). Thus, only during the rutting season, towards late autumn and early winter, both type of groups gather together (Granados, Pérez, et al., 2001).

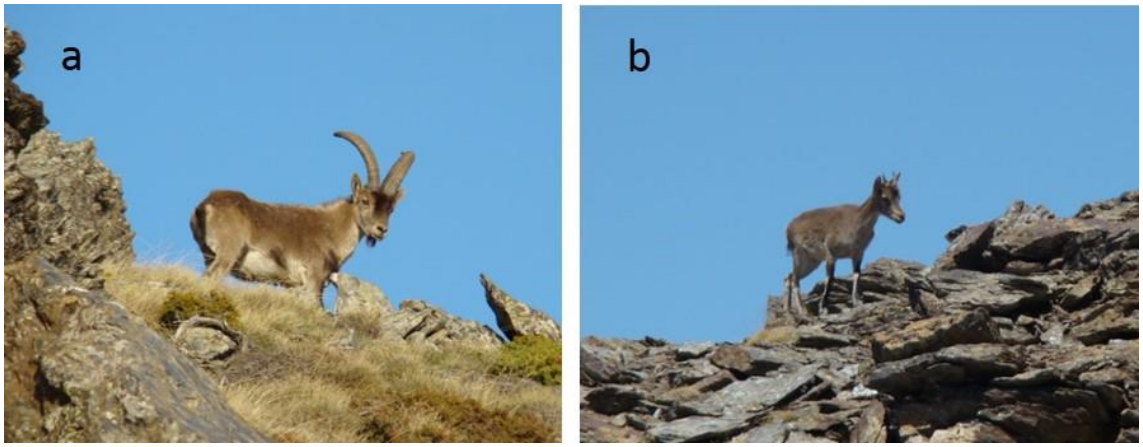

Figure 2.2. Iberian ibex male (a) and female (b).

2.1.3. Conservation threats

Iberian ibex distribution range is currently expanding and the population increasing and, therefore, it is not currently an endangered species (Herrero & Pérez, 2008). Nevertheless, Iberian ibex populations are exposed to conservation threats that may cause problems in the future, such as hybridisation between wild and domestic goat (Alasaad, Fickel, et al., 2012), human-caused habitat fragmentation, low genetic diversity, competition with domestic and others wild ungulates, selective hunting, inadequate management, and diseases, particularly sarcoptic mange (Herrero & Pérez, 2008; Pérez et al., 2002).

2.2. Sarcoptic mange

2.2.1. Aetiology and epidemiology

Sarcoptic mange is a contagious skin disease caused by the submacroscopic burrowing mite *Sarcoptes scabiei*. Worldwide distributed, sarcoptic mange has been reported in different domestic and wild mammal species, including humans. As a zoonosis, sarcoptic mange is more prevalent in developing countries, associated with poverty and overcrowded conditions (Romani, Steer, Whitfeld, & Kaldor, 2015; Walton, Holt, Currie, & Kemp, 2004). In livestock, it

can cause economic losses, but there are effective treatments available (Rehbein et al., 2003; Walton et al., 2004).

Adult females create tunnels in the epidermis in the *stratum granulosum*, where they lay two or three eggs per day for four to six weeks (Bornstein, Mörner, & Samuel, 2001; Walton & Currie, 2007), although less than 1% develop into adults (Mellanby, 1944). Development from egg to adult takes two weeks approximately, going through different life-phase stages: hexapodal larva, protonymph and tritonynmph (Arlian & Vyszenski-Moher, 1988; Bornstein et al., 2001) (Figure 2.3).

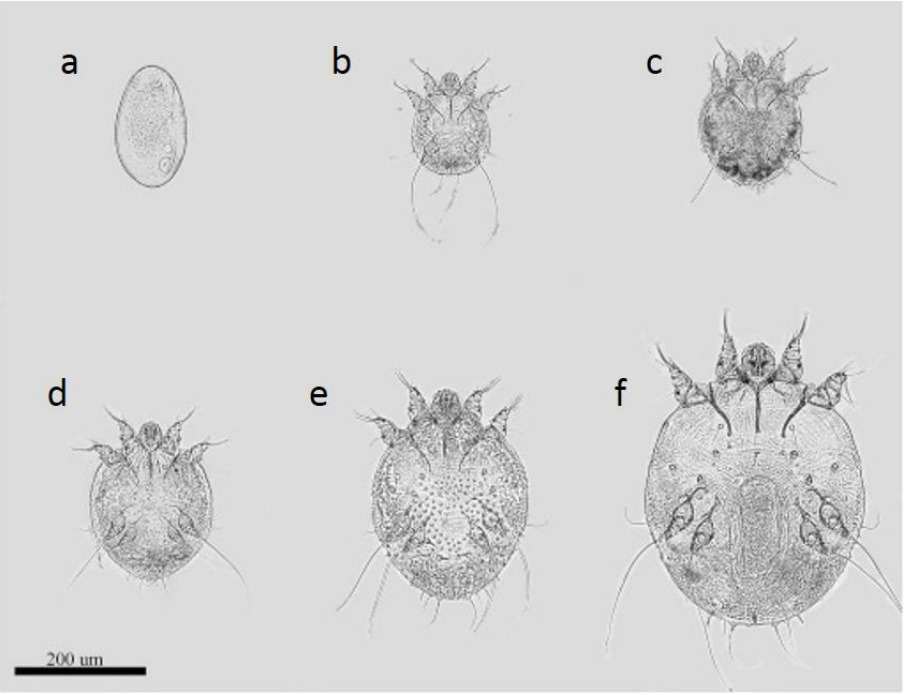

Figure 2.3. Life stages of *Sarcoptes scabiei*: egg (a), larva (b), protonymph (c), tritonymph (d), adult male (e), adult female (f) (Niedringhaus, Brown, Sweeley, & Yabsley, 2019).

Away from the host, *S. scabiei* can survive and remain capable of infestation for at least 24 hours at 20ºC and 25% relative humidity. Lower temperature (10-15ºC) and higher humidity prolong mite survival in all the stages (Arlian, Vyszenski-Moher, & Pole, 1989). Although transmission is mainly by close skin-to-skin contact, the mite capacity of surviving away from the host indicate that indirect transmission could also play a role in the spread of mites.

Different authors have discussed about the taxonomy of *S. scabiei*, but most support a single species with host-specific varieties. Mites from different host species are in general morphologically indistinguishable (Fain, 1968; Pence, Casto, & Samuel, 1975), but show a high degree of host specificity and low degree of cross infectivity (Arlian, Runyan, & Estes, 1984;

Fain, 1978; Pence & Ueckermann, 2002). Genetic studies also support that *S. scabiei* is only one species (Zahler, Essig, Gothe, & Rinder, 1999) with differences among mites from different host species (Rasero et al., 2010; Walton et al., 1999) and from different localizations (Berrilli, D'Amelio, & Rossi, 2002). Nevertheless, cross-transmission between different host species, including humans, has been demonstrated, although the disease is mostly self-limiting (Arlian et al., 1984; Mitra, Mahanta, Sen, Ghosh, & Hati, 1993; Morsy, Bakr, Ahmed, & Kotb, 1994; Rentería-Solís et al., 2014). Moreover, cross-infestation from prey to predator has been described, indicating that predation may be a factor for transmission of *S. scabiei* (Gakuya et al., 2011; Oleaga et al., 2011b).

2.2.2. Clinical signs and pathogenesis

The incubation period depends on host and mite load of exposure, ranging from six days in dogs experimentally infested to a month in other species (Bornstein, 1991; Bornstein & Zakrisson, 1993a; Bornstein, Zakrisson, & Thebo, 1995a; Mörner & Christensson, 1984). In humans, clinical presentation takes place four to six weeks after a primary infestation (Walton & Currie, 2007).

Clinical signs vary, depending on host species and immunological state (Pence & Ueckermann, 2002). General signs include intense pruritus, seborrhoea, erythematous eruptions, papule formation, and alopecia (Bornstein et al., 2001). In more severe cases, hyperkeratosis, thickening of the skin, fissuring and crusting are observed (Figure 2.4), allowing secondary infections to take hold (Nakagawa et al., 2009; Niedringhaus et al., 2019; Swe, Zakrzewski, Kelly, Krause, & Fischer, 2014).

Figure 2.4. Skin lesions of sarcoptic mange in an Iberian ibex.

In humans, two different presentations are reported: ordinary scabies and crusted (or Norwegian) scabies. Ordinary scabies is the most common form, and is characterised by a low number of mites and itching with localised pruritic papules (Bhat, Mounsey, Liu, & Walton, 2017; Walton & Currie, 2007). In crusted scabies, the mite load is higher and, therefore, it is considered more infectious (Walton & Currie, 2007), with the subsequent inflammatory and hyperkeratotic reaction (Roberts, Huffam, Walton, & Currie, 2005). Immunosuppression is considered a predisposing factor in crusted scabies, appearing in patients with immunodeficiency virus (HIV) infection (Drabick, Lupton, & Tompkins, 1987; Hulbert & Larsen, 1992), leukaemia (Suzumiya, Sumiyoshi, Kuroki, & Inoue, 1985), human T-lymphotropic virus type 1 (HTLV-1) infection (Einsiedel, Pepperill, & Wilson, 2014) and in patients undergoing organ transplantation (Paterson, Allen, & Beveridge, 1973; Sampathkumar, Mahaldar, Ramakrishnan, & Prabahar, 2010). Likewise, crusted scabies has also been noticed in patients with Down syndrome (Lee, Heresi, & Al Hammoud, 2019; Nagsuk, Moore, & Lopez, 2015) and in Aboriginal Australians (Gogna, Lee, & Howe, 1985; Roberts et al., 2005).

Apart from skin lesions, *S. scabiei* infestation has also systemic effects in the host, inducing changes in haematological and blood biochemical parameters (De & Dey, 2010; Kido et al., 2011; Pérez et al., 2015; Pérez, Granados, González, Ruiz-Martínez, & Soriguer, 1999; Saleh, Mahran, & Bassam Al-Salahy, 2011), oxidative stress status (De & Dey, 2010; Espinosa, Pérez, et al., 2017; Saleh et al., 2011), weight loss and body condition (Newman, Baker, & Harris, 2002; Ruykys, Taggart, Breed, & Schultz, 2009; Skerratt, 2003b) or even septicaemia (Nakagawa et al., 2009). Moreover, behavioural changes in the hosts were also described, since infested animals spend more time scratching, resulting in shorter resting time (Martin et al., 2018; Overskaug, 1994; Simpson, Johnson, & Carver, 2016; Skerratt, 2003b).

2.2.3. Immunology

The immune response to *S. scabiei* is still poorly understood. A large part of the pathogenesis of sarcoptic mange and the subsequent clinical course is a manifestation of hypersensitivity to the mites and their excretions and secretions, that increase the pruritus, and therefore, the hyperkeratosis, the alopecia, the inflammation and the self-inflicted injuries (Pence & Ueckermann, 2002). An imbalance between Th1/Th2 responses has been observed in severely affected hosts, with a pronounced Th2 allergic response and a low Th1 response (Mounsey et al., 2015; Walton et al., 2010). At local level in the skin, a predomination of cytotoxic T cells and a lack of B cells have been detected, resulting in the exacerbated dermal inflammatory

response (Bhat et al., 2017). Systemically, *S. scabiei* infestation results in both humoral and cellular immune responses. The host exhibits an increase of immunoglobulin G (IgG) and immunoglobulin E (IgE) concentrations, with differences among species and between primary and secondary infestations (Bhat et al., 2017; Rodríguez-Cadenas, Carbajal-González, Fregeneda-Grandes, Aller-Gancedo, & Rojo-Vázquez, 2010; Walton, 2010). Moreover, the immune and inflammatory systemic responses include the increase of white blood cells and inflammatory cytokines (Bhat et al., 2017). Other studies (Bernal et al., 2011; Rahman, Lecchi, Fraquelli, Sartorelli, & Ceciliani, 2010) observed that *S. scabiei* also caused the rise of some acute phase proteins (APPs). Overall, there are still gaps in the knowledge of the pathogenesis of sarcoptic mange.

Acute phase proteins are serum proteins whose concentration change as a response to inflammation, infection, trauma, neoplasia or stress (Murata, Shimada, & Yoshioka, 2004). Their production is controlled by cytokines released from the site of pathogenic or inflammatory damage, being the liver the main site of synthesis (Eckersall, 2008; Petersen, Nielsen, & Heegaard, 2004). Acute phase proteins are part of the innate immune response, and are aimed at the defence of the animal against the pathological damage and the restoration of homeostasis (Ceciliani, Ceron, Eckersall, & Sauerwein, 2012; Eckersall, 2008). Therefore, the quantification of these proteins can provide diagnostic and prognostic information, useful for monitoring disease evolution and treatment (Eckersall, 2000; Eckersall & Bell, 2010; Horadagoda et al., 1999; Murata et al., 2004; Petersen et al., 2004).

2.2.4. Diagnosis

Clinical signs can be used for diagnosis of sarcoptic mange, although they can appear similar to other skin diseases. Visual diagnosis of sarcoptic mange is the reference diagnostic method in the field (Arenas et al., 2002; Carvalho et al., 2015). However, the specificity of this method (60.71%) is lower than its sensitivity (87.14%), and the diagnosis must usually be confirmed of refused using laboratory tests (Valldeperes et al., 2019). Currently, different laboratory diagnostic techniques exist, but they are not completely efficient (Chandler & Fuller, 2019; Walton & Currie, 2007). The direct microscopical detection of mites, eggs, exuviae or faeces in skin scrapings or in skin biopsies is one hundred per cent specific, but presents a low sensitivity with low mite densities (Walter et al., 2011; Walton & Currie, 2007). In humans, some noninvasive techniques have been used such as videodermatoscopy, videomicroscope, dermatoscopy, reflectance confocal microscopy and optical coherence tomography (Micali,

Lacarrubba, Verzì, Chosidow, & Schwartz, 2016), although they are not been used in animals yet. Other diagnosis methods that have been used include the adhesive tape test (Katsumata & Katsumata, 2006; Walter et al., 2011), serological methods (Jayaraj et al., 2011; Rampton et al., 2013), polymerase chain reaction (PCR) and loop mediated isothermal amplification (LAMP) (Angelone-Alasaad et al., 2015; Fraser et al., 2018; Fukuyama et al., 2010), or even infrared thermography (Arenas et al., 2002; Cross et al., 2016) and trained sarcoptic mange detector dogs (Alasaad, Permunian, et al., 2012) in wildlife.

2.2.5. Sarcoptic mange and wildlife

Sarcoptic mange is considered an emerging wildlife disease (Astorga et al., 2018; Tompkins, Carver, Jones, Krkošek, & Skerratt, 2015) that affects different wild populations around the world, with epidemiological variations among species and areas (Bornstein et al., 2001; Pence & Ueckermann, 2002). Domestic animals are suspected to spread *S. scabiei* to wildlife in most of the cases, being responsible for different outbreaks (Ferroglio, Gortázar, & Vicente, 2011). Affected wild species exist in the five continents. In North America, most infestations are reported in wild canids (Niedringhaus et al., 2019), including wolves (*Canis lupus*) (Almberg, Cross, Dobson, Smith, & Hudson, 2012; Smith & Almberg, 2007), foxes (*Vulpes vulpes*) (Gosselink, Van Deelen, Warner, & Mankin, 2007; Little, Davidson, Howerth, Rakich, & Nettles, 1998) and coyotes (*Canis latrans*) (Brewster, Henke, Hilton, & Ortega-S., 2017; Pence & Windberg, 1994), although other species have also been affected (Fitzgerald, Cooley, Murphy, Cosgrove, & King, 2004; Schmitt, Cooley, Friedrich, & Schillhorn van Veen, 1987). Some other examples are vicuña (*Vicugna vicugna*) (Gómez-Puerta et al., 2013), capybaras (*Hydrochoerus hydrochaeris*) (Bernal et al., 2011), porcupines (*Coendou quichua*) (Gonzalez-Astudillo, Leon-Alvarado, Ossa-Lopez, Rivera-Paez, & Ramírez-Chaves, 2018) or wild canids (Deem et al., 2002; Díaz Luque, Berkunsky, Müller, & González, 2014) in Latin America, and in a wide range of species in Africa (Alasaad, Ndeereh, et al., 2012; Gakuya, Ombui, Maingi, et al., 2012; Hosni & Maghrbi, 2014; Kalema-Zikusoka, Kock, & Macfie, 2002; Zumpt, 1973). In Asia, different ruminants are affected (Chen, Pei, Lai, & Mortenson, 2012; Dagleish, Ali, Powell, Butz, & Woodford, 2007), as well as raccoon dogs (*Nyctereutes procyonoides*) (Ninomiya & Ogata, 2005) and Himalayan lynx (*Lynx lynx isabellinus*) (Hameed, Angelone-Alasaad, Din, Nawaz, & Rossi, 2016). Although sarcoptic mange has been recorded in different native Australia species, it has a great impact in wombats (*Vombatus ursinus*) (Fraser, Charleston, Martin, Polkinghorne, & Carver, 2016; Old, Sengupta, Narayan, & Wolfenden, 2017). In Europe, mange is widespread

in canids (Mörner, 1992; Oleaga et al., 2011), lagomorphs (Millán et al., 2012) and ruminants (Arenas et al., 2002; Fernández-Morán et al., 1997; Rossi, Fraquelli, Vesco, Permunian, Sommavilla, Carmignola, Da Pozzo, et al., 2007) in contrast with North America, where ruminants are barely affected.

Sarcoptic mange in wildlife species is usually characterized by an initial epizootic phase with high morbidity and mortality, followed by an enzootic period with lower prevalence (Astorga et al., 2018; Guberti & Zamboni, 2000; Pence & Windberg, 1994). Although sarcoptic mange in different wildlife species has been successfully treated (Rowe, Carver, & Whiteley, 2019), there are limitations such as the difficulty of capturing the animals and the stress that it can cause for wildlife. Oral drugs have been used in captive wild ruminants (Yeruham, Rosen, Hadani, & Nyska, 1996) and in an American black bear (*Ursus americanus*) (Van-Wick & Hashem, 2019), but further investigations are needed in order to assess the efficacy, the safety (a broad therapeutic margin is needed) and the possible side effects for the environment. For all these reasons, the effect of mange in isolated or in endangered species can be devastating.

In the Iberian Peninsula, sarcoptic mange has affected different wild ungulate species, with different population effects. Mange outbreaks have been described in Cantabrian chamois (*Rupicapra pyrenaica parva*) (Fernández-Morán et al., 1997), Iberian ibex (León-Vizcaíno et al., 1999) and in the introduced barbary sheep (*Ammotragus lervia*) (González-Candela, León-Vizcaíno, & Cubero-Pablo, 2004), significantly reducing their populations. Nevertheless, sarcoptic mange cases have been reported in the Iberian Peninsula in other ungulate species without apparent population impacts, like roe deer (*Capreolus capreolus*) (Oleaga, Balseiro, & Gortázar, 2008) and red deer (*Cervus elaphus*) (Oleaga, Casais, González-Quirós, Prieto, & Gortázar, 2008).

2.2.6. Sarcoptic mange and Iberian ibex

Sarcoptic mange has severely affected most Iberian ibex populations, although differences in morbidity and mortality have been registered among areas. In the late 1980s, mortality rates over 95% were registered in Iberian ibex populations from the Sierra de Cazorla, Segura y las Villas Natural Park (SCSVNP) population (Fandos, 1991; León-Vizcaíno et al., 1999). Since then, Iberian ibexes affected were detected in different Andalusian populations (A.J. Arenas et al., 2002). Among them, in 1992, sarcoptic mange was confirmed in the Sierra Nevada Natural and National Parks (SNNNP), although the mortality observed was lower than in the SCSVNP

(Pérez, Ruiz-Martínez, Granados, Soriguer, & Fandos, 1997). Other Iberian ibex populations affected by sarcoptic mange include those from Muela de Cortes (Valencia), from Murcia and, more recently, from Ports de Tortosa i Beseit (Catalonia) (Valldeperes et al., 2018). In a study performed in Iberian ibexes from the SNNNP with radio-collars, the authors observed ibexes affected with sarcoptic mange clinically recovered (Alasaad et al., 2013), which could explain the lower mortality registered in this population. However, the factors explaining the differences observed in the clinical evolution of sarcoptic mange in the affected ibexes, namely recovering or dead, are still unknown.

In addition, apart from the direct effects and mortality caused (Figure 2.5), sarcoptic mange has negative effects in Iberian ibex reproduction (Espinosa, Granados, et al., 2017; Sarasa et al., 2011), resulting in added negative population effects.

Figure 2.5. Dead male Iberian ibex affected by sarcoptic mange.

Since sarcoptic mange appeared in Iberian ibex populations, both researchers and managers have tried to prevent and control this disease to reduce the impact caused on the species. One of the measures was the creation in 1992 of a controlled stock reservoir in SNNNP that include a healthy and representative Iberian ibex group of the free-ranging population (Espinosa, López-Olvera, et al., 2017; Granados-Torres, Ruiz-Martínez, Pérez-Alvarado, & Pérez-Jiménez, 1996). Moreover, these facilities have been used to perform different scientific studies in order

to extend the knowledge of Iberian ibex (Espinosa, López-Olvera, et al., 2017; Pérez et al., 1999; Ráez-Bravo, Granados, Cano-Manuel, et al., 2016; Sarasa et al., 2009).

The individual clinical evolution of mange and the demographic effects caused in wildlife populations might be related to different extrinsic and intrinsic factors to the host. Extrinsic factors include seasonality (Pérez et al., 1997), parasitic load (Davis & Moon, 1990; Skerratt, 2003b) or genetic of the mite (Berrilli et al., 2002). Overabundance and low food availability also increase animal aggregation and, therefore, the possibility of transmission and loss of body condition (Gortázar, Acevedo, Ruiz-Fons, & Vicente, 2006). Various host-related factors could cause differences in individual host immune and inflammatory responses like sex (López-Olvera et al., 2015), previous exposure to the mite (Arlian, Morgan, Rapp, & Vyszenski-Moher, 1996; Arlian, Morgan, Vyszenskimoher, & Stemmer, 1994; Casais et al., 2014) and genetic variability, in terms of major histocompatibility complex (MHC) alleles (Harf & Sommer, 2005; S. Paterson, Wilson, & Pemberton, 1998; Schad, Ganzhorn, & Sommer, 2005). Although the epidemiological and population effects of sarcoptic mange in Iberian ibex have been extensively reported (Arenas et al., 2002; Fandos, 1991; León-Vizcaíno et al., 1999; Pérez et al., 1997; Sánchez-Isarria, Hermoso, Theureau de la Peña, et al., 2007; Valldeperes et al., 2018), there is little knowledge in the pathophysiology of this disease in this species, as well as the extrinsic and intrinsic factors explaining the observed differences in individual response and population trends.

The MHC is a multigene family that plays a key role in the vertebrate immune system (Bernatchez & Landry, 2003). Low levels of genetic diversity in the class II MHC DRB1 gene in Iberian ibex have been reported (Amills et al., 2004), although a new allele has been recently identified (Angelone et al., 2018). Moreover, the alleles identified differ among the Iberian ibex populations (Angelone et al., 2018) and, therefore, these differences could be related with the differences in the pathogenesis and epidemiology of the sarcoptic mange outbreaks occurred.

Given the importance of this parasitic disease in Iberian Ibex populations and other wild ruminants, in Spain it is legally required to test to discard sarcoptic mange, among other diseases, before translocating wild ruminants (RDL 1082/2009, de 3 de julio). The visual identification of mange compatible lesions is the main method used in the wild, although this method has serious flaws and limitation to diagnose sarcoptic mange in free-ranging Iberian ibexes (Valldeperes et al., 2019). Therefore, there is a need for an efficient and reliable diagnosis method to detect sarcoptic mange in Iberian ibexes as a tool to management.

Despite the multiple studies carried out about sarcoptic mange in Iberian ibexes and in other mammal species, there is still a lack of knowledge about the disease. Therefore, understanding the pathophysiology of sarcoptic mange and identifying the causes of the different evolutions among Iberian ibex populations is necessary to establish the best monitoring and management measures for the conservation of the species.

3. Hypotheses and objectives

More than 30 years after the first outbreak associated to sarcoptic mange in Iberian ibexes, the pathophysiology of sarcoptic mange in the Iberian ibex is mostly unknown. However, the impact of sarcoptic mange on Iberian ibex populations varies. There is a need to understand how the pathophysiology of the disease affects its epidemiology, and consequently, establish a scientific-based management of sarcoptic mange in Iberian ibex populations.

Therefore, the aim of this thesis is to provide more extensive knowledge about sarcoptic mange pathophysiology in Iberian ibex.

The hypotheses of the present thesis are:

1. Acute phase protein concentration are affected by *S. scabiei* infestation and disease severity in Iberian ibex (Study I).

2. An indirect enzyme-linked immunosorbent assay (ELISA) could improve traditional diagnostic methods to detect *S. scabiei* infestation in Iberian ibex (Study II and III).

3. Resistance to sarcoptic mange in Iberian ibex could depend on class II MHC alleles (Study III).

4. Skin local and systemic genomic response to *S. scabiei* infestation may explain differences in susceptibility and clinical outcome in Iberian Ibex (Study IV).

The objectives of this thesis are:

1. To determine the effect of sarcoptic mange in APP concentrations in Iberian ibex (Study I).

2. To develop, validate and evaluate an ELISA to diagnose sarcoptic mange in Iberian ibex (Study II).

3. To determine the effect of class II MHC alleles on the clinical evolution of sarcoptic mange in experimentally infested Iberian ibex (Study III).

4. To describe the pathophysiology of sarcoptic mange in experimentally infested Iberian ibex over time (Study III).

5. To characterize the local and systemic gene expression in Iberian ibexes experimentally infested with *S. scabiei* showing different clinical outcomes (Study IV).

4. Studies

4.1. Study 1

Acute phase proteins increase with sarcoptic mange status and severity in Iberian ibex (*Capra pyrenaica*, Schinz 1838)

Parasitology Research (2015) 114: 4005-4010

DOI: 10.1007/s00436-015-4628-3

4.2. Study 2

Evaluation of three enzyme-linked immunosorbent assays for sarcoptic mange diagnosis and assessment in the Iberian ibex, *Capra pyrenaica*

Parasites & Vectors (2016) 9: 558

4.2.1. Abstract

Sarcoptic mange is a contagious skin disease caused by the mite *Sarcoptes scabiei*, affecting different mammalian species worldwide including the Iberian ibex (*Capra pyrenaica*), in which mortalities over 90% of the population have been reported. No efficient diagnostic methods are available for this disease, particularly when there are low mite numbers and mild or no clinical signs. In this study, three enzyme-linked immunosorbent assays (ELISA) developed for dog (ELISA A), Cantabrian chamois (*Rupicapra pyrenaica parva*) (ELISA B) and Alpine chamois (*Rupicapra rupicapra*) (ELISA C), were evaluated to detect specific antibodies (IgG) to sarcoptic mange in Iberian ibex sera.

