UNIVERSIDAD DE LEÓN

FACULTAD DE VETERINARIA

DEPARTAMENTO DE SANIDAD ANIMAL

TESIS DOCTORAL

"SARNA SARCÓPTICA OVINA: ASPECTOS CLÍNICOS, MÉTODOS DE DIAGNÓSTICO Y PREVALENCIA EN LA COMUNIDAD AUTÓNOMA DE CASTILLA Y LEÓN"

Memoria que presenta el Licenciado en Veterinaria D. Fausto RODRÍGUEZ CADENAS para optar al grado de DOCTOR POR LA UNIVERSIDAD DE LEÓN.

Directores: Francisco Antonio ROJO VÁZQUEZ, José Miguel ALLER GANCEDO y Juan Miguel FREGENEDA GRANDES.

León, 27 de Febrero de 2012

A MIS PADRES

A MAITE

Agradecimientos

Quiero expresar mi más sincero agradecimiento a todas las personas que de una u otra manera han permitido que esta Tesis Doctoral se haya llevado a cabo, y en especial a:

- A mis directores, el Dr. Francisco Antonio Rojo Vázquez, el Dr. José Miguel Aller Gancedo y el Dr. Juan Miguel Fregeneda Grades, por la oportunidad que me brindaron, y por su confianza y dedicación durante todos estos años.
- Al Dr. John F. Huntley, investigador del Moredun Research Institute (Scotland, UK) y al Dr. Jens G. Mattsson, investigador del Statens Veterinärmedicinska Anstalt (Uppsala, Sweden) por haberme permitido realizar parte de esta Tesis Doctoral en sus laboratorios.
- A la Consejería de Educación de la Junta de Castilla y León y al Fondo Social Europeo, por la financiación tanto personal como material (proyecto Ref. LE010A05).
- A los Laboratorios Oficiales de Sanidad Animal de la Junta de Castilla y León, por el suministro de los sueros para el estudio de prevalencia.
- A Dña. Gloria Fernández Bayón, técnico del Departamento de Sanidad Animal de la Universidad de León, por toda la ayuda que me ha brindado durante estos años.
- A todos los veterinarios y ganaderos que desinteresadamente han colaborado, permitiendo, los primeros, la localización de rebaños adecuados para los objetivos de esta tesis y los últimos la recogida de las muestras necesarias.
- A todos los compañeros del Departamento de Sanidad Animal de la Universidad de León. Gracias por los buenos momentos tanto dentro, pero sobre todo fuera del laboratorio.
- A Maite, por su inestimable apoyo y compresión durante todo el tiempo que he dedicado a este trabajo.

- Finalmente, gracias a mi familia y amigos, que simpre han estado ahí cuando les he necesitado.

Índice

Capítulo I

Revisión

bibliográfica general

La explotación del ganado ovino se realiza, principalmente, para la obtención de carne, leche y lana, contribuyendo de esta manera a satisfacer necesidades básicas para la supervivencia humana, como son la disponibilidad de alimento y de vestido. Además, en España, se considera una producción que tiene otras connotaciones adicionales, ya que contribuye a mantener la actividad económica y la población en zonas desfavorecidas y aprovecha recursos naturales no utilizados por ninguna otra actividad. Todo ello justifica la explotación del ganado ovino y la necesidad de su promoción y puesta en valor. El censo de ganado ovino en España es de 18,5 millones de animales, que contribuyen con sus producciones al 8% de la Producción Final Ganadera (Ministerio de Medio Ambiente y Medio Rural y Marino, 2010).

Los principales pilares en los que se ha sustentado tradicionalmente la producción animal son la sanidad, la genética, la alimentación y el manejo. Otros factores, como el bienestar animal, la seguridad alimentaria, la salud pública y los aspectos medioambientales son cada vez más demandados por la sociedad, hasta el punto de que hoy ya casi se puede afirmar que se encuentran al mismo nivel que los anteriores. La misión de la sanidad animal es controlar los agentes patógenos potenciales (priones, virus, bacterias, hongos y parásitos) que afectan o están presentes en los animales y de esta manera mejorar su eficiencia biológica, su bienestar, así como la protección de la salud pública.

El ganado ovino padece numerosas enfermedades transmisibles de repercusión variable. Las enfermedades ocasionadas por ácaros se conocen con el nombre de sarnas, y en el ganado ovino tienen importancia la sarna sarcóptica, psoróptica, corióptica, demodécica y las causadas por *Psorergates ovis* y por ácaros de productos almacenados (familia

Acaridae=Tyroglyphidae) (Cordero del Campillo y Rojo-Vázquez, 1999; Meana y Aller, 2006). La sarna sarcóptica es consecuencia de la infección por *Sarcoptes scabiei* var. *ovis*, que es un ácaro excavador, que produce lesiones costrosas principalmente en la cabeza y se conoce con el nombre de "*sarna seca*". La sarna psoróptica está originada por el ácaro superficial *Psoroptes ovis*, que da lugar a lesiones a modo de capa amarillenta y gruesa en las áreas provistas de lana, y se denomina vulgarmente con el nombre de "*roña*" o "*sarna húmeda*". El agente etiológico de la sarna corióptica es *Chorioptes bovis ovis*, que es también superficial, pero afecta a las articulaciones carpianas y tarsianas. *Demodex ovis*, que es el agente causal de la sarna demodécica ovina, vive en los folículos pilosos y la infección normalmente tiene un curso subclínico. Las zonas del cuerpo más frecuentemente afectadas son la cabeza, el tronco y los muslos, donde la piel aparece arrugada, seca, engrosada y con cubiertas furfuráceas. La sarna causada por el ácaro excavador *Psorergates ovis* da lugar a la aparición de zonas con la lana aglutinada, mordida o arrancada.

La sarna sarcóptica es una enfermedad poco estudiada en el ganado ovino, a pesar de que ocasiona pérdidas económicas considerables derivadas de una disminución de las producciones y un aumento del gasto en tratamientos farmacológicos, da lugar a disminución de bienestar animal debido al prurito y, además puede tener repercusiones en la salud pública debido a su potencial transmisión al hombre.

1. El agente etiológico

El agente causal de la sarna sarcóptica es *S. scabiei*, que es un ectoparásito obligado. A continuación se describe brevemente su encuadre taxonómico, morfología y ciclo biológico, aportándose algunos datos sobre su genoma y transcriptoma.

1.1. Encuadre taxonómico

S. scabiei está taxonómicamente incluido en el filo Arthropoda, clase Arachnida, subclase Acari, orden Astigmata, superfamilia Sarcoptoidea y familia Sarcoptidae (NCBI Tax Browser). Los miembros de la clase Arachnida poseen cuatro pares de patas y tienen el cuerpo dividido en cefalotórax y abdomen. Los pertenecientes al orden Astigmata se caracterizan por no tener espiráculos ni sistema traqueal detectable, estar débilmente esclerotizados y ser de movilidad lenta. La superfamilia Sarcoptoidea incluye dos familias importantes desde un punto de vista veterinario: Psoroptidae y Sarcoptidae, que se caracterizan porque todos sus representantes son parásitos obligados. La familia Psoroptidae (parásitos superficiales) comprende *Psoroptes ovis*, que es el causante de la sarna psoróptica que afecta a varias especies, incluida la oveja; *Psoroptes cuniculi*, que es el agente de la otoacariosis de la oveja, cabra y conejo; *Chorioptes bovis*, agente de la sarna corióptica que afecta a ovinos y bovinos; y *Otodectes cynotis*, responsable de la otoacariosis del perro. En la familia Sarcoptidae (parásitos que excavan túneles en la piel), tienen importancia *S. scabiei*, agente de la sarna sarcóptica en varias especies animales, incluidos los ovinos, y *Notoedres cati*, agente etiológico de la sarna notoédrica del gato.

Otros ácaros de interés veterinario debido a que causan enfermedades en animales pertenecen a los ordenes Trombidiformes (géneros *Demodex*, *Cheyletiella* y *Psorergates*) y Mesostigmata (género *Dermanyssus*).

1.2. Morfología

S. scabiei es un ácaro de color marrón claro, con las patas y las partes circundantes a la boca de color más oscuro y esclerotizadas. El cuerpo se divide en varias partes (Figura 1).

El gnatosoma es cuadrangular y está constituido por un par de quelíceros y pedipalpos que utiliza para alimentarse y excavar túneles en la epidermis del hospedador. El idiosoma tiene forma ovalada, y es ventralmente plano y dorsalmente convexo. La superficie del idiosoma está cubierta por finas estrías y dorsalmente posee un número variable de campos con espinas que tienen importancia taxonómica (Fain 1968). Las hembras (300-500 μm de longitud por 230-420 μm de anchura) son de mayor tamaño que los machos (213-285 μm de largo por 162-210 μm de ancho) (Fain, 1968). El coxal, que es la unión de las patas con el cuerpo, está embebido en la superficie ventral del cuerpo. El primer y segundo par de patas son cortos, se extienden más allá del margen antero-lateral del idiosoma, se sitúan a ambos lados del gnatosoma y próximos al mismo, y terminan en forma de ventosa (Mellanby, 1972). El tercer y cuarto pares de patas son también cortos, pero no se proyectan más allá del borde del cuerpo. La parte terminal de los pares de patas 3º y 4º de la hembra y 3º del macho es un largo pelo quitinoso, mientras que el 4º par de patas del macho termina con una ventosa. El ano es posterior y terminal en ambos sexos.

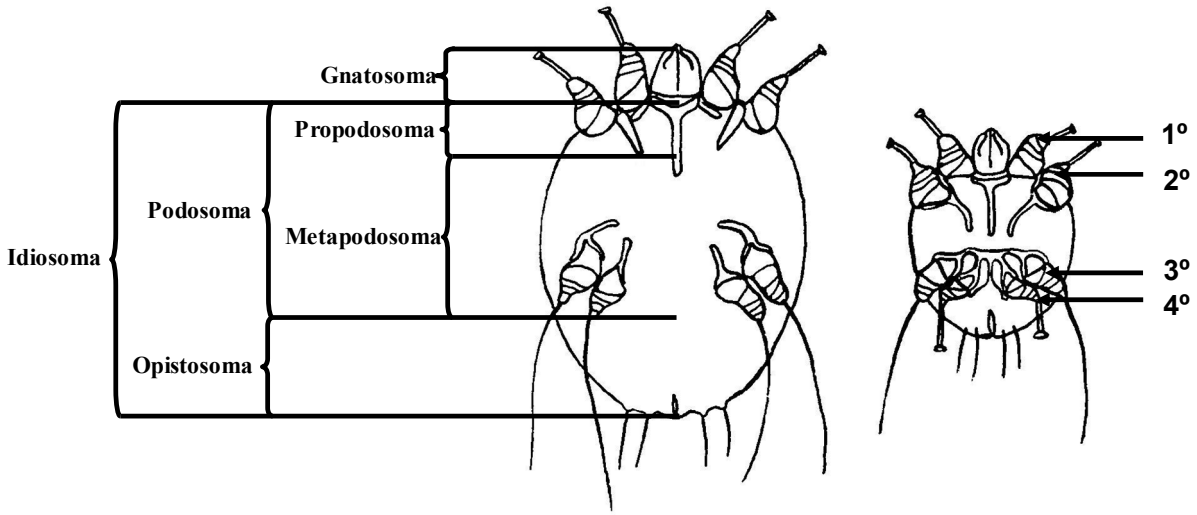


Figura. 1. Vista ventral esquemática de una hembra (izquierda) y de un macho (derecha) de *S. scabiei* en el que se indican las diferentes partes del cuerpo. Los números indican la denominación de los pares de patas.

Otras denominaciones que reciben las diferentes partes del cuerpo y que no se recogen en el esquema son: 1-Prosoma: incluye gnatosoma + podosoma; 2: Proterosoma: incluye gnatosoma + propodosoma y 3: Isterosoma: incluye metapodosoma + opistosoma.

1.3. Ciclo biológico

El ciclo biológico se compone de cinco etapas diferentes: huevo, larva, protoninfa, tritoninfa y adulto. La duración de cada una de las etapas y del ciclo biológico completo es diferente según la especie hospedadora de la que se ha obtenido el parásito.

S. scabiei es un ácaro excavador; durante todas las fases activas de su desarrollo penetra en el interior de la epidermis del hospedador en algún momento, formando túneles o galerías y alimentándose de linfa y células epidérmicas. Las hembras, una vez fecundadas, ponen 3-5 huevos al día dentro de los túneles, en cuyo interior se desarrolla una larva hexápoda, que eclosiona a los 3-5 días. Tras la eclosión algunas larvas salen a la superficie cutánea, otras pasan a los folículos pilosos o galerías preexistentes y otras continúan en la galería donde eclosionaron. A los 10-12 días se produce la muda a ninfas octópodas (protoninfa y tritoninfa), que en el caso de las larvas que permanecían en la superficie se realiza en pequeñas cavidadades que se conocen como"bolsas de muda". Las ninfas pueden permanecer en los túneles, las "bolsas de muda" o moverse por la superficie de la piel haciendo nuevos túneles. La muda de tritoninfa a adulto requiere 2-4 días adicionales. Las hembras adultas pueden ser fecundadas, comenzando a construir nuevas galerías, donde a los 4-5 días ponen los huevos. (Arlian y Vyszenski-Moher, 1988; Heilesen, 1946).

En la variedad humana, el tiempo necesario para el desarrollo desde huevo hasta adulto varía según los autores; por ejemplo, Mellanby (1944) lo cifró en 12-16 días y Heilesen

(1946) en 17-21 días. En la variedad porcina se necesitan 10-15 días para completar el ciclo biológico. En el modelo experimental en conejos, el desarrollo de huevo a adulto es más rápido y se completa en 10-13 días (Arlian y Vyszenski-Moher, 1988).

1.4. Genoma y transcriptoma

Recientemente se ha estimado que el genoma de *S. scabiei* contiene 96±7 millones de pares de bases que se distribuyen en 17 ó 18 cromosomas pequeños, sugiriendo que el sistema de determinación del sexo es del tipo XX/XO, de forma que las hembras (cariotipo XX) tendrían un cromosoma más que los machos (cariotipo XO) (Mounsey y col., 2012).

Un gran avance para el estudio de esta enfermedad ha sido la creación de librerías de ADNc de *S. scabiei*, lo que ha permitido establecer bases de datos con más de 50.000 marcadores de secuencia expresada (*Sarcoptes scabiei* DB), lo cual constituye una importante fuente de información para estudios moleculares sobre esta enfermedad (Fischer y col., 2003a y 2003b; Ljunggren y col., 2003).

2. Epidemiología

La epidemiología es la ciencia que se encarga del estudio de las enfermedades en las poblaciones y los factores que determinan su aparición. En este apartado aportamos información sobre cuales son las especies receptivas a la infección por *S. scabiei*, incluyendo datos sobre su prevalencia, el modo de transmisión de la infección y los factores que afectan a la misma, la especifidad de hospedador de *S. scabiei*, los factores de riesgo que determinan la presencia de la infección y el impacto económico de la enfermedad.

2.1. Especies receptivas y prevalencia

S. scabiei es un parásito de gran importancia veterinaria y médica ya que causa enfermedad en más de cien especies de mamíferos, incluido el hombre (Bornstein y col., 2001; Chakrabarti y col., 1981; Estes y col., 1983; Menzano y col., 2004; Pence y Ueckermann, 2002).

Entre los animales domésticos que pueden ser hospedadores de *S. scabiei* se incluyen la oveja (Rahbari y col., 2009), la cabra (Mulugeta y col., 2010), el cerdo (Rueda-López, 2006), la vaca (Urquhart y col., 1988), el perro (Feather y col, 2010), el gato (Malik y col., 2006), el conejo (Kaya y col., 2011) y los équidos (De Pennington y Colles, 2011; Osman y col., 2006). También afecta a animales de laboratorio como el ratón y el cobaya (Bornstein y col., 2001).

Un aspecto importante de la sarna sarcóptica es su impacto en la fauna silvestre, en la que se considera una enfermedad emergente/reemergente. En los últimos años se han descrito brotes de sarna sarcóptica en numerosas especies, como el zorro rojo y el lince en Europa (Bates, 2003; Ryser-Degiorgis y col., 2002; Sréter y col., 2003), mapache en América del Norte (Fitzgerald y col., 2004), camello en Asia (Al-Rawashdeh y col., 2000) y wombat en Australia (Martin y col., 1998). España no es una excepción, ya que se ha detectado la presencia de sarna sarcóptica en poblaciones de arruí, cabra montesa, rebeco, ciervo, corzo, conejo, zorro y lobo (Domínguez y col., 2008; Domínguez-Peñafiel y col., 2011; Fernández-Morán y col., 1997; González-Candela y col., 2004; Millán y col., 2012; Oleaga y col., 2008a, 2008b y 2011; Pérez y col., 2002).

Actualmente hay escasa información sobre la prevalencia de la sarna en los animales. La infección por *S. scabiei* es frecuente en España en el cerdo (prevalencia individual del 34%

y prevalencia de granja del 89% en el año 1996; Gutiérrez y col., 1996) y el conejo silvestre (seroprevalencia individual del 13%; Millán y col., 2012), así como en ovejas en Irán (prevalencia individual del 4,9%; Rahbari y col., 2009).

2.2. Modo de transmisión

Los animales con lesiones crónicas en la piel, que normalmente tienen una elevada carga parasitaria, constituyen la principal fuente de ácaros dentro de los rebaños. *S. scabiei* en todas las etapas activas de su desarrollo puede abandonar los túneles y situarse sobre la superficie cutánea. Por lo tanto, la transmisión es horizontal por contacto, ya sea pasando directamente de un animal a otro o contaminando el medio ambiente y a continuación pasando a otro animal (Arlian y Vyszenski-Moher, 1988).

2.3. Factores que afectan a la transmisión

La supervivencia de las distintas fases del desarrollo de *S. scabiei* fuera del hospedador depende de la humedad relativa (HR) y de la temperatura. Una HR elevada, combinada con una baja temperatura, prolonga la supervivencia de los ácaros, y consecuentemente temperaturas más altas y HR baja la reduce. *S. scabiei* sobrevive 24-36 horas en condiciones normales de laboratorio (21º C y 40-80% HR) (Arlian y col., 1984a). La supervivencia de las hembras adultas y de las ninfas es generalmente mayor que la de las larvas y machos adultos. La supervivencia más prolongada (2-3 semanas) se ha registrado en hembras adultas y ninfas mantenidas a 10º C y a una HR del 97% (Arlian y col., 1989). Por debajo de 20º C, *S. scabiei* permanece inmóvil, mientras que a 35º C tiene gran actividad. Cuando los ácaros se mantienen a una temperatura de 4º C y una HR del 95%, el tiempo medio de supervivencia es de 106 horas, por lo que estas condiciones se consideran un método adecuado para conservar

los inóculos destinados a infecciones experimentales, dando tiempo para estimar la cantidad de ácaros que hay en los mismos (Davis y Moon, 1987).

También se sabe que los ácaros aislados del perro tienen atracción selectiva hacia olores específicos de su hospedador y hacia estímulos térmicos, lo que le facilitaría el hallazgo de un nuevo hospedador (Arlian y col., 1984b).

El tiempo que necesita *S. scabiei* para penetrar la epidermis cuando previamente ha perdido contacto con su hospedador, y el tiempo que mantiene esa capacidad, son también factores importantes en la epidemiología de la enfermedad. En este sentido, Arlian y col. (1984a) observaron que a medida que se prolonga la falta de contacto de *S. scabiei* con su hospedador, las consecuencias son las siguientes; a) se inicia antes la penetración de la epidermis cuando se expone a un nuevo hospedador y b) se requiere más tiempo para completar la misma. Sin embargo, todos los ácaros que han sobrevivido tienen la capacidad de completar la penetración de la epidermis y, por lo tanto, de establecer una infección.

A pesar de la elevada resistencia de *S. scabiei* en ambientes fuera del hospedador, la atracción hacia estímulos térmicos y olores específicos del hospedador, y el mantenimiento de la capacidad infectante siempre que esté vivo, se ha visto que el riesgo real de transmisión de la infección a través del ambiente es bajo. Así, por ejemplo, se ha observado que no es necesaria la desinfección de las instalaciones para eliminar la sarna en explotaciones de cerdos (Jacobson y col., 1999). Mellanby (1944), en un estudio de transmisión de la enfermedad entre personas observó que únicamente cuatro de 272 habían adquirido la enfermedad después de utilizar camas que habían estado anteriormente ocupadas por pacientes gravemente afectados. En este sentido, parece que la principal ruta de transmisión de los ácaros es mediante contacto físico (Heilesen, 1946; Mellanby, 1972), aunque no debe

menospreciarse la posibilidad de contraer la infección a partir del ambiente en zonas con alto nivel de contaminación de ácaros y condiciones adecuadas para su supervivencia.