Serum samples from 131 Iberian ibexes (86 healthy and 45 scabietic) were collected from 2005 to 2012 in the Sierra Nevada Natural and National Parks (southern Spain). Based on visual inspection, ibexes were classified into one of three categories, namely healthy (without scabietic compatible lesions), mildly affected (skin lesions over less than 50% of the body surface) and severely affected (skin lesions over more than 50% of the body surface). The optimal cut-off point, specificity, sensitivity and the area under the curve (AUC) were calculated, and the agreement between tests was determined. Moreover, differences in the optical density (OD) related to scabies severity have been evaluated for the best test.

ELISA C showed better performance than the two other tests, reaching higher values of sensitivity (93.0%) and specificity (93.5%) against the visual estimation of the percentage of affected skin, chosen as the gold standard. Significantly higher concentrations of specific antibodies were observed with this test in the mildly and severely infested ibexes than in healthy ones.

Our results revealed that ELISA C was an optimal test to diagnose sarcoptic mange in the Iberian ibex. Further studies characterizing immune response during the course of the disease, including spontaneous or drug induced recovery, should follow in order to better understand sarcoptic mange in Iberian ibex populations.

Keywords: *Capra pyrenaica*, Diagnostic, ELISA, *Sarcoptes scabiei*, Serum

4.2.2. Introduction

Sarcoptic mange is a contagious skin disease caused by the mite *S. scabiei* (Linnaeus, 1758). This parasite is worldwide distributed (Bornstein et al., 2001; Pence & Ueckermann, 2002; Walton et al., 2004), causing disease in a wide range of mammalian species (Pence & Ueckermann, 2002). Wild ungulates are one of the most affected groups, as demonstrated by mange outbreak records in African and European ungulates (Gakuya, Ombui, Heukelbach, et al., 2012; Gakuya, Ombui, Maingi, et al., 2012; Rossi, Fraquelli, Vesco, Permunian, Sommavilla, Carmignola, Pozzo, et al., 2007). All wild ruminant species in Spain have also been affected by sarcoptic mange, even causing fatal epizootics, e.g. Iberian ibex (León-Vizcaíno, 1990; León-Vizcaíno et al., 1992, 1999), Cantabrian chamois (Fernández-Morán et al., 1997) and aoudad (González-Candela et al., 2004). The clinical signs and lesions in individuals infected by *S. scabiei* are characterized by pruritus, drying, peeling, alopecia, hyperkeratosis and scab formation. In population terms, this parasite is able to cause severe demographic downturns, up to above 90% on occasion (Fandos, 1991). In fact, stochastic simulations on population extinction have shown that the impact of sarcoptic mange on ungulate populations can be comparable to the impact observed for emerging viral diseases (Serrano et al., 2015).

Iberian ibex is a mountain ungulate endemic to the Iberian Peninsula (Pérez et al., 2002). Andalusia populations (southern Spain) have suffered sarcoptic mange outbreaks since 1987 (León-Vizcaíno et al., 1999), and recently the eastern populations have been also affected by this disease. Different methods exist for the diagnosis of sarcoptic mange, although none of them showed the ideal specificity and sensitivity (Walton & Currie, 2007). Amongst direct diagnostic methods, the gold standard is the microscopical detection of mites, exuviae, eggs or faeces in scrapings of infested skin. Although this method is deemed 100% specific, low sensitivity was found at low mite densities (Walter et al., 2011; Walton & Currie, 2007). Visual diagnosis of scabies compatible lesions has also been used for monitoring the disease in free-ranging ibexes (Pérez et al., 2011), and this disease has been included among those to check before translocating wild ruminants in Spain, visual inspection being the legally mandatory diagnostic method (RDL 1082/2009, de 3 de julio). A number of methods have been developed or proposed in an attempt to overcome such diagnostic deficiency, including the adhesive tape test (Katsumata & Katsumata, 2006; Walter et al., 2011), serological methods (Jayaraj et al., 2011; Rampton et al., 2013), polymerase chain reaction (PCR) (Angelone-Alasaad et al., 2015; Fukuyama et al., 2010), dermoscopy (Dupuy et al., 2007; Park, Kim, & Kim, 2012; Walter et al.,

2011), termography (Arenas et al., 2002), or trained disease-detector dogs (Alasaad, Permunian et al., 2012).

Sarcoptes scabiei stimulate E and G immunoglobulin production in infested hosts (Arlian, Morgan, Rapp, et al., 1996; Arlian et al., 1994; Bornstein & Zakrisson, 1993b; Bornstein, Zakrisson, & Thebo, 1995; Millán et al., 2013; Tarigan, 2004), including Iberian ibex (Lastras et al., 2000; Sarasa et al., 2010). Different commercial enzyme-linked immunosorbent assays (ELISA) have been evaluated to detect specific antibodies to *S. scabiei* in dogs (Bornstein, Thebo, & Zakrisson, 1996; Lower, Medleau, Hnilica, & Bigler, 2001), pigs (Hollanders, Vercruysse, Raes, & Bornstein, 1997; Van Der Heijden, Rambags, Elbers, Van Maanen, & Hunneman, 2000), wild boar (Haas, Rossi, Meier, & Ryser-Degiorgis, 2015) or chamois (Rambozzi, Menzana, Lavín, & Rossi, 2004). However, the use of ELISA tests to diagnose scabies in Iberian ibex has not yet been evaluated.

In this study, three IgG indirect ELISA tests were compared as diagnostic tools of sarcoptic mange in ibexes showing different lesional severity. In particular, the objectives of this study are: (i) to estimate the optimal cut-off points, specificity and sensitivity of the ELISA tests in Iberian ibex; (ii) to determine the agreement between tests; and (iii) to determine if a correlation between mange severity and the detected levels of humoral immune response (IgG) exists.

4.2.3. Materials and methods

Sample collection

Serum samples from 131 healthy and scabietic Iberian ibexes were collected from 2005 to 2012 in SNNNP (36°00'–37°10'N, 2°34'–3°40'W) (Table 4.2.1). The ibexes were chemically immobilized using a combination of ketamine and xylazine (3.0 + 3.0 mg/kg) (Casas-Díaz, Marco, López-Olvera, Mentaberre, & Lavín, 2011). After induction, blood samples were collected from the jugular vein and kept at 4 °C in a cold box. The age of the ibexes was estimated by horn-segment counts (Fandos, 1991), and ranged between one and 12 years.

Table 4.2.1. Number of Iberian ibexes (*Capra pyrenaica*) sampled in the Sierra Nevada Natural and National Parks according to their sex and sarcoptic mange status.

Sarcoptic mange status	Sex		Total
	Males	Females	
Healthy [a]	59	27	86
Mildly infested [b]	31	1	32
Severely infested [c]	9	4	13
Total	99	32	131

[a] Without scabietic compatible lesions
[b] Less than 50% of the body surface affected
[c] More than 50% of the body surface affected

Based on the visual estimation of the percentage of scabietic skin, each individual was classified into one of three categories (González-Quirós & Solano, 2009; Pérez et al., 2011): healthy (although the authors are aware that the absence of scabietic-compatible lesions is not a synonym of "uninfested", for the purpose of this study "healthy" will be used as "without visible scabietic-compatible lesions"), mildly infested (0–50% of the body surface affected by sarcoptic mange), and severely infested (more than 50% of the surface affected) (Table 4.2.2). This diagnostic method was considered as the "gold standard" to evaluate the three ELISA tests.

Table 4.4.2. Number of Iberian ibexes (*Capra pyrenaica*) sampled in the Sierra Nevada Natural and National Parks according the ELISA results and the categories based on the visually estimated percentage of affected skin (Pérez et al., 2011).

		Healthy [a]	Infested		
			Mildly [b]	Severely [c]	Total
ELISA A	Positive serology	9	10	4	14
	Negative serology	76	21	8	29
	Total	**85**	**31**	**12**	**43**
ELISA B	Positive serology	16	13	12	25
	Negative serology	58	17	1	18
	Total	**74**	**30**	**13**	**43**
ELISA C	Positive serology	5	27	13	40
	Negative serology	72	3	0	3
	Total	**77**	**30**	**13**	**43**

[a] Without scabietic compatible lesions
[b] Less than 50% of the body surface affected
[c] More than 50% of the body surface affected

Laboratory analyses

Blood samples were allowed to clot at room temperature. Within 24 hours from collection, serum was obtained by centrifugation at 4,750× *g* for ten minutes and stored at -20 °C until analysis.

The Iberian ibex sera were tested by three indirect ELISAs developed for dog (ELISA A, n = 128) (Puigdemont, Brazís, Fondati, & Ferrer, 2002), Cantabrian chamois (ELISA B, n = 117) (Casais et al., 2007) and Alpine chamois (ELISA C, n = 120) (in-house indirect ELISA, Istituto Zooprofilattico Sperimentale delle Venezie, modified from (Rambozzi, Menzana, et al., 2004)). ELISA A is a commercial ELISA (Univet, Barcelona, Spain) developed for the diagnosis in dog. The plates are coated with *S. scabiei* var. *canis* antigen. To adapt the ELISA A for its use in ibex, specific antibodies for goat (Donkey antigoat IgG-HRP SC-2020, Santa Cruz Biotechnology, Dallas, Texas, USA) were used. ELISA B is based on a structural antigen of the mite (SsλΔB3), whose encoding cDNA was identified by screening of *S. scabiei* var. *hominis* library using the sera from an infected chamois, and expressed in *Escherichia coli* as a unitary recombinant antigen (Casais et al., 2007). ELISA C is an in-house method, developed by the Istituto Zooprofilattico Sperimentale delle Venezie and validated for lung extract. ELISA C is a modification from (Rambozzi, Menzana, et al., 2004) by using commercial ELISA plates coated with *S. scabiei* var. *suis* antigen (Sarcoptes-Elisa 2001® PIG, AFOSA GmbH, Blankenfelde-Mahlow, Germany) instead of the red fox (*Vulpes vulpes*) *S. scabiei* antigens originally used (Rambozzi, Menzana, et al., 2004). This test uses the avidin-biotin detection system, as previously reported (Rambozzi, Menzana, et al., 2004).

Samples were analyzed in duplicate, and in triplicate in ELISA C.

The optical density (OD) was read at 450 nm in ELISA A and B, and at 405 nm in ELISA C, and was expressed as optical density percentage (OD%) using the following formula:

$$OD\% = \frac{(ODS - ODNcon)}{(ODPcon - ODNcon)} \times 100$$

where OD% is the optical density percentage; ODS is the mean optical density (OD) of the sample (two or three replicates); ODNcon is the mean OD of the negative control; and ODPcon is the mean OD of the positive control.

Ten sera from healthy Iberian ibexes (animals without skin lesions) without previous contact with mange were used as negative controls, whereas ten sera from actively sarcoptic mange-

infested Iberian ibexes were used as positive controls. The same negative and positive control sera were used for the three ELISA tests, and all controls consistently showed low (negative controls) and high (positive controls) values for the three ELISA tests.

Statistical analysis

The optimal cut-off points between positive and negative samples was estimated using the Youden index, maximizing the difference between true positive rate (sensitivity) and false positive rate (1 - specificity). Thereby, the maximum of sensitivity and specificity is achieved (Yin & Tian, 2014; Youden, 1950). Once the optimal cut-off points were established and positive and negative individuals assigned, the specificity and sensitivity of the three ELISA tests were then established using the following formulae (Parikh, Mathai, Parikh, Sekhar, & Thomas, 2008):

$$Specificity = \frac{true\ negative\ animals}{true\ negative\ animals + false\ positive\ animals} \times 100$$

$$Sensitivity = \frac{true\ positive\ animals}{true\ positive\ animals + false\ negative\ animals} \times 100$$

After that, the area under a receiver operating characteristic (ROC) curve, a graph of true positive rate (sensitivity) *versus* false positive rate (1 - specificity), was used to determine the diagnosis accuracy of the ELISA tests (Hanley & McNeil, 1982; Zou, O'Malley, & Mauri, 2007).

The agreement between the ELISA tests was evaluated by the Cohen's Kappa coefficient (Cohen, 1960) and Bland-Altman plots (Altman & Bland, 1983; Bland & Altman, 1999). The Cohen's Kappa coefficient ranges between 0 and 1, and values close to 1 indicate high correlation. Bland-Altman plot describes the agreement between two quantitative measurements by constructing limits of agreement, which are calculated by using the mean and the standard deviation of the differences between two measurements. The difference of the two paired measurements is plotted against the mean of the two measurements.

Since ELISA results did not fit a normal distribution, as assayed by Agostino skewness test and Bonett-Seier test for Geary kurtosis, Kruskal-Wallis with Mann-Whitney tests with Bonferroni correction were used to evaluate whether optical density (a proxy for IgG production) was influenced by mange severity (healthy, mildly and severely infested). This analysis was performed only with the optical density values from the ELISA test showing the highest sensitivity and specificity against the gold standard (ELISA C, see below).

Although sex and season determine sarcoptic mange severity and progression in Iberian ibex (Carvalho et al., 2015; López-Olvera et al., 2015; Pérez et al., 2011; Sarasa et al., 2010), the effects of these two factors on ELISA performance could not be evaluated due to insufficient sample size. Statistical analyses were performed using the R software version 3.2.4 (R Development Core Team 2016, The R Foundation, Vienna, Austria). Skewness and kurtosis tests were performed with the "*moments*" package (Komsta & Novomestky, 2015), Bland-Altman regressions with the "*BlandAltmanLeh*" package (Lehnert, 2014), the Kappa coefficients with the "*irr*" package (Gamer, Lemon, & Fellows-Puspendra, 2012), and the ROC-curves and the cut-off points were estimated with the "*OptimalCutpoints*" package (López-Ratón, Rodríguez-Álvarez, Cadarso-Suarez, & Gude-Sampedro, 2014).

4.2.4. Results

The cut-off points, sensitivity and specificity obtained for the three ELISA tests are shown in Table 4.2.3. The best results were obtained with ELISA C (cut-off = 92.0%OD; sensitivity = 93.0%; specificity = 93.5%), followed by ELISA B (cut-off = 10.4% OD; sensitivity = 58.1%; specificity = 78.4%) and ELISA A (cut-off = 45.5% OD; sensitivity = 32.6%; specificity = 89.4%). Consequently, the area under the curve (AUC) obtained for ELISA C was close to 1 (AUC = 0.971), indicating an excellent diagnostic accuracy. Conversely, the AUC for ELISA A and B was lower (0.594 and 0.691, respectively), indicating a poorer diagnosis accuracy of these two tests (Figure 4.2.1, Table 4.2.3). The false negative ibexes (i.e. ibexes with negative ELISA results but showing skin lesions compatible with sarcoptic mange) belonged to the mildly infested category in all cases (100%, 3 out of 3) for ELISA C and most cases for ELISA B (94.4%, 17 out of 18) and ELISA A (72.4%, 21 out of 29) (Table 4.2.2). Furthermore, the three mildly infested false negative cases for ELISA C, 15 out of 17 for ELISA B, and 13 out of 21 for ELISA A had less than 25% of the body surface affected, based on the visually estimated percentage of affected skin (González-Quirós & Solano, 2009; Pérez et al., 2011).

Table 4.2.3. Area under the curve (AUC) and its IC95%, cut-off, sensitivity and specificity values of the ELISA tests.

	ELISA A	**ELISA B**	**ELISA C**
AUC (IC95%)	0.594 (0.488-0.700)	0.691 (0.590-0.793)	0.971 (0.947-0.995)
Cut-off (OD%)	45.5	10.4	92.0
Sensitivity	32.6%	58.1%	93.0%
Specificity	89.4%	78.4%	93.5%

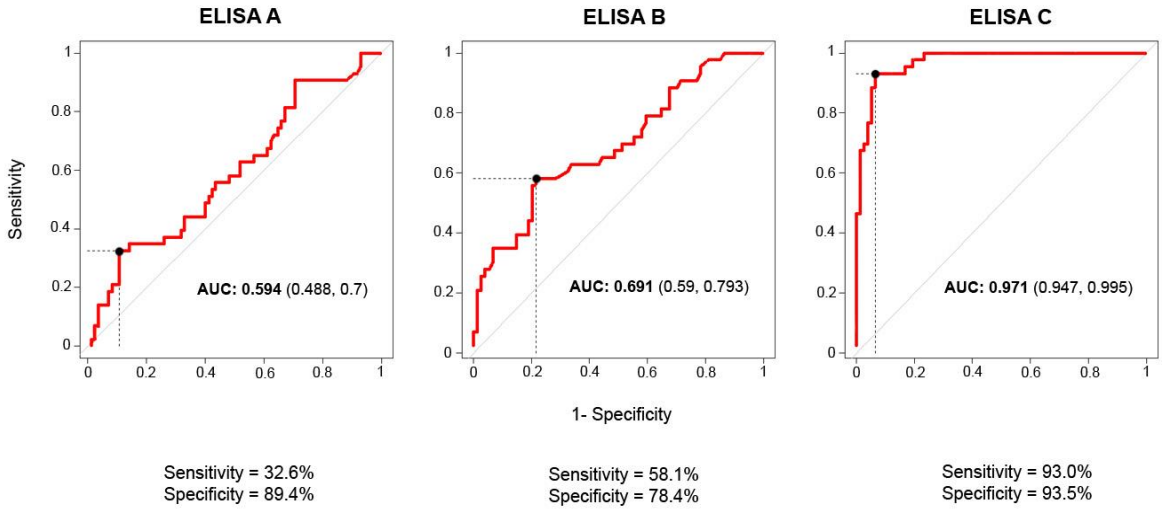

Figure 4.2.1. Receiver operating characteristic (ROC) curves for ELISA A (a), ELISA B (b) and ELISA C (c) for the detection of antibodies against *Sarcoptes scabiei* in Iberian ibex. The red line shows the mean area under the curve (AUC) plot, with the AUC value and the 95% confidence intervals in parentheses. The sensitivity and specificity values correspond to the points in the plots.

Bland-Altman plots show that the three ELISA tests are not interchangeable, especially at high values of OD (Figure 4.2.2). Along the same lines, the Cohen's Kappa coefficient (*k*) indicated that there is not a good strength of agreement between the three tests. The ELISA B and C showed the higher strength of agreement, which was only moderate, whereas the strength of agreement between the ELISA A and C was still lower (fair) and the ELISA A and B showed the lowest strength of agreement (slight) (Landis & Koch, 1977).

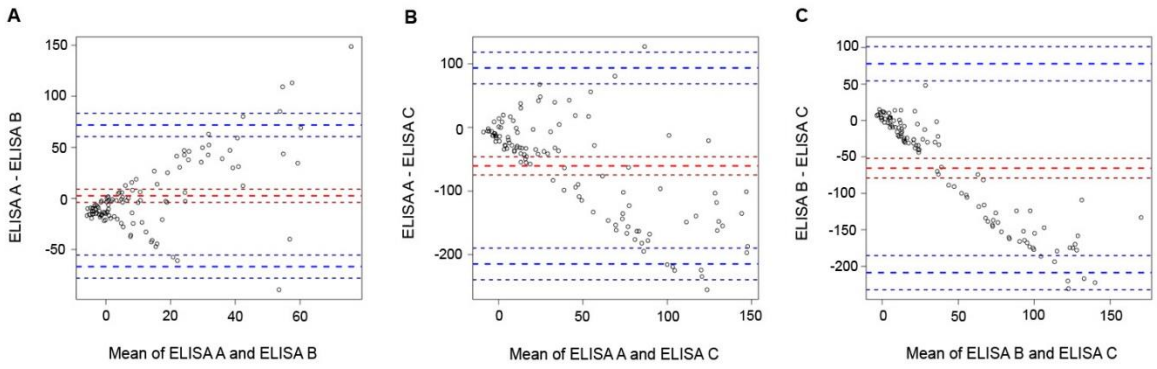

Figure 4.2.2. Bland-Altman plots comparing the ELISA tests. Dashed blue lines indicate the confidence interval limits for the agreement limits (±1.96*standard deviation); dashed red lines indicate the confidence interval limits for the mean bias difference. **(A)** Comparison of results for ELISA A and ELISA B: differences tended to increase with increasing mean OD% values. **(B)** Comparison of results for ELISA A and ELISA C: differences tended to decrease with increasing mean OD% values. **(C)** Comparison of results for ELISA B and ELISA C: differences decreased with increasing mean OD% values.

The relationship between sarcoptic mange severity and the OD% values was analyzed only for ELISA C, due to the better performance of this test as compared with the other two (Table 4.2.4). Significant differences in the OD% (Kruskal-Wallis test: $\chi^2 = 73.99$, $df = 2$, $p < 0.0001$) among the three categories of sarcoptic mange status were found. The healthy ibexes showed statistically significant lower OD% values than the mildly ($W = 90$, $p < 0.0001$) and severely ($W = 6$, $p < 0.0001$) infested ibexes. The difference between the higher OD% of the severely infested ibexes and the lower OD% of the mildly infested ones was close to significance ($W = 106$, $p = 0.058$).

Table 4.2.4. ELISA results (OD%) based on a visually estimated percentage of affected skin (Pérez et al., 2011) in Iberian ibex (*Capra pyrenaica*) sampled in the Sierra Nevada Natural and National Parks.

	Healthy			Mildly infested			Severely infested		
	n	**mean**	**sd**	**n**	**mean**	**sd**	**n**	**mean**	**sd**
ELISA A	85	9.50	28.55	31	19.46	33.49	12	24.28	41.43
ELISA B	74	7.63	9.45	30	13.76	19.61	13	34.71	28.72
ELISA C *	77	47.61 [a]	26.45	30	137.86 [b]	41.59	13	168.16 [b]	25.62

* Kruskal-Wallis test: $\chi^2 = 73.99$; $df = 2$, $p < 0.0001$; [a, b]: means with different superscript are statistically different
Abbreviation: *SD* standard deviation

4.2.5. Discussion

This study compared three ELISA tests for the detection of specific serum antibodies to *S. scabiei* in the Iberian ibex, which showed different performance in terms of specificity and sensitivity. Furthermore, the OD% varied among degrees of sarcoptic mange infestation for the best performing test.

To the best of our knowledge, this is the first time in which several ELISA tests are compared and evaluated for the diagnosis of sarcoptic mange in Iberian ibex. Based on results of this study, ELISA C was the best performing test to detect specific antibodies against *S. scabiei* in this species, showing higher sensitivity and specificity and an AUC close to 1. This test was validated for the detection of IgG in Alpine chamois lung extracts with a sensitivity of 100% and a specificity of 98.6% (data not published). Moreover, the ELISA C allowed the differentiation of healthy and mangy ibexes. ELISA B revealed lower specificity and sensitivity than previously

reported in Cantabrian chamois and red deer, with a specificity of 97 %, and a sensitivity of 100% and 75% in chamois and red deer (Casais et al., 2007; Oleaga, Casais, et al., 2008), respectively. ELISA A had never been used before this study for the detection of antibodies against *S. scabiei* in wild ungulates, and the sensitivity and specificity of the test in dog are 92.1% and 94.6%, respectively.

The avidin-biotin detection system used in the ELISA C could be one of the reasons explaining the best performance of ELISA C as compared to ELISAs B and C. This method has the potential to increase the sensitivity of the indirect ELISA, since avidin has four binding sites for biotin, resulting in an essentially irreversible complex which is stable in later washes and incubations (Kendall, Ionescu-Matiu, & Dreesman, 1983). Although serum cross-reactivity against different *S. scabiei* strains exists, each host species responds differently to different mite varieties (Arlian, Morgan, & Arends, 1996; Haas et al., 2005). Therefore, the differences in sensitivity among the three ELISA tests could also depend on the specific antigens used and the origin of mites.

False negative results (i.e. mangy ibexes in which no antibodies against *S. scabiei* were detected) could be explained by low levels of circulating IgG in recently infested ibexes or in chronic infestations. The clinical signs of sarcoptic mange may appear before IgG detection in different species (Arlian, Morgan, Rapp, et al., 1996; Bornstein et al., 1995; Casais et al., 2014; Tarigan, 2004; Van Der Heijden et al., 2000), and in experimentally infected Iberian ibex, IgG were detected no earlier than 18 to 27 days post infestation (Sarasa et al., 2010). This time lapse may explain at least some of the false negative ibexes within the mildly infested category. On the other hand, maximum OD values indicating specific IgG peaks occur between 50 days and 12–16 weeks post infestation in domestic goats, pigs and Iberian ibexes, to decline slightly or plateauing afterwards (Rampton et al., 2013; Sarasa et al., 2010; Tarigan, 2004). This could explain some false negative results in chronically affected ibexes.

A low test specificity (i.e. positive ELISA results in healthy ibexes) can be explained by a lack of detection of minute skin lesions (Haas et al., 2015; Pérez et al., 2011), subclinical infestations (Ippen, Nickel, & Schröder, 1995), or cross reactions with antigens from other related parasites (Arlian & Morgan, 2000; Arlian, Vyszenski-Moher, Ahmed, & Estes, 1991). Cross-reactivity against dust mites, ticks and *Trombicula* sp., a mite that affects the skin of different European wild ungulates like chamois (Walzer, 2008), have been previously assessed and discarded for ELISA A, B and C, respectively (Casais et al., 2007; Puigdemont et al., 2002; Rambozzi, Menzana, et al., 2004). Therefore, false positive results could be putatively related to cross-

reactions with other parasites reported to subclinically infest wild Iberian ibex, such as other mites (*Psoroptes* sp., *Trombicula* sp.) (Antón et al., 2002) or different tick species (Antón et al., 2002; Hueli & Díaz, 1989). ELISA C showed lower specificity than previously reported in Alpine chamois probably due to the application of the test on two different types of samples with different antibodies amount (serum *versus* lung extracted). However, false positive ibexes could also be due to individuals having had previous contact with *S. scabiei* and having recovered from the clinical phase of the disease. Hosts infested with *S. scabiei* develop resistance to re-infestation (Arlian et al., 1994) and healthy Iberian ibexes exposed to sarcoptic mange show detectable IgG levels (Lastras et al., 2000; Sarasa et al., 2010). In SNNNP, sarcoptic mange is endemic, thus some of the healthy Iberian ibexes of this study could have had previous contacts with the mite, thus developing an IgG response. However, the higher *S. scabiei*-positivity by ELISA in the severely infested ibexes suggests that IgGs are not protective against mange. In general, protection against mange has been associated with a cell-mediated immune response as well as with a humoral immune response (Arlian, Morgan, Rapp, et al., 1996; Arlian et al., 1994; Casais et al., 2014; Lalli et al., 2004; Tarigan, 2004; Walton, 2010). Therefore, further research appears to be needed to understand the specific role of both humoral and cellular immune responses. The significant differences in OD% found between healthy and infested ibexes for ELISA C are not consistent with a previous report, in which there were no OD% differences between infested and healthy ibexes from the same area (Lastras et al., 2000). However, results in this study fit the differences in two acute phase proteins (APP), namely alpha-1 acid glycoprotein (AGP) and serum amyloid A (SAA), observed in similarly ranked Iberian ibex. The higher APP level in severely affected ibexes was attributed to skin inflammation or the pathological secondary amyloidosis, leading to organ dysfunction in this category (Ráez-Bravo et al., 2015). The increasing OD% values with increasing sarcoptic mange severity found in this study could be explained by a higher intensity of infestation in severely affected ibexes, which in turn would stimulate more intensely the immune system and elicit a higher antibody production. Scabies-specific IgG rapidly decline in parallel with the disappearance of mites from the skin in goats (Tarigan, 2004), and the percentage of mange-damaged skin and mite load are related in Iberian ibex (Pérez et al., 2011). Moreover, although in some species skin lesions are reflective of delayed hypersensitivity reaction associated with few or no mites, in Iberian ibex mites are definitely abundant in the skin in the generalized stage of the disease (Pence & Ueckermann, 2002). Therefore, the OD% results obtained with ELISA C test would not only indicate the presence of viable *S. scabiei* in the skin, but could also correlate with mite load.

The evaluation of the ELISA tests carried out in this study and the identification of ELISA C as a reliable tool for sarcoptic mange diagnosis in Iberian ibex will be useful in future research. Nevertheless, the limitations in sensitivity and specificity should be considered while assessing the results, and more studies on (i) the dynamics of the antibody response after *S. scabiei* infestation in Iberian ibex; (ii) the correlation between parasitic load, mange severity and immune response; and (iii) the influence of sex (López-Olvera et al., 2015; Sarasa et al., 2010) and season (Carvalho et al., 2015; Pérez et al., 2011) on the OD% are necessary in order to better understand the immune response to sarcoptic mange in the Iberian ibex.

In conclusion, the ELISA developed for diagnose sarcoptic mange in Alpine chamois (Istituto Zooprofilattico delle Venezie, data not published) has been validated in this study as an effective test to be similarly applied in Iberian ibex. This can be helpful for completing the legal requirements for the transport of wild ungulates (RDL 1082/2009, de 3 de julio) and for seroepidemiological studies.

4.3. Study 3

Little effect of MHC class II diversity on the pathophysiology of Iberian ibexes experimentally infested with *Sarcoptes scabiei*

4.3.1. Abstract

Not all individuals of the same species show the same susceptibility to *Sarcoptes scabiei* infestation. Such inter-individual differences in the resistance to sarcoptic mange may be linked to polymorphism of the major histocompatibility complex (MHC). The Iberian Ibex (*Capra pyrenaica*) is a mountain ungulate endemic to the Iberian Peninsula with a deep history of sarcoptic mange outbreaks. Not all Iberian ibex populations show the same resistance to sarcoptic mange. Understanding the basis of interindividual variability in the resistance to sarcoptic mange infestation would be crucial for the management of this parasitic disease the Iberian ibex populations. In this study, an experimental infestation of Iberian ibexes with different MHC alleles was performed to determine the potential role of the MHC class II diversity on the resistance to sarcoptic mange and the pathophysiology of this parasitic disease over the course of the infestation. Additionally, we evaluated the use of immunoglogulin G (IgG) and immunoglobulin M (IgM) concentrations as indirect diagnosis for sarcoptic mange. The results obtained reveal that Iberian ibexes are able to recover naturally from sarcoptic mange independently of the MHC class II alleles. Two clinical pictures were identified in the infested ibexes: progression to severe and potentially lethal sarcoptic mange and recovery from the disease. The infested ibexes showed lymphopenia, and those developing severe sarcoptic mange also presented anaemia and neutrophilia. The IgG ELISA test was further confirmed as a complementary method when mite burdens are low and skin lesions small. Conversely, the IgM ELISA test needs to be further validated in order to be appropriate for sarcoptic mange diagnosis in the Iberian ibex.

Key words: *Capra pyrenaica*, Haematology, IgG, IgM, Resistance to infestation, Sarcoptic mange

4.3.2. Introduction

Sarcoptic mange outbreaks in wildlife are typically species-specific and population dependent (Bornstein et al., 2001; Pence & Ueckermann, 2002). In fact, not all mammal species are equally susceptible to *S. scabiei* infestation (Arlian & Morgan, 2017), and not all individuals of the same species show the same resistance to sarcoptic mange. Such inter-individual differences in the resistance to sarcoptic mange infestation are assumed to have a sexual or a genetic basis, but no work has tested their contribution under controlled conditions. On the other hand, environmental conditions drive the life cycle of the parasite (Castro et al., 2016) and hence the epidemiology of the disease (Davies, Moore, & Pointon, 1991) making the investigation of the inter-individual resistance to sarcoptic mange more difficult. As result, the impact of sarcoptic mange epidemics in wildlife is becoming unpredictable and a challenge for the development and progress of parasitic wildlife diseases management programmes worldwide (Astorga et al., 2018; Tompkins et al., 2015).

Different extrinsic and intrinsic factors have been linked to the progression of sarcoptic mange in wildlife. Among the extrinsic factors, weather conditions and therefore the season of the year appear to be one of the main determinants of sarcoptic mange epidemics (Pérez et al., 1997). Mite load also affects the clinical outcome of the affected species (Davis & Moon, 1990; Skerratt, 2003b). Mite virulence, however, has also been suggested to drive disease severity (Berrilli et al., 2002), as well as host density and food availability through changes in the parasite transmission or the resistance to disease progression (Gortázar et al., 2006). But differences in the epizootiology of the disease could also be related to intrinsic factors such as the sex of the host (López-Olvera et al., 2015) or the previous exposure to the mite (Arlian, Morgan, Rapp, et al., 1996; Arlian et al., 1994; Casais et al., 2014).

Differences in individual or population resistance to parasite diseases in wild mammal populations have been linked to polymorphism of the MHC (Harf & Sommer, 2005; Paterson et al., 1998; Schad et al., 2005). Major histocompatibility complex genes play a key role in the adaptive immune response of vertebrates (Bernatchez & Landry, 2003), and include two subgroups of molecules, namely MHC class I and II. The MHC I molecules are primarily associated with immune response to intracellular pathogens, whereas the class II reacts to extracellular parasites and pathogens (Piertney & Oliver, 2006). The role of MHC II activation in the successful immune response to scabies mites has been suggested more than two decades ago (Stemmer, Arlian, Morgan, Rapp, & Moore, 1996). Moreover, more recent studies found that *S. scabiei* inhibits the host immune response by supressing the expression of MHC class II

molecules on antigen presenting cells (Arlian, Fall, & Morgan, 2007; Arlian, Morgan, & Paul, 2006).

The Iberian Ibex is a mountain ungulate endemic to the Iberian Peninsula with a deep history of sarcoptic mange outbreaks (Pérez et al. 2002). Since 1987, southern and eastern Iberian ibex populations have been affected by repeated sarcoptic mange epidemics with contrasting consequences for the Iberian ibex population (Arenas et al., 2002; Fandos, 1991; León-Vizcaíno et al., 1999; Pérez et al., 2002, 1997; Valldeperes et al., 2018). In SCSVNP, a sarcoptic mange outbreak was responsible for the collapse of more than 90% of the Iberian ibex population (Fandos, 1991; León-Vizcaíno et al., 1999), but Iberian ibex mortality due to sarcoptic mange infestation in the nearby population of SNNNP was lower (Pérez et al., 1997). The SNNNP Iberian ibex population is the most genetically diverse, both when assessed through the cytochrome b (Márquez, Pérez, Granados, Soriguer, & Fandos, 2002) and through the MHC class II diversity (Angelone et al., 2018). That higher genetic diversity, particularly at the MHC class II, could provide some advantage to face the infestation and could explain, at least partially, the lower mortality observed in the SNNNP ibex population as compared to others. However, MHC class II diversity in Iberian ibex populations is typically low (Amills et al., 2004; Angelone et al., 2018), and thus whether the observed resistance of ibexes to scabies mite infestation has a genetic basis is under debate (Alasaad et al., 2013). On the other hand, physiologic response of ibexes to sarcoptic mange infestation is strongly seasonal (Carvalho et al., 2015; Pérez et al., 2015), hampering the investigation of the genetic basis of resistance to sarcoptic mange based on samples from free-ranging animals. Thus, experimental infestations under controlled conditions is the best approach to deal with this genetic issue. Understanding the basis of the interindividual variability in the resistance to sarcoptic mange infestation would be an important step to determine the best management measures of this parasitic disease the Iberian ibex populations.

In this study, scabies mites from a naturally parasitized wild ibex were collected to experimentally infest Iberian ibexes with different class II MHC alleles. The main objectives are two: (1) to describe the clinical evolution and the haematology of Iberian ibexes during the course of sarcoptic mange infestation; and (2) to determine the role of the MHC class II diversity on the resistance to sarcoptic mange. Additionally, we characterised the evolution of circulating antibodies (IgG, IgM) to determine their use as indirect diagnostic test for sarcoptic mange (objective 3).

4.3.3. Materials and methods

Experimental design

The experimental infestation was carried out in the "Las Mimbres" facilities from the Sierra de Huétor Natural Park (Andalusia, Southern Spain). Thirty-nine Iberian ibexes (22 males and 17 females), from one to 11 years old were selected from two stock reservoirs protected from the exposure to sarcoptic mange and placed in the SNNNP and in the SCSVNP. The ibexes were selected according to their alleles of the class II MHC DBR1 gene and at least one individual of each genetic group was kept as control (Table 4.3.1). The MHC haplotype was determine following Angelone et al. (2018) recommendations.

Table 4.3.1. Allele combination of the class II major histocompatibility complex DBR1 gene of the 39 Iberian ibexes experimentally infested with *Sarcoptes scabiei*.

Group	A (1,1)	B (1,3)	D (1,6)	E (3,3)	F (3,5)	G (3,6)	H (5,5)	I (5,6)	J (6,6)	K (1,7)	Undetermined	Total
Controls	3	1	1	1	1	1	2	1	1	2	-	14
Infested	4	2	3	2	3	2	4	2	-	2	1	25
- Recovered	-	-	-	-	-	-	2	-	-	-	-	2
- Reduced lesions	1	-	-	-	-	1	-	-	-	-	-	2
- Lesions < 50%	2	-	-	-	-	1	-	-	-	-	-	3
- Lesions > 50%	1	2	3	2	3	-	2	2	-	1	-	16

Recovered: ibexes that achieved the full recovery from the infestation; Reduced lesions: ibexes that experienced the reduction of skin lesions; Lesions < 50%: ibexes with lesions in less than 50% of the body surface at the end of the experimental infestation; Lesions > 50% lesions: ibexes with lesions in more than 50% of the body surface at the end of the experimental infestation.

Acepromazine (VedCo) and fluphenazine deconoate (Biopharma) were administered for the transportation and acclimatization of ibexes to the new pens. An antibiotic (enrofloxacin) and an inactivated vaccine (Cubolac®, CZ Veterinaria S.A.) against black disease, braxy, malignant oedema and enterotoxemias induced by *Clostridium chauvoei*, *Clostridium novyi* Type B, *Clostridium septicum*, *Clostridium sordellii* and *Clostridium perfringes* Types A, C and D were also applied before the transport, in order to prevent infections. Ibexes were kept in groups of three to five individuals in separate enclosures with *ad libitum* access to food and water.

Once adapted to the enclosure, 25 of the 39 ibexes were infested with *S. scabiei* with the skin pieces of a naturally parasitized free-ranging ibex, to standardize mite origin and therefore eliminate possible variations due to mite variability. Mite load was assessed with a stereomicroscope after digestion of six 2 x 2 cm patches of scabietic skin in 5% KOH solution at

40ºC overnight (Pérez et al., 2011). Then, skin pieces of the same size were dissected from the same body regions and stored in zip lock plastic bags. Parasite load in these skin patches was estimated in 750 ± 440 mites. Next, these skin pieces were fixed on a shaved area in the inter-scapular region of ibexes using adhesive bands (Figure 4.3.1).

Figure 4.3.1. Step sequence of the infestation of an Iberian ibex with *Sarcoptes scabiei* using skin pieces of a naturally parasitized free-ranging Iberian ibex.

The last day of the experimental period, the ibexes were anesthetized using an intramuscular combination of ketamine and xylacine (Casas-Díaz et al., 2011) and euthanized with intravenous T-61® (embutramide, mebezonium and tetracaine).

Sample collection

Clinical signs (e.g., skin showing compatible lesions with sarcoptic mange following Pérez et al. (2011) were monitored and recorded, and blood samples collected from the jugular vein placed in plain tubes without anticoagulant and tubes containing ethylenediaminetetraacetic acid tripotassium (EDTA K3) anticoagulant at 0, 4, 13, 26, 33, 46, 61, 75, 103 and 131 days post infestation (dpi). The blood samples without anticoagulant were allowed to clot and centrifuged at 3500 rpm for 15 minutes to obtain the serum, which was stored frozen at -20ºC until analysis. The blood samples with anticoagulant were kept refrigerated at 4ºC until analysis. The control ibexes were always handled first than the infested ones, and special care was taken to avoid any transmission of mites from the infested ibexes to the controls by using specific protective cloths for handling the animals. After the apparition of the early clinical signs of sarcoptic mange, animals were assigned to the mildly affected and severely affected categories described by Pérez et al. (2011).

Laboratory analyses

The haematological analyses were performed by the Prolab laboratory (Jaén) within 24 hours after the blood collection. A Mindray haematological analyser (A. Menarini Diagnostics) was used to determine red blood cell count (RBC), haematocrit, haemoglobin concentration, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and leukocyte count (white blood cell count, WBC). Additionally, at the end of the experimental infestation, basophil, eosinophil, monocyte, lymphocyte and neutrophil counts were also measured. The samples were run following the manufacturer's recommendations.

A validated in-house ELISA test was used to assess the IgG concentrations against *S. scabiei*, with a sensitivity of 91.7% and a specificity of 97.4%. Plates were coated with *S. scabiei* var. *canis* antigen (AFOSA GmbH, Germany) and serum samples were diluted 1:50 with PBS-T (phosphate buffered saline (PBS), pH 7.4 containing 1% of fish gelatin, 0.05% of Tween 20 detergent and 0.05% of sodium azide), and incubated at room temperature for 60 minutes. After the incubation, five washes with PBS-T diluted 1:10 with distilled water using the plate washer BioTek 405LS Washer®. The avidin-biotin detection increases the sensitivity of the ELISA test (Ráez-Bravo, Granados, Serrano, et al., 2016; Rambozzi, Menzana, et al., 2004), protecting from the impacts of washing and the incubation (Kendall et al., 1983). Hence, 100 µl of diluted (1:1000) biotinylated protein G (PierceTM Biotinylated Recombinant Protein G (1 µg/ml), Thermo Fisher Scientific Inc., USA) were added. After one hour of incubation, we performed three washes with PBS-T as described above. Then, we added 100 µl of diluted (1:57500) Immunopure® Avidin, Horseradish Peroxidase, Conjugated (0.1 µg/ml). Plates again incubated for an hour and washed three times more. The reaction was visualized by the addition of 100 µl of chromogenic substrate (TMB) for 15 minutes. The reaction was stopped with 100 µl of sulphuric acid. Absorbance at 450 nm was measured using a SUNRISE plate reader and the cut-off established at 27%OD.

To assess the concentration of IgM, the previous ELISA procedure was used, replacing the biotinylated secondary antibody for a Rabbit anti Goat IgM (heavy and light chains), conjugated with Biotin (Nordic-Mubio, The Netherlands). The test could not be validated, i.e., unestablished cut-off, due to the limited number of samples.

Statistical analysis

The role of the MHC class II alleles on the resistance to sarcoptic mange (i.e., proportion of showing scabietic compatible lesions) was evaluated by a Fisher Exact test.

Generalized additive mixed models (GAMM, Wood, 2017; Zuur, Saveliev, & Ieno, 2014) were used to investigate the effects of sarcoptic mange (infected *versus* control) on the haematological and immunological values overtime. In the GAMM, sarcoptic mange (infected *versus* control) was used as fixed factors whereas the dpi was used as a smoothed term. The ibex identity was used as random term GAMM. The model selection was based on the Akaike's Information Criterion (AIC, Akaike, 1973; Burnham & Anderson, 2002). The adjusted r-squared (i.e., proportion of observed variance explained by the model, R^2 hereafter) and the intraclass correlation coefficient (ICC, i.e., similarity between values from the same animal) for the best model were also calculated. Generalized additive mixed models requirements (normality and homocedasticity) were assessed before any model interpretation. Outliers without biological significance were excluded from the analysis following Zuur, Ieno, & Elphick (2010). Once described the dynamic of the haematological and immunological variables of the control and scabietic ibexes, the effects of mange severity (control, mildly affected and severely affected) on these variables were evaluated by a non-parametric Kruskal-Wallis test at 131 dpi, by the end of the experimental period when the two different clinical pictures were established. A Mann-Whitney test with Holm correction was used to account for multiple comparisons. Generalized additive mixed models modelling was performed with the "mgcv" package (Wood, 2017), and graphic representation was done with the "ggplot2" package (Wickham, 2009) of the R software 3.6.1 version (R Core Team, 2019).

4.3.4. Results

Clinical evolution of sarcoptic mange infestation

The 64% of infested ibexes (16 out of 25) presented lesions consistent with sarcoptic mange in the interscapular region at 13 dpi. By day 26, all the infested ibexes showed mange lesions in this area, including peeling and hyperkeratosis (Fig. 4.3.2).

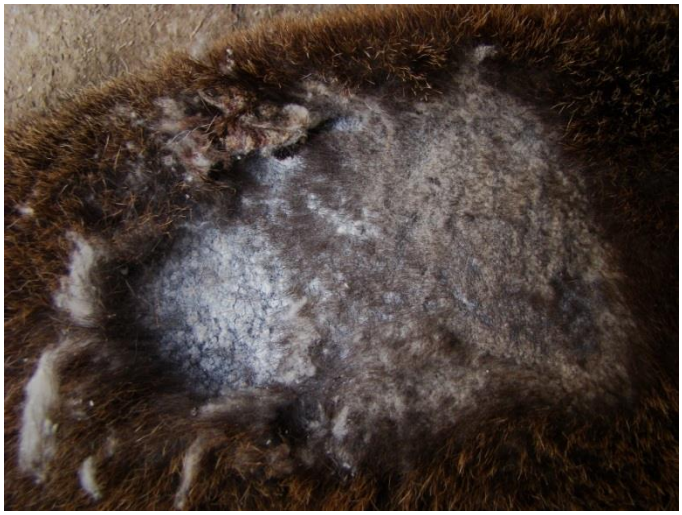

Figure 4.3.2. Iberian ibex showing sarcoptic mange compatible lesions in the interscapular region at day 26 post infestation.

Four of the infested ibexes experienced a reduction of the skin lesions during the infestation period, with presence of new hair growth (Figure 4.3.3). At 61 dpi and 103 dpi, two of them achieved the full recovery from the parasite infestation, respectively. Other three of the infested ibexes developed scabietic lesions in less than 50% of their body surface. The other 16 ibexes showed generalized mange lesions that extended over more than 60% of the body surface from 103 dpi onwards. Scabietic lesions in these ibexes affected the lumbar region, the ventral part of the body, limbs, neck and face, with severe crusting hyperkeratosis (scab formation), excoriations and deep fissures, eyelids inflammation and lips injuries (Figure 4.3.4).

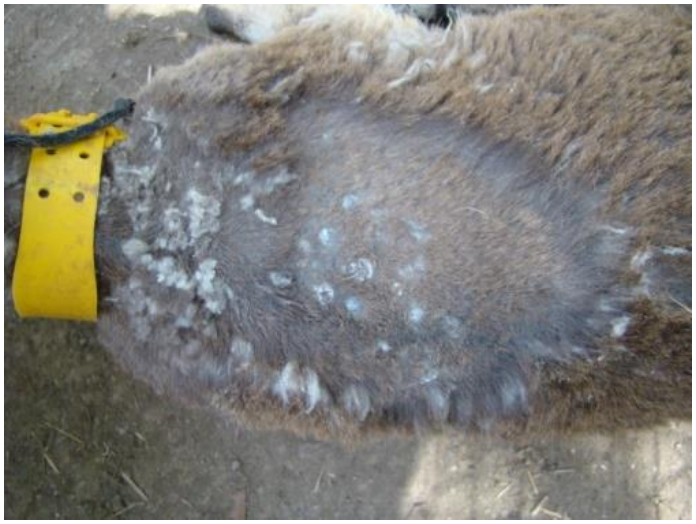

Figure 4.3.3. New hair growth in one of the recovered Iberian ibexes experimentally infested with *Sarcoptes scabiei*.

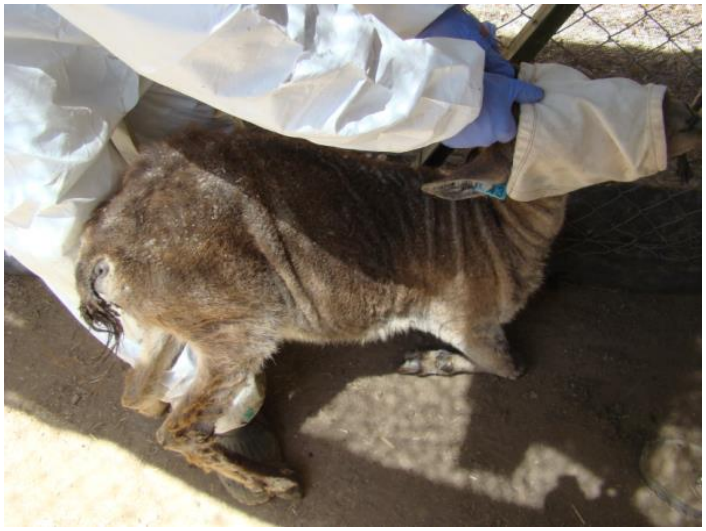

Figure 4.3.4. Iberian ibex showing widespread severe sarcoptic mange lesions with hyperkeratosis at day 131 post infestation.

The MHC class II allele combination had a non-significant effect (Fisher Exact test, $p = 0.06$), on the clinical evolution of the ibexes. In fact, the two recovered ibexes shared the same MHC allele combination (H (5,5)) as other two developing scabietic lesions in the entire body. Five other ibexes with self-limited lesions (with a reduction of the skin lesions or with lesions in less than 50% of the body surface) belonged to A (1,1) and G (6,3) allele combinations (Table 4.3.1). Furthermore, no significant differences in the trend of the infestation between males and females were observed (Fisher Exact test: $p = 0.4$). The two recovered ibexes were males, although two females showed a reduction of the lesions over the course of the experiment. Three other males developed lesions in less than 50% of the body surface.

Haematological variables

According to the model selection (Table 4.3.2), the models including the interaction between the time (dpi) and mange infestation (control or infested) were the best to explain the observed variability on RBC counts, haematocrit, haemoglobin concentration and MCV. The ICC between the infested and control ibexes did not differ, indicating similar variation among ibexes in each group. The RBC, haematocrit and haemoglobin concentration values remained stable over the course of the infestation in the control ibexes, but decreased in the scabietic counterparts since 60 dpi (Table 4.3.2, Figures 4.3.5 a, b, c). The MCV also decreased over the course of infestation but more in control than in the infested group (Figure 4.3.5 d). The MCH,

MCHC and the WBC values also varied over the infestation period, but non-significant differences between the control and in the infested ibexes were observed (Table 4.3.2, Figures 4.3.5 e, f, g). Descriptive statistics for the haematological variables in the control, recovered and severely infested ibexes at 131 dpi are shown in Table 4.3.3.

IgG and IgM concentrations

Two of the infested ibexes showed IgG against *S. scabiei* before infestation. The rest of the infested ibexes seroconverted and resulted positive to IgG between 13 dpi to 46 dpi (median=33 dpi). At that time, mange lesions in these ibexes ranged from 1 to 5% of the body surface (median = 3%). In the control group, six individuals were positive to the IgG ELISA test over the experiment. Another control ibex resulted positive to the ELISA test at 75 dpi, and two at 131 dpi. The concentrations of IgG in the control group remained stable over the experiment (Figure 4.3.6 a).

Table 4.3.2. Generalized additive mixed model (GAMM) selection to assess the effects of time (days post infestation, dpi) and mange infestation (control *versus* infested) on selected haematological variables in the 39 Iberian ibexes (*Capra pyrenaica*) experimentally infested with *Sarcoptes scabiei*. Ibex identity was considered the random term in the GAMM. The models with substantial support are indicated in bold.

Haematologic variable	Model	n	K	AIC	Δi AIC	L(gi/x)	wi	Information of the best model
RBC	**dpi * mange infestation**	376	7	1592.02	0.00	1.00	1.00	R^2: 11.9
	dpi	376	5	1618.71	26.69	0.00	0.00	ICCc: 0.569 / ICCi: 0.550
	dpi + mange infestation	376	6	1620.48	28.46	0.00	0.00	$F_{dpi * control}$ = 1.451; edf = 2.298; p-value = 0.34
	mange infestation	376	4	1688.23	96.21	0.00	0.00	$F_{dpi * infested}$ = 41.700; edf = 2.964; p-value = <2e-16
Haematocrit	**dpi * mange infestation**	376	7	2365.284	0.00	1.00	0.59	R^2: 23
	dpi	376	5	2366.626	1.34	0.51	0.30	ICCc: 0.433 / ICCi: 0.427
	dpi + mange infestation	376	6	2368.582	3.30	0.19	0.11	$F_{dpi * control}$ = 6.269; edf: 3.219; p-value = 0.000367
	mange infestation	376	4	2513.571	148.29	0.00	0.00	$F_{dpi * infested}$ = 62.233; edf: 3.048; p-value = < 2e-16
Haemoglobin concentration	**dpi * mange infestation**	376	7	1591.094	0.00	1.00	1.00	R^2: 20.7
	dpi	376	5	1606.404	15.31	0.00	0.00	ICCc: 0.54 / ICCi: 0.435
	dpi + mange infestation	376	6	1608.383	17.29	0.00	0.00	$F_{dpi * control}$ = 4.566; edf: 7.364; p-value = 0.000416
	mange infestation	376	4	1710.802	119.71	0.00	0.00	$F_{dpi * infested}$ = 37.251; edf: 4.510; p-value = < 2e-16
MCV	**dpi * mange infestation**	376	7	1353.926	0.00	1.00	0.91	R^2: 5.25
	dpi	376	5	1359.471	5.55	0.06	0.06	ICCc: 0.834 / ICCi: 0.726
	dpi + mange infestation	376	6	1360.547	6.62	0.04	0.03	$F_{dpi * control}$ = 38.89; edf: 1.638; p-value = 3.22e-13
	mange infestation	376	4	1433.272	79.35	0.00	0.00	$F_{dpi * infested}$ = 30.20; edf: 1; p-value = 7.06e-08
MCH	**dpi**	375	5	676.6736	0.00	1.00	0.62	R^2: 6.04
	dpi + mange infestation	375	6	677.7033	1.03	0.60	0.37	ICC: 62.6
	dpi * mange infestation	375	7	688.4615	11.79	0.00	0.00	F_{dpi} = 12.7; edf: 5.725; p-value = 1.75e-12
	mange infestation	375	4	727.9572	51.28	0.00	0.00	
MCHC	**dpi**	370	5	1115.42	0.00	1.00	0.65	R^2: 37.7
	dpi + mange infestation	370	6	1116.68	1.26	0.53	0.35	ICC: 20.6
	dpi * mange infestation	370	7	1127.852	12.43	0.00	0.00	F_{dpi} = 36.03; edf: 8.009; p-value = <2e-16
	mange infestation	370	4	1290.247	174.83	0.00	0.00	
WBC	**dpi**	373	5	1949.164	0.00	1.00	0.50	R^2: 1.61
	dpi + mange infestation	373	6	1950.383	1.22	0.54	0.27	ICC: 52.5
	dpi * mange infestation	373	7	1950.69	1.53	0.47	0.23	F_{dpi} = 14.83; edf: 1; p-value = 0.000138
	mange infestation	373	4	1960.858	11.69	0.00	0.00	

n: sample size; K: number of parameters; AIC: Akaike information criterion; Δi AIC: difference of AIC with respect to the best model; wi: Akaike weight; R^2 (%): variance explained by fixed factors; ICC: Intraclass correlation coefficient of measures from the same control (ICCc) or infested (ICCi) group; F: F-test, edf: effective degrees of freedom

Table 4.3.3. Descriptive statistics (n, median, minimum and maximum) of selected haematological variables in control, recovered and severely infested ibexes at day 131 post infestation. A different superscript indicates statistically significant differences among groups (Mann-Whitney test with Holm correction).