2.4. Especificidad de hospedador

Actualmente existe una gran controversia sobre la especificidad de hospedador de *S. scabiei*. Por un lado, la ausencia de diferencias morfológicas significativas entre aislados de *S. scabiei* obtenidos de distintas especies hospedadoras sugiere que *S. scabiei* es una única especie (Fain, 1968 y 1978).

En contraposición, otros autores mantienen que hay diferentes especies dentro del género *Sarcoptes*, las cuales infectarían a las diferentes especies hospedadoras (Kutzer y Grunberg, 1969). Esta afirmación se basa en estudios experimentales en los que, por ejemplo, no se consigue infectar al ratón, cobaya, cerdo, vaca, gato, cabra y oveja con aislados del perro, lo cual indicaría que los ácaros no se transmiten entre determinadas especies hospedadoras (Arlian y col., 1984c).

Sin embargo, Lavín y col. (2000) estudiaron la especificidad de hospedador de *S. scabiei* aislado de rumiantes y demostraron que es posible inducir sarna sarcóptica en rebecos utilizando *S. scabiei* de cabras domésticas. Estos autores observaron que los animales desarrollaban lesiones de sarna que se extendían progresivamente y hacían necesario el tratamiento para curar el proceso. Menzano y col. (2007) también describen un brote de sarna con un cuadro clínico grave en cabras domésticas ocasionado por *S. scabiei* procedente de rebecos. Además, la variedad ovina infecta con facilidad a la cabra (Abu-Samra y col., 1984; Ibrahim y Abu-Samra, 1985).

Arlian y col. (1996a) aportan datos que ponen en evidencia la existencia de diferentes cepas de *S. scabiei*. En sus experimentos utilizan tres variedades de *S. scabiei* para caracterizar la respuesta inmune humoral en varias especies hospedadoras y llegan a la conclusión de que realmente son variedades diferentes ya cada una de ellas contiene un conjunto de proteínas específicas. Sin embargo, el hecho de que también produzcan proteínas comunes indica que existe una relación filogenética próxima.

Las personas también pueden adquirir la sarna a partir de los animales, lo que hace que esta enfermedad tenga importancia en veterinaria de salud pública. Las personas con mayor riesgo a la infección zoonósica son las que manejan animales domésticos; por ejemplo, propietarios de perros, ganaderos, veterinarios y personal que trabaja en los mataderos (Burgess, 1994; Chakrabarti, 1990, Menzano y col., 2007). La forma más frecuente de infección humana se adquiere a partir del perro (Meijer y van Voorst Vader, 1990; Moriello, 2003; Orkin y Maibach, 1985). No obstante, se ha observado que este proceso es autolimitante en la mayor parte de los casos descritos (Burgess, 1994; Chakrabarti, 1990). Por esta razón, el tratamiento es innecesario ya que las personas afectadas están libres de los síntomas en pocos días. Sin embargo, se han observado casos atípicos que requieren de terapia específica ya que las lesiones persisten después de varias semanas (Menzano y col., 2007; Smith y Claypole, 1967). Por lo tanto, cuando se manipulen animales con sarna es recomendable llevar protección adecuada, como bata y guantes.

Estos estudios ponen de manifiesto la existencia de una limitada capacidad de infección cruzada de *S. scabiei*, por lo cual se considera que hay diferentes variedades "fisiológicas" que serían monoxenas u oligoxenas. Para hacer constar esta particularidad de *S. scabiei*, siempre hay que indicar de qué variedad estamos hablando, lo cual se hace añadiendo después

del nombre de la especie *S. scabiei* la abreviatura var., seguido del nombre del hospedador original del que proviene el ácaro; p.ej. *S. scabiei* var. *ovis* (oveja), *S. scabiei* var. *vulpes* (zorro), *S. scabiei* var. *canis* (perro) y *S. scabiei* var. *hominis* (hombre). El mecanismo responsable de la especificidad de hospedador no se conoce bien.

Sin embargo, si se tienen en cuenta estudios más recientes sobre genética de poblaciones encaminados a esclarecer la especificidad de hospedador de *S. scabiei*, el resultado es aún más ambiguo. Alasaad y col. (2009) y Zahler y col. (1999) no detectaron diferencias en un fragmento del espaciador interno transcrito 2 (ITS-2) del ADNr de ácaros de *S. scabiei* precedentes de varias especies de animales domésticos y silvestres. En otro estudio de variabilidad genética de la región ITS-2 y de un fragmento del gen mitocondrial ARNr 16S realizado con 28 ácaros aislados de rebeco y de zorros tampoco se detectaron diferencias genéticas según el hospedador de procedencia (Berrilli y col., 2002). Mediante el análisis de un fragmento del gen mitocondrial ARNr 12S tampoco se encontraron diferencias entre variedades de *S. scabiei* aislados de wombat, perro y hombre (Skerratt y col., 2002). Sin embargo, en este último estudio se detectó una división de las poblaciones de ácaros según su origen geográfico, lo que parece indicar un cierto aislamiento de las poblaciones. La conclusión de estos cuatro estudios es que *S. scabiei* es una única especie.

Walton y col. (1997 y 1999), mediante un análisis multisitio basado en el genotipado de tres marcadores microsatélites hipervariables, realizado en más de 700 ácaros recogidos de distintas áreas de Australia y América, han demostrado que existe divergencia entre las poblaciones de *S. scabiei* aisladas del perro y hombre. Además, en las poblaciones aisladas de un mismo hospedador, se detectó divergencia según su origen geográfico. En conjunto, estos resultados demuestran que los ácaros aislados del perro y del hombre de la misma región

geográfica son genéticamente diferentes y que, además, las poblaciones aisladas de un mismo hospedador pero de diferente región geográfica también lo son. Asimismo, no se produce intercambio de material genético entre ácaros procedentes de rebecos y zorros de la misma región geográfica, pero hay diferencias genéticas entre ácaros de la variedad del zorro procedentes de regiones geográficas diferentes (Soglia y col., 2007). Por lo tanto, estos estudios sugieren que las diferentes variedades serían en realidad especies diferentes. No obstante, un estudio de Walton y col. (2004a) detectó intercambio de material genético entre las poblaciones de *S. scabiei* var. *hominis* y las poblaciones de *S. scabiei* var. *canis* de la misma región geográfica, aunque esto es muy poco frecuente, lo cual indicaría que las dos variedades pertencen a la misma especie.

En conclusión, *S. scabiei* se considera en la actualidad una única especie con diferentes variedades "fisiológicas" debido al intercambio de material genético entre ellas, a que su morfología es similar y a su marcada especificidad de hospedador, pero existe cierta incertidumbre debido a las diferencias genéticas entre variedades simpátricas.

2.5. Factores de riesgo

Damriyasa y col. (2004) estudiaron los factores de riesgo que determinan la presencia de sarna sarcóptica en los rebaños porcinos, y observaron que la utilización de cama de paja es un factor de riesgo alto. Rahbari y col. (2009) han observado que la prevalencia de sarna en ovino es mayor en los machos y durante los meses de invierno. En los conejos silvestres, la prevalencia es mayor en las áreas de alta precipitación (Millán y col., 2012).

2.6. Impacto económico en ganado ovino

Las pérdidas económicas asociadas a la sarna ovina son cuantiosas. Se ha demostrado que cuando se administra moxidectina a ovejas con sarna sarcóptica la producción de leche aumenta un 22,4%, aunque posiblemente tal incremento no se deba exclusivamente a la eliminación de la infección por *S. scabiei* (Fthenakis y col., 2000). En corderos parasitados se ha observado que la administración de moxidectina produce un incremento de un 23,7% en la ganancia media diaria de peso y en un 15% el peso final al sacrificio (Fthenakis y col., 2000). También se sabe que en las ovejas con sarna sarcóptica la prolificidad es menor que en las sanas y el crecimiento de los corderos es más lento (Fthenakis y col., 2001). Otras pérdidas económicas, como las correspondientes a gastos en productos farmacológicos y su administración, también deben ser consideradas.

3. Patogenia

La patogenia de la infección por *S. scabiei* es compleja, y hay que considerar tanto los factores de patogenicidad de *S. scabiei* como la respuesta del hospedador tras el contacto con el parásito. A continuación se hace una breve descripción de la actividad enzimática de *S. scabiei* y su capacidad para inhibir el sistema del complemento y modular la producción de citocinas por diversos tipos celulares del hospedador, así como la respuesta de proteínas durante la fase aguda de la inflamación y las respuestas inmune celular y humoral que se observan en los hospedadores infectados. La infección por *S. scabiei* también produce cambios hemáticos y bioquímicos, y la colonización de las lesiones por bacterias es frecuente.

3.1. Actividad enzimática

Los extractos del parásito tienen actividad fosfatasa, esterasa, aminopeptidasa y glucosidasa, pero, a diferencia de otros ácaros, carecen de actividad serina peptidasa y no son capaces de hidrolizar la gelatina ni la caseína. Se cree que estas actividades catalíticas pueden contribuir a la inducción y manifestación de la respuesta inflamatoria e inmunitaria que se produce en los hospedadores infectados (Morgan y Arlian, 2006).

3.2. Inhibición del sistema del complemento

El sistema del complemento es uno de los componetes del sistema inmune y está formado por un conjunto de proteínas séricas que realizan diversas funciones como favorecer la fagocitosis, atraer neutrófilos y macrófagos y destruir células extrañas.

Se ha demostrado que *S. scabiei* produce parálogos de enzimas serina peptidasa que son inactivas, y se encuentran tanto en el interior como en el exterior del ácaro (Holt y col., 2003; Willis y col., 2006). Un estudio más profundo de estas proteínas ha puesto de manifiesto que son capaces de bloquear las tres rutas de la cascada del complemento, evitando, probablemente, que dicho sistema produzca daños en el aparato digestivo del parásito y por lo tanto actúa como un mecanismo de evasión de la respuesta inmune (Bergstrom y col., 2009).

3.3. Regulación de la producción de citocinas

Las citocinas, que son proteínas secretadas por diversos tipos celulares, tienen como función participar en la comunicación intercelular, regulando el mecanismo de la inflamación. *S. scabiei* libera moléculas que regulan su producción por varios tipos de células que participan en la respuesta inmunitaria, entre las que se encuentran los queratinocitos y

fibroblastos (Arlian y col., 2003; Mullins y col., 2009), las células mononucleares de sangre periférica (PBMCs) y las células dendríticas derivadas de éstas (NHDCs) (Arlian y col., 2004a), los linfocitos-T (Arlian y col., 2006), las células endoteliales de la microvasculatura de la dermis (HMVEC-D) (Elder y col., 2006 y 2009) y las células del bazo (Arlian y col., 2007).

Sin embargo, cuando se estudia la modulación de la producción de citocinas es preciso tener en cuenta las posibles interacciones celulares. Por ello, Morgan y Arlian (2010) han estudiado recientemente la respuesta de un cultivo celular similar a la piel del hombre a la estimulación con un extracto de *S. scabiei*, y han encontrado, a diferencia de lo que sucede en monocultivos de queratinocitos y fibroblastos, que produce niveles significativos de IL-1α, IL-1β y su inhibidor competitivo IL-1ra. La inducción de esta última citocina podría explicar el retraso de la reacción inflamatoria cutánea que se observa durante una infección primaria.

Se ha sugerido que la regulación de la producción de citocinas por *S. scabiei* permitiría al parásito evadir la respuesta inmune e inflamatoria del hospedador, y por lo tanto adaptarse a la vida a sus expensas (Morgan y Arlian, 2010).

3.4. Proteínas de respuesta aguda inflamatoria

Las proteínas de fase aguda son aquellas cuya concentación en el suero cambia tras un estímulo inflamatorio. La respuesta de proteínas durante la fase aguda de la inflamación es inducida por las citocinas, principalmente las IL-1 e IL-6, que actúan como moléculas mensajeras desde el sitio donde se produce la infección hasta los hepatocitos, que son las células encargadas de su síntesis y posterior liberación a la circulación (Petersen y col., 2004). Las proteínas de fase aguda producen, mediante diversos mecanismos, un estado de fiebre y

somnolencia que resulta beneficioso para la defensa del animal frente al agente infeccioso, y puede ser positivas (la concentración de la proteína aumenta tras el estímulo) o negativa (la concentración de la proteína disminuye) (Petersen y col., 2004).

En las cabras silvestres de los Alpes los niveles de amiloide A y de alfa 1 glucoproteína ácida en suero son 10 veces superiores en los animales infectados por *S. scabiei* que en los no infectados, mientras que los niveles de haptoglobina y ceruloplasmina son de dos a cinco veces superiores (Rahman y col., 2010). Los capibaras con sarna sarcóptica tienen unos niveles de haptoglobina y de alfa 1 glucoproteína ácida de 5 y 3 veces mayores que en los sanos. Sin embargo, el nivel de albúmina es un 13% menor en los animales infectados que en los no infectados (Bernal y col., 2011).

3.5. Respuesta inmune celular

El objetivo de la respuesta inmune celular es conseguir la estimulación y la proliferación de distintas poblaciones celulares para conferir protección. Los conejos que desarrollan resistencia a la reinfección por *S. scabiei* se caracterizan por tener una respuesta inmune humoral más baja y una respuesta inmune celular más elevada y rápida (Arlian y col., 1994a y 1994b), lo cual pone de manifiesto la importancia de la última en la inducción de inmunidad.

Las poblaciones celulares implicadas en la repuesta inmune celular a una infección primaria y a una prueba de desafío son diferentes. En el perro, en ambas infecciones se forman infiltrados celulares mixtos que contienen principalmente células mononucleares, neutrófilos, células plasmáticas y mastocitos. En la infección primaria predominan las células mononucleares y los mastocitos, mientras que la infección desafío se caracteriza por el

incremento de células mononucleares y neutrófilos. (Arlian y col., 1996b; Arlian y col., 1997; Stemmer y col., 1996). El rápido aumento y la mayor densidad de células CD3ε+ (Linfocitos T), CD11c+ (expresado en monocito/macrófago, granulocitos, linfocitos T citotóxicos, células de Langerhans y dendrocitos), CMH clase II+ (antígeno del complejo mayor de histocompatibilidad de clase II; expresado en células de Langerhans, dendrocitos dérmicos, monocito/macrófago, linfocitos B y linfocitos T) y CD1a+ (expresado en células de Langerhans de la epidermis) en la infección desafío comparado con la infección primaria indica que pueden jugar un papel importante en el desarrollo de resistencia (Stemmer y col., 1996). El posterior estudio de los linfocitos T implicados, ha demostrado que la subpoblación CD4+ es la que más aumenta durante la infección desafío en comparación con la infección primaria (Arlian y col., 1997).

3.6. Respuesta inmune humoral

En respuesta a la estimulación antigénica durante la infección primaria o la infección desafío, se induce una respuesta inmune de anticuerpos, que son moléculas que tienen como función neutralizar la capacidad de infección y facilitar la eliminación de patógenos. Los anticuerpos con isotipo IgG e IgE son los mayoritarios en la respuesta a la infección por *S. scabiei*, aunque también se han detectado niveles significativos de IgM (Arlian y col., 1994b; Arlian y col., 1996b, Arlian y Morgan, 2000; Arlian y col., 2004b; Bornstein y col., 1995; Rambozzi y col., 2007). También se ha observado que los animales que desarrollan resistencia a una prueba de desafío producen, durante la infección primaria, una respuesta inmune humoral más lenta y de menor intensidad que aquellos animales que eran receptivos (Arlian y col., 1994b, 1996b).

En una infección primaria experimental en cabras se ha observado que la seroconversión para la IgG se produce a los 30 días del contagio (Tarigan, 2004). Se han identificado cinco proteínas de 28-220 kDa del parásito que son reconocidas por las IgG a los 10 días postinfección, mientras que a los 50 días postinfección los sueros reconocían al menos 11 bandas del extracto antigénico. Las proteínas con pesos moleculares de 180, 135, 43 y 38 kDa son inmunodominantes para la reacción de IgG ya que son reconocidas por la mayor parte de los animales y con gran intensidad. Los niveles de IgE tras la infección primaria son más bajos, pero aumentan de forma progresiva. Se ha comprobado que dicha respuesta inmune se dirige frente a una banda de 130 kDa.

En la infección desafío en cabras (Tarigan, 2004) se ha detectado que los niveles de IgG aumentan más rápido. También se detecta una reacción inmunológica a bandas adicionales del antígeno y, en este caso, las proteínas con pesos moleculares 180, 60, 38, y 37 kDa son las inmunodominantes. Los niveles de IgE aumentan muy rápidamente y la reacción se dirige al menos a 10 antígenos de 25-130 kDa.

En conejos resistentes a la infección por *S. scabiei*, como consecuencia de una infección previa o de una inmunización con extractos preparados a partir de los ácaros del polvo *Dermatophagoides farinae* y *D. pteronyssinus*, se observa una respuesta inmune humoral más rápida pero de menor intensidad que en los que no desarrollan resistencia (Arlian y col., 1994b, 1995).

3.7. Cambios bioquímicos y hemáticos

El porcentaje de hemoglobina, el hematocrito, las concentraciones de eritrocitos, glutatión y albúmina, y la ratio albúmina/globulina son menores en las cabras infectadas que en las no infectadas. Sin embargo, las concentraciones de globulina, alanino aminotransferasas, bilirrubina, creatinina, nitrógeno ureico y nitrato, y la peroxidación de los lípidos de los eritrocitos son mayores en los animales infectados (De y Dey, 2010).

3.8. Infecciones sobreañadidas

La microbiota presente en las lesiones de animales y personas infectados por *S. scabiei* es amplia, destacando especies como *Staphylococcus aureus* o *Streptococcus* del grupo A (Brook, 1995; González-Candela y col., 2006; Steer y col., 2009). La colonización de las lesiones de sarna sarcóptica por estas bacterias se ha relacionado con consecuencias graves para la salud humana, como septicemia, gromerulonefritis, fiebre reumática y cardiopatía reumática (McDonald y col., 2004).

4. Signos clínicos y lesiones en ovino

En todas las especies hospedadoras, el signo clínico predominante de la sarna sarcóptica es el intenso prurito. En los ovinos, éste es el primero que se aprecia, lo que hace que los animales se muestren inquietos y se froten y muerdan las áreas afectadas. La piel aparece húmeda y caliente, presenta eritema intenso y además se forman numerosas pápulas y vesículas. A la semana de haber adquirido la infección, la piel se engrosa, está inflamada y se cubre de pequeñas costras secas y amarillentas. El persistente rascado y mordisqueo ocasiona

alopecia, exudación, y excoriaciones secundarias. A las tres o cuatro semanas, las zonas afectadas aparecen alopécicas y cubiertas de costras de 3-4 mm de espesor, con fisuras y agrietamientos que dan el aspecto de barro seco, por lo que el proceso se conoce vulgarmente con el nombre de "*sarna seca*". La sangre rezuma por las grietas y se aprecia un olor desagradable (Abu-Samra y col., 1981).

Las áreas más frecuentemente afectadas en la sarna sarcóptica ovina, se localizan principalmente en los labios superior e inferior, la nariz, las zonas periorbitales, la cara y la superficie externa de las orejas. En algunos animales también se observan lesiones en las extremidades, alrededor de las cuartillas y en el rodete coronario. Otras localizaciones menos frecuentes son la espalda, los flancos, el escroto, la zona inguinal, las nalgas y la cola. Los animales afectados comen menos y están emaciados. Las lesiones severas en los labios interfieren en la aprehensión del alimento y en la toma de agua, lo que puede incluso ocasionar la muerte por inanición (Abu-Samra y col., 1981).

En el examen histológico de las lesiones inducidas por *S. scabiei* en las ovejas se observa hiperqueratosis, acantosis, formación de una capa gruesa de costra debajo de la cual se encuentran los ácaros y cambios degenerativos en toda la epidermis. También se aprecian microabscesos y una marcada proliferación de tejido conectivo y de fibroblastos. La dermis está infiltrada con macrófagos, eosinófilos y linfocitos (Abu-Samra y col., 1981).

5. Diagnóstico

La identificación de la enfermedad que padece el animal es muy importante para la aplicación de un tratamiento y unas medidas de prevención adecuadas. En el caso de la sarna sarcóptica, los datos epidemiológicos y los resultados de diversas pruebas clínicas y de laboratorio aportan información que nos permiten diferenciarla de otros procesos, y por lo tanto llegar a diagnosticar infección por *S. scabiei*.