Haematologic variable	Control			Recovered			Severely infested		
	n	Median	Min-Max	n	Median	Min-Max	n	Median	Min-Max
RBC (10^{12}/L)	13	16.96 [a]	15.09 - 19.89	7	16.805 [a]	14.55 - 18.97	14	13.485 [b]	8.76 - 16.72
Haematocrit (%)	14	45.85 [a]	24.6 - 49.2	7	42.50 [a]	36.2 - 47	15	35.8 [b]	23.3 - 46
Haemoglobin concentration (g/dl)	14	16.6 [a]	8.0 - 18.8	7	15.8	12.4 - 17.7	15	12.9 [b]	7.6 - 17.1
MCV (fl)	14	26.55 [a]	21.7 - 33.3	7	25.6 [a]	23.9 - 27.1	15	28.6 [b]	25.5 - 35.3
MCH (pg)	14	9.65	8.3 - 10.9	7	9.3	8.2 - 9.7	15	9.8	9.1 - 12.5
MCHC (%)	14	36.35 [a]	32.6 - 38.2	7	36	34.2 - 37.7	14	35.3 [b]	32.6 - 36.6
WBC (10^{12}/L)	14	16.395	10.79 - 24.03	7	14.95	11.08 - 18.62	15	15.84	9.77 - 26.08
- **Eosinophils (10^{12}/L)**	14	0.49	0.15-2.07	7	0.31	0.19-0.77	15	0.48	0.10-1.02
- **Basophils (10^{12}/L)**	14	0.21	0.13-0.56	7	0.23	0.15-0.33	15	0.27	0.10-0.65
- **Neutrophils (10^{12}/L)**	14	6.09 [a]	3.19-9.06	7	6.66	4.93-10.61	15	10.02 [b]	5.28-15.13
- **Lymphocytes (10^{12}/L)**	14	8.17 [a]	4.96-14.66	7	5.78 [b]	3.62-7.77	15	3.95 [b]	2.29-8.49
- **Monocytes (10^{12}/L)**	14	0.68	0.38-1.61	7	0.61	0.46-1.64	15	0.94	0.49-2.09
- **Eosinophils (%)**	14	3	1 - 11	7	2	1 - 7	15	3	1 - 6
- **Basophils (%)**	14	1.5	1 - 3	7	2	1 - 3	15	2	1 - 3
- **Neutrophils (%)**	14	38.5 [a]	20 - 55	7	50 [b]	33 - 57	15	61 [c]	48 - 73
- **Lymphocytes (%)**	14	50.5 [a]	40 - 72	7	38 [b]	31 - 52	15	28 [c]	19 - 40
- **Monocytes (%)**	14	4.5	3 - 9	7	5	3 - 11	15	6	4 - 8

RBC: red blood cell count; MHC: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; WBC: white blood cell count.

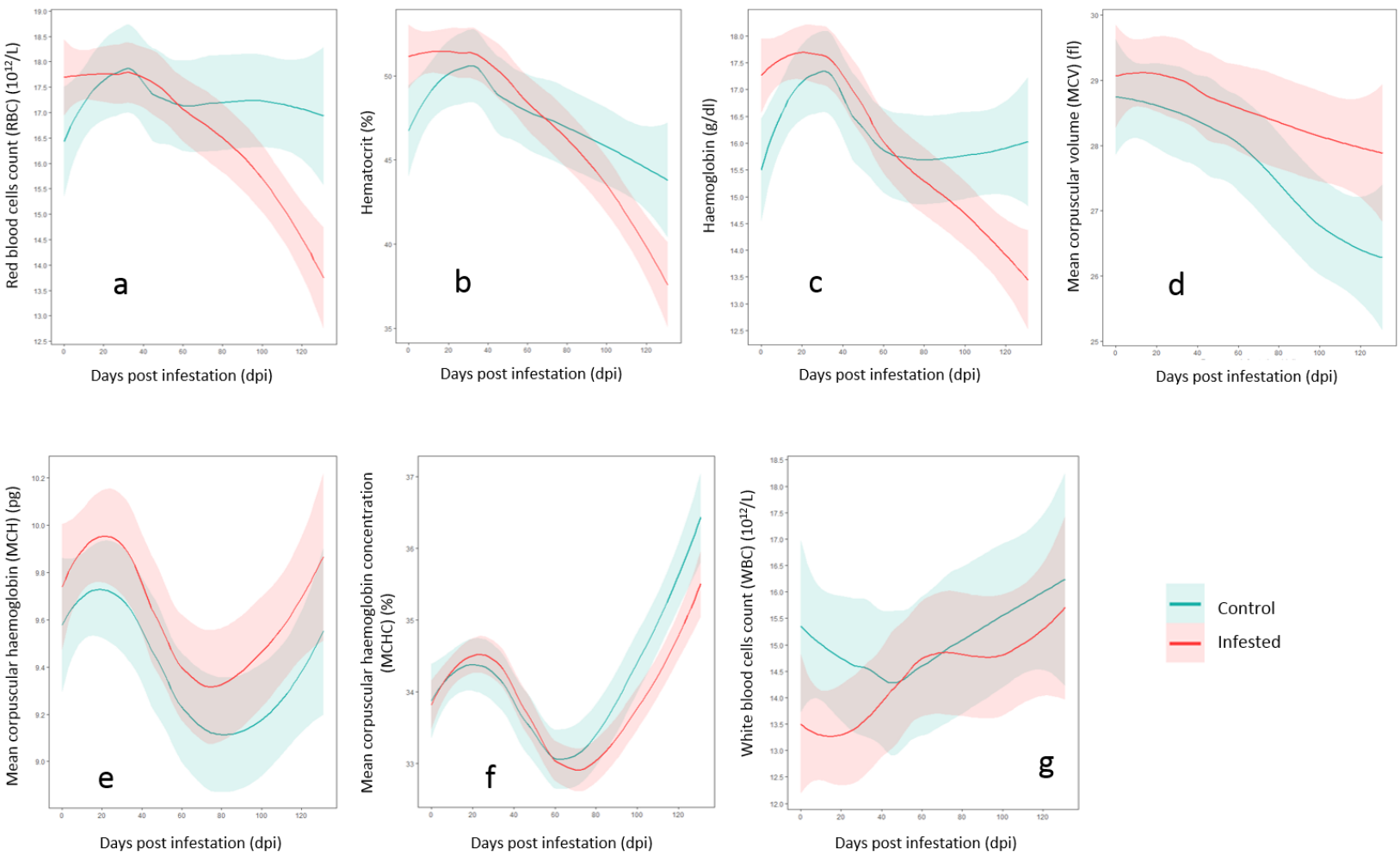

Figure 4.3.5. Red blood cell count (RBC, a), haematocrit (b), haemoglobin concentration (c), mean corpuscular haemoglobin (MCH, d), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and white blood cell count (WBC) over time (days post infestation, dpi), in the control (blue) and infested (red) Iberian ibexes experimentally infested with *Sarcoptes scabiei*.

Table 4.3.4. Generalized additive mixed model (GAMM) selection to assess the effects of time (days post infestation, dpi) and mange infestation (control *versus* infested) on the IgG and IgM concentrations in the 39 Iberian ibexes (*Capra pyrenaica*) experimentally infested with *Sarcoptes scabiei*. Ibex identity was considered the random term in the GAMM. The models with substantial support are indicated in bold.

Immunoglobulin	Model	n	K	AIC	Δi AIC	L(gi/x)	wi	Information of the best model
IgG	**dpi * mange infestation**	379	7	3573.424	0.00	1.00	1.00	R^2: 51.7
	dpi + mange infestation	379	6	3773.197	199.77	0.00	0.00	ICCc: 0.897 / ICCi: 0.487
	dpi	379	6	3802.309	228.89	0.00	0.00	$F_{dpi * control}$ = 0.844; p-value = 0.268
	mange infestation	379	4	3993.659	420.24	0.00	0.00	$F_{dpi * infested}$ = 244.083; p-value = <2e-16
IgM	**dpi * mange infestation**	379	7	3297.503	0.00	1.00	1.00	R^2: 11.6
	dpi	379	5	3313.637	16.13	0.00	0.00	ICCc: 0.740 / ICCi: 0.362
	dpi + mange infestation	379	6	3315.581	18.08	0.00	0.00	$F_{dpi * control}$ = 2.656; p-value = 0.104
	mange infestation	379	4	3359.077	61.57	0.00	0.00	$F_{dpi * infested}$ = 15.708; p-value = 6.89e-15

n: sample size; K: number of parameters; AIC: Akaike information criterion; Δi AIC: difference of AIC with respect to the best model; wi: Akaike weight; R^2 (%): variance explained by fixed factors; ICC: Intraclass correlation coefficient of measures from the same control (ICCc) or infested (ICCi) group; F: F-test, edf: effective degrees of freedom

Table 4.3.5. Descriptive statistics (n, median, minimum and maximum) of the IgG and IgM concentrations in the control, recovered and severely infested ibexes at 131 dpi. A different superscript indicates statistical differences between sarcoptic mange categories (Mann-Whitney test with Holm correction).

Immunoglobulin	**Control**			**Recovered**			**Severely infested**		
	n	Median	Min - Max	n	Median	Min - Max	n	Median	Min - Max
IgG (OD%)	14	27.680 [a]	6.84 - 95.46	7	125.395 [b]	60.17 - 188.06	15	154.900 [c]	117.11 - 222.26
IgM (OD%)	14	21.61	8.04 - 121.09	7	32.81	21.51 - 159.40	15	33.08	19.76 - 62.83

According to GAMM selection (Table 4.3.4), the models including the interaction between dpi and mange status (control or infested) were the best to explain the observed IgG and IgM concentration variability. Regarding IgG concentration, 51.7% of the observed variability was explained by the effect of mange infestation over time. In fact, IgG concentrations in the control ibexes remained stable over the course of the infestation, but increased in the infested ibexes from the beginning of the infestation (Figure 4.3.6 a). In the case of IgM, 11.6% of the observed variability was explained by the interaction of mange and dpi. The IgM concentrations in the control ibexes stayed more stable over time, and increased in the infested ones (Figure 4.3.6 b). The ICC in the control group was higher for both immunoglobulins, since the variation among the control ibexes was lower than in the infested group. At the end of the experimental infestation, significant differences between IgG concentrations were observed among disease categories (Table 4.3.5). For IgM, however, there was no significant differences among groups (Table 4.3.5).

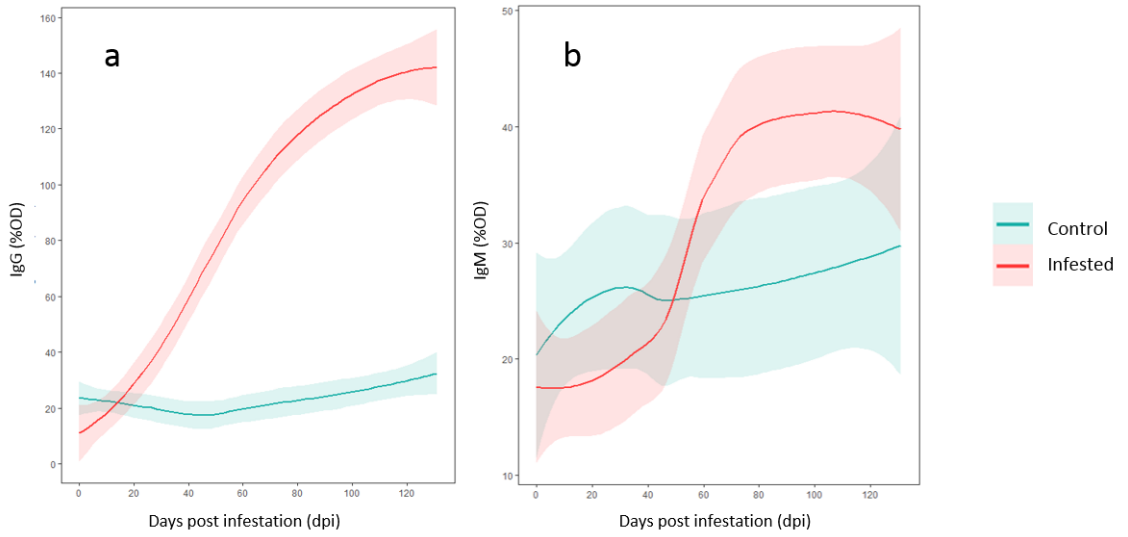

Figure 4.3.6. Immunoglobulin G (a) and Immunoglobulin M (b) concentrations over time (days post infestation, dpi), in the control (blue) and infested (red) Iberian ibexes experimentally infested with *Sarcoptes scabiei*.

4.3.5. Discussion

In this study, individual differences in the clinical manifestations of ibexes experimentally infested with *S. scabiei* were described. Though the total recovering from sarcoptic mange in free-ranging ibexes has previously been reported by Alasaad et al. (2013), to the best of our knowledge, this is the first description under controlled conditions. In fact, this experimental

trial revealed that individual differences in the resistance to sarcoptic mange exists beyond the influence of mite load, mite origin, the season of the year or the food availability.

In line with the observations made in other mammal species (Bornstein, 1991; Bornstein, Frössling, Näslund, Zakrisson, & Mörner, 2006; Bornstein et al., 1995; Bornstein & Zakrisson, 1993a; Casais et al., 2014; Mörner & Christensson, 1984), the first clinical manifestations of sarcoptic mange occurred two weeks after infestation.

Although different authors have observed an association between MHC alleles and resistance to parasite infestation (Harf & Sommer, 2005; Paterson et al., 1998; Schad et al., 2005), no relationships between the MHC alleles and the clinical outcome of sarcoptic mange in Iberian ibex was observed in the experiment. In fact, the higher MHC class II polymorphism found in the SNNNP ibex population could be the consequence of a lower mortality rate, as suggested in other mountain ungulate species (Cavallero, Marco, Lavín, D'Amelio, & López-Olvera, 2012), and not an infestation driver.

Conversely to previous studies reporting higher severity of sarcoptic mange in free-ranging Iberian ibex males than in females (López-Olvera et al., 2015), no differences between males and females in the course of sarcoptic mange were found in this study. This suggests that the differences between sexes found in the free-ranging ibexes might be related to differences in factors non-controlled in this experiment, such as free-ranging behaviour of both sexes and/or environmental factors. Adult males live together in larger groups than females for most of the year, except for the rutting season toward late autumn and early winter (Alados & Escós, 1996; Granados, Pérez, et al., 2001), increasing the infestation risk. Such differences could also be related to a seasonal immunosuppression of males related to testosterone (Decristophoris, Von Hardenberg, & McElligott, 2007; Klein, 2004) and higher loss of body condition during the rut season (Brivio, Grignolio, & Apollonio, 2010; López-Olvera et al., 2015) in free-ranging conditions, which were absent in the controlled conditions during the experimental infestation.

Regarding the haematological red cell values, the differences between the control and the infested ibexes were associated to the severity of mange, in particular at the end of the infestation period. At that time, RBC, haematocrit and haemoglobin concentration in the severely infested ibexes were lower than in the other two categories of infestation. These alterations indicate anaemia, as previously reported in wild mammals affected by sarcoptic mange, including free-ranging Iberian ibexes (Arlian, Ahmed, and Vyszenski-Moher 1988; Pérez

et al. 2015). The higher MCV and lower MCHC concentration in the severely infested ibexes is compatible with macrocytic and hypochromic anaemia and the active regeneration of erythrocytes in the bone marrow of scabietic individuals (Tvedten, 2010). Therefore, the anaemia associated to sarcoptic mange could be due to the erythrocyte lysis induced by inflammatory cytokines (Nemeth & Ganz, 2014) produced by the action of mites (Bhat et al., 2017). This pattern has also been observed in Iberian ibexes affected by sarcoptic mange, where a low RBC and a high MCV and MCH were observed, but no differences in the remaining haematological parameters were identified (Pérez et al., 1999). The differences in the results between both studies could be explain by the severity of mange and the duration of the infestation, as the controlled conditions of the experimental infestation allowing a finer assessment of the clinical and clinical-pathological effects of sarcoptic mange.

Concerning white blood cells, the neutrophilia and lymphopenia at 131 dpi found in the severely infested ibexes are also in agreement to previous research (Beigh, Soodan, & Bhat, 2016; Kido et al., 2011; Pérez et al., 2015, 1999; Skerratt, Middleton, & Beveridge, 1999). These results could be linked to the bacterial secondary infections observed in the severely infested ibexes (Espinosa, Ráez-Bravo, et al., 2017). However, no anaemia or neutrophilia was observed in the ibexes that clinically recovered from sarcoptic mange (Table 4.3.3), demonstrating that these ibexes not only recovered from the disease, but also overcame the pathophysiological consequences of the infestation and the associated secondary bacterial infections.

Resistance to *S. scabiei* reinfestation has been described in several wild mammal species (Arlian, Morgan, Rapp, et al., 1996; Arlian et al., 1994; Casais et al., 2014). In this study, two experimentally infested ibexes showed IgG antibodies against *S. scabiei* before the infestation, probably due to a previous encounter with the mite (Lastras et al., 2000). However, other studies on domestic (Tarigan, 2004) and wild Caprinae species (Sarasa et al., 2010) have found that IgG concentrations drop sharply after sarcoptic mange infestation. Therefore, since IgG do not seem to provide protection against *S. scabiei* infestation, and even if they were they disappear fast after the infestation, initial IgG detection suggesting previous contact with the mite does not affect the ability of the host to deal with new infestations. On the other hand, the increase of IgG concentrations in the infested ibexes over the course of the infection is probably the consequence of an overstimulated antigenic response to scabies mites rather than the reflect of an effective adaptive immune response, as previously was reported in Iberian ibexes (Ráez-Bravo, Granados, Serrano, et al., 2016). Moreover, the positive ELISA test

results found in three control ibexes later in the experimental period could indicate an accidental contact with *S. scabiei* despite the control measures adopted, with low parasitic loads and no lesions.

In line with other mammals suffering from sarcoptic mange, IgG were detected once the first skin lesions were visible (Casais et al., 2014; Tarigan, 2004; Van Der Heijden et al., 2000). This fact appear to limit the use of the IgG ELISA test to detect previous expositions to the scabies mite, but would be useful for sarcoptic mange diagnosis in ibexes showing small lesions or affected by other skin diseases. Hence, the IgG ELISA tests would be used in combination with the ordinary diagnosis methods, in particular when ibexes are translocated for reintroduction purposes (RDL 1082/2009, de 3 de julio), since a recent study showed that visual diagnosis had accuracy limitations in free-ranging Iberian ibexes (Valldeperes et al., 2019).

On the contrary, IgM concentration did not seem to be useful for sarcoptic mange diagnosis in Iberian ibexes. In fact, the low affinity of this immunoglobulin for antigens (Eisen, 2014) could explain the high IgM variability observed in the ibexes experimentally infested. Hence, the huge variability of the IgM concentrations hampers the use of the IgM ELISA tests for sarcotic mange diagnosis. Further studies should be carried out to validate IgM detection before it could be considered useful in the assessment of sarcoptic mange in the Iberian ibex.

In conclusion, the Iberian ibex is able to recover naturally from sarcoptic mange independently of the class II MHC alleles. Ibexes developing sarcoptic mange showed evident pathophysiological alterations affecting not only the skin surface, but also the haematological profiles. Although the IgM ELISA test appears to be inappropriate for sarcoptic mange diagnosis in the Iberian ibex, the IgG ELISA test would be useful as complementary method when mite burdens are low and skin lesions small.

4.4. Study 4

Clinical outcome of sarcoptic mange is related to local and systemic gene expression in Iberian ibex

4.4.1. Abstract

Sarcoptic mange is a contagious skin disease caused by the mite *Sarcoptes scabiei*, affecting different mammalian species worldwide, including wild ungulates. Iberian ibex (*Capra pyrenaica*) is a medium-sized ungulate endemic to the Iberian Peninsula, whose populations have suffered different mortalities due to sarcoptic mange. The aim of this study is to characterize the gene expression in Iberian ibexes experimentally infested with *S. Scabiei* and to identify the biological processes that determine the differences in the clinical response to sarcoptic mange.

Twelve Iberian ibexes were experimentally infested with *S. scabiei*, whereas other six ibexes were maintained as controls. Clinical signs were monitored periodically during the infestation period. Four of the infested ibexes developed a mild stage of the disease, and even recovered completely, whereas sarcoptic mange progressed to severe stages in the other eight ibexes. At day 131 post infestation, whole blood samples and skin biopsies were collected with solutions to stabilize and protect ribonucleic acid (RNA). The samples were stored at -80ºC until analysis. RNA was extracted and expression was measured with microarrays using the Ovine Gene 1.0 ST array (Affymetrix) to detect differences in blood and skin gene expression. Gene Set Enrichment Analysis (GSEA) was used in order to retrieve functional pathways using the GO biological process and the KEGG collection. The differences in the functional pathways between the control and the infested ibexes (as a whole and for each clinical outcome separately) were studied, as well as between the infested ibexes that recovered and those severely infested.

Severe infestation with *S. scabiei* promoted an immune and inflammatory genomic response, consistent with an ineffective response against the mite and with the response against secondary infections, and a lower expression of cell division processes, attributed to consumption due to the higher metabolic demands caused by to the disease. Conversely, the upregulated immune pathways found in the skin of the recovered ibexes as compared to the severely infested suggest a significant role of local immunity in controlling sarcoptic mange in Iberian ibex, related with an effective recognition of *S. scabiei* and the subsequent activation of T cells. The recovered ibexes also showed lower inflammation activation than the severely infested ones.

Keywords: *Capra pyrenaica*, *Sarcoptes scabiei*, Microarray, RNA sequencing, Skin and blood, Genomic response, GSEA, Cytoscape, Enrichment Map, Gene Ontology, KEGG

4.4.2. Introduction

Sarcoptic mange is a contagious skin disease caused by the mite *S. scabiei*. The clinical signs and lesions are characterized by pruritus, peeling, alopecia, hyperkeratosis and scab formation. Distributed worldwide, *S. scabiei* affects a wide range of wild and domestic mammalian species as well as humans (Pence & Ueckermann, 2002). In Europe, different wild ungulate species have been affected by sarcoptic mange, including chamois (*Rupicapra* spp.) (Fernández-Morán et al., 1997b; Rossi, Fraquelli, Vesco, Permunian, Sommavilla, Carmignola, Da Pozzo, et al., 2007), both Alpine and Iberian ibex (León-Vizcaíno et al., 1999; Ondersheka, Kutzer, & Richter, 1968; Schaschl, 2003), Barbary sheep (González-Candela et al., 2004), red and roe deer (Oleaga, Balseiro, et al., 2008; Oleaga, Casais, et al., 2008) and wild boar (*Sus scrofa*) (Haas et al., 2018). However, the epidemiology, mortality and demographic consequences of sarcoptic mange in wildlife populations differs among the species and even among different populations of the same species (Bornstein et al., 2001).

Iberian Ibex is a mountain ungulate valuable as an Iberian Peninsula endemism and as a big game species (Fandos et al., 2010; Granados, Pérez, Soriguer, Fandos, & Ruiz-Martínez, 1997). Although high mortality rates related to sarcoptic mange epizootics have been reported in Iberian ibex populations, the demographic consequences of such epizootics have varied among the populations affected (Fandos, 1991; Pérez et al., 2002, 1997). Moreover, resistance and survival to sarcoptic mange have been reported in free-ranging naturally infested Iberian ibexes in SNNNP (Alasaad et al., 2013). However, the causes for such resistance and the demographic population consequences have not been investigated up to date in Iberian ibex.

The individual clinical expression and demographic effects of wildlife diseases in free-ranging populations might be related to differences in the host, the pathogen and/or the environment (Hudson, Rizzoli, Grenfell, Heesterbeek, & Dobson, 2002). Among the host-related factors, host immune and inflammatory responses determine the clinical manifestation of mange (Bhat et al., 2017). Genomic studies have contributed to advance in the knowledge of the pathogenesis of different diseases in domestic and wild animals (Galindo et al., 2012; Nam et al., 2018; Ojaimi, Qanud, Hintze, & Recchia, 2007; Tao & Mallard, 2007), but available genomic information on host response to sarcoptic mange is limited (Arlian et al., 2007; Morgan et al., 2013). Therefore, there is a need for gene expression studies to provide insight on the pathogenesis of sarcoptic mange in Iberian ibex.

The aims of the study are: (1) characterizing the gene expression in Iberian ibexes experimentally infested with *S. scabiei*; and (2) identifying the biological processes and pathways determining the differences in the clinical response to sarcoptic mange in Iberian ibex.

4.4.3. Materials and methods

Eighteen Iberian ibexes from a stock reservoir protected from exposure to sarcoptic mange in SNNNP (Espinosa, López-Olvera, et al., 2017; Granados-Torres et al., 1996) were moved to "Las Mimbres" building facilities at Sierra de Huétor Natural Park. After an adaption period, twelve of the ibexes were infested with *S. scabiei* using skin pieces of a naturally parasitized wild ibex, whereas the remaining six ibexes were maintained as controls. The skin pieces were fixed onto the ibex previously shaved inter-scapular region using adhesive bands, thereby inducing contact between the mites and the host skin. The estimated mite load in the skin pieces fixed to each ibex was 750 ± 440 mites.

Clinical signs, including the presence of skin sarcoptic mange lesions and their extension, were monitored during the infestation period.

By day 131 post infestation, when two different clinical pictures (recovering and severe infestation) were shown by the infested ibexes (Study III), whole blood samples were collected from the jugular vein in commercially available tubes with ribonucleic acid (RNA) preservation buffer (PAXgene™ blood RNA tubes). Skin biopsies were also obtained the same day using an 8 mm diameter punch and were placed in disposable tubes with RNA*later*® solution. The samples were stored at -80ºC until analysis.

Ribonucleic acid was extracted using PAXgene kit (QIAGEN) for blood samples and RNeasy mini kit (QIAGEN) for skin samples. The samples were processed according to the following Affymetrix protocols: GeneChip WT PLUS Reagent kit (P/N 703174 Rev. 2) and Expression Wash, Stain and Scan User Manual (P/N 702731 Rev. 3) (Affymetrix Inc., Santa Clara, CA, USA). Gene expression was measured with microarrays using the Ovine Gene 1.0 ST array (Affymetrix), and genes were noted using the annotation file provided by Affymetrix for the OviGene-1 0-st array and the UCSC database (Nov. 2016 hg38, GRCh38) to complement Affymetrix annotations. The location of each probeset (Start, Stop, Strand and Chromosome)

was obtained with the Affymetrix annotation file (NetAffx na36), and this information was used to map in the UCSC database genes matching the same coordinates.

After processing, data were quality-controlled and normalized using the Robust Multi-array Average algorithm (RMA) (Irizarry et al., 2003) included in aroma.affymetrix package (Bengtsson, Irizarry, Carvalho, & Speed, 2008). The statistical analyses were performed using R programming (Version 3.3.2), Bioconductor packages (Gentleman et al., 2004) and the Comprehensive R Archive Network (CRAN). The Differential Expression analysis was performed with the limma package (Ritchie et al., 2015). The sva package (Leek et al., 2017) from Bioconductor was used in order to estimate batch and other artefacts into surrogate variables and to adjust them as covariates into the limma model.

Differences in gene expression were studied between the control and the infested ibexes (as a whole and for each clinical outcome separately), and between the infested ibexes that recovered and those severely infested. Genes were considered differentially expressed when the p-value was < 0.05 and the fold-change was > 0.58 or < -0.58.

Gene Set Enrichment Analysis (GSEA) was used in order to retrieve functional pathways (Subramanian et al., 2005). This method links the obtained microarray expression profile with gene sets available in the Molecular Signatures Database V5.0 (MSigDB). Gene sets in MSigDB are grouped in eight collections, from which C2 KEGG (Kyoto Encyclopedia of Genes and Genomes) curated gene sets and C5 GO biological process were used in this study. Gene Set Enrichment Analysis software was used with the option Pre-Ranked Analysis to rank genes using the *p*-values obtained in the Differential Expression analysis with limma. The ranked list of genes was generated using the score [-log (*p*-value)*sign(fold change)] for each gene. The gene sets were filtered by a nominal p-value of 0.05 and a false discovery rate (FDR, q-value) of 0.1. The differences in the functional pathways were investigated among the same groups as the differences in gene expression using the GO collection, whereas the KEGG collection was used to further investigates the differences between the infested ibexes that recovered and those severely infested.

4.4.4. Results

The control ibexes did not develop skin lesions. Four of the infested ibexes developed lesions in less than 25% of the body surface and even recovered completely (recovered ibexes), whereas the other eight ibexes developed lesions in more than 70% of the body surface (severely infested ibexes).

Table 4.4.1 shows the number of upregulated and downregulated genes found for each of the studies performed: infested *versus* control ibexes; recovered *versus* control ibexes, severely infested *versus* control ibexes; and, finally, recovered *versus* severely infested ibexes.

The Tables 4.4.2 and 4.4.3 summarise the number and relative importance of the significantly enriched gene sets using GO biological process collection with involvement in different biological processes. The significantly enriched gene sets using GO biological process collection and their classification in functional categories are presented in Figure 4.4.1. Table 4.4.4 shows the pathways identified by the KEGG analysis as significant enriched in the ibexes that recovered as compared to the severely infested ones in both skin and blood. Table 4.4.5 shows the pathways significantly enriched in the ibexes that recovered and the ibexes that developed severe infestation as compared to the control ones, according to the KEGG analysis, in both skin and blood.

Table 4.4.1. Number of differentially expressed genes among the different groups of Iberian ibexes according to sarcoptic mange evolution.

Expression change as compared to controls				
	SKIN		**BLOOD**	
	Up	Down	Up	Down
Infested altogether	159	323	137	107
- Recovered	304	162	63	276
- Severely infested	149	97	67	83

Expression change as compared to severely infested				
	SKIN	**BLOOD**	**SKIN**	**BLOOD**
	Up	Down	Up	Down
Recovered	266	543	339	168

Table 4.4.2. Number of differentially enriched genes sets and percentage included in pathogenically significant processes between the control and the infested ibexes (both as a whole and for each of the different clinical outcomes). Data obtained using the GO biological process collection (nominal *p-value* < 0.05 and FDR < 0.1) both in skin and blood.

		Expression change as compared to controls			
		SKIN		**BLOOD**	
		n	Biological categories	n	Biological categories
Infested altogether	Up	26	Immunity and inflammation (65%): - Response to cytokine (23%) - Response to other organism (15%) - Immune system processes (15%) - Cytokine production (8%) - Inflammatory response (4%)	383	Immunity and inflammation (22%): - Response to cytokine (1%) - Response to other organism (2%) - Immune system processes (9%) - Cytokine production (5%) - Inflammatory response (2%) Response to wounding (3%)
	Down	209	Cell division (32%): - Chromosome organization (10%) - DNA metabolic processes (12%) - RNA metabolic processes (1%) - Cell cycle (8%) - Cell division (1%) Pigmentation (4%) Response to radiation (1%) Immunoglobulin production (2%)	316	Cell division (52%): - Chromosome organization (8%) - DNA metabolic processes (14%) - RNA metabolic processes (20%) - Cell cycle (3%) - Gene expression (5%) Response to radiation (3%)
- Recovered	Up	21	Immunity and inflammation (24%): - Leukocyte differentiation (19%) - Response to chemokine (5%) Cell division - Gene expression (14%) Protein folding (19%) Response to topologically incorrect protein (19%)	87	Immunity and inflammation (2%): - Leukocyte migration (1%) - Response to other organism (1%) Response to wounding (2%) Coagulation (5%)

	Down	50	Cell division (16%): - Chromosome organization (8%) - DNA metabolic processes (8%) Metabolic processes (62%) Pigmentation (12%)	306	Cell division (41%): - Chromosome organization (7%) - DNA metabolic processes (7%) - RNA metabolic processes (21%) - Cell cycle (1%) - Gene expression (5%) Response to radiation (1%) Immunity and inflammation (11%): - Immune system process (9%) - Cytokine production (2%)
- Severely infested	Up	14	Immunity and inflammation (79%): - Response to cytokine (29%) - Cytokine production (14%) - Response to other organism (29%) - Lymphocyte migration (7%)	249	Immunity and inflammation (31%): - Cytokine production (8%) - Leukocyte processes (7%) - Innate immune response (3%) - Inflammatory response (2%) - Response to other organism (2%) Response to wounding (2%) Coagulation (1%)
	Down	209	Cell division (36%): - Cell cycle (11%) - Cell division (1%) - Chromosome organization and segregation (11%) - DNA metabolic processes (10%) - RNA metabolic processes (3%) Immune system processes (2%) Pigmentation (4%)	700	Cell division (62%): - Chromosome organization (9%) - DNA metabolic processes (20%) - RNA metabolic processes (24%) - Cell cycle (4%) - Gene expression (5%) Metabolic processes (13%) Immune system processes (4%) Response to radiation and UV (3%) Pigmentation (1%)

Table 4.4.3. Number of differentially enriched genes sets and percentage included in pathogenically significant processes between the infested ibexes that recovered and those severely infested. Data obtained using the GO biological process collection (nominal *p-value* < 0.05 and FDR < 0.1) both in skin and blood.

		Expression change as compared to severely infested			
		SKIN		**BLOOD**	
		n	Biological categories	n	Biological categories
Recovered	Up	79	Cell division (48%): - Chromosome organization (11%) - DNA metabolic processes (15%) - RNA metabolic processes (13%) - Cell cycle (6%) - Cell division (3%) Immunity – B cell proliferation (1%) Pigmentation (3%)	0	
	Down	48	Immunity and inflammation (27%): - Cytokine production (13%) - Response to cytokine (2%) - Response to other organism (6%) - Immune system processes (4%) - Inflammatory response (2%) Metabolic processes (56%): - Lipid (21%) - Carbohydrate (10%) Lipid storage (4%)	330	Immunity and inflammation (36%): - Cytokine production (12%) - Response to cytokine (2%) - Response to other organism (2%) - Immune system processes (11%) - Innate immune response (5%) - Adaptive immune response (1%) - Inflammatory response (2%) Response to wounding (1%)

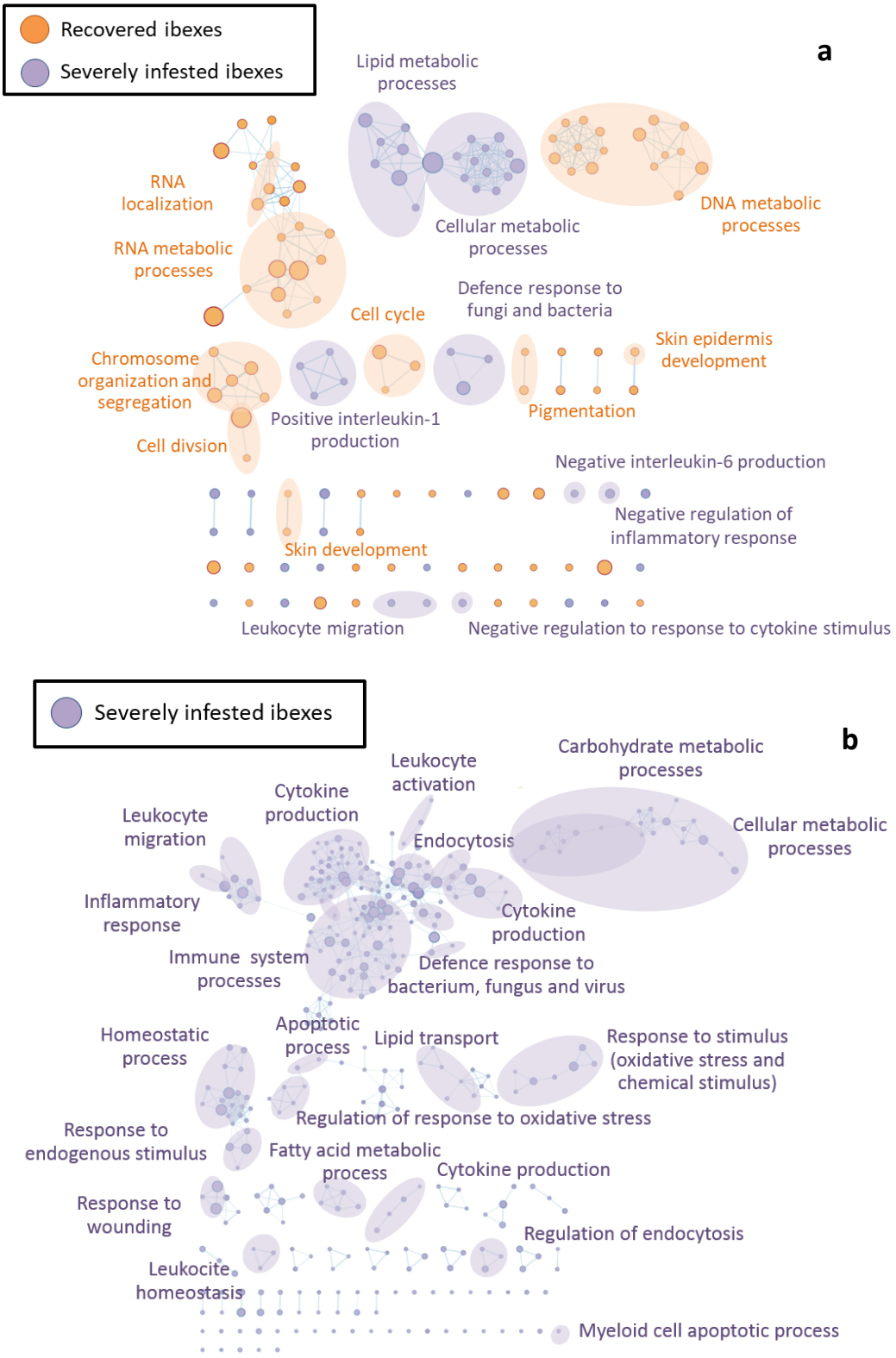

Figure 4.4.1. Networks of enriched gene sets ($p < 0.05$, FDR Q < 0.1) in the skin using GO biological process collection comparing the recovered and the severely infested ibexes in skin (a) and in blood (b). Cytoscape (v3.3) and Enrichment Map (Merico, Isserlin, Stueker, Emili, & Bader, 2010) were used for visualization the results. Nodes representing enriched gene sets are grouped and annotated by their similarity according to the biological category. Node size is proportional to the total number of genes within each gene set. Proportion of shared genes between gene sets is represented as the thickness of the line between nodes.

Table 4.4.4. Differentially enriched pathways between the recovered and the severely infested ibexes observed using the KEGG collection (nominal *p-value* < 0.05 and FDR < 0.1) in skin and blood. In bold, pathways related with immunity and inflammation.

		Expression change as compared to severely infested			
		SKIN		BLOOD	
		n	Pathways	n	Pathways
Recovered	Up	5	- **Antigen processing and presentation** - Cell cycle - Mismatch repair - Spliceosome - Homologous recombination	0	
	Down	12	- Peroxisome - PPAR signalling pathway - Valine leucine and isoleucine degradation - Galactose metabolism - Fatty acid metabolism - Fructose and mannose metabolism - Glycosaminoglycan degradation - Starch and sucrose metabolism - Tryptophan metabolism - Glycolysis gluconeogenesis - Adipocytokine signalling pathway - Olfactory transduction	21	- Leishmania infection - **Toll like receptor signalling pathway** - **NOD-like receptor signalling pathway** - **Fc gamma R mediated phagocytosis** - ERBB signalling pathway - Vibrio cholera infection - **Chemokine signalling pathway** - Epithelial cell signalling in *Helicobacter pylori* infection - **Cytokine-cytokine receptor interaction** - Lysosome - Glycerophospholipid metabolism - JAK/STAT signalling pathway - Glutathione metabolism - Ether lipid metabolism - Prostate cancer - Renal cell carcinoma - N glycan biosynthesis - Non-small cell lung cancer - **Fc epsilon RI signalling pathway** - Glioma - Acute myeloid leukaemia

Table 4.4.5. Comparison of the pathways upregulated in the skin and blood of the ibexes that recovered and the severely infested ones as compared to the controls, using the KEGG collection (nominal *p*-value < 0.05 and FDR < 0.1) in skin and blood. In bold, pathways related with immunity and inflammation.

	Upregulation as compared to controls			
	SKIN		**BLOOD**	
	n	Pathways	n	Pathways
Recovered	12	- **Antigen processing and presentation** - **Cytokine-cytokine receptor interaction*** - Autoimmune thyroid disease - **NOD-like receptor signalling pathway*** - JAK/STAT signalling pathway - **T cell receptor signalling pathway** - Prion diseases - **Intestinal immune network for IgA production** - Graft versus host disease - Allograft rejection - Type I diabetes mellitus - Asthma	1	- Olfactory transduction
Severely infested	0		8	- **Cytokine-cytokine receptor interaction*** - Leishmania infection - **NOD-like receptor signalling pathway*** - **Toll-like receptor signalling pathway** - Epithelial cell signalling in *Helicobacter pylori* infection - **Complement and coagulation cascades** - JAK/STAT signalling pathway - Lysosome

* Pathways upregulated in the skin of the recovered ibexes and in the blood of the severely infested ones as compared to the controls.

4.4.5. Discussion

To the authors' knowledge, this is the first time genomic response to sarcoptic mange has been studied in Iberian ibexes. Moreover, it identifies differences in both local and systemic gene expression related to the clinical expression of sarcoptic mange. Up to date, the modulation of gene expression by *S. scabiei* has been previously studied only in mice spleen (Arlian et al., 2007) and in human skin equivalents (Morgan et al., 2013). *Sarcoptes scabiei* infestation promoted immune and inflammatory response in Iberian ibexes, both in skin and in blood, and was associated with a lower expression of genes related to cell division. The ibexes that recovered from mange showed lower local and systemic inflammatory and immune responses and higher cell activity in skin that the severely affected ibexes. In addition, genes related to immunity were more expressed in the skin in the ibexes that recovered and in the blood of the severely infested ibexes, both when comparing both groups between them and with the control ibexes.

This study further confirms that Iberian ibexes can spontaneously control *S. scabiei* infestation and even recover under experimental infestation conditions, something already reported in free-ranging Iberian ibexes in the SNNNP (Alasaad et al., 2013). However, the pathogenesis of sarcoptic mange and the immune mechanisms leading to resistance against *S. scabiei* in Iberian ibexes is still not yet fully understood.

As compared with the recovered and the control ibexes, the overall pro-inflammatory and immune response profile found in the severely infested Iberian ibexes are consistent with an ineffective immune response against the mite and with the activation of defence response against secondary infections (Espinosa, Ráez-Bravo, et al., 2017; Nakagawa et al., 2009; Swe, Zakrzewski, Kelly, Krause, & Fischer, 2014). Beyond defence against bacteria, the processes up-regulated in the severely infested ibexes included innate immune response gene sets. Severe scabies clinical manifestations have been associated with a pronounced Th2 allergic immune response against the mite and its secretory products and eggs in humans (Mounsey et al., 2015; Walton et al., 2010). The up-regulated gene sets associated with inflammation, production and response to cytokines, mast cells and interferons found in the severely affected ibexes agree with this link between the allergic and inflammatory responses and the lesions (Bhat et al., 2017; Walton, Beroukas, Roberts-Thomson, & Currie, 2008). These results are consistent with the pro-inflammatory gene expression found in keratinocytes and fibroblasts of human skin equivalents in response to *S. scabiei* (Morgan et al., 2013).

Increasing serum immunoglobulins levels with mange severity have been reported in Iberian ibexes, but they seem to indicate exposition to the mite rather than have a protective effect (Ráez-Bravo, Granados, Serrano, et al., 2016). The increased expression of genes related to immunity found in the skin of the ibexes that recovered as compared to the enriched pathways of the severely infested ibexes (Tables 4.4.4 and 4.4.5) suggest that local cellular immunity plays a significant role in controlling the extension of mange. This agrees with previous studies suggesting that the effective immune response to sarcoptic mange is cell-mediated (Arlian et al., 1994; Bhat et al., 2017). Particularly, the most enriched pathways in the skin of the ibexes that recovered, "Antigen processing and presentation" and "T cell receptor signalling pathway" (Tables 4.4.4 and 4.4.5), further suggest that the appropriate recognition of *S. scabiei* by the host cellular immune system is essential to control the infestation. Sarcoptic mange-related changes in immune cellular populations in skin may vary among species and clinical pictures, and include effective immune response mediated by CD4+ T cells in humans (Bhat et al., 2017) or a significantly increased number of B lymphocytes in the skin of the most affected Alpine chamois (Salvadori et al., 2016). Conversely, in other species like foxes (Nimmervoll et al., 2013), free-living wombats (Skerratt, 2003a) or even in humans with crusted scabies (Walton et al., 2008), no plasma cells or B lymphocytes were found. These findings agree with the lower number of plasma cell previously reported in the infested ibexes of this study (Espinosa, Ráez-Bravo, et al., 2017).

On the other hand, the increased expression of inflammatory and immune processes in the blood of the severely infested ibexes suggest that, if the infestation is not controlled by the local cellular immune response in the skin, the systemic immune response seems not to achieve an efficient control of mange. To avoid immune cellular recognition, *S. scabiei* mites stimulate the secretion of cytokines (Arlian et al., 2006; Lalli et al., 2004) and down-regulate the gene expression of the immune response in the spleen, including the antigen-presenting cells (Arlian et al., 2007). This agrees with the up-regulation of cytokine production gene sets found in this study in the severely infested ibexes as compared with the control and the recovered ones.

Higher energetic demands have been reported in mange-infested animals and attributed to different effects, including scratching, that hamper the conservation of energy because of interrupted rest periods, and heat loss caused by alopecia (Martin et al., 2018). In Iberian ibex, sarcoptic mange have been reported to disrupt bottom-up regulation of body condition due to the detrimental effects of the disease overcoming the consumption of available food resources

(Carvalho et al., 2015). The up-regulation of the immune system observed in the severely infested ibexes could induce higher metabolic demands (Lochmiller & Deerenberg, 2000; Martin et al., 2018; Zuk & Stoehr, 2002), causing the reduction in resources allocated to other biological functions. Since cell division is a complex process that requires the production of large amounts of energy, new proteins, lipids and nucleic acids (Buchakjian & Kornbluth, 2010; Kalucka et al., 2015; Lee & Finkel, 2013; Salazar-Roa & Malumbres, 2017), the metabolic impact caused by sarcoptic mange could explain the higher percentage of processes associated with cell division found in the control ibexes, as well as in the skin of the recovered ibexes as compared to the severely ones. The fact that the ibexes in the study showed the effects of this negative energetic balance despite being fed *ad libitum* (Study III) suggests that the increase in energetic demands induced by sarcoptic mange is impossible to compensate in natural free-ranging conditions.

The up-regulated processes related with pigmentation found in the control ibexes, as well in the recovered as compared to the severely ones, are in accordance with the depigmentation observed in crusted scabies (Mounsey, McCarthy, & Walton, 2013). This find is consistent with the skin damage caused by *S. scabiei* in the most severe infested ibexes, where the different processes involved in skin pigmentation (Yamaguchi, Brenner, & Hearing, 2007) could be affected. Accordingly, processes related with response to radiation were also found in the control and in the recovered ibexes, since pigmentation is influenced by UV radiation (Slominski & Pawelek, 1998).

To summarize, this study provides the first insights in the genomic response of Iberian ibex to the infestation by *S. scabiei*, which modulates gene expression in this species. The infested ibexes showed an increased expression of inflammatory and immune processes both in skin and blood, and a downregulation of processes related with cell division. The upregulation of pathways related to immune response in the skin was related to the control of the infestation, suggesting a local effective immune response probably related with the antigen processing and presentation function and, therefore, with the activation of T cells. Conversely, an increased systemic inflammatory and immune response was present in the ibexes with severe infestation, showing a failure to effectively respond to *S. scabiei* infestation.

However, further research is required to clarify the immune mechanisms providing protection and resistance against sarcoptic mange. Such studies should include the investigation of the cellular and humoral immune response to sarcoptic mange, both locally in the skin and the

systemic response in blood, as well as determining both the genomic and immune responses in earlier stages of the disease.

5. General discussion

This thesis provides significant knowledge on the pathophysiological response of Iberian ibexes to sarcoptic mange, in both free-ranging ibexes and those experimentally infested with *S. scabiei*. In addition to the direct effect that the burrowing mite caused in the skin, the host immune response against *S. scabiei* is the primary cause of the clinical manifestation of the disease (Pence & Ueckermann, 2002). The acute phase proteins, immunoglobulin levels, haematological variables and gene expression of Iberian ibexes infested by *S. scabiei* were investigated in this thesis. The combined results obtained allow for a complete overview of the host response, and may serve as a basis for deepening in the understanding of the mechanisms explaining the differences observed in the individual clinical trend of the affected ibexes, from recovering to severe disease and mortality (Alasaad et al., 2013).

Acute phase protein concentration varies in relation to inflammation, infection or stress, among other causes, induced by cytokines (Cray et al., 2009; Petersen et al., 2004) and, consequently, *S. scabiei* are expected to alter APP levels (Bernal et al., 2011; Rahman et al., 2010). As seen in the **Study I**, the levels of two of the APPs studied, SAA and AGP, increased in Iberian ibexes affected by sarcoptic mange. According the classification previously used (Cerón et al., 2005), SAA could be considered as moderate APP in Iberian ibexes affected by sarcoptic mange, as it increased from two to ten times in the infested ibexes. However, the increase observed in the AGP, despite being significant, was lower. Moreover, the increases of both SAA and AGP correlated with the severity of mange, with higher response in the more severely affected individuals. In Alpine ibex (*Capra ibex*), a closely related species, SAA and AGP rose also with sarcoptic mange, but the increase was higher in both APPs (Rahman et al., 2010). The differences found between both species reinforce the need to stablish APPs reference values for each species, but also for each condition, since APP variation is related with the inflammation caused in the host. Higher increases in SAA concentration, deserving the classification as major APP, have been reported in Iberian ibex experimentally-induced inflammation with turpentine, in spite of lower SAA concentrations in the control ibexes as compared to the healthy ibexes of the **Study I** (Pastor et al., in press). Therefore, not only the nature of the inflammation but also differences in the basal values, related either to the capture method (physical restraint *versus* chemical restraint) or the management (free-ranging *versus* captivity) must be considered when interpreting APP reference values and variations.

The **Study I** suggested the possibility of a relation between SAA concentration and amyloidosis. A positive relation between SAA concentration and the number of organs with reactive amyloidosis was later identified in the experimentally infested ibexes, coupled with increased SAA values in the severely affected ibexes (Espinosa, Ráez-Bravo, et al., 2017). Therefore, SAA

concentration could be used as a prognostic tool in Iberian ibexes affected by sarcoptic mange, since secondary amyloidosis causes organ dysfunction in the severely affected ibexes.

The review of the methods currently used to diagnose sarcoptic mange revealed the lack of a reliable standardised method, since visual diagnosis of mange-compatible lesions is the most employed method in Iberian ibex. However, it has been recently demonstrated that this method has limited accuracy (Valldeperes et al., 2019). Furthermore, diagnostic confirmation was traditionally carried out by looking for the mite in skin scrapings or biopsies, but these methods have a low sensitivity, especially with low parasitic load (Walter et al., 2011; Walton & Currie, 2007) and differences in the mite densities between body areas have been observed in Iberian ibexes (Castro et al., 2018), which further hampers their use as a diagnostic method. Although the development of a real-time PCR has increased the sensitivity to detect the mite (Angelone-Alasaad et al., 2015), it still depends on getting the mite in the piece of skin sampled. For all these reasons, one of the objectives of the present thesis is to validate an optimal test to diagnose sarcoptic mange in Iberian ibexes.

In the **Study II**, the results of the ELISA tests evaluation to detect IgG in free-ranged Iberian ibexes showed higher specificity and sensitivity of the test developed for Alpine chamois (Rambozzi, Menzano, Lavin, & Rossi, 2004). This test used the avidin-biotin system, which improves the sensitivity since the complex formed is stable for the washes and incubations performed during the ELISA test procedure (Kendall et al., 1983). Based on this finding, in the **Study III** a validated ELISA test with the avidin-biotin system was used to determine the evolution of serum IgG concentrations in the experimental infestation of Iberian ibexes with *S. scabiei*. The detection of positive IgG levels from day 13 to 46 post infestation in all the experimentally infested ibexes further confirmed the usefulness of this indirect ELISA to diagnose sarcoptic mange in Iberian ibexes. This test can be helpful to confirm *S. scabiei* as the cause of compatible skin lesions in the first stages of the disease or before translocating Iberian ibexes. Moreover collecting the blood sample needed for the ELISA test is less invasive that performing skin scrapings or a obtaining a biopsy, so the ELISA test is also superior from an animal welfare approach. Therefore, this thesis achieved to validate and standardise a reliable, repeatable, sensitive and specific test to diagnose sarcoptic mange.