5.1. Estudio clínico-epidemiológico

El mayor problema para el diagnóstico clínico de las enfermedades cutáneas es que las manifestaciones no suelen ser patognomónicas. Por ello, el diagnóstico de la sarna sarcóptica ha supuesto tradicionalmente un reto importante tanto para los veterinarios como para los médicos debido a la dificultad para diferenciarla de otros procesos cutáneos por los signos clínicos que produce (Burgess, 1994). En las ovejas, la presencia de una dermatosis limitada principalmente a la cabeza, que ocasiona prurito y que es de elevada contagiosidad hace sospechar sarna sarcóptica. Dos pruebas clínicas en el perro y en el cerdo, enfocadas a la detección de prurito, permiten avanzar en el diagnóstico:

- El reflejo de rascado de la extremidad posterior al frotar la punta de la oreja se considera un elemento de ayuda en el diagnóstico de la sarna sarcóptica en el perro (Mueller y col., 2001).
- El estudio del comportamiento de rascado en los cerdos permite establecer el índice de rascado, que mide la frecuencia del mismo y está relacionado con el grado de infección de las piaras por *S. scabiei* (Hollanders y col., 1995). Este método de diagnóstico es también útil para monitorizar la enfermedad (Loewenstein y col., 2006).

Sin embargo, no hay datos sobre estas dos pruebas clínicas en la sarna sarcóptica ovina. En la sarna psoróptica, se sabe que los animales se rascan, se frotan y se muerden las lesiones, aunque no se ha establecido ningún índice para conocer el grado de afectación de un rebaño (Corke y Broom, 1999). Además, también se muerden los labios y hay protrusión de la lengua en ausencia de estímulos externos. Los animales pueden tener hiperestesia y pueden morder en respuesta a estímulos táctiles (Sargison, 1995).

A pesar de haber realizado estas pruebas clínicas todavía puede haber dudas, ya que la sarna sarcóptica puede confundirse con otros procesos cutáneos como el eczema facial, reacciones alérgicas, dermatofitosis, dermatofilosis, ectima contagioso, dermatitis por *Pelodera* spp., estafilococias, tembladera y enfermedades causadas por piojos u otros ácaros, con los que hay que hacer el diagnóstico diferencial utilizando métodos de laboratorio.

Finalmente, en el contexto del diagnóstico clínico y aunque no es un procedimiento muy ortodoxo, se ha utilizado el tratamiento con acaricidas como prueba de diagnóstico diferencial de la sarna (diagnóstico a través del tratamiento) y otras enfermedades cutáneas causadas por otros agentes patógenos (Folz y col., 1984).

5.2. Demostración del agente patógeno

Diversas técnicas parasitológicas contribuyen al diagnóstico de la sarna sarcóptica. Todas ellas se basan en la visualización de adultos de *S. scabiei*, ninfas, larvas o huevos. Uno de los inconvenientes es que todas tienen una baja sensibilidad, sobre todo cuando el grado de parasitación es bajo, lo cual es muy frecuente en las fases iniciales de la infección. De forma resumida, se exponen a continuación los métodos utilizados.

- La técnica más común para confirmar el diagnóstico de la sarna sarcóptica en animales es el examen microscópico de raspados cutáneos profundos recogidos de los bordes de las lesiones (OIE, 2011). Es una técnica rápida, sencilla y barata. El estudio se puede hacer mediante examen de las costras sin procesar, donde se buscan los ácaros vivos, o bien, de las costras procesadas, donde los ácaros siempre se observan muertos.

En el primer caso, las muestras se incuban durante ocho horas a una temperatura de 30º C y a continuación se observan al microscopio para detectar los ácaros, o bien se observan directamente sin incubación previa.

En el segundo caso, se hace una digestión de las costras con hidróxido potásico durante un determinado tiempo y, tras centrifugar, se observa el sedimento al microscopio para detectar los ácaros, o se resuspende en una solución saturada de sacarosa y se centrifuga de nuevo para concentrar los ácaros en la parte superior del tubo, desde donde se recogen mediante adhesión a un portaobjetos y se observan al microscopio.

El examen microscópico de los raspados tiene una especificidad del 100%, ya que para considerar una muestra positiva hay que visualizar los ácaros e identificarlos, lo cual es un indicador inequívoco de que el animal está infectado. Sin embargo, hay que prestar atención para evitar la contaminación durante la recogida, transporte y procesado de las muestras. Sin embargo, esta técnica tiene, en general, baja sensibilidad, y depende mucho de la fase en que se encuentra la enfermedad, de forma que la sensibilidad es más alta en infecciones crónicas y baja al inicio de la infección (Bornstein y col., 2006). Con esta técnica se ha observado que la sensibilidad es menor del 50% en perros (Morris y Dunstan, 1996) y del 80% en ovejas para el examen con concentración de ácaros (Papadopoulos y Fthenakis., 1999).

- Las biopsias de piel no siempre permiten la identificación de ácaros adultos, ninfas, larvas o huevos (Morris y Dunstan, 1996). La visualización de un corte de un huevo eclosionado, que da una imagen típica en la que uno de los polos aparece roto, es un signo indicativo de sarna sarcóptica (Kristjansson y col., 2007; Reinig y col., 2011).

Otras técnicas que se usan en medicina humana pero para las que no hemos encontrado referencias en veterinaria son las siguientes:

- El método tradicional para diagnosticar la sarna ha sido el examen de la piel en busca de un túnel y con la punta de una aguja tratar de extraer al ácaro e identificarlo (Ramos-e-Silva, 1998, Roncalli, 1987).
- La aplicación de una cinta adhesiva en la lesión y su posterior observación al microscopio también permite la detección de los ácaros (Katsumata y Katsumata, 2006)
- Mediante dermoscopía se examina la superficie de la piel con una lupa para identificar estructuras del propio ácaro (Neynaber y Wolff, 2008; Prins y col., 2004). La sensibilidad es del 91% y la especificidad del 86% (Dupuy y col., 2007).
- También se ha utilizado la microscopía confocal, que permite visualizar *in vivo* la epidermis y la dermis superficial, y así se pueden detectar ácaros en el interior de los túneles (Turan y col., 2011).

5.3. Reacción en Cadena de la Polimerasa (PCR)

- La Reacción en Cadena de la Polimerasa (PCR), en la cual se detecta un fragmento específico del ADN de *S. scabiei* (Bezold y col., 2001; Fukuyama y col., 2010). Se ha demostrado su utilidad en el hombre cuando hay manifestaciones clínicas atípicas y en

infecciones subclínicas, así como para valorar la eficacia de un tratamiento. No se ha descrito su utilización en animales.

5.4. Diagnóstico lesional

- En la prueba de tinción de los túneles con tinta (Woodley y col., 1981), se esparce tinta sobre el área de piel a examinar y se frota, para a continuación eliminarla con alcohol. Si hay túneles, la tinta penetra en ellos y aparecen como caminos oscuros y tortuosos. Esta técnica tiene una alta frecuencia de falsos negativos y no se ha descrito su utilización en animales.
- En las biopsias de piel no se ha conseguido identificar alguna característica histopatológica que pueda servir para confirmar la infección por *S. scabiei* (Morris y Dunstan, 1996).

5.5. Pruebas serológicas

Debido a que los signos clínicos de la sarna sarcóptica se parecen a los observados en otros procesos cutáneos, unido a la dificultad de demostrar la presencia de los ácaros de *S. scabiei*, se han desarrollado métodos de diagnóstico basados en la detección de anticuerpos específicos en el suero. Como ya se dijo anteriormente, se ha demostrado que *S. scabiei* induce una respuesta inmune humoral específica basada en IgG, IgE e IgM (Arlian y col., 1994b; Arlian y col., 1996b, Arlian y Morgan, 2000; Arlian y col., 2004b; Bornstein y col., 1995; Rambozzi y col., 2007). Por ahora, la técnica más utilizada ha sido el ELISA (ensayo de inmunoabsorción ligada a enzima), debido a su sencillez, rapidez, sensibilidad y baja cantidad de antígeno que se requiere. Recientemente, se han realizado investigaciones de la

respuesta de proteínas durante la fase aguda de la infección por *S. scabiei* y se han obtenido buenos resultados, pero aún no se ha establecido ningúna aplicación práctica.

5.5.1 Detección de anticuerpos específicos

5.5.1.1 Técnicas basadas en antígenos crudos

Actualmente, hay técnicas ELISA indirecto para la detección de IgG específicas basadas en la utilización de antígenos crudos de *S. scabiei*, que están validadas y tienen buenas propiedades para el diagnóstico de la sarna sarcóptica en el cerdo, perro, zorro rojo y rebeco (Bornstein y col., 1996 y 2006; Bornstein y Wallgren, 1997; Jacobson y col., 1999; Rambozzi y col., 2004).

El principal problema de estas técnicas ELISA es la necesidad de disponer de un suministro regular y abundante de ácaros de *S. scabiei*. En la actualidad no existe ninguna técnica de cultivo *in vitro* de *S. scabiei*, por lo que hay que obtener los ácaros de animales infectados. También se ha observado que algunos antígenos son comunes en las distintas variedades de *S. scabiei* (Arlian y col., 1996a), lo que a veces hace posible detectar anticuerpos en una especie hospedadora utilizando antígenos preparados a partir de *S. scabiei* de una variedad heteróloga. Esto ha permitido el desarrollo de técnicas ELISA con alta sensibilidad para detectar la infección por *S. scabiei* en cerdos, perro y rebeco infectados con su variedad específica utilizando un antígeno preparado a partir de la variedad parásita del zorro rojo (Hollanders y col., 1997; Rambozzi y col., 2004; Wallgren y Bornstein, 1997).

Sin embargo, se ha investigado mediante ELISA la reacción cruzada de las IgG en suero de personas infectadas por *S. scabiei* frente a un extracto antigénico preparado a partir de la variedad del zorro rojo, y se ha demostrado que existe reacción cruzada pero la

sensibilidad de la técnica es baja (48%), indicando que en este caso se necesitaría una purificación del mismo para incluir únicamente los componentes más sensibles y específicos (Haas y col., 2005).

Por lo tanto, la variedad de zorro rojo, *S. scabiei* var. *vulpes*, ha sido la principal fuente de ácaros utilizada para producir antígeno, lo cual se debe a que a partir de un solo individuo muerto de sarna sarcóptica se pueden obtener miles de ácaros. Recientemente, Mounsey y col. (2010a) han desarrollado un modelo de infección experimental en el cerdo, en el cual se alcanzan densidades superiores a 6.000 ácaros por gramo de piel, permitiendo la obtención regular de gran cantidad de antígeno de *S. scabiei* var. *suis*.

En el caso de la sarna sarcóptica, la utilización del Western blot de antígenos crudos para el diagnóstico de la enfermedad carece de aplicación práctica ya que se requiere gran cantidad de antígeno. Sin embargo, sí puede resultar útil con vista a la identificación de los componentes más sensibles y específicos, y su posterior producción como antígenos recombinantes para el desarrollo de un test de inmunodiagnóstico.

En infecciones experimentales en cabras (Tarigan, 2004) se ha observado que cuatro proteínas de 180, 135, 43 y 38 kDa son reconocidas por las IgG a partir de los 10 días postinfección, con alta frecuencia y con gran intensidad y, por lo tanto, podrían resultar útiles para el desarrollo de un test de diagnóstico inmunológico.

Rambozzi y col. (2007), demostraron que en el caso del cerdo, dos proteínas con pesos moleculares de >220 y 36 kDa pueden ser utilizadas para el desarrollo de un test de detección de sarna sarcóptica altamente específico.

5.5.1.2 Técnicas basadas en antígenos recombinantes

El desarrollo de una técnica de inmunodiagnóstico basada en proteínas recombinantes solucionaría el problema actual de utilización de animales para la obtención de los ácaros, generaría una fuente constante de antígeno, y además sería más fácil de estandarizar. En los últimos años se ha hecho un gran esfuerzo en este sentido por varios grupos de investigación. La técnica utilizada para la identificación de estas proteínas ha sido el inmunoescrutinio de librerías de ADNc con suero obtenido de hospedadores infectados o inmunizados con extractos de *S. scabiei*, unas veces completos y otras veces únicamente con las fracciones identificadas previamente como mejores candidatos mediante caracterización de la respuesta inmune. Además, también se han identificado directamente varios alérgenos de *S. scabiei* debido a su alta homología con los de los ácaros del polvo.

A continuación se describen brevemente algunas de las proteínas recombinantes disponibles en la actualidad y su validez diagnóstica:

- MSA1: antígeno mayor de *S. scabiei*. El ADN que codifica este antígeno se aisló mediante inmunoescrutinio de una librería de ADNc de *S. scabiei* var. *vulpes* con suero de conejo inmunizado con extractos antigénicos de dicha variedad. Este antígeno posee una alta homología con las proteínas incluidas en el grupo 14 de los alérgenos de los ácaros de polvo. Mediante estudios de Western blot con sueros de perro se demostró que en esta especie la sensibilidad es del 85,5% y la especificidad del 100% (Ljunggren y col., 2006). Posteriormente, se aislaron dos fragmentos de este mismo antígeno (denominados Ssag1 y Ssag2) mediante inmunoescrutinio de una librería de ADNc de *S. scabiei* var. *hominis* con suero de conejo infectado con *S. scabiei* var. *canis* (Harumal y col., 2003).

- La paramiosina, antígeno aislado mediante inmunoescrutinio con suero de conejo inmunizado con *S. scabiei* var. *vulpes* de una librería de ADNc de la misma variedad de ácaro (Mattsson y col., 2001), reacciona con sueros de perro y cerdo infectados por *S. scabiei*. Asimismo, se ha producido un fragmento de la misma que incluye la región más variable y se ha demostrado que reacciona con suero de conejo inmunizado con *S. scabiei* var. *vulpes*.

- También se ha utilizado la proteína rSsGST01 (glutatión-S-transferasa de clase-μ) para la detección de anticuerpos en personas con sarna (Dougall y col., 2005). Se ha demostrado que los niveles de IgE e IgG_4 frente a esta proteína en pacientes con la forma costrosa de la sarna son mucho más elevados que en los pacientes con la forma ordinaria y los sanos.

- El ASA1, es un antígeno atípico de *S. scabiei* aislado mediante inmunoescrutinio con suero de perro infectado por *S. scabiei* var. *canis* de una librería de ADNc de *S. scabiei* var. *vulpes* (Lejungren y col., 2006). Tiene la particularidad de que posee un dominio conservado denominado MADF, que normalmente está implicado en procesos de transcripción. La capacidad diagnóstica de este antígeno en el perro ha sido evaluada mediante Western blot y se ha demostrado que posee una alta especificidad (98%) pero baja sensibilidad (23%).

- El Ssλ20 es un antígeno aislado mediante inmunoescrutinio con suero de un rebeco infectado por *S. scabiei* de la librería de ADNc de *S. scabiei* var. *hominis* (Casáis y col., 2007). Se ha demostrado que es muy eficaz para el diagnóstico de la sarna sarcóptica del ciervo y del rebeco, ya que cuando se utiliza en un ELISA con el punto de corte óptimo se consigue el 100% de sensibilidad y especificidad. Este antígeno se ha utilizado varias veces

para estimar la seroprevalencia de anticuerpos frente a *S. scabiei* en varias especies animales (Millán y col., 2012; Oleaga y col., 2008b y 2011).

- Un estudio de inmunoescrutinio con suero de personas infectadas por *S. scabiei* var. *hominis* de una librería de ADNc de *S. scabiei* var. *suis* ha permitido el aislamiento de varios clones positivos, de los cuales seis se produjeron como proteínas recombinantes (Kuhn y col., 2008). Uno de ellos era homólogo a la D-titina de *Drosophila melanogaster*, y otro de una enzima dihidrogenasa/reductasa de *Aedes aegypti*. Otro de los clones se identificó como parálogo de proteasa inactivada de *S. scabiei* var. *hominis*. El valor diagnóstico de los seis fragmentos clonados se determinó con sueros de cerdo y de personas y ninguno de ellos resulto ser útil para el desarrollo de una técnica ELISA con alta sensibilidad y especificidad.

- Recientemente, Jayaraj y col. (2011) han producido y probado la proteína rSar s 14.3, que es un fragmento de la proteína de *S. scabiei* homóloga al alérgeno 14 de los ácaros del polvo. Para ello, utilizaron la técnica de fluoroinmunoanálisis por marcador lantánido (DELFIA) y demostraron que tiene un 100% de sensibilidad y 93,8% de especificidad en personas.

5.5.2 Detección de proteínas de la respuesta aguda inflamatoria

Las proteínas de fase aguda se pueden utilizar como marcadores inespecíficos de infección clínica y subclínica, para discriminar entre infecciones agudas y crónicas, y para realizar el pronóstico de la infección ya que la duración y magnitud de la respuesta están relacionadas con la gravedad del proceso y la eficacia del tratamiento (Petersen y col., 2004).

Una posible utilidad de la determinación de proteínas de la fase aguda de la inflamación como una herramienta de diagnóstico en la sarna sarcóptica, es que cuando se detectan anticuerpos específicos en animales clínicamente sanos permite diferenciar entre un animal con infección subclínica y un animal no infectado. La detección de anticuerpos específicos indicaría que el animal está infectado o ha estado en contacto previamente con *S. scabiei*, mientras que la detección de proteínas de fase aguda indicaría que hay una infección activa (Wells y col., 2012). Ello podría constituir una buena herramienta para la monitorización de la eficacia del tratamiento, ya que se podrían detectar antes los casos de inficacia.

En el caso de *S. scabiei*, los niveles de amiloide A y de alfa 1 glucoproteína ácida en la cabra silvestre de los Alpes son 10 veces mayores en los animales infectados que en los no infectados, por lo que podrían ser útiles como marcadores de infección activa (Rahman y col., 2010).

6. Prevención y control

El control incluye todas las actividades encaminadas a reducir la mortalidad y la morbilidad causada por las enfermedades (Thrusfield, 2005). En el caso de la sarna, los programas sanitarios deberían combinar medidas de prevención de la infección con el tratamiento de los animales infectados.

6.1. Medidas preventivas generales

Una buena medida consiste en conocer la situación sanitaria del rebaño de procedencia antes de incorporar animales nuevos en el rebaño. Si se sospecha sarna, se debe administrar un tratamiento acaricida y mantener a los animales en cuarentena.

En el caso de realizar pastoreo, una buena práctica es evitar compartir áreas con rebaños infectados y rumiantes silvestres.

Otra medida conveniente es el aislamiento de los animales infectados y sospechosos hasta que se hayan recuperado o se haya descartado la infección, reduciendo las posibilidades de contagio a otros animales del rebaño. Dicha práctica resulta más eficaz cuanto más pronto se haga, por lo que es muy deseable un diagnóstico precoz de la enfermedad.

Cuando hay pocos animales afectados, se deben aislar y tratar a los enfermos, a la vez que se aconseja intensificar la vigilancia para detectar animales sospechosos, su aislamiento y tratamiento, aunque la eficacia de esta medida para evitar la difusión de la enfermedad depende del grado de contagiosidad de la misma. En el caso de que la infección afecte a numerosos animales es recomendable tratar a todo el rebaño.

La limpieza y desinfección de las instalaciones y fómites es recomendable para evitar cualquier riesgo de reinfección. Los animales con lesiones crónicas que no responden al tratamiento son una fuente importante de ácaros por lo que es conveniente su eliminación.

6.2. Inmunoprofilaxis

El uso inadecuado de productos químicos para el control de los ectoparásitos puede favorecer el desarrollo de resistencia en los organismos diana; otros inconvenientes son la

presencia de residuos en productos procedentes de los animales tratados, los efectos nocivos sobre organismos del medio ambiente y los efectos tóxicos sobre las personas que los manipulan. Además, por diversas razones, cada vez resulta más difícil para la industria farmacéutica el desarrollo de nuevas sustancias activas eficaces. Todo esto ha conducido a la necesidad de buscar métodos alternativos de control, entre los que se encuentra la vacunación. La inmunoprofilaxis tiene las ventajas de mayor seguridad, al dar menos problemas de residuos y ser más específica, y menor probabilidad de desarrollo de resistencias (Willadsen, 1997).

El hecho de que los animales que se han recuperado de una infección por *S. scabiei* muestren resistencia a las reinfecciones pone en evidencia que es posible el desarrollo de una vacuna frente a *S. scabiei* (Arlian y col, 1994b; Arlian y col., 1996b; Tarigan, 2002). En un estudio experimental con conejos a los que se les había administrado un extracto de *Dermatophagoides farinae* y *Dermatophagoides pteronyssinus*, se observó inmunidad protectora cruzada ya que se redujo la carga parasitaria en el 71% de los animales (Arlian y col., 1995).