With regard to IgM, although a previous study suggest that the diagnosis test for sarcoptic mange should be based on the detection of this immunoglobulin due to it was produced before IgG (Arlian, Feldmeier, & Morgan, 2015), the results obtained in the **Study III** reveal that the ELISA test performed is not an efficient method to diagnose sarcoptic mange in Iberian

ibexes, probably due to the low affinity of this immunoglobulin to antigens (Eisen, 2014). Nonetheless, this ELISA could not be validated.

Differences in the epidemiology and the effects on population of sarcoptic mange among wild species, populations and areas have been widely reported (Bornstein et al., 2001; Pence & Ueckermann, 2002). Indeed, even within the same species, namely Iberian ibex, different epidemiological patterns of the disease have been reported (Fandos, 1991; León-Vizcaíno et al., 1999; Pérez et al., 1997) and, also individual differences in the clinical outcome have been observed in the same ibex population of the SNNNP, ranging from death to clinical recovering (Alasaad et al., 2013). As opposite to these previous reports, performed on free-ranging ibexes and therefore under the influence of a series of uncontrolled factors, the experimental infestation carried out in this thesis (**Study III**) allowed the identification of the factors determining the individual evolution of sarcoptic mange in the Iberian ibexes under controlled conditions of parasitic load, mite origin, climatology and other extrinsic factors.

The experimental infestation was designed to investigate the effect of the variability in the MHC, associated to the immune system (Bernatchez & Landry, 2003). The diversity in the class II MHC *DRB1* gene is low in the Iberian ibex populations, with the highest diversity detected in the SNNNP with four alleles (Amills et al., 2004; Angelone et al., 2018). However, the results failed to demonstrate an effect of the MHC on the Iberian ibex response to sarcoptic mange, since different clinical outcomes were identified for the same MHC allele combination, and severe infestations were registered for different MHC allele combinations.

The **Study III** provided further evidence that Iberian ibex can spontaneously recover from sarcoptic mange, as previously reported on the field in free-ranging conditions (Alasaad et al., 2013). Conversely to previous studies reporting higher severity of sarcoptic mange in free-ranging Iberian ibex males than in females (López-Olvera et al., 2015), no differences between males and females in the course of sarcoptic mange were found in the experimentally infested ibexes. This suggests that the differences between sexes found in the free-ranging ibexes might be related to behavioural or extrinsic factors, related to a higher infestation risk of males by living together in large groups and females only join in the rutting season (Alados & Escós, 1996; Granados, Pérez, et al., 2001; Pérez, Granados, & Soriguer, 1994), or a seasonal immunosuppression of males related to testosterone (Decristophoris et al., 2007; Klein, 2004) and higher loss of body condition during the rut season (Brivio et al., 2010; López-Olvera et al., 2015) in free-ranging conditions. The avoidance of these factors due to the controlled

conditions in the experimental infestation carried out in this thesis would therefore explain the lack of sex-related differences in susceptibility and severity of sarcoptic mange observed.

Resistance to sarcoptic mange has been associated with cellular immunity (Arlian et al., 1994; Bhat et al., 2017). The **Studies II** and **III** confirmed the non-protective role of the humoral immune response previously observed by other authors (Arlian et al., 1994; Casais et al., 2014), with higher levels associated to the severity of the mange lesions, and, therefore, to the parasitic load (Pérez et al., 2011). In addition, the gene expression found in the recovered ibexes in the **Study IV** suggests the role of pathways related with the recognition of the mite and the T cells activity in controlling the infestation in the skin at a local level.

The genomic response analysis carried out in the **Study IV** showed an increased expression of genes related to inflammation and immune response in the severely infested ibexes, which establish an ineffective host response against *S. scabiei* and a defence response against the secondary infections found in this ibexes (Espinosa, Ráez-Bravo, et al., 2017). Conversely, the ibexes that recovered achieved a local immune response at the skin that prevented a systemic inflammatory response, probably through an increased antigen recognition. Furthermore, the inflammatory systemic consequences caused directly or indirectly by the mite in the severe stages of the disease negatively affected the physical condition of the ibexes, causing anaemia among other changes, as previously reported (Arlian et al., 1988; Kido et al., 2011; Pérez et al., 2015). This anaemia could be related to erythrocyte lysis caused by inflammatory cytokines (Nemeth & Ganz, 2014), agreeing with the overall increased gene expression of inflammatory cytokines observed in the most affected ibexes (**Study IV**).

Management recommendations and future directions

Although Iberian ibex is not currently an endangered species, the economic and ecological importance of this species and the consequences of sarcoptic mange in ibex populations require management strategies based on scientific evidences to prevent and control the disease.

Different management strategies mostly based on empiric approaches have been implemented in the different ibex populations affected by mange, but none has achieved the eradication of the disease. As previously observed in different wild mammals, the disease remains endemic in the population, with cycles of variable mortality (Astorga et al., 2018; Guberti & Zamboni, 2000; Pence & Windberg, 1994). The management strategies used include

treatment with ivermectin and culling of diseased individuals, either any animal affected by sarcoptic mange or only those more severely affected (Fandos, 1991; Granados, Montes, et al., 2001; Junta de Andalucía. Consejería de Agricultura Pesca y Medio Ambiente, 2013; Sánchez-Isarria, Hermoso, Thereau de la Peña, et al., 2007; Sánchez-Isarria, Hermoso, Theureau de la Peña, et al., 2007).

However, the advances in knowledge derived from this thesis should be helpful to implement new management strategies or at least to rethink the current ones. The diagnostic and prognostic tools developed and validated in this thesis, namely the ELISA test and APPs, allow refining the management by using an individual decision-taking procedure for each affected ibex, according to the probability to recover. Although these tools are difficult to extensively apply in free-ranging populations, they can be used before performing translocations of Iberian ibexes from a population where sarcoptic mange is endemic. The ELISA would complement the visual detection of compatible lesions in early stages of the disease, when lesions are difficult to detect but the ibex can carry the mite, in order to prevent the introduction of *S. scabiei* in a naïve population.

The confirmation that Iberian ibexes can spontaneously recover from sarcoptic mange must drive to a reflection on the usefulness of ivermectin treatment, as well as the criteria of culling any single affected individual, since it could be a resistant one. Given the systemic consequences that *S. scabiei* produce in the host, the survival and recover of the ibexes with extensive lesions is unlikely. Therefore, eliminating only the severely infested ibexes would make sense for animal welfare reasons, but previous experiences have demonstrated that it has no effect on sarcoptic mange trend within the affected population.

Finally, the results suggesting that local skin immune response drives resistance to sarcoptic mange extension within the host open a research field for further studies directed to elucidate the factors that determine the recovering from sarcoptic mange. Specifically, determining the cellular immune response in the skin through immunohistochemical studies would shed light on the resistance mechanisms, allowing an even finer tuning of the management strategies for sarcoptic mange. Genomic and immune humoral and cellular analysis in early stages of the infestation would also provide relevant information on the resistance mechanisms to *S. scabiei* in Iberian ibexes.

6. Conclusions

1. Alpha-1-acid glycoprotein (AGP) and serum amyloid A (SAA) increase with sarcoptic mange infestation severity in Iberian ibex. Serum amyloid A can be considered a moderate APP and could be used as a prognostic indicator of sarcoptic mange in Iberian ibex.

2. The indirect ELISA test with the avidin-biotin system to detect specific IgG antibodies is an optimal diagnosis method to confirm the infestation with *S. scabiei* in Iberian ibex.

3. Class II MHC DRB-1 gene alleles are not related to resistance to sarcoptic mange in Iberian ibex.

4. Iberian ibex can spontaneously recover from sarcoptic mange.

5. *Sarcoptes scabiei* infestation induces a non-protective humoral immune response in Iberian ibex.

6. Sarcoptic mange induces anaemia, neutrophilia, and lymphopenia in the Iberian ibexes severely infested.

7. Sarcoptic mange severe infestation in Iberian ibex increases the gene expression of immune and inflammatory processes, resulting in an ineffective response against the mite and higher metabolic demands.

8. Resistance to *S. scabiei* infestation in Iberian ibex may be related with the effective recognition of the mite and the subsequent activation of T cells in the skin.

7. Acknowledgements

This thesis would not been possible without José Espinosa and Jesús María Pérez, from the Departamento de Biología Animal, Biología Vegetal y Ecología of the Universidad de Jaén; Francisco Javier Cano Manuel, from SNNNP; Ramón Soriguer, from the Estación Biológica de Doñana (CSIC); Paulino Fandos, from the Agencia de Medio Ambiente y Agua; José Joaquín Cerón, from the Departmento de Medicina y Cirugía Animal of the Universidad de Murcia; Rosa Casais, from the Servicio Regional de Investigación y Desarrollo Agroalimentario (SERIDA); Luca Rossi, from the Dipartimento di Scienze Veterinarie of Università di Torino; Debora Dellamaria and Carlo Citterio, from the Istituto Zooprofilattico Sperimentale delle Venezie; Anna Puigdemont, from the Departament de Farmacologia of the Universitat Autònoma de Barcelona; Lara Nonell, Elulalia Puigdecanet and Marta Bódalo, from the Servei d'Anàlisi de Microarrays of the Institut Hospital del Mar d'Investigacions Mèdiques.

It is also acknowledged the support of the institutional authorities of the SNNNP, the contributions of the SCSVNP and of the Sierra de Huétor Natural Park, to the transfer of their experimental facilities.

Ibex capture and sample collection would have not been possible without the aid of Apolo Sánchez, José López, Isidro Puga, Elías Martínez, Manuela Cárdenas, Francisco Casado and Antonio Rodríguez.

Thanks are also due to Rafaela Cuenca and Ester Bach, from the Servei d'Hematologia Clínica Veterinària of the Universitat Autònoma de Barcelona, and to Emanuela Cristelli, Francesca Denardi and Silvia Simonato for their support in the laboratory analyses.

8. References

Acevedo, P., & Cassinello, J. (2009). Biology, ecology and status of Iberian ibex *Capra pyrenaica*: a critical review and research prospectus. *Mammal Review*, *39*(1), 17–32.

Akaike, H. (1973). Information theory and an extension of the maximum likelihood principle. In B. N. Petrov & F. Csaki (Eds.), *Proceedings of the 2nd International Symposium on Information Theory* (pp. 267–281). Budapest: Akademiai Kiado.

Alados, C. L., & Escós, J. (1996). *Ecología y comportamiento de la cabra montés: consideraciones para su gestión.* (C. Martín-Escorza & A. Salvador-Milla, Eds.). Madrid: Museo Nacional de Ciencias Naturales, Consejo Superior de Investigaciones Científicas.

Alados, C. L., & Escós, J. (2012). Cabra montés – *Capra pyrenaica*. In A. Salvador & J. Cassinello (Eds.), *Enciclopedia Virtual de los Vertebrados Españoles*. Museo Nacional de Ciencias Naturales, Madrid.

Alasaad, S., Fickel, J., Rossi, L., Sarasa, M., Benítez-Camacho, B., Granados, J. E., & Soriguer, R. C. (2012). 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), 1–6.

Alasaad, S., Granados, J. E., Fandos, P., Cano-Manuel, F. J., Soriguer, R. C., & Pérez, J. M. (2013). The use of radio-collars for monitoring wildlife diseases: a case study from Iberian ibex affected by *Sarcoptes scabiei* in Sierra Nevada, Spain. *Parasites & Vectors*, *6*(1), 242.

Alasaad, S., Ndeereh, D., Rossi, L., Bornstein, S., Permunian, R., Soriguer, R. C., & Gakuya, F. (2012). The opportunistic *Sarcoptes scabiei*: A new episode from giraffe in the drought-suffering Kenya. *Veterinary Parasitology*, *185*, 359–363.

Alasaad, S., Permunian, R., Gakuya, F., Mutinda, M., Soriguer, R. C., & Rossi, L. (2012). Sarcoptic-mange detector dogs used to identify infected animals during outbreaks in wildlife. *BMC Veterinary Research*, *8*, 110.

Almberg, E. S., Cross, P. C., Dobson, A. P., Smith, D. W., & Hudson, P. J. (2012). Parasite invasion following host reintroduction: a case study of Yellowstone's wolves. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *367*, 2840–2851.

Altman, D. G., & Bland, J. M. (1983). Measurement in Medicine : the Analysis of Method Comparison Studies. *Statistician*, *32*, 307–317.

Amills, M., Jiménez, N., Jordana, J., Riccardi, A., Fernández-Arias, A., Guiral, J., … Sànchez, A. (2004). Low diversity in the major histocompatibility complex class II DRB1 gene of the Spanish ibex, *Capra pyrenaica*. *Heredity*, *93*(3), 266–272.

Angelone-Alasaad, S., Min, A. M., Pasquetti, M., Alagaili, A. N., Amelio, S. D., Berrilli, F., … Rossi, L.

(2015). Universal conventional and real-time PCR diagnosis tools for *Sarcoptes scabiei*. *Parasites & Vectors*, *8*(587).

Angelone, S., Jowers, M. J., Rita, A., Min, M., Fandos, P., Prieto, P., … Granados, J. E. (2018). Hidden MHC genetic diversity in the Iberian ibex (*Capra pyrenaica*). *BMC Genetics*, *19*(28).

Antón, J. M., Boticario, D., Granados, J. E., Habela, M. A., Márquez, F. J., Meana, A., … Sangenis, J. (2002). Estudio de las enfermedades parasitarias de la cabra montés. In J. M. Pérez (Ed.), *Distribución, genética y status sanitario de las poblaciones andaluzas de cabra montés* (pp. 117–153). Jaén, Spain: Universidad de Jaén–Consejería de Medio Ambiente de la Junta de Andalucía.

Arenas, A. J., Astorga, R. J., Carrasco, P. A., Chirosa, M., Gaina, C., Granados, J. E., … Ruiz-Martínez, I. (2002). Estudio epizootiológico de la sarcoptidosis. In J. M. Pérez-Jiménez (Ed.), *Distribución, genética y estatus sanitario de las poblaciones andaluzas de cabra montés* (pp. 69–115). Universidad de Jaén.

Arenas, A. J., Gómez, F., Salas, R., Carrasco, P., Borge, C., Maldonado, A., … Martínez-Moreno, F. J. (2002). An evaluation of the application of infrared thermal imaging to the tele-diagnosis of sarcoptic mange in the Spanish ibex (*Capra pyrenaica*). *Veterinary Parasitology*, *109*, 111–117.

Arlian, L. G. (1989). Biology, host relations, and epidemiology of *Sarcoptes scabiei*. *Annual Review of Entomology*, *34*, 139–161.

Arlian, L. G., Ahmed, M., & Vyszenski-Moher, D. L. (1988). Effects of *S. scabiei* var. *canis* (Acari: Sarcoptidae) on blood indexes of parasitized rabbits. *Journal of Medical Entomology*, *25*(5), 360–369.

Arlian, L. G., Bruner, R. H., Stuhlman, R. A., Ahmed, M., & Vyszenski-Moher, D. L. (1990). Histopathology in host parasitized by *Sarcoptes scabiei*. *The Journal of Parasitology*, *76*, 889–894.

Arlian, L. G., Fall, N., & Morgan, M. S. (2007). In vivo evidence that *Sarcoptes scabiei* (Acari: Sarcoptidae) is the source of molecules that modulate splenic gene expression. *Journal of Medical Entomology*, *44*(6), 1054–1063.

Arlian, L. G., Feldmeier, H., & Morgan, M. S. (2015). The potential for a blood test for scabies. *PLoS Neglected Tropical Diseases*, *9*(10).

Arlian, L. G., & Morgan, M. S. (2000). Serum antibody to *Sarcoptes scabiei* and house dust mite prior to and during infestation with *S. scabiei*. *Veterinary Parasitology*, *90*, 315–326.

Arlian, L. G., & Morgan, M. S. (2017). A review of *Sarcoptes scabiei*: past, present and future. *Parasites & Vectors*, *10*(297).

Arlian, L. G., Morgan, M. S., & Arends, J. J. (1996). Immunologic cross-reactivity among various strains of

Sarcoptes scabiei. *The Journal of Parasitology*, *82*, 66–72.

Arlian, L. G., Morgan, M. S., & Paul, C. C. (2006). Evidence that scabies mites (Acari: Sarcoptidae) influence production of interleukin-10 and the function of T-regulatory cells (Tr1) in humans. *Journal of Medical Entomology*, *43*(2), 283–287.

Arlian, L. G., Morgan, M. S., Rapp, C. M., & Vyszenski-Moher, D. L. (1996). The development of protective immunity in canine scabies. *Veterinary Parasitology*, *62*, 133–142.

Arlian, L. G., Morgan, M. S., Vyszenskimoher, D. L., & Stemmer, B. L. (1994). *Sarcoptes scabiei*: The circulating antibody response and induced immunity to scabies. *Experimental Parasitology*, *78*, 37–50.

Arlian, L. G., Runyan, R. A., & Estes, S. A. (1984). Cross infestivity of *Sarcoptes scabiei*. *Journal of the American Academy of Dermatology*, *10*(6), 979–986.

Arlian, L. G., & Vyszenski-Moher, D. L. (1988). Life cycle of *Sarcoptes scabiei* var. *canis*. *The Journal of Parasitology*, *74*(3), 427–430.

Arlian, L. G., Vyszenski-Moher, D. L., & Pole, M. J. (1989). Survival of adults and development stages of *Sarcoptes scabiei* var. *canis* when off the host. *Experimental & Applied Acarology*, *6*(3), 181–187.

Arlian, L. G., Vyszenski-Moher, L., Ahmed, S. G., & Estes, S. A. (1991). Cross-antigenicity between the scabieis mite, *Sarcoptes scabiei*, and the house dust mite, *Dermatophagoides pteronyssinus*. *Journal of Investigative Dermatology*, *96*, 349–354.

Astorga, F., Carver, S., Almberg, E. S., Sousa, G. R., Wingfield, K., Niedringhaus, K. D., … Escobar, L. E. (2018). International meeting on sarcoptic mange in wildlife, June 2018, Blacksburg, Virginia, USA. *Parasites and Vectors*, *11*(1).

Bach, E. (2012). *Acute phase proteins in wild boar, Pyrenean chamois and Spanish ibex: method validation and reference values*. Universitat Autònoma de Barcelona (Spain).

Beigh, S. A., Soodan, J. S., & Bhat, A. M. (2016). Sarcoptic mange in dogs: Its effect on liver, oxidative stress, trace minerals and vitamins. *Veterinary Parasitology*, *227*, 30–34.

Bengtsson, H., Irizarry, R., Carvalho, B., & Speed, T. P. (2008). Estimation and assessment of raw copy numbers at the single locus level. *Bioinformatics*, *24*(6), 759–767.

Bernal, L., Feser, M., Martínez-Subiela, S., García-Martínez, J. D., Cerón, J. J., & Tecles, F. (2011). Acute phase protein response in the capybara (*Hydrochoerus hydrochaeris*). *Journal of Wildlife Diseases*, *47*(4), 829–835.

Bernatchez, L., & Landry, C. (2003). MHC studies in nonmodel vertebrates: what have we learned about

natural selection in 15 years? *Journal of Evolutionary Biology*, *16*(3), 363–377.

Berrilli, F., D'Amelio, S., & Rossi, L. (2002). Ribosomal and mitochondrial DNA sequence variation in *Sarcoptes* mites from different hosts and geographical regions. *Parasitology Research*, *88*(8), 772–777.

Bhat, S. A., Mounsey, K. E., Liu, X., & Walton, S. F. (2017). Host immune responses to the itch mite, *Sarcoptes scabiei*, in humans. *Parasites & Vectors*, *10*(385).

Bland, J. M., & Altman, D. G. (1999). Measuring agreement in method comparison studies. *Statistical Methods in Medical Research*, *8*, 135–160.

Bornstein, S. (1991). Experimental infection of dogs with *Sarcoptes scabiei* derived from naturally infected wild red foxes (*Vulpes vulpes*): clinical observations. *Veterinary Dermatology*, *2*(3/4), 151–159.

Bornstein, S., Frössling, J., Näslund, K., Zakrisson, G., & Mörner, T. (2006). Evaluation of a serological test (indirect ELISA) for the diagnosis of sarcoptic mange in red foxes (*Vulpes vulpes*). *Veterinary Dermatology*, *17*(6), 411–416.

Bornstein, S., Mörner, T., & Samuel, W. M. (2001). *Sarcoptes scabiei* and sarcoptic mange. In W. M. Samuel, M. J. Pybus, & A. A. Kocan (Eds.), *Parasitic Diseases of Wild Mammals* (2nd ed., pp. 107–119).

Bornstein, S., Thebo, P., & Zakrisson, G. (1996). Evaluation of an enzyme-linked immunosorbent assay (ELISA) for the serological diagnosis of canine sarcoptic mange. *Veterinary Dermatology*, *7*, 21–28.

Bornstein, S., & Zakrisson, G. (1993a). Clinical picture and antibody response in pigs infected by *Sarcoptes scabiei* var. *suis*. *Veterinary Dermatology*, *4*(3), 123–131.

Bornstein, S., & Zakrisson, G. (1993b). Humoral antibody response to experimental *Sarcoptes scabiei* var. *vulpes* infection in the dog. *Veterinary Dermatology*, *4*, 107–110.

Bornstein, S., Zakrisson, G., & Thebo, P. (1995). Clinical picture and antibody response to experimental *Sarcoptes scabiei* var. *vulpes* infection in red foxes (*Vulpes vulpes*). *Acta Veterinaria Scandinavica*, *36*, 509–519.

Brewster, K., Henke, S. E., Hilton, C., & Ortega-S., A. (2017). Use of remote cameras to monitor the potential prevalence of sarcoptic mange in southern Texas, USA. *Journal of Wildlife Diseases*, *53*(2), 377–381.

Brivio, F., Grignolio, S., & Apollonio, M. (2010). To feed or not to feed? Testing different hypotheses on rut-induced hypophagia in a mountain nngulate. *Ethology*, *116*, 406–415.

Buchakjian, M. R., & Kornbluth, S. (2010). The engine driving the ship: Metabolic steering of cell proliferation and death. *Nature Reviews Molecular Cell Biology*, *11*, 715–727. https://doi.org/10.1038/nrm2972

Cabrera, A. (1911). The subspecies of the Spanish ibex. *Proceedings of the Zoological Society of London*, *81*(4), 963–977.

Carvalho, J., Granados, J. E., López-Olvera, J. R., Cano-Manuel, F. J., Pérez, J. M., Fandos, P., … Serrano, E. (2015). Sarcoptic mange breaks up bottom-up regulation of body condition in a large herbivore population. *Parasites & Vectors*, *8*(572).

Casais, R., Dalton, K. P., Millán, J., Balseiro, A., Oleaga, Á., Solano, P., … Parra, F. (2014). Primary and secondary experimental infestation of rabbits (*Oryctolagus cuniculus*) with *Sarcoptes scabiei* from a wild rabbit: Factors determining resistance to reinfestation. *Veterinary Parasitology*, *203*, 173–183.

Casais, R., Prieto, M., Balseiro, A., Solano, P., Parra, F., & Martín-Alonso, J. M. (2007). Identification and heterologous expression of a *Sarcoptes scabiei* cDNA encoding a structural antigen with immunodiagnostic potential. *Veterinary Research*, *38*, 435–450.

Casas-Díaz, E., Marco, I., López-Olvera, J. R., Mentaberre, G., & Lavín, S. (2011). Comparison of xylazine-ketamine and medetomidine-ketamine anaesthesia in the Iberian ibex (*Capra pyrenaica*). *European Journal of Wildlife Research*, *57*, 887–893.

Castro, I., De La Fuente, A., Fandos, P., Cano-Manuel, F., Granados, J. E., Soriguer, R. C., … Pérez, J. M. (2016). On the population biology of *Sarcoptes scabiei* infesting Iberian ibex (*Capra pyrenaica*). *International Journal of Acarology*, *42*(1).

Castro, I., Espinosa, J., Granados, J. E., Cano-Manuel, F. J., Fandos, P., Ráez-Bravo, A., … Pérez, J. M. (2018). Characterizing the growth of *Sarcoptes scabiei* infrapopulations. *Experimental and Applied Acarology*, *76*(1), 41–52.

Cavallero, S., Marco, I., Lavín, S., D'Amelio, S., & López-Olvera, J. R. (2012). Polymorphisms at MHC class II DRB1 exon 2 locus in Pyrenean chamois (*Rupicapra pyrenaica pyrenaica*). *Infection, Genetics and Evolution*, *12*(5), 1020–1026.

Ceciliani, F., Ceron, J. J., Eckersall, P. D., & Sauerwein, H. (2012). Acute phase proteins in ruminants. *Journal of Proteomics*, *75*(14), 4207–4231.

Ceciliani, F., Giordano, A., & Spagnolo, V. (2002). The systemic reaction during inflammation: the acute phase proteins. *Protein and Peptide Letters*, *9*(3), 211–223.

Cerón, J. J., Eckersall, P. D., & Martínez-Subiela, S. (2005). Acute phase proteins in dogs and cats: current

knowledge and future perspectives. *Veterinary Clinical Pathology*, *34*(2), 85–99.

Chandler, D. J., & Fuller, L. C. (2019). A Review of Scabies: An Infestation More than Skin Deep. *Dermatology*, *235*(2), 79–80.

Chen, C. C., Pei, K. J. C., Lai, Y. C., & Mortenson, J. A. (2012). Participatory epidemiology to assess sarcoptic mange in serow of Taiwan. *Journal of Wildlife Diseases*, *48*(4), 869–875.

Cohen, J. (1960). A coefficient of agreement for nominal scales. *Educational and Psychological Measurement*, *20*, 37–46.

Conner, J., Eckersall, P., Ferguson, J., & Douglas, T. (1988). Acute phase response in the dog following surgical trauma. *Research in Veterinary Science*, *45*, 107–110.

Crampe, J., Sourp, E., & Cavailhes, J. (2015). Reintroduction du bouquetin ibérique dans les Pyrenees, quand le reve devient realite. In *33èmes Rencoontres du GEEFSM* (p. 38). Balme (Italy).

Cray, C., Zaias, J., & Altman, N. H. (2009). Acute phase response in animals: A review. *Comparative Medicine*, *59*(6), 517–526.

Cross, P. C., Almberg, E. S., Haase, C. G., Hudson, P. J., Maloney, S. K., Metz, M. C., … Smith, D. W. (2016). Energetic costs of mange in wolves estimated from infrared thermography. *Ecology*, *97*(8), 1938–1948.

Dagleish, M. P., Ali, Q., Powell, R. K., Butz, D., & Woodford, M. H. (2007). Fatal *Sarcoptes scabiei* infection of blue sheep (*Pseudois nayaur*) in Pakistan. *Journal of Wildlife Diseases*, *43*(3), 512–517.

Davies, P., Moore, M., & Pointon, A. (1991). Seasonality of sarcoptic mange in pigs in South Australia. *Australian Veterinary Journal*, *68*(12), 390–392.

Davis, D. P., & Moon, R. D. (1990). Density of itch mite, *Sarcoptes scabiei* (Acari: Sarcoptidae) and temporal development of cutaneous hypersensitivity in swine mange. *Veterinary Parasitology*, *36*(3–4), 285–293.

De, U. K., & Dey, S. (2010). Evaluation of organ function and oxidant/antioxidant status in goats with sarcoptic mange. *Tropical Animal Health and Production*, *42*(8), 1663–1668.

Decristophoris, P. M. A., Von Hardenberg, A., & McElligott, A. G. (2007). Testosterone is positively related to the output of nematode eggs in male Alpine ibex (*Capra ibex*) faeces. *Evolutionary Ecology Research*, *9*(8), 1277–1292.

Deem, S. L., Noss, A. J., Cuéllar, R. L., Villarroel, R., Linn, M. J., & Forrester, D. J. (2002). Sarcoptic mange in free-ranging pampas foxes in the Gran Chaco, Bolivia. *Journal of Wildlife Diseases*, *38*(3), 625–628.

Díaz Luque, J. A., Berkunsky, I., Müller, H., & González, L. (2014). Clinical signs suggestive of mange infestation in a free-ranging maned wolf (*Chrysocyon brachyurus*) in the Moxos savannahs of Beni, Bolivia. *Mastozoologia Neotropical*, *21*(1), 135–138.

Drabick, J. J., Lupton, G. P., & Tompkins, K. (1987). Crusted scabies in human immunodeficiency virus infection. *Journal of the American Academy of Dermatology*, *17*(1), 142.

Dupuy, A., Dehen, L., Bourrat, E., Lacroix, C., Benderdouche, M., Dubertret, L., … Petit, A. (2007). Accuracy of standard dermoscopy for diagnosing scabies. *Journal of the American Academy of Dermatology*, *56*, 53–62.

Eckersall, Peter David Duthie, S., Safi, S., Moffatt, D. A., Horadagoda, N. U., Doyle, S., Parton, R., … Fitzpatrick, J. L. (1999). An automated biochemical assay for haptoglobin: prevention of interference from albumin. *Comparative Haematology International*, *9*, 117–124.

Eckersall, P. D. (2000). Acute phase proteins as markers of infection and inflammation: Monitoring animal health, animal welfare and food safety. *Irish Veterinary Journal*, *53*(6), 307–311.

Eckersall, P. D. (2008). Proteins, proteomics, and the dysproteinemias. In J. J. Kaneko, J. W. Harvey, & M. L. Bruss (Eds.), *Clinical Biochemistry of Domestic Animals* (6th ed., pp. 117–155). San Diego: Academic Press.

Eckersall, P. D., & Bell, R. (2010). Acute phase proteins: Biomarkers of infection and inflammation in veterinary medicine. *The Veterinary Journal*, *185*, 23–27.

Eckersall, P. D., Lawson, F. P., Bence, L., Waterston, M. M., Lang, T. L., Donachie, W., & Fontaine, M. C. (2007). Acute phase protein response in an experimental model of ovine caseous lymphadenitis. *BMC Veterinary Research*, *3*, 35.

Einsiedel, L. J., Pepperill, C., & Wilson, K. (2014). Crusted scabies: A clinical marker of human t-lymphotropic virus type 1 infection in central Australia. *Medical Journal of Australia*, *200*(11), 633–634.

Eisen, H. N. (2014). Affinity enhancement of antibodies: how low-affinity antibodies produced early in immune responses are followed by high-affinity antibodies later and in memory B-cell responses. *Cancer Immunology Research*, *2*(5), 381–392.

Espinosa, J., Granados, J. E., Cano-Manuel, F. J., López-Olvera, J. R., Ráez-Bravo, A., Romero, D., … Fandos, P. (2017). *Sarcoptes scabiei* alters follicular dynamics in female Iberian ibex through a reduction in body weight. *Veterinary Parasitology*, *243*, 151–156.

Espinosa, J., López-Olvera, J. R., Cano-Manuel, F. J., Fandos, P., Pérez, J. M., López-Graells, C., … Granados, J. E. (2017). Guidelines for managing captive Iberian ibex herds for conservation

purposes. *Journal for Nature Conservation*, *40*(September), 24–32.

Espinosa, J., Pérez, J. M., López-Olvera, J. R., Ráez-Bravo, A., Cano-Manuel, F. J., Fandos, P., … Romero, D. (2017). Evaluation of oxidant/antioxidant balance in Iberian ibex (*Capra pyrenaica*) experimentally infested with *Sarcoptes scabiei*. *Veterinary Parasitology*, *242*, 63–70.

Espinosa, J., Ráez-Bravo, A., López-Olvera, J. R., Pérez, J. M., Lavín, S., Tvarijonaviciute, A., … Velarde, R. (2017). Histopathology, microbiology and the inflammatory process associated with *Sarcoptes scabiei* infection in the Iberian ibex, *Capra pyrenaica*. *Parasites & Vectors*, *10*(596).

Fain, A. (1968). Etude de la Variabilité de *Sarcoptes scabiei* avec une Révision des Sarcoptidae. *Acta Zoologica et Pathologica Antverpiensia*, *47*, 1–196.

Fain, A. (1978). Epidemiological problems of scabies. *International Journal of Dermatology*, *17*(1), 20–30.

Fandos, P. (1991). *La cabra montés (*Capra pyrenaica*) en el Parque Natural de las Sierras de Cazorla, Segura y Las Villas*. Madrid, Spain: Icona-CSIC Colección Técnica.

Fandos, P. (1995). Factors affecting horn growth in male Spanish ibex (*Capra pyrenaica*). *Mammalia*, *59*(2), 229–235.

Fandos, P., Arcenegui, P., Lora, M. A., Buron, D., Granados, J. E., & Cadenas, R. (2010). Evolución deográfica de la cabra montés en Andalucía en los últimos 100 años. *Galemys*, *22*, 347–358.

Fernández-Morán, J., Gómez, S., Ballesteros, F., Quirós, P., Benito, J., Feliu, C., & Nieto, J. (1997). Epizootiology of sarcoptic mange in a population of cantabrian chamois (*Rupicapra pyrenaica parva*) in Northwestern Spain. *Veterinary Parasitology*, *73*, 163–171.

Ferroglio, E., Gortázar, C., & Vicente, J. (2011). Wild ungulate diseases and the risk for livestock and public health. In R. Putman, M. Apollonio, & R. Andersen (Eds.), *Ungulate management in Europe: problems and practices* (pp. 192–214). Cambridge Univerity Press.

Fitzgerald, S. D., Cooley, T. M., Murphy, A., Cosgrove, M. K., & King, B. A. (2004). Sarcoptic mange in raccoons in Michigan. *Journal of Wildlife Diseases*, *40*(2), 347–350.

Fraser, T. A., Carver, S., Martin, A. M., Mounsey, K., Polkinghorne, A., & Jelocnik, M. (2018). A *Sarcoptes scabiei* specific isothermal amplification assay for detection of this important ectoparasite of wombats and other animals . *PeerJ*, *6*(e5291).

Fraser, T. A., Charleston, M., Martin, A., Polkinghorne, A., & Carver, S. (2016). The emergence of sarcoptic mange in Australian wildlife: an unresolved debate. *Parasites and Vectors*, *9*(316).

Fukuyama, S., Nishimura, T., Yotsumoto, H., Gushi, A., Tsuji, M., Kanekura, T., & Matsuyama, T. (2010). Diagnostic usefulness of a nested polymerase chain reaction assay for detecting *Sarcoptes scabiei*

DNA in skin scrapings from clinically suspected scabies. *British Journal of Dermatology*, *163*, 892–894.

Gakuya, F., Ombui, J., Heukelbach, J., Maingi, N., Muchemi, G., Ogara, W., … Alasaad, S. (2012). Knowledge of mange among Masai pastoralists in Kenya. *PLoS ONE*, *7*(8).

Gakuya, F., Ombui, J., Maingi, N., Muchemi, G., Ogara, W., Soriguer, R. C., & Alasaad, S. (2012). Sarcoptic mange and cheetah conservation in Masai Mara (Kenya): epidemiological study in a wildlife/livestock system. *Parasitology*, *139*, 1587–1595.

Gakuya, F., Rossi, L., Ombui, J., Maingi, N., Muchemi, G., Ogara, W., … 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), 1–7.

Galindo, R. C., Falconi, C., López-Olvera, J. R., Jim??nez-Clavero, M. ??ngel, Fern??ndez-Pacheco, P., Fern??ndez-Pinero, J., … de la Fuente, J. (2012). Global gene expression analysis in skin biopsies of European red deer experimentally infected with bluetongue virus serotypes 1 and 8. *Veterinary Microbiology*, *161*, 26–35.

Gamer, M., Lemon, J., & Fellows-Puspendra, I. (2012). irr: Various coefficients of interrater reliability and agreement. R package version 0.84

Gentleman, R., Carey, J., Bates, D., Bolstad, B., Dettling, M., Dudoit, S., … Zhang, J. (2004). Bioconductor: Open software development for computational biology and bioinformatics. *Genome Biology*, *5*(10), R80.

Gogna, N., Lee, K., & Howe, D. (1985). Norwegian scabies in Australian aborigines. *The Medical Journal of Australia*, *142*(2), 140–142.

Gómez-Puerta, L., Olazábal, J., Taylor, C., Cribillero, N., López-Urbina, M., & González, A. (2013). Sarcoptic mange in vicuña (*Vicugna vicugna*) population in Perú. *Veterinary Record*, *173*(11), 269–269.

Gonzalez-Astudillo, V., Leon-Alvarado, O. D., Ossa-Lopez, P. A., Rivera-Paez, F. A., & Ramírez-Chaves, H. E. (2018). Sarcoptic mange in wild quichua porcupines (*Coendou quichua Thomas*, 1899) in Colombia. *International Journal for Parasitology: Parasites and Wildlife*, *7*(1), 95–98.

González-Candela, M., León-Vizcaíno, L., & 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*(3), 456–465.

González-Quirós, P., & Solano, S. (2009). Monitoring of an outbreak of sarcoptic mange in Cantabrian chamois (*Rupicapra pyrenaica parva*) in the game reserves of Asturias (north of Spain). In F. J.

Pérez-Barbería & B. Palacios (Eds.), *El rebeco cantábrico* Rupicapra pyrenaica parva*: conservación y gestión de sus poblaciones* (pp. 292–319). Madrid: Ministerio de Medio Ambiente y Medio Rural y Marino.

González, F. H. ., Tecles, F., Martínez-Subiela, S., Tvarijonaviciute, A., Soler, L., & Cerón, J. J. (2008). Acute phase protein response in goats. *Journal of Veterinary Diagnostic Investigation*, *20*, 580–584.

Gortázar, C., Acevedo, P., Ruiz-Fons, F., & Vicente, J. (2006). Disease risks and overabundance of game species. *European Journal of Wildlife Research*, *52*(2), 81–87.

Gosselink, T., Van Deelen, T., Warner, R., & Mankin, P. (2007). Survival and Cause-Specific Mortality of Red Foxes in Agricultural and Urban Areas of Illinois. *Journal of Wildlife Management*, *71*(6), 1862–1873.

Granados-Torres, J. E., Ruiz-Martínez, I., Pérez-Alvarado, M. C., & Pérez-Jiménez, J. M. (1996). Experiencias de mantenimiento de cabra montés (*Capra pyrenaica*) en cautividad. Incidencias sanitarias y demográficas. *Bulletin d'Information Sur La Pathologie Des Animaux Sauvages En France*, *15*, 59–67.

Granados, J. E., Cano-Manuel, J., Castillo, A., Fandos, P., Pérez, J. M., Alasaad, S., … Soriguer, R. C. (2008). Evolución de la sarcoptidosis en la población de cabra montés de Sierra Nevada. In: II Congreso Internacional del género Capra en Europa, Granada, Spain, November 20–23.

Granados, J. E., Montes, J., Pérez, J. M., Serrano, E., Fandos, P., & Soriguer, R. C. (2001). Plan de gestión de la cabra montés (*Capra pyrenaica*) en el espacio natural protegido de Sierra Nevada. In *III Congreso forestal*. Granada (Spain).

Granados, J. E., Pérez, J. M., Márquez, E. J., Serrano, E., Soriguer, R. C., & Fandos, P. (2001). La cabra montés (*Capra pyrenaica*, SCHINZ 1838). *Galemys*, *13*(1), 3–37.

Granados, J. E., Pérez, J. M., Soriguer, R. C., Fandos, P., & Ruiz-Martínez, I. (1997). On the biometry of the Spanish ibex, *Capra pyrenaica*, from Sierra Nevada (Southern Spain). *Folia Zoologica*, *46*(1), 9–14.

Guberti, V., & Zamboni, L. (2000). Can the host resistance hypothesis explain the cyclic patterns observed in *Sarcoptes scabiei* in chamois (*Rupicapra rupicapra*)? *Parassitologia*, *42*(Suppl.1), 72.

Haas, C., Origgi, F. C., Rossi, S., López-Olvera, J. R., Rossi, L., Castillo-Contreras, R., … 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*(117).

Haas, C., Rossi, S., Meier, R., & Ryser-Degiorgis, M. P. (2015). Evaluation of a commercial ELISA for the

detection of antibodies to *Sarcoptes scabiei* in wild boar (*Sus scrofa*). *Journal of Wildlife Diseases*, *51*(3).

Haas, N., Wagemann, B., Hermes, B., Henz, B. M., Heile, C., & Schein, E. (2005). Crossreacting IgG antibodies against fox mite antigens in human scabies. *Archives of Dermatological Research*, *296*, 327–331.

Hameed, K., Angelone-Alasaad, S., Din, J. U., Nawaz, M. A., & Rossi, L. (2016). The threatening but unpredictable *Sarcoptes scabiei*: first deadly outbreak in the Himalayan lynx, *Lynx lynx isabellinus*, from Pakistan. *Parasites & Vectors*, *9*, 402.

Hanley, J. A., & McNeil, B. J. (1982). The meaning and use of the area under a receiver operating characteristic (ROC) curve. *Radiology*, *143*, 29–36.

Harf, R., & Sommer, S. (2005). Association between major histocompatibility complex class II DRB alleles and parasite load in the hairy-footed gerbil, *Gerbillurus paeba*, in the southern Kalahari. *Molecular Ecology*, *14*(1), 85–91.

Herrero, J., & Pérez, J. M. (2008). *Capra pyrenaica*. The IUCN Red List of Threatened Species 2008.

Hollanders, W., Vercruysse, J., Raes, S., & Bornstein, S. (1997). Evaluation of an enzyme-linked immunosorbent assay (ELISA) for the serological diagnosis of sarcoptic mange in swine. *Veterinary Parasitology*, *69*, 117–123.

Horadagoda, N. U., Knox, K. M. G., Gibbs, H. A., Reid, S. W. J., Horadagoda, A., Edwards, S. E. R., & Eckersall, P. D. (1999). Acute phase proteins in cattle: discrimination between acute and chronic inflammation. *Veterinary Record*, *144*, 437–441.

Hosni, M. M., & Maghrbi, A. A. (2014). Ectoparasites infestation of free-ranging hedgehog (*Etelerix algirus*) in north western Libya. *Open Veterinary Journal*, *4*, 12–15.

Hudson, P., Rizzoli, A., Grenfell, B., Heesterbeek, H., & Dobson, A. (2002). *The ecology of wildlife diseases*. Oxford, UK: Oxford University Press.

Hueli, L. E., & Díaz, V. (1989). Ixódidos (Acarina: Ixodidae) parásitos de *Capra pyrenaica* en la provincia de Granada. In *Resúmenes del VI Congreso Nacional y I Congreso Ibérico de Parasitología* (p. 223). Cáceres, Spain.

Hulbert, T. V., & Larsen, R. A. (1992). Hyperkeratotic (Norwegian) scabies with gram- negative bacteremia as the initial presentation of AIDS. *Clinical Infectious dDseases*, *14*(5), 1164–1165.

Ippen, R., Nickel, S., & Schröder, H. D. (1995). Krankheiten des jagdbaren Wildes. *Deutscher Landwirtschaftsverlag Berlin*, 189–195.

Irizarry, R. A., Hobbs, B., Collin, F., Beazer-Barclay, Y. D., Antonellis, K. J., Scherf, U., & Speed, T. P. (2003). Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics*, *4*(2), 249–264. h

Jayaraj, R., Hales, B., Viberg, L., Pizzuto, S., Holt, D., Rolland, J. M., … Walton, S. F. (2011). A diagnostic test for scabies: IgE specificity for a recombinant allergen of *Sarcoptes scabiei*. *Diagnostic Microbiology and Infectious Disease*, *71*, 403–407.

Junta de Andalucía. Consejería de Agricultura Pesca y Medio Ambiente. (2013). *Programa de gestión de la cabra montés en Andalucía*.

Kalema-Zikusoka, G., Kock, R., & Macfie, E. (2002). An outbreak of sarcoptic mange in free-ranging mountain gorillas (*Gorilla gorilla berengei*) in Bwindi Impenetrable National Park, South Western Uganda. *Veterinary Record*, *150*, 12–15.

Kalucka, J., Missiaen, R., Georgiadou, M., Schoors, S., Lange, C., De-Bock, K., … Carmeliet, P. (2015). Metabolic control of the cell cycle. *Cell Cycle*, *14*(21), 3379–3388.

Katsumata, K., & Katsumata, K. (2006). Simple method of detecting *Sarcoptes scabiei* var *hominis* mites among bedridden elderly patients suffering from severe scabies infestation using an adhesive-tape. *Internal Medicine (Tokyo, Japan)*, *45*(14), 857–859.

Kendall, C., Ionescu-Matiu, I., & Dreesman, G. R. (1983). Utilization of the biotin/avidin system to amplify the sensitivity of the enzyme-linked immunosorbent assay (ELISA). *Journal of Immunological Methods*, *56*(3), 329–339.

Kido, N., Kamegaya, C., Omiya, T., Wada, Y., Takahashi, M., & Yamamoto, Y. (2011). Hematology and serum biochemistry in debilitated, free-ranging raccoon dogs (*Nyctereutes procyonoides*) infested with sarcoptic mange. *Parasitology International*, *60*(4), 425–428.

Klein, S. L. (2004). Hormonal and immunological mechanisms mediating sex differences in parasite infections. *Parasite Immunology*, *26*, 247–264.

Komsta, L., & Novomestky, F. (2015). moments: Moments, cumulants, skewness, kurtosis and related tests. R package version 0.14.

Lalli, P. N., Morgan, M. S., & Arlian, L. G. (2004). Skewed Th1 / Th2 immune response to *Sarcoptes scabiei*, *90*, 711–714.

Landis, J. R., & Koch, G. G. (1977). The measurement of observer agreement for categorical data. *Biometrics*, *33*, 159–174.

Lastras, M. E., Pastor, J., Marco, I., Ruiz, M., Viñas, L., & Lavín, S. (2000). Effects of sarcoptic mange on serum proteins and immunoglobulin G levels in chamois (*Rupicapra pyrenaica*) and Spanish ibex

(*Capra pyrenaica*). *Veterinary Parasitology*, *88*, 313–319.

Lee, I. H., & Finkel, T. (2013). Metabolic regulation of the cell cycle. *Current Opinion in Cell Biology*, *25*(6).

Lee, K., Heresi, G., & Al Hammoud, R. (2019). Norwegian scabies in a patient with Down Syndrome. *Journal of Pediatrics*, *209*, 253.

Leek, J. T., Johnson, W. E., Parker, H. S., Fertig, E. J., Jaffe, A. E., Storey, J. D., … Torres, L. C. (2017). sva: Surrogate Variable Analysis. R package version 3.26.0.

Lehnert, B. (2014). BlandAltmanLeh: Plots (slightly extended) Bland-Altman plots. R package version 0.1.0.

León-Vizcaíno, L. (1990). Patología de la sarna en la Cabra montés en Cazorla. *Quercus*, *50*, 22.

León-Vizcaíno, L., Astorga, R., Escos, J., Alonso, F., Alados, C., Contreras, A., & Cubero, M. J. (1992). Epidemiología de la sarna sarcóptica en el Parque Natural de las Sierras de Cazorla, Segura y Las Villas. In *Proceedings of the International Congress on the Genus Capra in Europe* (pp. 95–99). Sevilla (Spain): Junta Rectora del Parque Natural Sierra de las Nieves, Consejería de Medio Ambiente, Junta de Andalucía.

León-Vizcaíno, L., Ruíz de Ybáñez, M. R., Cubero, M. J., Ortíz, J. M., Espinosa, J., Pérez, L., … Alonso, F. (1999). Sarcoptic mange in Spanish ibex from Spain. *Journal of Wildlife Diseases*, *35*(4), 647–659.

Little, S. E., Davidson, W. R., Howerth, E. W., Rakich, P. M., & Nettles, V. F. (1998). Diseases diagnosed in red foxes from the southeastern United States. *Journal of Wildlife Diseases*, *34*(3), 620–624.

Lochmiller, R. L., & Deerenberg, C. (2000). Trade-offs in evolutionary immunology: Just what is the cost of immunity? *Oikos*, *88*(1), 87–98.

López-Olvera, J. R., Serrano, E., Armenteros, A., Pérez, J. M., Fandos, P., Carvalho, J., … Granados, J. E. (2015). Sex-biased severity of sarcoptic mange at the same biological cost in a sexually dimorphic ungulate. *Parasites & Vectors*, *8*(583).

López-Ratón, M., Rodríguez-Álvarez, M. X., Cadarso-Suarez, C., & Gude-Sampedro, F. (2014). OptimalCutpoints: an R package for selecting optimal cutpoints in diagnostic tests. *Journal of Statistical Software*, *61*(8).

Lower, K. S., Medleau, L. M., Hnilica, K., & Bigler, B. (2001). Evaluation of an enzyme-linked immunosorbant assay (ELISA) for the serological diagnosis of sarcoptic mange in dogs. *Veterinary Dermatology*, *12*, 315–320.

Manceau, V., Crampe, J., Boursot, P., & Taberlet, P. (1999). Identification of evolutionary significant units in the Spanish wild goat, *Capra pyneraica* (Mammalia, Artiodactyla). *Animal Conservation*, *2*, 33–

39.

Márquez, F. ., Pérez, J. M., Granados, J. E., Soriguer, R. C., & Fandos, P. (2002). Estudios genéticos de las poblaciones de cabra montés. In J. M. Pérez-Jiménez (Ed.), *Distribución, genética y estatus sanitario de las poblaciones andaluzas de cabra montés* (pp. 257–276). Universidad de Jaén.

Martin, A. M., Fraser, T. A., Lesku, J. A., Simpson, K., Roberts, G. L., Garvey, J., … Carver, S. (2018). The cascading pathogenic consequences of *Sarcoptes scabiei* infection that manifest in host disease. *Royal Society Open Science*, *5*(180018).

Meling, S., Bårdsen, K., & Ulvund, M. J. (2012). Presence of an acute phase response in sheep with clinical classical scrapie. *Veterinary Research*, *8*(113).

Mellanby, K. (1944). The development of symptoms, parasitic infection and immunity in human scabies. *Parasitology*, *35*(4), 197–206.

Merico, D., Isserlin, R., Stueker, O., Emili, A., & Bader, G. D. (2010). Enrichment map: A network-based method for gene-set enrichment visualization and interpretation. *PLoS ONE*, *5*(11).

Micali, G., Lacarrubba, F., Verzì, A. E., Chosidow, O., & Schwartz, R. A. (2016). Scabies: Advances in noninvasive diagnosis. *PLoS Neglected Tropical Diseases*, *10*(6).

Millán, J., Casais, R., Colomar, V., Bach, E., Prieto, J. M., & Velarde, R. (2013). Experimental infection of wild-caught European rabbits (*Oryctolagus cuniculus*) with *Sarcoptes scabiei* from a naturally infected wild rabbit. *Medical and Veterinary Entomology*, *27*, 232–235.

Millán, J., Casáis, R., Delibes-Mateos, M., Calvete, C., Rouco, C., Castro, F., … Villafuerte, R. (2012). Widespread exposure to *Sarcoptes scabiei* in wild European rabbits (*Oryctolagus cuniculus*) in Spain. *Veterinary Parasitology*, *183*(3–4), 323–329.

Mitra, M., Mahanta, S. K., Sen, S., Ghosh, C., & Hati, A. K. (1993). *Sarcoptes scabiei* in animals spreading to man. *Tropical and Geographical Medicine*, *45*(142–143).

Moço, G., Guerreiro, M., Ferreira, A. F., Rebelo, A., Loureiro, A., Petrucci-Fonseca, F., & Pérez, J. M. (2006). The ibex *Capra pyrenaica* returns to its former Portuguese range. *Oryx*, *40*(3), 351–354.

Morgan, M. S., & Arlian, L. G. (1994). Serum antibody profiles of *Sarcoptes scabiei* infested or immunized rabbits. *Folia Parasitologica*, *41*, 223–227.

Morgan, M. S., Arlian, L. G., & Markey, M. P. (2013). *Sarcoptes scabiei* mites modulate gene expression in human skin equivalents. *PLoS ONE*, *8*(8).

Mörner, T. (1992). Sarcoptic mange in Swedish wildlife. *Revue Scientifique et Technique (International Office of Epizootics)*, *11*(4), 1115–1121.

Mörner, T., & Christensson, D. (1984). Experimental infection of red foxes (*Vulpes vulpes*) with *Sarcoptes scabiei* var. *vulpes*. *Veterinary Parasitology*, *15*, 159–164.

Morsy, G. H., & Gaafar, S. M. (1989). Responses of immunoglobulin-secreting cells in the skin of pigs during *Sarcoptes scabiei* infestation. *Veterinary Parasitology*, *33*, 165–175.

Morsy, T. A., Bakr, M. E., Ahmed, M. M., & Kotb, M. M. (1994). Human scabies acquired from a pet puppy. *Journal of the Egyptian Society of Parasitology*, *24*, 305–308.

Mounsey, K. E., McCarthy, J. S., & Walton, S. F. (2013). Scratching the itch: new tools to advance understanding of scabies. *Trends in Parasitology*, *29*(1), 35–42.

Mounsey, K. E., Murray, H. C., Bielefeldt-Ohmann, H., Pasay, C., Holt, D. C., Currie, B. J., … McCarthy, J. S. (2015). Prospective study in a porcine model of *Sarcoptes scabiei* indicates the association of Th2 and Th17 pathways with the clinical severity of scabies. *PLoS Neglected Tropical Diseases*, *9*(3).

Murata, H., Shimada, N., & Yoshioka, M. (2004). Current research on acute phase proteins in veterinary diagnosis: an overview. *The Veterinary Journal*, *168*, 28–40.

Nagsuk, P., Moore, R., & Lopez, L. (2015). A case report of crusted scabies in an adult patient with Down syndrome. *Dermatology Online Journal*, *21*(8), 13.