Con objeto de conocer la capacidad de conferir inmunidad de los antígenos Ssag1 y Ssag2 de *S. scabiei* se han realizado pruebas en conejos (Harumal y col., 2003). Estos autores, observaron que a pesar de inducir altos niveles de IgG no se desarrolla resistencia a la infección, aunque se apreciaron cambios en los signos clínicos.

Tarigan y Huntley (2005), realizaron un ensayo de inmunización de cabras con diferentes fracciones de un extracto soluble de *S. scabiei* var. *caprae* y, observaron, durante la prueba desafío, que los animales no habían desarrollado resistencia a la infección a pesar de haber inducido un elevado nivel de IgG específica. Sin embargo, se observó que la vacuna no

inducía la producción de IgE, a diferencia de los altos niveles que aparecen en respuesta a una infección, lo cual sugiere que la IgE puede tener un papel fundamental en el desarrollo de resistencia a la infección.

En la actualidad, las únicas vacunas disponibles comercialmente frente a ectoparásitos son TickGARD™ (Intervet Australia Pty. Ltd., Bendigo, Australia) y Gavacv™ (Heber Biotec S.A., La Habana, Cuba) que se utilizan para el control de las infecciones por *Boophilus microplus* y actúan mediante el llamado mecanismo del antígeno oculto. Se conocen bajo esta denominación las vacunas cuyo mecanismo inmunológico de acción nunca aparece en el desarrollo de una infección natural, ya que se basan en antígenos somáticos que no entran en contacto con el sistema inmune (Willadsen y col., 1997). Los antígenos ocultos se localizan en los órganos internos del parásito, tienen un papel fisiológico crítico y dan lugar a una fuerte respuesta inmunitaria. Los anticuerpos específicos generados tras la inmunización son ingeridos por el ectoparásito al alimentarse y ocasionan un daño en el mismo, que da lugar a un crecimiento más lento, reducción de la fecundidad o la muerte.

Para que sea posible el desarrollo de una vacuna basada en antígenos ocultos es necesario que el ectoparásito ingiera inmunoglobulinas del hospedador y que lleguen a los órganos internos del parásito manteniendo su actividad. Rapp y col. (2006), Tarigan (2005) y Willis y col. (2006) demostraron que *S. scabiei*, a pesar de que no es un parásito hematófago, sí ingiere inmunoglobulinas que llegan al esófago y al intestino medio, lo cual hace posible el desarrollo de una vacuna frente a *S. scabiei* que actúe mediante el mecanismo del antígeno oculto.

Otro posible mecanismo inmunológico frente a *S. scabiei* es mediante el sistema del complemento del hospedador. Como se expuso anteriormente, *S. scabiei* inhibe las rutas del

complemento. Sin embargo, recientemente, se han detectado en el sistema digestivo del parásito tanto los factores del complemento como las peritrofinas, que son proteínas que podrían actuar como diana de la ruta de la lectinas (Mika y col., 2011). Por lo tanto, el estudio de los mecanismos moleculares implicados en la inactivación del complemento puede dan lugar al desarrollo de nuevas estrategias de control de la sarna sarcóptica.

6.3. Tratamiento

6.3.1 Tratamientos tradicionales

A lo largo de la historia se han utilizado distintas preparaciones para el tratamiento tópico de la sarna en los animales. Una mezcla de desechos de aceite de oliva, extracto de altramuz y vino ya se utilizaba en la antigüedad. También se ha descrito el uso de raíces de narcisos y de mezclas de alquitrán y grasa y de orina de asno y tierra (Roncalli, 1987).

A principios del siglo XIX se comenzaron a aplicar los baños de arsénico, azufre y nicotina, en los años 40 del siglo XX los compuestos organoclorados y en los años 50 los organofosforados (Roncalli, 1987). Sin embargo, estas sustancias prácticamente ya no se utilizan debido a su elevada toxicidad para los animales domésticos, el hombre y los organismos del medio ambiente.

6.3.2 Tratamientos actuales

Existe una amplia gama de sustancias activas para el tratamiento de la sarna sarcóptica en los ovinos que se administran por vía parenteral o tópica. Los grupos de fármacos más frecuentemente utilizados son las lactonas macrocíclicas, los piretroides y las amidinas. Los extractos de plantas también pueden constituir una buena alternativa para el tratamiento. Para evaluar la eficacia de los acaricidas frente a los ácaros de los rumiantes, la Asociación

Mundial para el Avance de la Parasitología Veterinaria ha publicado una guía en la que aconseja sobre el modo de realizar las estudios con animales y de preparar la documentación necesaria para su registro ante las autoridades (Vercruysse y col., 2004).

Como paso previo a la aplicación del tratamiento hay que tener presente que si la infección afecta a una alta proporción de animales es recomendable tratar a todo el rebaño. Antes de la administración de acaricidas de uso tópico se recomienda reblandecer y eliminar escamas y costras, así como cortar el pelo en las zonas afectadas, para facilitar el contacto del acaricida con los parásitos (Cordero del Campillo y Rojo-Vázquez, 1999). La utilización de queratinolíticos es frecuente en el tratamiento de los casos severos de la enfermedad en el hombre (Currie y col., 2004). En algunos casos puede ser necesaria la administración de terapia adyuvante, como los antibióticos para el tratamiento de las infecciones sobreañadidas, pero en este apartado nos vamos a referir únicamente al tratamiento específico de la causa primaria, es decir la administración de acaricidas.

6.3.2.1 Productos farmacológicos

6.3.2.1.1.Vía parenteral

Las sustancias acaricidas que se administran por vía parenteral son las lactonas macrocíclicas, de las cuales las que más se utilizan para el tratamiento de la sarna sarcóptica son la ivermectina y la moxidectina. Estas dos sustancias carecen de actividad ovicida, por lo que para alcanzar la curación parasitaria es necesario mantener niveles terapéuticos durante un periodo de tiempo que permita actuar frente a las larvas procedentes de los huevos que van eclosionando.

A continuación se exponen las pautas de tratamiento recomendadas y la eficacia descrita para ambas sustancias:

- Ivermectina – Es eficaz frente a la sarna sarcóptica de los ovinos, aunque puede haber diferencias entre las distintas formulaciones (Dia y Diop, 2005). La pauta de tratamiento recomendada en ovino es de dos dosis de 200 µg/kg de peso vivo, por vía subcutánea, con un intervalo de siete días. También es preciso reseñar que en algunos casos graves de la forma costrosa de la enfermedad en el hombre ha sido necesario administrar hasta siete dosis para conseguir la curación, incluso asociando la ivermectina con otros fármacos acaricidas (Currie y col., 2004; Huffam y Currie, 1998; Roberts y col., 2005).

- Moxidectina – Se recomienda administar dos dosis de 200 µg/kg de peso vivo, por vía subcutánea, con un intervalo de 7-10 días. El signo clínico de rascado desaparece a los siete días de iniciado el tratamiento, y a los 35-56 días no se observan lesiones clínicas de sarna ni ácaros en los raspados cutáneos (Čorba y col., 1995; Fthenakis y col., 2000; Hidalgo-Argüello y col., 2001). También se ha demostrado que la administración de una única dosis de moxidectina previene de la infección por *S. scabiei* durante al menos 25 días, y por lo tanto es útil cuando se quiere evitar la infección de ovejas sanas que se incorporan a rebaños con problemas de sarna (Papadopoulos y col., 2000). Recientemente, se ha desarrollado una formulación de moxidectina de acción prolongada, y se ha demostrado que una única dosis de 1 mg/kg de peso vivo es suficiente para conseguir la curación clínica en 40 días (Astiz y col., 2011).

6.3.2.1.2.Vía tópica

Sharma y col. (1997) probaron el benzoato de bencilo para el tratamiento de la sarna sarcóptica ovina y observaron que tras la aplicación tópica en días alternos las lesiones de los animales curaban a los 14 días.

Rahbari y col. (2009) probaron la eficacia de cipermetrina, amitraz y propetamfos en el tratamiento de la sarna sarcóptica en ovejas. Se observó que a los dos meses tras el tratamiento el 80% de los animales no mostraban lesiones clínicas de sarna, pero en algunos animales se detectaron recidivas.

De las sustancias activas enumeradas anteriormente, el propetamfos, perteneciente al grupo de los organofosforados, no está autorizado en la Unión Europea para animales de abasto (Reglamento UE 37/2010).

6.3.2.2 Extractos de plantas

La utilización de extractos naturales de plantas en el tratamiento de la sarna sarcóptica es una alternativa valiosa para los ganaderos de regiones que no tienen acceso a las sustancias farmacológicas. También puede ser una herramienta útil en los modernos sistemas de producción ganadera ecológica donde se limita la utilización de fármacos. Además, las investigaciones en este campo pueden tener como objetivo el descubrimiento de nuevos fármacos acaricidas.

Se ha demostrado que el aceite de *Cedrus deodara* (cedro del Himalaya) es más eficaz que el benzoato de bencilo para el tratamiento de la sarna sarcóptica ovina. La recuperación clínica es más rápida y no se detectan ácaros tras cinco aplicaciones con dos días de intervalo,

frente a las siete aplicaciones que se necesitan para eliminar los ácaros con benzoato de bencilo (Sharma y col., 1997).

El tratamiento de ovinos con sarna sarcóptica mediante la aplicación diaria de aceite de *Jatropha curcas* (piñón de tempate) tiene una eficacia del 50% a los cinco días (Dimri y Sharma, 2004a y 2004b). Si a la vez se administra ácido ascórbico por vía intramuscular la eficacia aumenta al 75% y además la recuperación es más rápida ya que se adelanta a los dos días de iniciado el tratamiento.

Pasay y col. (2010) probaron *in vitro* la eficacia acaricida del aceite de clavo, de nuez moscada y de canangay y observaron que el aceite de clavo es el más eficaz de todos. A continuación probaron la eficacia del principal componente del aceite de clavo, el eugenol, y otros componentes menores derivados del eugenol, y detectaron que el eugenol, el isoeugenol y el acetileugenol tienen elevada eficacia acaricida, y que esta es similar a la del benzoato de bencilo, pero presentan resistencia cruzada con la permetrina. Estos resultados demuestran que estas sustancias son buenos candidatos para posteriores estudios de seguridad y eficacia *in vivo*.

La aplicación de un extracto metanólico-acuoso de aceite de nim al 20% en vaselina conduce a la curación parasitaria de la sarna sarcóptica ovina a los 16 días y la curación clínica a los 20 días (Tabassam y col., 2008). El champú con extracto de semilla de nim tiene actividad frente a la sarna sarcóptica en el perro, ya que aplicándolo diariamente durante 14 días, se consiguió que 8 de 10 animales se curaran de la enfermedad (Abdel-Ghaffar y col., 2008). Una microemulsión de aceite de nim en dodecil benceno sulfonato de sodio, Tween-80 y alcohol hexílico es eficaz *in vitro* frente a las larvas de *S. scabiei* var. *cuniculi* (Xu y col., 2010).

Para intentar conocer los componentes acaricidas del aceite de nim, se han obtenido y probado un extracto de éter de petróleo, un extracto de cloroformo y un extracto de éter acético. El primero de ellos es el más eficaz *in vitro* frente a larvas de *S. scabiei* var. *cuniculi*, mientras que la eficacia de los otros dos es similar. (Du y col., 2008). Una posterior purificación del extracto de cloroformo permitió identificar una sustancia con alta actividad frente a las citadas larvas (Du y col., 2009).

El aceite esencial de eucalipto es eficaz para el tratamiento de la sarna sarcóptica humana ya que se consigue una curación completa en un plazo de entre 5 y 10 días (Morsy y col., 2003). Walton y col. (2004b) probaron la eficacia frente a *S. scabiei* del aceite del árbol del té y encontraron alta eficacia *in vitro,* siendo un componente activo el terpinen-4-ol. El extracto etanólico de *Eupatorium adenophorum* (sándara) también tiene alta actividad frente a *S. scabiei* (Nong y col., 2012).

6.3.3 Resistencia a los acaricidas

El progresivo desarrollo de resistencia a los antibióticos y antihelmínticos utilizados en el tratamiento contra los agentes biológicos es ampliamente reconocido. La resistencia a los insecticidas y a los acaricidas no es una excepción. Hasta ahora, se han descrito, tanto en el hombre como en los animales, los casos de resistencia de *S. scabiei* a los acaricidas que aparecen recogidos en el Cuadro 1.

Cuadro 1. Casos descritos de resistencia de *S. scabiei* a los acaricidas.

Variedad de *S. scabiei*	Fármaco acaricida	Referencia bibliográfica
hominis	Permetrina	Speare y McConnell, 1997; Walton y col., 2000
	Ivermectina	Currie y col., 2004; Mounsey y col., 2009
	Lindano	Roth, 1991
	Crotamitón	Roth, 1991
canis	Ivermectina	Terada y col., 2010

La valoración de la resistencia de *S. scabiei* a los fármacos se puede realizar mediante pruebas clínicas (*in vivo*), mediante pruebas de exposición del ácaro al fármaco (*in vitro*) o poniendo de manifiesto los mecanismos responsables de la resistencia.

Las pruebas *in vivo* se basan en la valoración de la respuesta clínica al tratamiento con un fármaco (Currie y col., 2004; Terada y col., 2009) y las pruebas *in vitro* en la valoración de la actividad o supervivencia del ácaro tras la exposición a los fármacos, que están inversamente relacionadas con la sensibilidad al mismo (Brimer y col., 2004; Walton y col., 2000).

El desarrollo de resistencia a los acaricidas puede ser debido a una modificación del sitio de acción que da lugar a insensibilidad al fármaco o a un aumento de la capacidad para metabolizarlo. Respecto al primer mecanismo, ya se han desarrollado métodos para el genotipado de algunas dianas moleculares de la ivermectina (Mounsey y col., 2006 y 2007) y de la permetrina (Pasay y col., 2006). En el caso de la permetrina, se ha identificado un polimorfismo de nucleótido simple relacionado con la sensibilidad/tolerancia al fármaco y se ha desarrollado una técnica rápida de genotipado, lo cual permite el diagnóstico precoz de la

resistencia y su monitorización (Pasay y col., 2008). Respecto al segundo mecanismo, el incremento de la actividad glutatión S-tranferasa, esterasa y citocromo P-450 monooxigenasa en *S. scabiei* aumenta su resistencia a permetrina mediante una mayor metabolización (Pasay y col., 2009; Mounsey y col., 2010b). No obstante, este último tipo de resistencia se puede revertir utilizando el fármaco junto con un sinergista (Pasay y col., 2009).

Capítulo II

Trabajos publicados

Introducción

La revisión bibliográfica de la sarna sarcóptica de los ovinos nos permitió identificar las lagunas existentes sobre esta parasitosis, y en consecuencia abordar el estudio de algunos aspectos de utilidad para su control.

Un aspecto fundamental para el control de la sarna en los rebaños es el diagnóstico temprano de la enfermedad. La sospecha clínico-epidemiológica requiere la confirmación posterior mediante el análisis de raspados cutáneos (Cordero del Campillo y Rojo-Vázquez, 1999). Este procedimiento es útil en animales con lesiones crónicas, pero tiene escaso valor en infecciones recientes en las que el número de ácaros es escaso (Bornstein y col., 2006). Varios estudios han demostrado que la detección de anticuerpos específicos constituye una herramienta útil para el diagnóstico y la realización de estudios epidemiológicos (Falconi y col., 2002; Jacober y col., 2006; Jacobson y col., 1999; Rueda-López, 2006). Sin embargo, hasta la fecha no se ha desarrollado ninguna técnica para la sarna sarcóptica ovina.

Por todo ello, se planteó como primer objetivo el desarrollo de una técnica ELISA para la detección de IgG en suero de oveja frente a un antígeno crudo de *S. scabiei* var. *ovis* y su validación para el diagnóstico de la sarna sarcóptica ovina en los rebaños de la Comunidad Autónoma de Castilla y León. También nos propusimos recabar datos sobre los animales que se utilizarían para validar la técnica, ya que de este modo se podrían identificar los factores biológicos que determinan sus propiedades diagnósticas, y de esta forma la técnica se podría aplicar con mayor eficiencia en cualquier población ovina. Los resultados de este estudio constituyeron el primer artículo de esta tesis doctoral, y se titula: **"Development and**

evaluation of an antibody ELISA for sarcoptic mange in sheep and a comparison with the skin-scraping method".

Un aspecto importante es el conocimiento de la prevalencia de las enfermedades en el ganado ovino, ya que de este modo los programas de control se pueden enfocar a las de mayor repercusión y así mejorar la relación coste-beneficio de los recursos invertidos en la lucha contra las enfermedades. Por ello, creímos conveniente realizar un estudio para conocer la prevalencia de la infección por *S. scabiei* en los rebaños de Castilla y León. Teniendo en cuenta que los sueros para el estudio se iban a recoger de los Laboratorios de Sanidad Animal de Castilla y León, se decidió recabar también todos los datos posibles para realizar un análisis de factores de riesgo, ya que permitiría diseñar mejor los programas de control. En el segundo de los artículos de la tesis doctoral, titulado: **"Cross-sectional sero-epidemiological survey of sarcoptic mange in sheep of Western Castile and Leon, Spain"** se describe un estudio sobre la estimación de prevalencia de sarna sarcóptica en las provincias de León, Zamora y Salamanca (oeste de Castilla y León), junto con un análisis de factores de riesgo.

La técnica ELISA desarrollada está basada en un antígeno crudo de *S. scabiei*. Actualmente, los ácaros de *S. scabiei* hay que obtenerlos a partir de animales infectados ya que no es posible su cultivo *in vitro*. Esto implica que sólo se pueden conseguir pequeñas cantidades y, por lo tanto, la técnica ELISA no se puede utilizar a gran escala. Ante esta limitación, se planteó la necesidad de caracterizar la respuesta inmune humoral frente a la infección por *S. scabiei*, y así identificar los antígenos más sensibles y específicos, que posteriormente podrían ser producidos en forma recombinante. Para ello, se decidió realizar infecciones experimentales de forma que se identificarían proteínas potencialmente útiles para un diagnóstico precoz de la enfermedad. La realización de este estudio abrió la

posibilidad de realizar una prueba de desafío tras la recuperación de la infección primaria. Esto nos permitiría determinar si las ovejas desarrollan resistencia durante la infección primaria y también caracterizar la respuesta inmune humoral tras el desafío. Este estudio clínico constituye el tercer artículo de la tesis, y se titula: **"Clinical evaluation and antibody responses in sheep after primary and secondary experimental challenges with the mange mite *Sarcoptes scabiei* var. *ovis*"**.

A continuación, se adjuntan copias de cada una de las tres publicaciones, en las que el autor de la tesis doctoral es el primer firmante y los directores, coautores.

Publicación nº 1

Development and evaluation of an antibody ELISA for sarcoptic mange in sheep and a comparison with the skin-scraping method

Rodríguez-Cadenas, F., **Carbajal-González, M.T., Fregeneda-Grandes, J.M., Aller-Gancedo, J.M., Huntley, J.F., Rojo-Vázquez, F.A.**

Preventive Veterinary Medicine 96 (2010) 82–92

Preventive Veterinary Medicine 96 (2010) 82–92

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

journal homepage: www.elsevier.com/locate/prevetmed

Development and evaluation of an antibody ELISA for sarcoptic mange in sheep and a comparison with the skin-scraping method

F. Rodríguez-Cadenas[a], M.T. Carbajal-González[a], J.M. Fregeneda-Grandes[a], J.M. Aller-Gancedo[a], J.F. Huntley[b], F.A. Rojo-Vázquez[a,*]

[a] Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad de León, Campus de Vegazana s/n, E-24071, León, Spain
[b] Parasitology Division, Moredun Research Institute, Pentlands Science Park, Bush Loan, EH26 0PZ, Penicuik, Midlothian, Scotland, United Kingdom

ARTICLE INFO

Article history:
Received 9 December 2008
Received in revised form 4 May 2010
Accepted 14 May 2010

Keywords:
Sensitivity
Specificity
Sarcoptes scabiei
ELISA
Skin-scraping
ROC analysis
Latent-class analysis
Biological factors
Repeatability

ABSTRACT

In this work an indirect ELISA for detecting serum-specific IgG antibodies in sheep was developed using a crude saline extract from *Sarcoptes scabiei* var. *ovis* mites and then the repeatability of the ELISA outcomes was estimated. Subsequently, its diagnostic accuracy was evaluated by Receiver Operating Characteristics (ROC) analysis using a sample collected from the entire sheep population of western Castile and Leon region in Spain, and then compared with that of the skin-scraping method. The reference method used was a combination of clinical examination, skin-scraping analysis and epidemiological surveys, but it introduced selection and probably information biases. Furthermore, we attempted to identify biological factors useful to predict the sensitivity or specificity of the ELISA as determined by comparison with the reference method. Additionally, conventional latent-class analysis [Hui, S.L., Walter, S.D., 1980. Estimating the error rates of diagnostic tests. Biometrics 36, 167–171] was also used to estimate accuracy parameters.

The between-run coefficient of variation (CV) for a standard serum was 8.8% and the within-run CV 4.3%. No significant deviation between the OD% means and strength positive correlation between the OD% values ($r = 0.98$) were found for the results from two different batches of antigen. When compared to the reference method, the Area Under the ROC curve (AUC) for the reference population was 0.967 (95% CI: 0.949–0.985) for the ELISA and 0.915 (95% CI: 0.863–0.968) for the skin-scraping method. By logistic regression analysis, one explanatory biological factor—result to the skin-scraping method—and four explanatory biological factors–Tyroglyphidae individual status, *Trichophyton verrucosum* individual status, *Oestrus ovis* status of the flock and presence of adjacent animals with a clinical disease neighbour to *S. scabiei* infection—were found for diagnostic sensitivity and specificity of the ELISA, respectively, although this depended on the OD% cut-off value used. Latent-class analysis, carried out for the ELISA at 17.8 OD% cut-off value (mean plus 3 SDs of sheep considered negative to anti-*S. scabiei* antibodies), showed a marked difference between the estimated diagnostic sensitivity of the ELISA (87.6%) and the skin-scraping method (62.8%), but closer diagnostic specificities (95.9% vs. 100%, respectively). These results demonstrate that the developed ELISA is valid for different applications in clinical as well as in epidemiological contexts.

Abbreviations: AUC, area under the receiver operating characteristics curve; CV, coefficient of variation; LR, likelihood ratio; ROC, receiver operating characteristics; SENS, individual-level diagnostic sensitivity; SPEC, individual-level diagnostic specificity; TG-ROC, two-graph receiver operating characteristics curve.

* Corresponding author. Current address: Instituto de Ganadería de Montaña (CSIC-ULE), Finca Marzanas s/n, E-24346, Grulleros, León, Spain. Tel.: +34 987 291 338; fax: +34 987 291 304.
E-mail address: francisco.rojo@unileon.es (F.A. Rojo-Vázquez).

0167-5877/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.prevetmed.2010.05.009

1. Introduction

The burrowing mite *Sarcoptes scabiei* causes a parasitic skin disease in animals known as sarcoptic mange. This disease is found worldwide and affects several species including sheep, in which the lesions are mainly located on the head. Clinical manifestations are crust formation and intense pruritus (Cordero del Campillo and Rojo-Vázquez, 1999). Affected ewes have a reduced reproductive performance and also produce less meat and milk (Fthenakis et al., 2000, 2001). The prevalence of sarcoptic mange in sheep of western Castile and Leon region in Spain is considerable, as 23% of sheep flocks and 7% of individual sheep are infected (Rodríguez-Cadenas et al., submitted for publication). Moreover, as the authors know from experience, in this region many of the flocks of sheep with sarcoptic mange are endemically infected and treatments are periodically administered at intervals sufficient to control the disease, which leads to considerable financial losses and may be favouring the development of *S. scabiei*-resistance to the acaricidal drugs.

At present, the diagnosis of sarcoptic mange in sheep is based on suspicion by clinical examination and subsequent confirmation by demonstrating *S. scabiei* mites in skin-scrapings collected from the lesion (Cordero del Campillo and Rojo-Vázquez, 1999). The individual-level diagnostic sensitivity (SENS) of the skin-scraping method is high in animals severely affected, but not in recent infections where there may be only a few mites (Bornstein et al., 2006). As a consequence, when *S. scabiei* infection is detected by this classic procedure the mites have often already spread out into the flock, thus hampering its control. Moreover, the collection and analysis of a large number of skin-scrapings is tedious and impractical.

A test to detect anti-*Psoroptes ovis* antibodies in serum has been successfully used in control programmes to identify *P. ovis*-infected flocks accurately and quickly (Jacober et al., 2006), as well as in large sero-epidemiological surveys (Falconi et al., 2002). Similarly, the detection of anti-*S. scabiei* serum antibodies has been employed as a valuable additional tool to monitor the efficacy of eradication programmes for sarcoptic mange in swine (Jacobson et al., 1999; Rueda-López, 2006). However, no studies have been reported about the diagnostic utility in sheep of the detection of anti-*S. scabiei* antibodies. In Spain, as in some other countries, *S. scabiei* var. *ovis* and *P. ovis* mites coexist (Čorba et al., 1995; Razmi and Sharifi, 2007, personal observations), which, together with the reported strong cross-reactivity between *P. ovis* antisera and *S. scabiei* antigens (Matthes et al., 1996) would suggest that it is not possible to develop an immunological test based on crude mite extracts which has high accuracy for diagnosing sarcoptic mange in sheep from these areas.

The validity of a diagnostic test result depends on its precision and accuracy (Greiner and Gardner, 2000). Precision refers to the closeness of multiple determinations of the level of target analyte and it is improved by controlling the technical factors which interfere during the measurements leading to different values, whereas accuracy refers to the closeness of the inference from the test result, which is made from the accuracy parameters SENS and SPEC of the test, to the true infection status. However, it is known that the level of antibodies is influenced by a number of explanatory biological factors, including the specific factor (positive/negative to the disease) and unspecific factors (e.g. stage of infection, other diseases, age, sex, breed), and thus SENS and SPEC of a test are also population-specific parameters conditioned by the distribution of the unspecific factors in the positive and negative populations, respectively (Greiner et al., 1997; Greiner and Gardner, 2000). Its identification enables readjust the SENS and SPEC of the test and therefore increasing the accuracy of the inference from the test results.

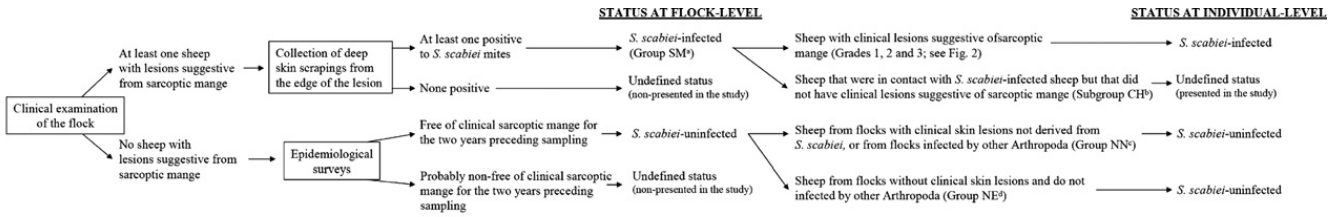
The evaluation of a diagnostic test can be accomplished by comparing it with a reference method which determines infection and non-infection more accurately than the test to be evaluated (Greiner and Gardner, 2000). However, the availability of a perfect reference method (gold standard) which classifies infection and non-infection certainly is questionable for most of the diseases. Enøe et al. (2000) reviewed the existing methods to determine accuracy parameters of diagnostic tests in the absence of a gold standard. Within these, latent-class models, which consider the true infection status as latent (present but not observable), have been developed and are based on a set of important assumptions.

In the present study an ELISA to detect IgG antibodies against *S. scabiei* var. *ovis* in sheep serum was developed, and then the repeatability of multiple measurements of the antibody levels on sera investigated. Subsequently, it was evaluated against a reference method utilising samples collected from the entire sheep population from western Castile and Leon region in Spain, and compared with the classical skin-scraping method. Furthermore, we attempted to identify explanatory biological factors for SENS and SPEC, as determined by comparison with the reference method, of the techniques evaluated. Finally, we used latent-class analysis for estimation of the SENS and SPEC of both methods.

2. Materials and methods

2.1. Reference method

The approach used as reference method to define the status of sheep in relation to *S. scabiei* at both flock and individual-level is shown in Fig. 1. The epidemiological surveys were focused principally upon determining the likelihood of subclinical infection by *S. scabiei*, and included two personal interviews per flock which were performed through questionnaires; one filled out with the farmer's declaration and another one with the declaration of his veterinary practitioner. They were asked about the presence of sheep with crust lesions on the head into the flock, its symptoms, the degree of diffusion within the flock, and the treatments administered and its efficacy, in the previous two years. Thus, the flock status was defined as being *S. scabiei*-uninfected when there was no evidence from the two questionnaires of potential subclinical infection, determined as absence of clinical-epidemiological indicatives of sarcoptic mange during the preceding two years.



[a]: Group SM = Group of flocks with Sarcoptic Mange.
[b]: Subgroup CH = Subgroup of Clinically Healthy sheep.
[c]: Group NN = Group of flocks with diseases "Near Neighbour" to sarcoptic mange. Non-uniform skin samples were collected in order to determine the alternative disease.
[d]: Group NE = Group of flocks considered NEgative to anti-*S. scabiei* antibodies.

Fig. 1. Protocol used as reference method to determine the status of sheep in relation to *S. scabiei* infection at both flock-level and individual-level.

2.2. Sampling strategy

A cluster sampling design was used to select the serum and skin samples for the validation study. In every flock surveyed at least eight serum samples were collected, and in the flocks suspicious of being *S. scabiei*-infected upon clinical examination at least five skin-scrapings from sheep with clinical lesions suggestive of sarcoptic mange (if present) were also taken. This minimum number of skin-scrapings per flock was considered enough to prevent a significant incorporation bias during the evaluation of the SENS of the skin-scraping method through comparison to the reference method, as assuming a conservative *a priori* SENS of the technique of 60% (less than the 80% of samples positive to the mite reported by Papadopoulos and Fthenakis, 1999), the resulting flock-level diagnostic sensitivity of the technique in the reference method is already close to 100%.

2.3. Estimation of the required sample size

2.3.1. Precision study

The number of sera needed for the evaluation of the precision of ELISA results was calculated to detect significant differences of OD% values (percentage of Optical Density) between two independent runs using a two-tailed paired-samples *t*-test. It was assumed a mean of the paired-difference variable of 2 OD% and a standard deviation of 5 OD%, the Type I error (α) was set up at 0.05 and the Type II error (β) at 0.01. The software MedCalc® 9.6.2.0 was used and the resulting sample size (n) was 115.

2.3.2. Accuracy study

The sample size required to assess the accuracy of the two techniques, as determined by comparison with the reference method, was calculated to estimate the parameters SENS and SPEC with an appropriate error using the following formulae (Greiner and Gardner, 2000):

$$n = \left(\frac{z_{1-\alpha/2}}{e}\right)^2 \theta(1-\theta)$$

It was assumed $\alpha = 0.05$, $e = 0.08$ (desired error margin on the estimate), and $\theta = 0.8$ (*a priori* estimate of SENS or SPEC). The intended sample size (n) was 96.

2.4. Origin of the samples

The present study was conducted on western Castile and Leon region in Spain between the years 2004 and 2006. The sheep flocks held in this area are usually managed under intensive or semi-extensive systems for milk production, or under semi-extensive systems for the production of meat lambs.

In the flocks surveyed, diagrams of clinical skin lesions reflecting distribution patterns were drawn for all animals showing them. These diagrams allowed the animals defined as *S. scabiei*-infected by the reference method to be graded in accordance with the extent of the clinical lesions (Fig. 2). Blood samples were taken by jugular puncture

Grade 1: a defined focal lesion; Grade 2: large lesions on the face and/or ears; and Grade 3: very extensive lesions on the head.

Fig. 2. Grading, in accordance to the extent of the clinical lesions, of sheep defined *S. scabiei*-infected by the reference method (Fig. 1 shows the reference method used).

from all sampled animals. As *S. scabiei* inhabits the deeper parts of the epidermis, in the sheep suspected to have sarcoptic mange by clinical examination a deep skin-scraping (until a homogeneous bleeding surface was observed) was taken from an area of approximately one square centimetre (1 cm^2) at the edge of lesions. Non-uniform skin samples such as skin-scrapings, exudates, fleece, hairs, skin biopsies and bedding samples were also collected in an attempt to diagnose other skin conditions different from sarcoptic mange (Group NN), which had lesions consisting of ulceration, crust formation, proliferative formation, and erythematous or alopoecic areas.

2.5. Analysis of skin samples

The skin-scrapings collected from sheep suspected of having *S. scabiei* infection upon clinical examination were analysed using a digestion-concentration technique. Briefly, the crusts were digested in a tube with 10% KOH for 24 h at room temperature, centrifuged for 10 min at 1100 × *g* and then resuspended in saturated sucrose solution. After another centrifuging for 2 min at 250 × *g*, more sucrose solution was added until it reached the upper part of the tube. A cover-slip was then placed on top of it and left there for 15 min to recover any mites present in the solution. Three cover-slip mounts were prepared from each skin-scraping and subsequently observed under the microscope. The detection of one *S. scabiei* mite was enough to classify an animal as positive, while if none were detected on any of the three mounts the classification was negative.

Non-uniform methods were used to analyse the skin samples collected from Group NN. The techniques used depended on the skin condition suspected and were those previously described as suitable (Cordero del Campillo and Rojo-Vázquez, 1999; Kane et al., 1997; Quinn et al., 1995; Ramos et al., 1996; Yeruham et al., 2000). The techniques used were the following: (a) histopathological studies were performed by examination of haematoxylin-eosin stained sections, (b) fungal and bacterial studies were performed by microscopic examination, culture (in Sabouraud agar with chloramphenicol, gentamicin and cycloheximide for isolating fungi, and sheep blood agar for bacteria) and subsequent identification by morphological characteristics for fungi or biochemistry tests (API® Staph, Biomérieux, France) for bacteria, (c) free-living nematode studies were performed by isolating, identifying and counting larvae from bedding samples, (d) ectoparasites studies were carried out by direct examination of the skin samples under the stereomicroscope or by the procedure described previously for detecting *S. scabiei* mites.

2.6. Collection of S. scabiei var. ovis mites and preparation of the mite extract

S. scabiei var. *ovis* mites were obtained from a sheep with extensive lesions from sarcoptic mange. The ewe was euthanized with an intravenous injection of embutramide plus mebezonium (T-61®, Intervet International B.V.) and then the lesion crusts were removed with a scalpel and chopped to produce particles of approximately 5 mm diameter. For mite isolation, the technique described by Nöckler et al. (1992) was used. Briefly, the crusts with mites were placed on a filter paper and then incubated in a wet chamber for 48 h at 37 °C. Then, the mites which had migrated from the crust onto the Petri dish were collected with the aid of a vacuum pump system and stored frozen at −20 °C until being processed as described in Ref. Rodríguez-Cadenas et al. (2010).

2.7. ELISA procedure

High-binding microtitre plates (Costar®, Corning Incorporated, USA) were coated overnight at 4 °C with 50 μl per well of a solution of the mite extract adjusted to 1 μg ml^{-1} of protein with carbonate-bicarbonate buffer (70 mM $NaHCO_3$, 30 mM Na_2CO_3, 0.2 g l^{-1} NaN_3, pH 9.6). Then a three cycle washing step with PBS-T20 (PBS, 0.5 ml l^{-1} Tween 20) was performed. Each cycle consisted of filling the wells manually with a washing comb (Nunc-Immuno™ wash 12) and leaving the washing solution for 5 min before removing it. Then the plates were incubated for 1 h at 37 °C with 50 μl per well of sheep sera diluted 1/100 in PBS-T80-NaCl (PBS, 5 ml l^{-1} Tween 80, 0.5 M NaCl) and added in duplicate. A further washing was performed, then the plates were incubated for 1 h at 37 °C with 50 μl per well of biotin-labelled anti-sheep IgG monoclonal antibody (clone VPM6; Bird et al., 1995) at 0.5 μg ml^{-1} in PBS-T80-NaCl. After another washing, the plates were incubated for 30 min at 37 °C with 50 μl per well of streptavidin-HRP conjugate (GE Healthcare, UK) diluted 1/40,000 in PBS-T80-NaCl. Following a further wash, peroxidase activity was visualized using OPD chromogen substrate (OPD Tablets, Dako, Denmark) according to the manufacturer's instructions (incubation in the dark for 15 min at room temperature). After the reaction had been stopped with 50 μl per well of 0.5 M H_2SO_4, the OD was measured at 492 nm using a spectrophotometer and the OD for each serum calculated as the mean of the ODs in the duplicate wells. The results were expressed as normalized data in terms of OD%, obtained by linear inter/extrapolation from the results of the positive (OD = 0.692 ± 0.095 [mean ± S.D. in 20 runs]; assigned OD% = 100) and negative (OD = 0.060 ± 0.010; OD% = 0) control sera which were included in each plate. This transformation makes the ELISA results from different runs more repeatable, thus enables more valid inferences from the outcomes (Sánchez et al., 2002). A sheep was deemed seropositive when its OD% result was higher than the OD% cut-off value; otherwise it was considered seronegative.

2.8. Precision evaluation of the ELISA

The repeatability of the ELISA results was estimated in three ways. First, a standard serum was tested in 10 different runs of the assay, and second, the same standard serum was tested 10 times within a run. Then, the between-run and within-run coefficients of variation (CV_b and CV_w, respectively) were calculated from the normalized outcomes. Third, repeatability of the ELISA was evaluated by re-testing a new aliquot of 120 sera using a different batch of the mite extract. The OD% values of these sera in the first run were representative of the full range of expected

OD% values in the sheep population. The OD% agreement between the results from the two runs was presented in a Bland–Altman plot (Bland and Altman, 1986). Correlation between the OD% values, deviation between the OD% means, mean dichotomized test results agreement and difference in the proportions of positive/negative and negative/positive sera were used as indicators of repeatability.

2.9. Accuracy evaluation of the ELISA and skin-scraping method

2.9.1. ROC analysis

The accuracy of the ELISA and skin-scraping method, as compared to the reference method, was evaluated by means of the Receiver Operating Characteristics (ROC) approach. The Area Under the ROC curve (AUC), which ranges from 0.5 for a test with non-ability to discriminate between the positive and negative reference status to 1.0 for a test with perfect discrimination, was used as a measure of the overall accuracy. All possible combinations of the SENS and SPEC of the ELISA obtained by changing the OD% cut-off value were presented as two-graph ROC (TG-ROC) plot (Greiner et al., 1995). Likelihood ratios (LRs), which are measures indicating how many more times likely a test outcome is from an infected animal than from one uninfected, were also used as indicators of accuracy (Choi, 1998).

2.9.2. Latent-class analysis

The latent-class analysis was carried out using maximum likelihood estimation. It was used the conventional model proposed by Hui and Walter (1980) which is based on a set of requirements known as the Hui and Walter paradigm: (a) two (or more) tests applied to two (or more) populations with different true prevalences, (b) the tests have the same SENS and SPEC in all populations, and (c) the tests are conditionally independent given the true status.

2.10. Statistical analysis

2.10.1. Descriptive and comparative statistics

The one-sample Kolmogorov–Smirnov test was used to determine whether the requirement of normality needed to run parametric tests was fulfilled or not, and the Levene test to determine homogeneity of variances. The chi-squared test was used to compare the SENS and SPEC of the techniques between groups. The independent-samples *t*-test/one-way ANOVA or the Mann–Whitney/Kruskal–Wallis test was used to determine whether there was significant difference between the OD% means or medians, respectively, in the different groups. Confidence intervals were calculated following the normal approximation to the binomial distribution.

In the repeatability estimation using two different batches of the mite extract, the Pearson correlation coefficient was used to determine the correlation between the OD% values in the two runs, the paired-samples *t*-test to determine whether there was significant difference between the OD% means, and the Binomial test to determine if there was significant difference between the proportions of positive/negative and negative/positive sera in the discordant results.

2.10.2. Logistic regression model

Logistic regression models were constructed using as dependent variable the ELISA dichotomous results at a specific OD% cut-off value and as independent variables the explanatory biological factors. The general model was the following:

$$\text{Logit } Pr(Y = 1|X_1, X_2, X_3, \ldots, X_k) = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \ldots + \beta_k X_k$$

where Y is the ELISA dichotomous result ($Y = 1$ if seropositive and $Y = 0$ if seronegative), X denote the different explanatory variables and β the coefficients.

As the investigated biological factors were different in the sheep positive and negative to the reference method, different models were constructed for each population (Coughlin et al., 1992). Thus, when used the positive reference population we modelled, within a logit, the SENS of the ELISA determined by comparison with the reference method, whereas when used the negative reference population we modelled, within a logit, 1 – SPEC of the ELISA determined by comparison with the reference method. Variables significant in the univariate analysis ($p < 0.25$) were offered to the model. The initial model was reduced using a stepwise backwards elimination procedure with variables retained based on its significance (two-tailed Wald's test).