Nakagawa, T. L. D. R., Takai, Y., Kubo, M., Sakai, H., Masegi, T., & Yanai, T. (2009). A pathological study of sepsis associated with sarcoptic mange in raccoon dogs (*Nyctereutes procyonoides*) in Japan. *Journal of Comparative Pathology*, *141*(2–3), 177–181.

Nam, G.-H., Mishra, A., Gim, J.-A., Lee, H.-E., Jo, A., Yoon, D., … Kim, H.-S. (2018). Gene expression profiles alteration after infection of virus, bacteria, and parasite in the Olive flounder (*Paralichthys olivaceus*). *Scientific Reports*, *8*(1), 18065.

Nemeth, E., & Ganz, T. (2014). Anemia of inflammation. *Hematol Oncol Clin North Am.*, *28*(4), 671–681.

Newman, T. J., Baker, P. J., & Harris, S. (2002). Nutritional condition and survival of red foxes with sarcoptic mange. *Canadian Journal of Zoology*, *80*(1), 154–161.

Niedringhaus, K. D., Brown, J. D., Sweeley, K. M., & Yabsley, M. J. (2019). A review of sarcoptic mange in North American wildlife. *International Journal for Parasitology: Parasites and Wildlife*, *9*, 285–297.

Nimmervoll, H., Hoby, S., Robert, N., Lommano, E., Welle, M., & Ryser-Degiorgis, M. P. (2013). Pathology of sarcoptic mange in red foxes (*Vulpes vulpes*): macroscopic and histologic characterization of three disease stages. *Journal of Wildlife Diseases*, *49*(1), 91–102.

Ninomiya, H., & Ogata, M. (2005). Sarcoptic mange in free-ranging raccoon dogs (*Nyctereutes procyonoides*) in Japan. *Veterinary Dermatology*, *16*(3), 177–182.

Ojaimi, C., Qanud, K., Hintze, T. H., & Recchia, F. A. (2007). Altered expression of a limited number of genes contributes to cardiac decompensation during chronic ventricular tachypacing in dogs. *Physiological Genomics*, *29*, 76–83.

Old, J. M., Sengupta, C., Narayan, E., & Wolfenden, J. (2017). Sarcoptic mange in wombats—A review and future research directions. *Transboundary and Emerging Diseases*, *65*(2), 399-407.

Oleaga, A., Balseiro, A., & Gortázar, C. (2008). Sarcoptic mange in two roe deer (*Capreolus capreolus*) from northern Spain. *European Journal of Wildlife Research*, *54*(1), 134–137.

Oleaga, A., Casais, R., Balseiro, A., Espí, A., Llaneza, L., Hartasánchez, A., & Gortázar, C. (2011). New techniques for an old disease: sarcoptic mange in the Iberian wolf. *Veterinary Parasitology*, *181*(2–4), 255–266.

Oleaga, A., Casais, R., González-Quirós, P., Prieto, M., & Gortazar, C. (2008). Sarcoptic mange in red deer from Spain: Improved surveillance or disease emergence? *Veterinary Parasitology*, *154*, 103–113.

Ondersheka, K., Kutzer, E., & Richter, H. E. (1968). Die raude der gemse und ihre bekampfung. *Zeitschrift Für Jagdwissenschaft*, *14*, 12–27.

Overskaug, K. (1994). Behavioural changes in free-ranging red foxes (*Vulpes vulpes*) due to sarcoptic mange. *Acta Veterinaria Scandinavica*, *35*, 457–459.

Parikh, R., Mathai, A., Parikh, S., Sekhar, G. C., & Thomas, R. (2008). Understanding and using sensitivity, specificity and predictive values. *Indian Journal of Opthalmology*, *56*(1), 45–50.

Park, J. H., Kim, C. W., & Kim, S. S. (2012). The diagnostic accuracy of dermoscopy for scabies. *Annals of Dermatology*, *24*(2), 194–199.

Pastor, J., Bach-Raich, E., Ráez-Bravo, A., López-Olvera, J. R., Tvarijonaviciute, A., Granados, J. E., … Cuenca, R. (in press). Method validation, reference values and characterization of acute phase proteins response to experimentally induced inflammation and bluetongue virus infection in Iberian ibex. *Veterinary Clinical Pathology*.

Paterson, S., Wilson, K., & Pemberton, J. M. (1998). Major histocompatibility complex variation associated with juvenile survival and parasite resistance in a large unmanaged ungulate population (*Ovis aries L.*). *Proceedings of the National Academy of Sciences of the United States of America*, *95*(7), 3714–3719.

Paterson, W., Allen, B., & Beveridge, G. (1973). Norwegian scabies during immunosuppressive therapy. *British Medical Journal*, *4*, 211–212.

Pence, D. B., Casto, S. D., & Samuel, W. M. (1975). Variation in the chaetotaxy and denticulation of *Sarcoptes scabiei* (Acarina: Sarcoptidae) from wild canids. *Acarologia*, *17*(1), 160–165.

Pence, D. B., & Ueckermann, E. (2002). Sarcoptic mange in wildlife. *Revue Scientifique et Technique (International Office of Epizootics)*, *21*(2), 385–398.

Pence, D. B., & Windberg, L. A. (1994). Impact of a sarcoptic mange epizootic on a coyote population. *The Journal of Wildlife Management*, *58*(4), 624–633.

Pérez, J. M., González, F. J., Serrano, E., Granados, J. E., Fandos, P., Carro, F., & Soriguer, R. C. (2006). Is blood collected from shot Iberian ibex (*Capra pyrenaica*) useful for monitoring their physiological status? *European Journal of Wildlife Research*, *52*, 125–131

Pérez, J. M., Granados, J. ., Sarasa, M., & Serrano, E. (2011). Usefulness of estimated surface area of damaged skin as a proxy of mite load in the monitoring of sarcoptic mange in free-ranging populations of Iberian wild goat, *Capra pyrenaica*. *Veterinary Parasitology*, *176*, 258–264.

Pérez, J. M., Granados, J. E., González, F. J., Ruiz-Martínez, I., & Soriguer, R. C. (1999). Hematologic parameters of the Spanish ibex (*Capra pyrenaica*). *Journal of Zoo and Wildlife Medicine*, *30*, 550–554.

Pérez, J. M., Granados, J. E., & Soriguer, R. C. (1994). Population dynamic of the Spanish ibex *Capra pyrenaica* in Sierra Nevada Natural Park (southern Spain). *Acta Theriologica*, *39*(3), 289–294.

Pérez, J. M., Granados, J. E., Soriguer, R. C., Fandos, P., Márquez, F. J., & Crampe, J. P. (2002). Distribution, status and conservation problems of the Spanish ibex, *Capra pyrenaica* (Mammalia: Artiodactyla). *Mammal Review*, *32*(1), 26–39.

Pérez, J. M., Ruiz-Martínez, I., Granados, J. ., Soriguer, R. C., & Fandos, P. (1997). The dynamics of sarcoptic mange in the ibex population of Sierra Nevada in Spain - Influence of climatic factors. *Journal of Wildlife Reserch*, *2*(1), 86–89.

Pérez, J. M., Serrano, E., Soriguer, R. C., González, F. J., Sarasa, M., Granados, J. E., … Fandos, P. (2015). Distinguishing disease effects from environmental effects in a mountain ungulate: seasonal variation in body weight, hematology, and serum chemistry among Iberian ibex (*Capra pyrenaica*) affected by sarcoptic mange. *Journal of Wildlife Diseases*, *51*(1), 148–156.

Petersen, H. H., Nielsen, J. P., & Heegaard, P. M. H. (2004). Application of acute phase protein measurements in veterinary clinical chemistry. *Veterinary Research*, *35*, 163–187.

Piertney, S. B., & Oliver, M. K. (2006). The evolutionary ecology of the major histocompatibility complex. *Heredity*, *96*(1), 7–21.

Puigdemont, A., Brazís, P., Fondati, S., & Ferrer, L. (2002). Diagnóstico serológico de la sarna sarcóptica en el perro. *Consulta de Difusión Veterinaria*, *89*, 71–73.

Putman, R., Apollonio, M., & Andersen, R. (Eds.). (2011). *Ungulate management in Europe: problems*

and practices. Cambridge Univerity Press.

R Core Team. (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

R Development Core Team 3.2.1. (2015). R: a language and environment for statistical computing. R Foundation for Statistical Computing. Wien, Austria.

Ráez-Bravo, A., Granados, J. E., Cano-Manuel, F. J., Soriguer, R. C., Fandos, P., Pérez, J. M., … Romero, D. (2016). Toxic and essential element concentrations in Iberian ibex (*Capra pyrenaica*) from the Sierra Nevada Natural Park (Spain): Reference intervals in whole blood. *Bulletin of Environmental Contamination and Toxicology*, *96*, 276–280.

Ráez-Bravo, A., Granados, J. E., Cerón, J. J., Cano-Manuel, F. J., Fandos, P., Pérez, J. M., … López-Olvera, J. R. (2015). Acute phase proteins increase with sarcoptic mange status and severity in Iberian ibex (*Capra pyrenaica*, Schinz 1838). *Parasitology Research*, *114*, 4005–4010.

Ráez-Bravo, A., Granados, J. E., Serrano, E., Dellamaria, D., Casais, R., Rossi, L., … López-Olvera, J. R. (2016). Evaluation of three enzyme-linked immunosorbent assays for sarcoptic mange diagnosis and assessment in the Iberian ibex, *Capra pyrenaica*. *Parasites & Vectors*, *9*(558).

Rahman, M. M., Lecchi, C., Fraquelli, C., Sartorelli, P., & Ceciliani, F. (2010). Acute phase protein response in Alpine ibex with sarcoptic mange. *Veterinary Parasitology*, *168*, 293–298.

Rambozzi, L., Menzano, A., Lavin, S., & Rossi, L. (2004). Biotin-avidin amplified ELISA for detection of antibodies to *Sarcoptes scabiei* in chamois (*Rupicapra* spp.). *Veterinary Research*, *35*, 701–708.

Rampton, M., Walton, S. F., Holt, D. C., Pasay, C., Kelly, A., Currie, B. J., … Mounsey, K. E. (2013). Antibody responses to *Sarcoptes scabiei* apolipoprotein in a porcine model: relevance to immunodiagnosis of recent infection. *PLoS ONE*, *8*(6).

Rasero, R., Rossi, L., Soglia, D., Maione, S., Sacchi, P., Rambozzi, L., … Alasaad, S. (2010). Host taxon-derived *Sarcoptes* mite in European wild animals revealed by microsatellite markers. *Biological Conservation*, *143*(5), 1269–1277.

Real Decreto 1082/2009, de 3 de julio, por el que se establecen los requisitos de sanidad animal para el movimiento de animales de explotaciones cinegéticas, de acuicultura continental y de núcleos zoológicos, así como de animales de fauna silvestre (2009). España: Boletín Oficial del Estado.

Rehbein, S., Visser, M., Winter, R., Trommer, B., Matthes, H. F., Maciel, A. E., & Marley, S. E. (2003). Productivity effects of bovine mange and control with ivermectin. *Veterinary Parasitology*, *114*(4), 267–284.

Rentería-Solís, Z., Min, A. M., Alasaad, S., Müller, K., Michler, F. U., Schmäschke, R., … Wibbelt, G. (2014).

Genetic epidemiology and pathology of raccoon-derived *Sarcoptes* mites from urban areas of Germany. *Medical and Veterinary Entomology*, *28*(Suppl.1), 98–103.

Ritchie, M. E., Phipson, B., Wu, D., Hu, Y., Law, C. W., Shi, W., & Smyth, G. K. (2015). *limma* powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research*, *43*(7).

Roberts, L. J., Huffam, S. E., Walton, S. F., & Currie, B. J. (2005). Crusted scabies: clinical and immunological findings in seventy-eight patients and a review of the literature. *Journal of Infection*, *50*(5), 375–381.

Rodríguez-Cadenas, F., Carbajal-González, M. T., Fregeneda-Grandes, J. M., Aller-Gancedo, J. M., & Rojo-Vázquez, F. A. (2010). Clinical evaluation and antibody responses in sheep after primary and secondary experimental challenges with the mange mite *Sarcoptes scabiei* var. *ovis*. *Veterinary Immunology and Immunopathology*, *133*, 109–116.

Romani, L., Steer, A. C., Whitfeld, M. J., & Kaldor, J. M. (2015). Prevalence of scabies and impetigo worldwide: a systematic review. *The Lancet Infectious Diseases*, *15*, 960–967.

Rossi, L., Fraquelli, C., Vesco, U., Permunian, R., Sommavilla, G. M., Carmignola, G., … Meneguz, P. G. (2007). Descriptive epidemiology of a scabies epidemic in chamois in the Dolomite Alps, Italy. *European Journal of Wildlife Research*, *53*, 131–141.

Rossi, L., Fraquelli, C., Vesco, U., Permunian, R., Sommavilla, G. M., Carmignola, G., … Meneguz, P. G. (2007). Descriptive epidemiology of a scabies epidemic in chamois in the Dolomite Alps, Italy. *European Journal of Wildlife Research*, *53*, 131–141.

Rowe, M. L., Carver, S., & Whiteley, P. L. (2019). The treatment of sarcoptic mange (*Sarcoptes scabiei*) in wildlife : a systematic review. *Parasites & Vectors*, 1–14.

Ruykys, L., Taggart, D. A., Breed, W. G., & Schultz, D. (2009). Sarcoptic mange in southern hairy-nosed wombats (*Lasiorhinus latifrons*): distribution and prevalence in the Murraylands of South Australia. *Australian Journal of Zoology*, *57*(2), 129–138.

Salazar-Roa, M., & Malumbres, M. (2017). Fueling the cell division cycle. *Trends in Cell Biology*, *27*(1), 69–81.

Saleh, M. A., Mahran, O. M., & Bassam Al-Salahy, M. (2011). Circulating oxidative stress status in dromedary camels infested with sarcoptic mange. *Veterinary Research Communications*, *35*(1), 35–45.

Salvadori, C., Rocchigiani, G., Lazzarotti, C., Formenti, N., Trogu, T., Lanfranchi, P., … Poli, A. (2016). Histological lesions and cellular response in the skin of alpine chamois (*Rupicapra r. rupicapra*)

spontaneously affected by sarcoptic mange. *BioMed Research International*, *2016*(2).

Sampathkumar, K., Mahaldar, A. R., Ramakrishnan, M., & Prabahar, S. (2010). Norwegian scabies in a renal transplant patient. *Indian Journal of Nephrology*, *20*(2), 89–91.

Sánchez-Isarria, M. A., Hermoso, J., Thereau de la Peña, J., Casanova, G., Burgui, J. M., Sanchís, G., … Sánchez, R. (2007). La ivermctina como tratamiento de la sarna sarcóptica en el medio natural: vías de aplicación. In *Tendencias actuales en el Estudio y Conservación de los Caprinos Europeos. II Congreso Internacional del género Capra en Europa* (pp. 255–268). Granada (Spain).

Sánchez-Isarria, M. A., Hermoso, J., Theureau de la Peña, J., Casanova, G., Burgui, J. M., Sanchís, G., … Sánchez, R. (2007). Metodología empleada en la estrategia del control de la sarna sarcótica en la cabra montés de la reserva valenciana de la muela de cortes entre los años 2002 y 2007 (Valencia). In *Tendencias actuales en el Estudio y Conservación de los Caprinos Europeos. II Congreso Internacional del género Capra en Europa* (pp. 255–268). Granada (Spain).

Sarasa, M., Rambozzi, L., Rossi, L., Meneguz, P. G., Serrano, E., Granados, J. E., … Pérez, J. M. (2010). *Sarcoptes scabiei*: Specific immune response to sarcoptic mange in the Iberian ibex *Capra pyrenaica* depends on previous exposure and sex. *Experimental Parasitology*, *124*, 265–271.

Sarasa, M., Serrano, E., Gonzalez, G., Granados, J. E., Soriguer, R. C., Pérez, J. M., & Joachim, J. (2009). Pseudoectoparasites: a new tool for exploring the relationship between host behaviour and ectoparasites. *Animal Behaviour*, *77*(5), 1351–1356.

Sarasa, M., Serrano, E., Soriguer, R. ., Granados, J. E., Fandos, P., González, G., … Pérez, J. M. (2011). Negative effect of the arthropod parasite, *Sarcoptes scabiei*, on testes mass in Iberian ibex, *Capra pyrenaica*. *Veterinary Parasitology*, *175*, 306–312.

Schad, J., Ganzhorn, J. U., & Sommer, S. (2005). Parasite burden and constitution of major histocompatibility complex in the Malagasy mouse lemur, *Microcebus murinus*. *Evolution*, *59*(2), 439–450.

Schaschl, L. F. (2003). *Gamsräude. Österreichischer Jagd- und Fischereiverlag*. Wien, Austria.

Schmitt, S. M., Cooley, T. M., Friedrich, P. D., & Schillhorn van Veen, T. W. (1987). Clinical mange of the black bear (*Ursus americanus*) caused by *Sarcoptes scabiei* (Acarina, Sarcoptidae). *Journal of Wildlife Diseases*, *23*(1), 162–165.

Serrano, E., Colom-Cadena, A., Gilot-Fromont, E., Garel, M., Cabezón, O., Velarde, R., … Marco, I. (2015). Border disease virus: an exceptional driver of chamois populations among other threats. *Frontiers in Microbiology*, *6*(1307), 1–9.

Serrano, E., Granados, J. E., & Pérez, J. M. (2007). Sarcoptic mange and metapodial development in

growing male Iberian ibex (*Capra pyrenaica*). *Veterinary Parasitology*, *144*(3–4), 375–379.

Shackleton, D., & IUCN/SSC Caprinae Specialist Group. (1997). *Wild sheep and goats and their relatives. Status survey and conservation action plan for Caprinae*. (D. Shackleton, Ed.). IUCN, Gland, Switzerland and Cambridge, UK.

Simpson, K., Johnson, C. N., & Carver, S. (2016). *Sarcoptes scabiei*: The mange mite with mighty effects on the common wombat (*Vombatus ursinus*). *PLoS ONE*, *11*(3), 1–17.

Skerratt, L. F. (2003a). Cellular response in the dermis of common wombats (*Vombatus ursinus*) infected with *Sarcoptes scabiei* var. *wombati*. *Journal of Wildlife Diseases*, *39*(1), 193–202.

Skerratt, L. F. (2003b). Clinical response of captive common wombarts (*Vombatus ursinus*) infected with *Sarcoptes scabiei* var. *wombati*. *Journal of Wildlife Diseases*, *39*(1), 179–192.

Skerratt, L. F., Middleton, D., & Beveridge, I. (1999). Distribution of life cycle stages of *Sarcoptes scabiei* var. *wombati* and effects of severe mange on common wombats in Victoria. *Journal of Wildlife Diseases*, *35*(4), 633–646.

Slominski, A., & Pawelek, J. (1998). Animals under the sun: effects of ultraviolet radiation on mammalian skin. *Clinics in Dermatology*, *16*, 503–515.

Smith, D. W., & Almberg, E. (2007). Wolf diseases in Yellowstone National Park. *Yellowstone Science*, *185*(2), 17–19.

Stemmer, B. L., Arlian, L. G., Morgan, M. S., Rapp, C. M., & Moore, P. F. (1996). Characterization of antigen presenting cells and T-cells in progressing scabietic skin lesions. *Veterinary Parasitology*, *67*(3–4), 247–258.

Subramanian, A., Tamayo, P., Mootha, V. K., Mukherjee, S., Ebert, B. L., Gillette, M. A., … Mesirov, J. P. (2005). Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. *Proceedings of the National Academy of Sciences*, *102*(43), 15545–15550.

Suffredini, A. F., Fantuzzi, G., Badolato, R., Oppenheim, J. J., & O'Grady, N. P. (1999). New insights into the biology of the acute phase response. *Journal of Clinical Immunology*, *19*(4), 203–214.

Suzumiya, J., Sumiyoshi, A., Kuroki, Y., & Inoue, S. (1985). Crusted (Norwegian) scabies with adult T-cell leukemia. *Archives of Dermatology*, *121*(7), 903–904.

Swe, P. M., Zakrzewski, M., Kelly, A., Krause, L., & Fischer, K. (2014). Scabies mites alter the skin microbiome and promote growth of opportunistic pathogens in a porcine model. *PLoS Neglected Tropical Diseases*, *8*(5).

Tao, W., & Mallard, B. (2007). Differentially expressed genes associated with *Staphylococcus aureus*

mastitis of Canadian Holstein cows. *Veterinary Immunology and Immunopathology*, *120*, 201–211.

Tarigan, S. (2004). Antibody responses in naïve and sensitised goats infested by *Sarcoptes scabiei*. *Indonesian Journal of Animal and Veterinary Science*, *9*(4), 258–265.

Tataruch, F., Steineck, T., & Onderscheka, K. (1985). Investigations on the metabolism of chamois suffering from sarcoptic mange. In S. Lovari (Ed.), *The biology and management of mountain ungulates* (pp. 250–255). London: Croom- Helm.

Tecles, F., Fuentes, P., Martínez Subiela, S., Parra, M. D., Muñoz, A., & Cerón, J. J. (2007). Analytical validation of commercially available methods for acute phase proteins quantification in pigs. *Research in Veterinary Science*, *83*(1), 133–139.

Tompkins, D. M., Carver, S., Jones, M. E., Krkošek, M., & Skerratt, L. F. (2015). Emerging infectious diseases of wildlife: a critical perspective. *Trends in Parasitology*, *31*(4), 149–159.

Tvedten, H. (2010). Laboratory and clinical diagnosis of anemia. In D. Weiss & K. Wardrop (Eds.), *Schalm's Veterinary Hematology* (6th ed., pp. 152–161). Wilew-Blackwell.

Valldeperes, M., Granados, J. E., Gregorio, M., Prieto, P., Escribano, F., Cardells, J., … López-Olvera, J. R. (2018). Serological survey of sercoptic mange in Mediterranean Iberian ibex (*Capra pyrenaica*) populations. In *13th EWDA Conference*.

Valldeperes, M., Granados, J. E., Pérez, J. M., Castro, I., Ráez-Bravo, A., Fandos, P., … Mentaberre, G. (2019). How sensitive and specific is the visual diagnosis of sarcoptic mange in free-ranging Iberian ibexes? *Parasites & Vectors*, *12*(405), 1–7.

Van-Wick, M., & Hashem, B. (2019). Treatment of sarcoptic mange in an American black bear (*Ursus americanus*) with a single oral dose of fluralaner. *Journal of Wildlife Diseases*, *55*(1), 250–253.

Van Der Heijden, H. M. J. F., Rambags, P. G. M., Elbers, A. R. W., Van Maanen, C., & Hunneman, W. A. (2000). Validation of ELISAs for the detection of antibodies to *Sarcoptes scabiei* in pigs. *Veterinary Parasitology*, *89*, 95–107.

Walter, B., Heukelbach, J., Fengler, G., Worth, C., Hengge, U., & Feldmeier, H. (2011). Comparison of dermoscopy, skin scraping, and the adhesive tape test for the diagnosis of scabies in a resource-poor setting. *Archives of Dermatology*, *147*(4), 468–473.

Walton, S. F. (2010). The immunology of susceptibility and resistance to scabies. *Parasite Immunology*, *32*(8), 532–540.

Walton, S. F., Beroukas, D., Roberts-Thomson, P., & Currie, B. J. (2008). New insights into disease pathogenesis in crusted (Norwegian) scabies: the skin immune response in crusted scabies. *British Journal of Dermatology*, *158*(6), 1247–1255.

Walton, S. F., Choy, J. L., Bonson, A., Valle, A., McBroom, J., Taplin, D., … Kemp, D. J. (1999). Genetically distinct dog-derived and human-derived *Sarcoptes scabiei* in scabies-endemic communities in northern Australia. *The American Journal of Tropical Medicine and Hygiene*, *61*(4), 542–547.

Walton, S. F., & Currie, B. J. (2007). Problems in diagnosing scabies, a global disease in human and animal populations. *Clinical Microbiology Reviews*, *20*(2), 268–279.

Walton, S. F., Holt, D. C., Currie, B. J., & Kemp, D. J. (2004). Scabies: new future for a neglected disease. *Advanges in Parasitology*, *57*, 309–376.

Walton, S. F., Pizzutto, S., Slender, A., Viberg, L., Holt, D., Hales, B. J., … O'Hehir, R. (2010). Increased allergic immune response to *Sarcoptes scabiei* antigens in crusted versus ordinary scabies. *Clinical and Vaccine Immunology*, *17*(9), 1428–1438.

Walzer, C. (2008). Diseases of chamois. In *Zoo and wild animal medicine. Current therapy* (Sixth Edit, pp. 408–415). Elsevier Inc.

Wells, B., Innocent, G. T., Eckersall, P. D., McCulloch, E., Nisbet, A. J., & Burgess, S. T. G. (2013). Two major ruminant acute phase proteins, haptoglobin and serum amyloid A, as serum biomarkers during active sheep scab infestation. *Veterinary Research*, *44*(103).

Wickham, H. (2009). ggplot2: Elegant graphics for data analysis. *Springer-Verlag New York*.

Wood, S. (2017). *Generalized Additive Models: An Introduction with R* (2nd ed.). Chapman and Hall/CRC.

Yamaguchi, Y., Brenner, M., & Hearing, V. J. (2007). The regulation of skin pigmentation. *Journal of Biological Chemistry*, *282*(38), 27557–27561.

Yeruham, I., Rosen, S., Hadani, A., & Nyska, A. (1996). Sarcoptic mange in wild ruminants in zoological gardens in Israel. *Journal of Wildlife Diseases*, *32*(1), 57–61.

Yin, J., & Tian, L. (2014). Joint inference about sensitivity and specificity at the optimal cut-off point associated with Youden index. *Computational Statistics and Data Analysis*, *77*, 1–13.

Youden, W. J. (1950). Index for rating diagnostic tests. *Cancer*, *3*(1), 32–35.

Zahler, M., Essig, A., Gothe, R., & Rinder, H. (1999). Molecular analyses suggest monospecificity of the genus *Sarcoptes* (Acari: Sarcoptidae). *International Journal for Parasitology*, *29*(5), 759–766.

Zou, K. H., O'Malley, A. J., & Mauri, L. (2007). Receiver-operating characteristic analysis for evaluating diagnostic tests and predictive models. *Circulation*, *115*, 654–657.

Zuk, M., & Stoehr, A. M. (2002). Immune defense and host life history. *The American Naturalist*, *160*(Suppl.), S9–S22.

Zumpt, J. A. (1973). Present epidemiological problems of sarcoptic mange in wild and domestic animal. *South African Journal of Wildlife Research*, *3*(2), 119–120.

Zuur, A. F., Ieno, E. N., & Elphick, C. S. (2010). A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution*, *1*, 3–14.

Zuur, A. F., Saveliev, A. A., & Ieno, E. . (2014). *A Beginner's Guide to Generalised Additive Mixed Models with R*. Highland Statistics Ltd.