The level of significance (alpha) was set at 0.05, except for the univariate analysis. All statistical tests and mathematical calculations were performed with SPSS 15.0 for Windows®, MedCalc® 9.6.2.0 and Microsoft® Office Excel 2003.

3. Results

3.1. Analysis of skin samples and sera

Once skin samples had been analysed, the flocks and individual sheep were allocated into the different subpopulations. A summary of the samples analysed within each one and the results obtained is shown in Table 1. In Group NN, the epidemiological surveys determined there was endemic infection by *O. ovis* in one flock, and in combination with clinical examination and the analysis of the skin samples led to the diagnosis of seven different skin infections.

The Group NE was assumed to be representative of the sheep from the field population negative to anti-*S. scabiei* antibodies, and so was taken as negative reference in order to determine significant level of cross-reacting antibodies. It was found that the OD% median in sheep with pelodera dermatitis lesions and in sheep clinically infected by the orf virus was significantly higher than that in Group NE ($p < 0.01$ and $p < 0.05$, respectively, one-tailed Mann–Whitney test). In Group SM, the four subpopulation of sheep had OD% medians significantly higher than those in Groups NE and NN ($p < 0.01$, one-tailed Mann–Whitney test), and the OD% means at the different positive subpop-

Table 1
Serum and skin samples collected from western Castile and Leon region in Spain used in the validation of the ELISA and skin-scraping method, and results obtained in their analysis.

Subpopulations (see Fig. 1)	N° flocks	N° samples		Detection of *S. scabiei* in skin-sample analysis	ELISA OD% (box = first quartil, median and third quartile; line = minimum and maximun values, excluding outliyers [○] and extreme values [*])
		Skin	Sera		0 20 40 60 80 100 120 140
Group NE	11	n.c.[a]	89	-	
Group NN[b]	18	100	172	0% (0/100)	
Oestrus ovis-infected flock[1]	1	-	14	-	
Psoroptes ovis	3	-	33	-	
Tyroglyphidae	1	-	3	-	
Pelodera strongyloides	2	-	15	-	
Trichophyton verrucosum	1	-	2	-	
Staphylococcus aureus	3	-	15	-	
Contagious ecthyma	3	-	17	-	
Bovicola ovis	3	-	25	-	
Unsuccessful diagnosis	1	-	6	-	
Others[2]	16	-	42	-	
Group SM	13	71	186	-	
Subgroup CH	11	n.c.	73	-	
Grade 1	9	14	29	64.3% (9/14)	
Grade 2	13	49	76	85.7% (42/49)	
Grade 3	4	8	8	100% (8/8)	

[a] Not collected.
[b] All subgroups within Group NN refer to sheep clinically infected by the corresponding etiological agent, except: (1) due to the inability to make individual diagnosis for *O. ovis* infection all sheep sampled from the *O. ovis*-infected flock were classified equally, and (2) which includes sheep from Group NN not included in the other subgroups.

Table 2
Dichotomised ELISA result agreement of 120 sheep sera tested with two different batches of the *S. scabiei* extract on independent runs.

Cut-off value (OD%)	Sero+ 1st run		Sero− 1st run		Agreement (%)
	Sero+ 2nd run	Sero− 2nd run	Sero+ 2nd run	Sero− 2nd run	
5	113	1	0	6	99
15	98	3	0	19	98
35	73	2	1	44	98
60	36	2	8	74	92
80	13	3	4	100	94

ulations (Grades 1, 2 and 3) were significantly different ($p < 0.001$, one-way ANOVA test).

3.2. Precision evaluation of the ELISA

The evaluation of the variability of the ELISA results by means of re-testing the standard serum ten times yielded a CV_b value of 8.8% (OD% = 89.0 ± 7.8 [mean ± S.D.]) and a CV_w value of 4.3% (OD% = 88.8 ± 3.6). The repeatability of the OD% values of the 120 sera analysed with two different batches of the mite extract is shown in Fig. 3. The OD% means obtained in the two runs were similar ($p > 0.05$, two-tailed paired-samples *t*-test). It was also found a strength positive correlation between the OD% results in the two runs ($r = 0.98$, $p < 0.001$, one-tailed). The mean agreement of the dichotomized test results on the two runs was 96%

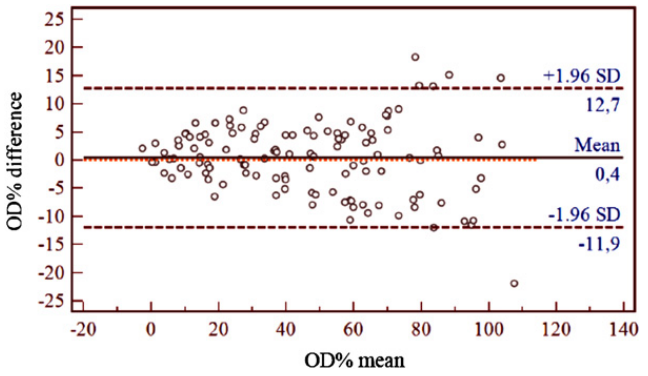


Fig. 3. Bland Altman plot showing the repeatability of OD% values of 120 sheep serum samples tested by the *S. scabiei* ELISA using two different batches of the mite extract.

Table 3
Cross-tabulation of results from ELISA vs. skin-scraping method in the two populations of sheep from western Castile and Leon, Spain, used in the latent-class analysis.

		ELISA[a]			
		Population 1[b] (n = 184/239)		Population 2[c] (n = 148/166)	
		Sero+	Sero−	Sero+	Sero−
Skin-scraping method	*S. scabiei* positive	41/41	5/5	12/12	1/1
	S. scabiei negative[d]	13/31	125/162	7/13	128/140

[a] Cut-off value of 17.8 OD% (mean plus 3 SDs of OD% in sheep from Group NE, see Fig. 1).
[b] Population 1: n = 184 are 1/2 of sheep from Groups NN + NE plus 3/4 of sheep from Group SM (n = 71; only the sheep subjected to both ELISA and skin-scraping method were included) except Subgroup CH, whereas n = 239 are the same sheep plus 3/4 of sheep from Subgroup CH.
[c] Population 2: n = 148 are the other 1/2 of sheep from Group NN + NE plus the other 1/4 of sheep from Group SM except Subgroup CH, whereas n = 166 are the same sheep plus the other 1/4 of sheep from Subgroup CH.
[d] All sheep from Groups NN and NE and from Subgroup CH were assumed to be *S. scabiei* negative to the skin-scraping method.

(Table 2), and the proportions of positive/negative and negative/positive sera in the 24 discordant results detected were similar ($p > 0.05$, two-tailed Binomial test).

3.3. Accuracy evaluation of the ELISA and the skin-scraping method

3.3.1. ROC analysis

The aim of the present study was the evaluation of the accuracy of the two techniques to diagnose sarcoptic mange in sheep from the entire ovine population of western Castile and Leon region in Spain. Based on the reference method (see Fig. 1), the SENS was therefore estimated from the positive reference population comprised by sheep in Group SM with Grades 1, 2 or 3 (n = 71 for the skin-scraping method, and n = 113 for the ELISA) and the SPEC from the negative reference population formed by the sheep included in Groups NE and NN (n = 261 for the ELISA). Therefore, for the population considered and as compared with the reference method the accuracy parameters were the following. The SENS of the skin-scraping method was 83.1% (95% CI: 74.4–91.8), while the SPEC was safely assumed to be 100%. The resulting LR− (95% CI) of this technique was 0.17 (0.08–0.26) and the LR+ was ∞. Fig. 4 shows the TG-ROC plot indicating the estimated SENS and SPEC of the ELISA at the different OD% cut-off values. Interval LRs (95% CI) were calculated for the ELISA: <5.0 OD% = 0.02 (0.01–0.09); 5.1–15.0 OD% = 1.22 (0.73–2.03); 15.1–35.0 OD% = 3.47 (1.73–6.95); >35.1 OD% = 57.0 (18.3–177). The AUC for the skin-scraping method was 0.915 (95% CI: 0.863–0.968, it was considered that the negative reference population included 100 sheep negative to *S. scabiei* mites) and for the ELISA 0.967 (95% CI: 0.949–0.985).

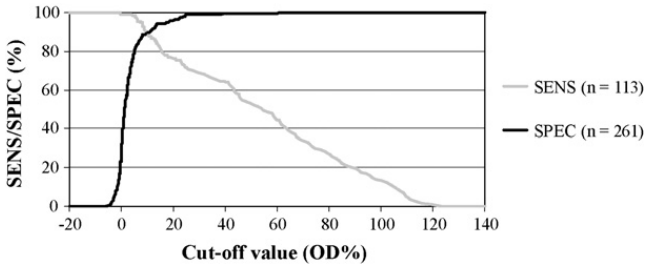


Fig. 4. TG-ROC plot of the *S. scabiei* ELISA estimated using a serum sample collected form the entire sheep population of western Castile and Leon region in Spain (AUC = 0.967; 95% CI: 0.949–0.985; Fig. 1 shows the reference method used).

3.3.2. Latent-class analysis

Latent-class analysis was carried out with and without the inclusion of sheep from Subgroup CH. New populations of sheep were formed for latent-class analysis in order to achieve the requirements of the Hui and Walter model. Table 3 shows the cross-tabulation of results from ELISA vs. skin-scraping method and Table 4 the estimated accuracy

Table 4
Estimated disease prevalences, and accuracy parameters of the *S. scabiei* ELISA and the skin-scraping method for sheep from the entire ovine population of western Castile and Leon region, Spain, using two different analytical approaches.

Estimated parameter	Analytical approach[a]	
	ROC analysis[b]	Latent-class analysis for independent tests[c]
SENS skin-scraping method (%)	83.1/n.a.[d]	82.5/62.8
SENS ELISA (%)	85.9/n.a.	87.9/87.6
SPEC skin-scraping method (%)	100/n.a.	100/100
SPEC ELISA (%)	95.0/n.a.	96.6/95.9
Prevalence in Population 1 (%)	28.8/n.a.	30.7/31.2
Prevalence in Population 2 (%)	12.2/n.a.	11.2/13.2

[a] See Table 3. Data shown as Population 1 (n = 184) – Population 2 (n = 148)/Population 1 (n = 239) – Population 2 (n = 166).
[b] Fig. 1 shows the reference method used.
[c] Hui and Walter (1980).
[d] Not applicable.

Table 5
Variables identified as being significantly associated ($p < 0.25$; chi-squared test) in the univariate analysis of potential factors which condition the result to the *S. scabiei* ELISA in sheep positive and negative to the reference method (Fig. 1 shows the reference method used).

Population	Variables and levels (See Fig. 1 and Table 1)	Cut-off value (OD%)					
		5		15		35	
		% Sero+	*p*-value	% Sero+	*p*-value	% Sero+	*p*-value
S. scabiei-infected sheep	Extent of mange lesion						
	Grade 1	93.1	0.052	69.0	0.075	55.2	0.061
	Grade 2	100	(d.f. = 2)	84.2	(d.f. = 2)	65.8	(d.f. = 2)
	Grade 3	100		100		100	
	Result to skin-scraping method						
	S. scabiei positive	100	n.s.[a] (1.00)	93.2	0.008	83.1	0.012
	S. scabiei negative	100	(d.f. = 1)	66.7	(d.f. = 1)	50.0	(d.f. = 1)
S. scabiei-uninfected sheep	Disease neighbour to *S. scabiei* infection						
	No disease (Group NE)	19.1	0.094	3.4	<0.001	0.0	0.160
	Oestrus ovis-infected flock	35.7	(d.f. = 10)	28.6	(d.f. = 10)	7.1	(d.f. = 10)
	Psoroptes ovis	18.2		0.0		0.0	
	Tyroglyphidae	33.3		33.3		0.0	
	Pelodera strongyloides	33.3		13.3		6.7	
	Trichophyton verrucosum	50.0		50.0		0.0	
	Staphylococcus aureus	6.7		0.0		0.0	
	Contagious ecthyma	35.3		11.8		5.9	
	Bovicola ovis	24.0		8.0		0.0	
	Unsuccessful diagnosis	16.6		0.0		0.0	
	Others	4.8		0.0		0.0	

[a] Non significant.

and prevalence parameters. Interestingly, the introduction of Subgroup CH led to a considerable reduction in the estimated SENS of the skin-scraping method, keeping all the other accuracy parameters similar.

3.4. Identification of explanatory biological factors for SENS and SPEC, determined by comparison with the reference method, of the ELISA

The SENS of the skin-scraping method on the different grades of clinical mange was similar ($p > 0.05$, chi-squared test, d.f. = 2). The results of univariate analysis of potential factors for the ELISA outcomes are show in Table 5. Four logistic regression models were constructed (Table 6).

4. Discussion

The ELISA developed in this survey is based on a crude antigen prepared from the specific variety of *S. scabiei* that infects sheep, and uses a protocol similar to others devised to detect anti-*S. scabiei* antibodies in other species (Bornstein et al., 1996, 2006; Hollanders et al., 1997; Rambozzi et al., 2004). At present, several *S. scabiei* recombinant-antigens have been produced and it has been demonstrated that some of them are potentially useful for immunodiagnosis (Casais et al., 2007; Ljunggren et al., 2006). However, it is known that *S. scabiei* infection may produce a range of host-specific antibody responses (Arlian et al., 1996), and to the authors knowledge none of those antigens has been tested with sheep sera. Further-

Table 6
Logistic regression models of SENS and 1 − SPEC, as determined by comparison with the reference method (Fig. 1), of the *S. scabiei* ELISA.

Model	Explanatory variable	Cut-off value (OD%)					
		5		15		35	
		β	*p*-value[a]	β	*p*-value	β	*p*-value
SENS	Intercept	n.s.[b]		0.69		0.00	
	Result to skin-scraping method[c]			1.93	0.016	1.59	0.018
1 − SPEC	Intercept	−1.24		−3.25		n.s.	
	Tyroglyphidae[d]			2.56	0.044		
	T. verrucosum[e]			3.25	0.025		
	O. ovis-infected flock[f]			2.34	0.001		
	Others[g]	−1.75	0.018				

[a] Two-tailed Wald's test.
[b] Non significant model.
[c] Coded as positive to *S. scabiei* = 1, otherwise = 0.
[d] Coded as infected by Tyroglyphidae = 1, otherwise = 0.
[e] Coded as infected by *T. verrucosum* = 1, otherwise = 0.
[f] Coded as sheep from an *O. ovis*-infected flock = 1, otherwise = 0.
[g] Coded as others = 1, otherwise = 0 (See Table 1).

more, in a recent study it has been shown that obtaining efficient *S. scabiei* recombinant-antigens for specific assays in pigs and humans is not easy (Kuhn et al., 2008). As a consequence, the use of whole mite antigen to detect antibodies in sheep could be beneficial in the meantime until tests based on recombinant-antigens are available.

We have demonstrated that the ELISA produces results with high repeatability between-run, within-run, and between the different batches of antigen, as all of the indicators of variability showed that the multiple measurements of antibodies on sera fall within acceptable limits of tolerance. These issues indicate that the protocol of ELISA was sufficiently standardized to produce repeatable results, and therefore more valid inferences from test outcomes can be made. Nevertheless, a more extensive study of this topic would be desirable.

In the present study, a reference method based on a combination of clinical examination, epidemiological surveys and analysis of skin-scrapings was employed to define the status of sheep in relation to *S. scabiei* infection. The reference method was based on a primary screening by clinical examination, as it is assumed to have high SPEC (it classifies as negatives all uninfected sheep except those with lesions resembling sarcoptic mange: lesions usually restricted to the head, with the first clinical lesions appearing on the lips or nostrils, Rahbari et al., 2009) and high SENS (it classifies as positive all infected sheep except the subclinically infected ones). Then, the combinations in serial with the skin-scraping method to obtain the positives and in parallel with the epidemiological surveys to obtain the negatives were used to mitigate those limitations and therefore to improve the positive and negative predictive values. The epidemiological surveys consisted of open-ended questions and were performed to people who work in close contact with the animals. In the region of study the presence of sarcoptic mange into a flock has no legal consequences, and hence the declarations of the farmer were therefore considered reliable. Nevertheless, to further increase the negative predictive value, another questionnaire filled out with the declarations of the farmer's veterinary practitioner was included. Despite our best efforts, the authors admit that the reference method used leaves small residues of doubt in both the defined positive and negative; this may have introduced some information bias in the study, leading to an underestimation of the SENS and SPEC.

Furthermore, the reference method also left three subpopulations of sheep with undefined status in relation to *S. scabiei* infection. They include sheep whose accurate classification would have required costly methods, this being not feasible under the economic conditions. Of these subpopulations, the results of analysis of sera from Subgroup CH were included, as they may be the most significant source of selection bias. These sheep were mixed with *S. scabiei*-infected ones and had an OD% median significantly higher than that detected in sheep negative to the reference method, suggesting that these antibodies had been elicited by *S. scabiei*. This subpopulation may comprise both *S. scabiei*-infected and uninfected sheep. The absence of this subpopulation in the estimation of accuracy parameters of the ELISA for the entire sheep population of western Castile and Leon region in Spain has probably led to an overestimation of both SENS and SPEC. The former because subclinically *S. scabiei*-infected sheep highly-likely have lower antibody level than the defined infected by the reference method, and the latter because some of the *S. scabiei*-uninfected sheep in this subpopulation probably have an antibody level higher than the sheep defined uninfected by the reference method due to persistent antibodies following infection resolution. The skin-scraping method presumably has in this subpopulation a SENS close to 0% and therefore AUC $\approx$ 0.5 (non-informative test), but unfortunately the ROC analysis could not determine if the ELISA improves over this.

Regarding the accuracy of the tests, as determined by comparison with the reference method, both skin-scraping method and ELISA can be conceived as highly-accurate methods (AUCs were between 0.9 and 1, Greiner et al., 2000) for diagnosing sarcoptic mange in sheep from the entire ovine population from western Castile and Leon region in Spain, but the authors acknowledge that these values may be biased due to the limitations discussed previously. Furthermore, both techniques seem to have similar overall accuracy (overlapping of the 95% CI of their AUCs, Greiner et al., 2000). It is also provided LRs for multiple levels of ELISA OD% results and for the dichotomous outcomes of the skin-scraping method. The LRs presented are the link between the pre-test likelihood of *S. scabiei* infection of a sheep from the entire ovine population in western Castile and Leon region in Spain (or true prevalence) and the pos-test likelihood, and therefore enable producing post-test quantitative results for interpretation of outcomes from that population. Interestingly, it was also found that ELISA results from that population falling within the interval 5-15 OD% are non-informative (LR $\approx$ 1).

The present study also enabled the identification of biological factors which condition the SENS or SPEC, determined by comparison with the reference method, of the ELISA at specific OD% cut-off values. However, the interaction between the factors could not be investigated in the model for 1 − SPEC as there were not, in the negative reference sample, any animals with more than one explanatory biological factor. Nevertheless, the information provided facilitates, through the readjustment of these accuracy parameters, a more adequate extrapolation of the ELISA to other populations/subpopulations of sheep. In addition, the possible conditional dependence of the SENSs of both techniques should be also taken into account during interpretation of results produced by the combination of both tests. In case the ELISA is used at a OD% cut-off value in which their SENSs are conditionally dependent (for instance 15 and 35 OD%, Table 5), then the resulting combined SENS will be less than that obtained assuming conditional independence for a parallel-interpretation scheme, but higher for a series-interpretation scheme (Gardner et al., 2000).

Due to the exposed limitations of the ROC analysis we decided to carry out latent-class analysis. This approach allows the elimination of selection and information biases but it is based on a set of important requisites; if these are not fulfilled the derived estimates are biased (Toft et al., 2005). The first assumption of the conventional model used (Hui and Walter, 1980) is that the tests should be applied to populations with different prevalences. The populations

used had different proportions of sheep indefinable or considered positive by the reference method, and therefore it is a reasonable supposition that they have different true prevalences. Another assumption is that the tests need to have the same SENS and SPEC in the different populations considered; however, these should also be the same to those in the intended target population of the tests. The reorganization of sera into two new populations was made in order to ensure similar SENS of the techniques across the two populations; stratification by solely the result to the reference method probably had led to a failure of this requisite as stage of infection is known to be a major explanatory factor for SENS. However, as the sera of the two populations were collected from the same area, the assumption of constant SPEC is acceptable. Inclusion of Subgroup CH allowed elimination of selection bias, hence these derived estimates of SENS and SPEC would be the nearest to the true ones for the entire ovine population of western Castile and Leon, the intended target population. Finally, the tests should be conditionally independent. The two techniques evaluated have different biological base (detection of antibodies vs. mites), and therefore there seems reasonable to assume that they are conditionally independent. This is true for their SPECs, as the SPEC of the skin-scraping method is 100%. Regarding the SENSs, we found by ROC analysis, at the specific OD% cut-off value used for the ELISA in the latent-class analysis, a sensitivity covariance (γ_{SENS}) of 0.03 (28% of maximum possible value), indicating low dependence of their SENSs. However, probable information bias in the positive reference population would have overestimated it, whereas selection bias would have underestimated it.

In summary, ROC analysis determines that the ELISA developed and the skin-scraping method are internally equally valid for determining the status of sheep of the entire ovine population from western Castile and Leon region in Spain in relation to *S. scabiei* infection, and in addition, explanatory biological factors of the accuracy parameters SENS and SPEC of the ELISA have been found out, allowing an adequate extrapolation of this technique to other populations/subpopulations of sheep. Conversely, the latent-class analysis without selection bias, which may have produced the results nearest to the true ones for the intended target population of the tests, determined that the ELISA, at 17.8 OD% cut-off value, is markedly more sensitive than the skin-scraping method, but however, they have closer specificities. Possible applications of the ELISA may be "confirmation" of cases of sarcoptic mange, demonstration of "freedom" from infection, estimation of seroprevalence as a basis for determining true prevalence or for risk-analysis surveys, and in the context of disease surveillance to determine the efficacy of control/eradication programmes.

Acknowledgements

The present study was financially supported by grant LE010A05 from the Education Bureau of the Castile and Leon Regional Government (Consejería de Educación de la Junta de Castilla y León). FRC and MTCG hold fellowships from the Education Bureau of the Castile and Leon Regional Government, co-financed by the European Social Fund. We should like to express our deep gratitude to Dra. Maite Trobajo, from the Department of Mathematics at the University of Leon, for her help with the statistical analysis, and to the veterinary practitioners and farmers who collaborated in this study.

References

Arlian, L.G., Morgan, M.S., Arends, J.J., 1996. Immunologic cross-reactivity among various strains of *Sarcoptes scabiei*. J. Parasitol. 82, 66–72.

Bird, P., Jones, P., Allen, D., Donachie, W., Huntley, J., McConnell, I., Hopkins, J., 1995. Analysis of the expression and secretion of isotypes of sheep B cell immunoglobulins with a panel of isotype-specific monoclonal antibodies. Res. Vet. Sci. 59, 189–194.

Bland, J.M., Altman, D.G., 1986. Statistical methods for assessing agreement between two methods of clinical measurements. Lancet 327, 307–310.

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*). Vet. Dermatol. 17, 411–416.

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

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. Vet. Res. 38, 435–450.

Choi, B.C.K., 1998. Slopes of the receiver operating characteristics curve and likelihood ratios for a diagnostic test. Am. J. Epidemiol. 148, 1127–1132.

Čorba, J., Várady, M., Prasličkа, J., Tomašovičová, O., 1995. Efficacy of moxidectin against mixed (*Psoroptes ovis* and *Sarcoptes scabiei* var. *ovis*) mange infestation in sheep. Vet. Parasitol. 56, 339–344.

Cordero del Campillo, M., Rojo-Vázquez, F.A., 1999. Parasitología Veterinaria. McGraw-Hill Interamericana, Madrid, pp. 968.

Coughlin, S.S., Trock, B., Criqui, M.H., Pickle, L.W., Browner, D., Tefft, M.C., 1992. The logistic modeling of sensitivity, specificity, and predictive value of a diagnostic test. J. Clin. Epidemiol. 45, 1–7.

Enøe, C., Georgiadis, M., Johnson, W.O., 2000. Estimation of sensitivity and specificity of diagnostic tests and disease prevalence when the true disease state is unknown. Prev. Vet. Med. 45, 61–81.

Falconi, F., Ochs, H., Deplazes, P., 2002. Serological cross-sectional survey of psoroptic sheep scab in Switzerland. Vet. Parasitol. 109, 119–127.

Fthenakis, G.C., Karagiannidis, A., Alexopoulos, C., Brozos, C., Papadopoulos, E., 2001. Effects of sarcoptic mange on the reproductive performance of ewes and transmission of *Sarcoptes scabiei* to newborn lambs. Vet. Parasitol. 95, 63–71.

Fthenakis, G.C., Papadopoulos, E., Himonas, C., Leontides, L., Kritas, S., Papatsas, J., 2000. Efficacy of moxidectin against sarcoptic mange and effects on milk yield of ewes and growth of lambs. Vet. Parasitol. 87, 207–216.

Gardner, I.A., Stryhn, H., Lind, P., Collins, M.T., 2000. Conditional dependence between tests affects the diagnostic and surveillance of animal diseases. Prev. Vet. Med. 45, 107–122.

Greiner, M., Gardner, I.A., 2000. Epidemiologic issues in the validation of veterinary diagnostic tests. Prev. Vet. Med. 45, 3–22.

Greiner, M., Pfeiffer, D., Smith, R.D., 2000. Principles and practical application of the receiver-operating characteristic analysis for diagnostic tests. Prev. Vet. Med. 45, 23–41.

Greiner, M., Shivarama, T., Patzelt, R.J., Kakaire, D., Schares, G., Dietz, E., Böhning, D., Zessin, K., Mehlitz, D., 1997. Impact of biological factors on the interpretation of bovine trypanosomosis serology. Prev. Vet. Med. 30, 61–73.

Greiner, M., Sohr, D., Göbel, P., 1995. A modified ROC analysis for the selection of cut-off values and the definition of intermediate results of serodiagnostic tests. J. Immunol. Methods 185, 123–132.

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

Hui, S.L., Walter, S.D., 1980. Estimating the error rates of diagnostic tests. Biometrics 36, 167–171.

Jacober, P., Ochs, H., Torgerson, P.R., Schnyder, M., Deplazes, P., 2006. A method for sheep scab control by applying selective treatment based on flock serology. Vet. Parasitol. 136, 373–378.

Jacobson, M., Bornstein, S., Wallgren, P., 1999. The efficacy of simplified eradication strategies against sarcoptic mange mite infec-

tions in swine herds monitored by an ELISA. Vet. Parasitol. 81, 249–258.
Kane, J., Summerbell, R., Sigler, L., Krajden, S., Land, G., 1997. Laboratory handbook of dermatophytes: a clinical guide and laboratory handbook of dermatophytes and other filamentous fungi from skin, hair and nails. Star Publishing Company, Belmont, pp. 344.
Kuhn, C., Lucius, R., Matthes, H.F., Meusel, G., Reich, B., Kalinna, B.H., 2008. Characterisation of recombinant immunoreactive antigens of the scab mite *Sarcoptes scabiei*. Vet. Parasitol. 153, 329–337.
Ljunggren, E.L., Bergstrom, K., Morrison, D.A., Mattsson, J.G., 2006. Characterization of an atypical antigen from *Sarcoptes scabiei* containing an MADF domain. Parasitology 132, 117–126.
Matthes, H.F., Harrison, G.B.L., Shaw, R.J., Heath, A.C.G., Pfeffer, A., Hiepe, T.H., 1996. Cross-reacting antibodies to *Sarcoptes suis*, Chorioptes bovis and Notoedres cati and anti-*P. ovis* IgE in sera from sheep infested naturally with *Psoroptes ovis*. Int. J. Parasitol. 26, 437–444.
Nöckler, K., Matthes, H.F., Hiepe, T., Ziegler, H., 1992. Nachweis von Anti-*Sarcoptes suis*-IgG im Blutserum neonatal mit Sarkoptesmilben infizierter Ferkel mit dem indirekten ELISA. Monatsh. Veterinarmed. 47, 415–421.
Papadopoulos, E., Fthenakis, G.C., 1999. Administration of moxidectin for treatment of sarcoptic mange in a flock of sheep. Small Rumin. Res. 31, 165–168.
Quinn, P.J., Carter, M.E., Markey, B., Carter, G.R., 1995. Clinical Veterinary Microbiology. Wolfe Publishing, Arizona, pp. 648.
Rahbari, S., Nabian, S., Bahonar, A.R., 2009. Some observations on sheep sarcoptic mange in Tehran province, Iran. Trop. Anim. Health Prod. 41, 397–401.
Rambozzi, L., Menzano, A., Lavin, S., Rossi, L., 2004. Biotin-avidin amplified ELISA for detection of antibodies to *Sarcoptes scabiei* in chamois (*Rupicapra* spp.). Vet. Res. 35, 701–708.
Ramos, J.J., Luco, D.F., Verde, M.T., Lucientes, J., Fernández, A., 1996. Pelodera dermatitis in sheep. Vet. Rec. 138, 474–475.
Razmi, G.R., Sharifi, K., 2007. A report of ovine acariasis due to mixed Sarcoptes scabiei and *Psoroptes ovis* in a sheep flock. In: Proceedings of the 21st Conference of WAAVP, Gent, Belgium, p. 630.
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*. Vet. Immunol. Immunopathol. 133, 109–116.
Rodríguez-Cadenas, F., Carbajal-González, M.T., Fregeneda-Grandes, J.M., Aller-Gancedo, J.M., Rojo-Vázquez, F.A. Cross-sectional seroepidemiological survey of sarcoptic mange in sheep of Western Castile and Leon, Spain. Prev. Vet. Med. submitted for publication.
Rueda-López, M., 2006. Elimination of mange due to *Sarcoptes scabiei* var. *suis* from a 1800-sow farrow-to-finish farm. Vet. Rec. 159, 595–597.
Sánchez, J., Dohoo, I.R., Markham, F., Leslie, K., Conboy, G., 2002. Evaluation of the repeatability of a crude adult indirect *Ostertagia ostertagi* ELISA and methods of expressing test results. Vet. Parasitol. 109, 75–90.
Toft, N., Jørgensen, E., Højsgaard, S., 2005. Diagnosing diagnostic tests: evaluating the assumptions underlying the estimation of sensitivity and specificity in the absence of a gold standard. Prev. Vet. Med. 68, 19–33.
Yeruham, I., Perl, S., Abraham, A., 2000. Orf infection in four sheep flocks. Vet. J. 160, 74–76.

Publicación nº 2

Cross-sectional sero-epidemiological survey of sarcoptic mange in sheep of Western Castile and Leon, Spain

Rodríguez-Cadenas, F., Carbajal-González, M.T., Fregeneda-Grandes, J.M., Aller-Gancedo, J.M., Rojo-Vázquez, F.A., 2010

Preventive Veterinary Medicine 96 (2010) 226–231

Preventive Veterinary Medicine 96 (2010) 226–231

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

journal homepage: www.elsevier.com/locate/prevetmed

Cross-sectional sero-epidemiological survey of sarcoptic mange in sheep of Western Castile and Leon, Spain

F. Rodríguez-Cadenas, M.T. Carbajal-González, J.M. Fregeneda-Grandes, J.M. Aller-Gancedo, F.A. Rojo-Vázquez*

Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad de León, Campus de Vegazana s/n, E-24071 León, Spain

ARTICLE INFO

Article history:
Received 6 August 2009
Received in revised form 4 June 2010
Accepted 27 June 2010

Keywords:
Sarcoptes scabiei
Mange
Sheep
ELISA
Prevalence
Risk factors

ABSTRACT

The aim of the present study was to determine the prevalence of *Sarcoptes scabiei*-infection of ovine livestock in three provinces (Leon, Zamora and Salamanca) in the Western part of the Castile and Leon region in Spain, and to determine the association between different variables and seropositivity. A total of 3730 sheep sera from 373 flocks (10 sera from each flock) collected from May to September over the course of the years 2006 and 2007 were individually analysed by an indirect antibody ELISA validated for diagnosing sarcoptic mange in sheep. The overall flock-level true prevalence was 22.6% (95% CI: 17.8–27.4), the overall individual-level true prevalence within the total flocks was 7.2% (95% CI: 6.1–8.3) and the overall individual-level true prevalence within the seropositive flocks was 31.3% (95% CI: 27.2–35.4). The apparent prevalences, at flock-level and at individual-level within the total flocks and within the seropositive flocks, were not statistically different ($p > 0.05$) when the primary production objective of the flock is milk vs. meat, or in smaller ($\leq$276 sheep, 50th percentile) vs. larger flocks (>276 sheep). The apparent prevalences, at flock-level and at individual-level within the seropositive flocks, were, likewise, not statistically different between the three provinces, but the individual-level apparent prevalence within the total flocks showed significant variation from one province to another ($p \leq 0.05$). Sheep maintained in the Provinces of Zamora and Salamanca had greater odds (OR = 1.7, 95% CI: 1.2–2.6; OR = 1.9, 95% CI: 1.3–2.8, respectively) of being seropositive than those located in Leon Province (OR = 1.0). The findings of the present study clearly show the need to implement in this region effective control measures against sarcoptic mange in sheep.

1. Introduction

Sarcoptic mange is a parasitic skin disease of animals caused by the mite *Sarcoptes scabiei*. This infection is found worldwide and at least 104 animal species are susceptible to the infection (Bornstein et al., 2001). In sheep, the lesions are mainly located on the head, giving rise to the formation of crusts up to 1 cm in thickness, and with scratching and rubbing as the main clinical manifestations (Cordero del Campillo and Rojo-Vázquez, 1999). The infection is more frequent in rams and during the winter months (Rahbari et al., 2009). Furthermore, it is responsible for reduced reproductive performance and a decrease in milk and meat production (Fthenakis et al., 2000, 2001).

Spain is home to around 20 million ewes whose products contribute 5% to the Final Agricultural Production, with meat (75% sheep) and milk (15% sheep) as the main outputs (Anonymous, 2007). To the authors' knowledge, at present

Abbreviations: HSENS, Flock-level diagnostic sensitivity; HSPEC, Flock-level diagnostic specificity; OR, Odds ratio; SENS, Individual-level diagnostic sensitivity; SPEC, Individual-level diagnostic specificity.

* Corresponding author. Current address: Instituto de Ganadería de Montaña (CSIC-ULE), Finca Marzanas s/n, 24346 Grulleros, León, Spain. Tel.: +34 987 291 338; fax: +34 987 291 304.
E-mail address: francisco.rojo@unileon.es (F.A. Rojo-Vázquez).

0167-5877/$ – see front matter © 2010 Published by Elsevier B.V.
doi:10.1016/j.prevetmed.2010.06.014

Fig. 1. Location within Spain of the three provinces in which the sero-epidemiological study of sarcoptic mange in sheep was completed (1: Leon; 2: Zamora; 3: Salamanca).

there are no reported large epidemiological studies on sarcoptic mange in sheep. This may in part be due to the fact that the only available technique for diagnosing this disease hitherto was by means of the visual observation of *S. scabiei* mites in skin scrapings, which makes it impractical to analyse a large number of samples. However, we have recently developed an ELISA to detect specific antibodies in serum and following validation have demonstrate that it constitutes an accurate diagnostic tool facilitating epidemiological studies (Rodríguez-Cadenas et al., 2010).

The aim of the present work was to determine the prevalence of exposure to *S. scabiei* of ovine livestock of the Western part of the Castile and Leon region in Spain. In addition, risk factors for seropositivity were investigated using the data available.

2. Materials and methods

2.1. Area of study

The sero-epidemiological survey was performed in the ovine population located in Western Castile and Leon region in Spain (Fig. 1), lying between 40°16′ and 43°14′ North latitudes and between 4°44′ and 7°5′ West longitudes. In this region the climate is continental, with a mean annual temperature of 12.1 °C and a mean annual precipitation of 452 mm (Instituto Nacional de Meteorología [Spanish Institute for Meteorology], 2005). The territory has a total surface area of 38,478 square kilometres (km^2), includes the Provinces of Leon, Zamora and Salamanca, and is home to 6301 flocks of sheep (census taken on 25th November 2005) with approximately 2 million sheep, representing around 9% of the total sheep population of Spain (Anonymous, 2008). The flocks are usually managed under a semi-extensive system for meat production and under semi-extensive or intensive systems for milk production.

2.2. Study design

The study was designed to determine the overall flock-level true prevalence by analysing sera with an indirect antibody ELISA recently demonstrated to have high overall accuracy to diagnose sarcoptic mange in sheep from the region of study (Rodríguez-Cadenas et al., 2010). For this purpose, high values of flock-level diagnostic sensitivity (HSENS) and flock-level diagnostic specificity (HSPEC) of the ELISA were required. The procedure used to calculate the HSENS and HSPEC was the described by Martin et al. (1992).

The within-flock true prevalence was assumed to be the mean prevalence we had observed in the flocks declared *S. scabiei*-infected in the ELISA validation study by applying the reference method (Group SM of Ref. Rodríguez-Cadenas et al., 2010), which was 40% (rounded from 43.2%). A spreadsheet was then used to calculate the HSENS and HSPEC of the ELISA for each coordinate of the Receiver Operating Characteristic (ROC) plot (Fig. 4 of Ref. Rodríguez-Cadenas et al., 2010) and for several within-flock sample—sizes, assuming as seropositive flock when at least one animal tested within the flock was seropositive. Adequate HSENS (94.9%, 95% CI: 91.8–96.9 [CI for flock-level accuracy parameters were calculated as described in Christensen and Gardner, 2000]) and HSPEC (96.2%, 95% CI: 89.2–100) accuracy parameters or the ELISA were obtained when 10 sera per flock were analysed and interpreted at the 41.2 OD% cut-off value, which corresponded to 63.7% (95% CI: 54.9–72.6) of SENS and 99.6% (95% CI: 98.9–100) of SPEC.

2.3. Estimation of the required flock-level sample size

As the test used to analyse the sera was imperfect, the flock-level sample size (HN) needed to determine the overall flock-level true prevalence in the target population with an appropriate error was calculated using the formula (2) of Humphry et al. (2004), which accounts for misclassifications.

It was assumed: $Z = 1.96$ (95% confidence level); $L = 0.05$ (required error); HSENS = 0.949; HSPEC = 0.962; HTP = 0.31 (*a priori* estimated overall flock-level true prevalence). The latter was calculated as the proportion of *S. scabiei*-infected flocks (Group SM: 13 flocks) within the entire group of flocks whose status in relation to *S. scabiei* could be defined (Group SM + Group NN + Group NE: 42 flocks in total) in the ELISA validation report (Rodríguez-Cadenas et al., 2010). It was obtained from a result for HN of 370 flocks.

2.4. Origin of serum samples and sampling approach

Serum samples were gathered from May to September over the course of the years 2006 and 2007 from the Animal Health Laboratories. The sera had been collected during the execution of the ovine brucellosis eradication programme, which encompasses the whole Spanish sheep population, and for the sheep population target of the present study included for that time at least one diagnosis per year of every sheep above 6 months age (Veterinary Health Alert Network: http://rasve.mapa.es). The calculated HN value of 370 sheep flocks was rounded up to 400, and then proportionally stratified according to the flock census in each of the 39 administrative subdivisions (veterinary units) of the area of study in the Livestock Regional Map of Spain (Spanish Ministry of the Environment and Rural and Marine Affairs: www.marm.es). Flocks within each

veterinary unit were selected through computer generated random sequences (MedCalc® 9.6.2.0) after numbering every eligible flock in the census list. Within the flock, as the order of collection of sera by the veterinary teams is not predefined, the sera could be considered essentially randomly collected and therefore any group of 10 sera were valid as a simple random sample. The completed serum bank comprised a total of 3730 sheep sera from 373 different flocks (10 sera each), and each stratum had at least 80% of the assigned flocks.

Additionally, flock information (if available) regarding the geographical location, the primary production objective and the census of sheep numbers was compiled.

2.5. Statistical analysis

2.5.1. Calculation of true prevalences and confidence limits

The Rogan–Gladen estimator was used to calculate the true prevalences from the apparent prevalences (Greiner and Gardner, 2000). The error on the estimators was expressed as 95% confidence interval (95% CI). Errors on the odds ratio (OR) and prevalence (true and apparent) estimators were calculated using the logarithmic and normal approximations, respectively.

2.5.2. Descriptive and comparative statistics

The one-sample Kolmogorov–Smirnov test was used to determine if the requirement needed to run parametric tests was fulfilled. Dependence between the individual observations among the flocks was estimated through the intra-class correlation coefficient (ρ). The Spearman correlation coefficient (rho) was used to determine whether there was correlation between the estimated individual-level seroprevalence within the flock and the flock-size. The Pearson chi-squared test was used to analyse the association between variables and seropositivity.

All statistical analyses were performed using SPSS 15.0 for Windows® and Win Episcope 2.0, and the alpha value was set at 0.05.

3. Results

The overall and stratum-specific results following analysis of sera by ELISA are summarised in Tables 1–3. Fig. 2 shows the distribution of the seropositive flocks according to the individual-level apparent prevalence. It was observed that it is ≤20% in around 75% of the seropositive flocks. No correlation was found between the number of positive sera within the flock and the flock-size, neither for the total flocks (rho = −0.0077; $p > 0.05$, two-tailed) nor for the seropositive flocks (rho = 0.078; $p > 0.05$, two-tailed). Likewise, no intra-flock correlation for seropositivity was found, neither among the sheep from the total flocks ($\rho = -0.00049$; $p > 0.05$) nor among the sheep from the seropositive flocks ($\rho = -0.0011$; $p > 0.05$).

The results of the univariate analysis at flock-level and at individual-level within the seropositive flocks are shown in Tables 2 and 3. The seroprevalence, at flock-level and individual-level within the total flocks and within the seropositive flocks, were similar in flocks with a primary production objective milk vs. meat, and in smaller (≤276 sheep, 50th percentile) vs. larger flocks ($p > 0.05$, chi-squared test, d.f. = 1). The flock-level apparent prevalence and the individual-level apparent prevalence within the seropositive flocks were similar over the various provinces ($p > 0.05$, chi-squared test, d.f. = 2). However, the individual-level apparent prevalence within the total flocks was significantly different across the various provinces ($p < 0.01$, chi-squared test, d.f. = 2). A sheep of Zamora or Salamanca Province was found to be more likely (OR = 1.7, 95% CI: 1.2–2.6; OR = 1.9, 95% CI: 1.3–2.8, respectively) to be seropositive than if it was from Leon Province (OR = 1.0).

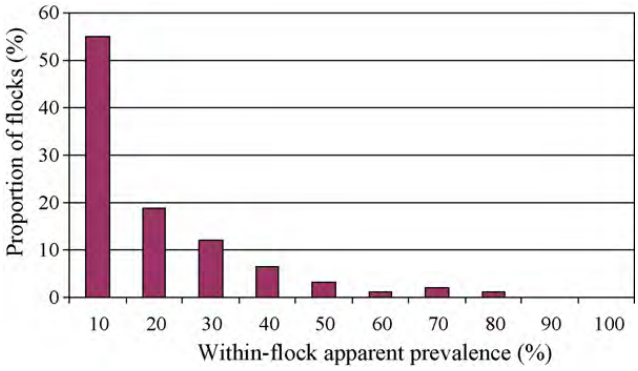


Fig. 2. Distribution, according to the detected within-flock apparent prevalence, of the *S. scabiei*-seropositive flocks identified in the cross-sectional survey carried out on sheep in Western Castile and Leon, Spain.

4. Discussion

The present study investigated the prevalence of *S. scabiei*-infection in sheep of Western Castile and Leon region, Spain, and was based on the analysis of a high number of serum samples.

The validity of an estimator depends on its precision and accuracy. Regarding the precision (defined as the magnitude of the error on the prevalence estimators), a source of error on the apparent and true prevalence estimators is due to the analysis of only a random sample collected from the target population. In the present study this error was small as the 95% confidence intervals were narrow. The software and formulae used calculate the error assuming simple random sampling. This supposition was true at flock-level, whereas at individual-level it was carried out a two-stage random sampling approach; the first stage was a cluster random sampling and the second stage a simple random sampling. The procedure used at individual-level may lead to a need of variance inflation (with the relevant impact on the error) as observations within-flock may be more similar than between-flock (McDermott and Schukken, 1994). This effect is measured through the intra-class correlation coefficient, but as it was not found a significant intra-flock correlation for seropositivity there was not necessary to inflate the variance. Another source of additional error on the true prevalence estimators is the incorporation of the error on the accuracy parameters of the test (Greiner and Gardner, 2000; Christensen and Gardner, 2000). However, this error was not propagated to the present study and therefore the error on the true prevalence estimators would

Table 1
Overall results from the cross-sectional serum survey of sarcoptic mange carried out in sheep livestock of Western Castile and Leon, Spain.

	Flock-level	Individual-level within the total flocks	Individual-level within the seropositive flocks
Number tested by ELISA	373	3730	910
Number seropositive	91	184	184
True prevalence (95% CI)[a]	22.6% (17.8–27.4)	7.2% (6.1–8.3)	31.3% (27.2–35.4)

[a] Errors on the SENS, SPEC, HSENS and HSPEC accuracy parameters of the ELISA for the target population were not propagated during the calculation of the 95% CI of the true prevalence estimators.

Table 2
Stratum-specific flock-level prevalences of anti-*S. scabiei* antibodies in sheep flocks of Western Castile and Leon, Spain, and results of the univariate analysis.

Variables and levels	*n*	Seropositives[a]	Seroprevalence (95% CI)	Chi-squared	*p*-Value
Primary production objective of the flock					
Milk	187	44	24% (18–30)	0.27; d.f. = 1	0.60
Meat	162	42	26% (19–33)		
Flock-size					
Small flocks (≤276[b])	175	43	25% (18–32)	0.00; d.f. = 1	1.00
Large flocks (>276)	175	43	25% (18–32)		
Province where the flock was situated					
Leon	129	26	20% (14–28)	2.20; d.f. = 2	0.33
Zamora	123	31	25% (18–34)		
Salamanca	121	34	28% (20–37)		

[a] At least one of the 10 sera from the flock that were tested had OD% ≥41.2 (ELISA accuracy parameters: HSENS = 94.9% [95% CI: 91.8–96.9] and HSPEC = 96.2% [95% CI: 89.2–100]).
[b] 50th percentile.

have been underestimated. Nevertheless, this additional error would be low as the accuracy parameters of the test were estimated with adequate precision (see Section 2.2).

In relation to the accuracy of the estimators (defined as the closeness of the prevalence estimators to the actual values), one of the possible sources of bias in the true prevalence estimators is the misclassification (or information) bias. In the present study this bias was avoided through correction of the apparent prevalences to the true prevalences using the accuracy parameters of the diagnostic test. The efficacy of this procedure depends on the availability of unbiased estimators of the accuracy parameters of the test. The individual-level accuracy parameters used had been obtained from ROC analysis using a reference method which introduced selection and probably information biases but in opposite directions (Rodríguez-Cadenas et al., 2010). However, the latent-class analysis, although was carried out at an OD% cut-off value different to the one used in the present study, determined individual-level accuracy parameters for the test similar to those found by ROC analysis, and therefore both validation approaches would have produce approximate results in the present study. Regarding the flock-level accuracy parameters, in the present study their accuracy depends on the unbiasedness of both the individual-level accuracy parameters (see previous comments) and the *a priori* estimation of the within-flock individual-level true prevalence used to derive them, as well as the absence of considerable variation between the infected flocks of the individual-level true prevalence. For the *a priori* estimation of the within-flock individual-level true prevalence, it had been assumed to be 40% and therefore approximated adequately to the individual-level true prevalence within the seropositive flocks obtained in this study (31.3%), and for the variation between the infected flocks of the individual-level true prevalence, Fig. 2 shows that the individual-level seropos-

Table 3
Stratum-specific individual-level prevalences of anti-*S. scabiei* antibodies within the seropositive sheep flocks (tested 10 sera from each flock; see Table 2) of Western Castile and Leon, Spain, and results of the univariate analysis.

Variables and levels	*n*	Seropositives[a]	Seroprevalence (95% CI)	Chi-squared	*p*-Value
Primary production objective of the flock					
Milk	440	92	21% (17–25)	0.17; d.f. = 1	0.68
Meat	420	83	20% (16–24)		
Flock-size					
Small flocks (≤276[b])	430	81	19% (15–23)	1.21; d.f. = 1	0.27
Large flocks (>276)	430	94	22% (18–26)		
Province where the flock was situated					
León	260	42	16% (12–21)	3.73; d.f. = 2	0.15
Zamora	310	68	22% (18–27)		
Salamanca	340	74	22% (18–27)		

[a] Sheep with OD% ≥41.2 (ELISA accuracy parameters: SENS = 63.7% [95% CI: 54.9–72.6] and SPEC = 99.6% [95% CI: 98.9–100]).
[b] 50th percentile.

itivity had little variation between the seropositive flocks (10–20% in ~75% of the seropositive flocks). Another potential source of inaccuracy is selection bias, which may occur when the sample collected is not representative of the target population, and would affect both apparent and true prevalence estimators. In the present study the sampling approach at individual-level consisted of sampling a fixed number of sheep within the flock irrespective of the flock-size and therefore the sample was not fully representative of the target population. However, as there was no correlation between the number of the seropositive sheep within the flock and the flock-size it did not introduce any bias in the individual-level prevalence estimators. This would also imply that the overall individual-level prevalences equal the overall individual-level mean prevalences within the flocks.

The overall flock-level true prevalence observed (23%) was higher than those reported by Bisdorff et al. (2006) in Great Britain (9%) and by Falconi et al. (2002) in Switzerland (12%) for the sheep scab mite *Psoroptes ovis*. In contrast, the overall individual-level true prevalence (7.2%) was lower than the findings of Falconi et al. (2002) for sheep scab (10%). Both overall individual-level and flock-level true prevalences were lower than the reported by Gutiérrez et al. (1996) concerning mange in pigs in Spain (34% and 89%, respectively). The overall individual-level true prevalence (31%) within the seropositive flocks was higher than the findings of Rahbari et al. (2009) relative to *S. scabiei*-infected sheep flocks in Iran, where the reported value was 4.9%.

Regarding the univariate analysis, in the present study it was performed on the result of an imperfect test and not on the true infection status (possible misclassification bias). The importance of this is that the actual ORs would have been underestimated if the ELISA does not make differential misclassification between the variable categories, while if it does the actual ORs may have been over- or underestimated (Greiner and Gardner, 2000). By this analysis, it was found that the individual-level apparent prevalence within the total flocks was significantly higher in some provinces than in others. This may be the consequence of differing management practices employed out in the different areas. For instance, it has been reported that the use of straw bedding is a major risk factor for the presence of sarcoptic mange in a herd of pigs (Damriyasa et al., 2004). In the same study, it was also demonstrated that the use of high-pressure cleaning and disinfection of the boxes markedly reduces the risk of infection by lice. Moreover, the management of the pig herds into pasture was quoted as a major risk factor relative to the infection of a herd by lice. Regarding the main production objective and the flock-size, in the present study they were found not to be associated with the presence of sarcoptic mange in sheep. In the area of study the management system is associated with the primary production objective; intensive systems focus mainly on milk production whereas semi-extensive systems focus on meat or milk productions. This issue would suggest that the management system is not either associated with the *S. scabiei* status. In a recent study, Rahbari et al. (2009) reported that sarcoptic mange in sheep is more prevalent in rams and during the winter months. The latter finding would imply that the prevalence of *S. scabiei*-infection in this period may be higher than the one reported in the present study, as it was carried out during the spring and summer months. Anyway, more extensive studies are needed to figuring out the epidemiological factors associated with the presence of this disease in sheep.

In summary, this study has demonstrated a considerable flock-level and individual-level true prevalences of *S. scabiei*-infection in ovine livestock of Western Castile and Leon region in Spain. These findings evidence the need to search for effective control measures against sarcoptic mange in sheep and implement them in the infected flocks.

Acknowledgements

The present study was financially supported by grant LE010A05 of the Education Bureau of the Castile and Leon Regional Government (Consejería de Educación de la Junta de Castilla y León). FRC and MTCG are fellowship holders co-financed by the Education Bureau of the Castile and Leon Regional Government and the European Social Fund. We are extremely grateful to the Agriculture and Livestock Bureau of the Castile and Leon Regional Government (Consejería de Agricultura y Ganadería de la Junta de Castilla y León) and the Animal Health Laboratories for having allowed and facilitated the collection of sheep sera, and to Dr. J.F. Huntley for the correction of English.

References

Anonymous, 2007. Hechos y cifras de la Agricultura, la Pesca y la Alimentación en España. Ministry of Agriculture Fisheries and Food, http://www.mapa.es.

Anonymous, 2008. Estadísticas agrarias de Castilla y León. Junta de Castilla y León, http://www.jcyl.es.

Bisdorff, B., Milnes, A., Wall, R., 2006. Prevalence and regional distribution of scab, lice and blowfly strike in Great Britain. Vet. Rec. 158, 749–752.

Bornstein, S., Mörner, T., Samuel, W.M., 2001. Sarcoptes scabiei and sarcoptic mange. In: Samuel, W.M., Pybus, M.J., Kocan, A.A. (Eds.), Parasitic diseases of wild mammals, 2nd Ed. Iowa University Press, Ames, pp. 107–119.

Christensen, J., Gardner, I.A., 2000. Herd-level interpretation of test results for epidemiologic studies of animal diseases. Prev. Vet. Med. 45, 83–106.

Cordero del Campillo, M., Rojo-Vázquez, F.A., 1999. Parasitología Veterinaria. McGraw-Hill Interamericana, Madrid, 968 pp.

Damriyasa, I.M., Failing, K., Volmer, R., Zahner, H., Bauer, C., 2004. Prevalence, risk factors and economic importance of infestations with *Sarcoptes scabiei* and *Haematopinus suis* in sows of pig breeding farms in Hesse, Germany. Med. Vet. Entomol. 18, 361–367.

Falconi, F., Ochs, H., Deplazes, P., 2002. Serological cross-sectional survey of psoroptic sheep scab in Switzerland. Vet. Parasitol. 109, 119–127.

Fthenakis, G.C., Karagiannidis, A., Alexopoulos, C., Brozos, C., Papadopoulos, E., 2001. Effects of sarcoptic mange on the reproductive performance of ewes and transmission of *Sarcoptes scabiei* to newborn lambs. Vet. Parasitol. 95, 63–71.

Fthenakis, G.C., Papadopoulos, E., Himonas, C., Leontides, L., Kritas, S., Papatsas, J., 2000. Efficacy of moxidectin against sarcoptic mange and effects on milk yield of ewes and growth of lambs. Vet. Parasitol. 87, 207–216.

Greiner, M., Gardner, I.A., 2000. Application of diagnostic tests in veterinary epidemiologic studies. Prev. Vet. Med. 45, 43–59.

Gutiérrez, J.F., Méndez de Vigo, J., Castellá, J., Muñoz, E., Ferrer, D., 1996. Prevalence of sarcoptic mange in fattening pigs sacrified in a slaughterhouse of northeastern Spain. Vet. Parasitol. 61, 145–149.

Humphry, R.W., Cameron, A., Gunn, G.J., 2004. A practical approach to calculate sample size for herd prevalence surveys. Prev. Vet. Med. 65, 173–188.

Instituto Nacional de Meteorología, 2005. Guía resumida del clima en España 1971–2000. Ministerio de Medio Ambiente, Madrid, 257 pp.
Martin, S.W., Shoukri, M., Thorburn, M.A., 1992. Evaluating the health status of herds based on tests applied to individuals. Prev. Vet. Med. 14, 33–43.
McDermott, J.J., Schukken, Y.H., 1994. A review of methods used to adjust for cluster effects in explanatory epidemiological studies of animal populations. Prev. Vet. Med. 18, 155–173.
Rodríguez-Cadenas, F., Carbajal-González, M.T., Fregeneda-Grandes, J.M., Aller-Gancedo, J.M., Huntley, J.F., Rojo-Vázquez, F.A., 2010. Development and evaluation of an antibody ELISA for sarcoptic mange in sheep and a comparison with the skin-scraping method. Prev. Vet. Med. 96, 82–92.
Rahbari, S., Nabian, S., Bahonar, A.R., 2009. Some observations on sheep sarcoptic mange in Tehran province, Iran. Trop. Anim. Health Prod. 41, 397–401.

Publicación nº 3

Clinical evaluation and antibody responses in sheep after primary and secondary experimental challenges with the mange mite *Sarcoptes scabiei* var. *ovis*

Rodríguez-Cadenas, F., Carbajal-González, M.T., Fregeneda-Grandes, J.M., Aller-Gancedo, J.M., Rojo-Vázquez, F.A.

Veterinary Immunology and Immunopathology 133 (2010) 109–116

Veterinary Immunology and Immunopathology 133 (2010) 109–116

Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

journal homepage: www.elsevier.com/locate/vetimm

Research paper

Clinical evaluation and antibody responses in sheep after primary and secondary experimental challenges with the mange mite *Sarcoptes scabiei* var. *ovis*

F. Rodríguez-Cadenas, M.T. Carbajal-González, J.M. Fregeneda-Grandes, J.M. Aller-Gancedo, F.A. Rojo-Vázquez *

Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad de León, Campus de Vegazana s/n, E-24071, León, Spain

ARTICLE INFO

Article history:
Received 16 September 2008
Received in revised form 16 April 2009
Accepted 1 July 2009

Keywords:
Sheep
Sarcoptes scabiei
ELISA: Western-blotting
Clinical-aspects
Resistance

ABSTRACT

In this work the clinical evolution and the specific serum IgG and IgE antibody responses in sheep after primary ($n = 10$) and secondary ($n = 4$) experimental challenges with the mange mite *Sarcoptes scabiei* var. *ovis* were studied. The primary infection was characterized by the development of mange lesions in all sheep, a detection of live *S. scabiei* mites in 70% skin scrapings taken in week 10 post-challenge (PC), strongly raised and sustained specific IgG levels and a more moderate but continuous rise in specific IgE levels. Seroconversion was detected for IgG and IgE by ELISA in 90% and 60% of the sheep in week 8 PC, respectively. By Western-blotting (WB), ten IgG-reactive bands (36–120 kDa) and four IgE-reactive bands (90–180 kDa) were observed in week 8 PC. Following the secondary challenge the ewes developed a smaller area of mange lesion than that seen following primary challenge and live *S. scabiei* mites were not detected in skin scrapings collected in week 8 PC, suggesting that sheep had developed immunity to re-infection. Compared to primary infection, the specific IgG secondary antibody levels were transient, but in contrast there was an anamnestic IgE response, resulting in an elicitation of specific serum IgE levels in week 2 PC significantly higher than those demonstrated after primary infection. WB analysis revealed one additional IgG-reactive band (180 kDa) and no additional IgE-reactive bands. Determining the immunodiagnostic or vaccination value of the IgG-reactive antigens and IgE-reactive allergens detected requires further studies.

1. Introduction

Sarcoptic mange is a parasitic skin disease due to infection by the mite *Sarcoptes scabiei*. The lesions produced in sheep, characterized by formation of crust of up to 1 cm in thickness, are mainly located on the head (Cordero del Campillo and Rojo-Vázquez, 1999). The main clinical signs are rubbing and scratching. It also causes important financial losses due to decreases in milk production, reproductive performance and the growth of lambs born from affected ewes (Fthenakis et al., 2000, 2001).

Currently, diagnosis of sarcoptic mange in sheep is performed by visual observation of the mites in skin scrapings. The detection of specific serum antibodies against *S. scabiei* by ELISA is routinely used to diagnose sarcoptic mange in dogs (Curtis, 2001; Lower et al., 2001) and has also been successfully used to monitor the effectiveness of eradication programmes for sarcoptic mange in pigs (Jacobson et al., 1999; Rueda-López, 2006). Recently, the authors have developed and validated an antibody ELISA to

Abbreviations: OD, optical density; PC, post-challenge; RT, room temperature.

* Corresponding author. Instituto de Ganadería de Montaña (CSIC-ULE), Finca Marzanas s/n, E-24346, Grulleros, León, Spain.
Tel.: +34 987 291 338; fax: +34 987 291 304.
E-mail address: francisco.rojo@unileon.es (F.A. Rojo-Vázquez).

0165-2427/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetimm.2009.07.004

diagnose sarcoptic mange in sheep using a crude saline extract from *S. scabiei* var. *ovis*, and demonstrated that it is highly accurate (Rodríguez-Cadenas et al., submitted). However, this test is unsuitable for large scale use, owing to limitations on the amount of mites which can be collected, as there is currently no *in vitro* culture technique for breeding *S. scabiei*. Identification of the major antigens and allergens of *S. scabiei* would assist in the development of an immunodiagnostic test based on recombinant proteins (Kuhn et al., 2008). The antibody response against *S. scabiei* has already been characterized in dogs, foxes and goats (Bornstein et al., 1995; Arlian and Morgan, 2000; Tarigan, 2004), showing differences between species. These inter-species antibody profile differences are in agreement with the findings of Arlian et al. (1996a), who reported that each variety of *S. scabiei* may produce a range of proteins comprised of both those which are variety-specific, and those which are immunologically identical and shared by the different mite sub-types.

Currently, control of sarcoptic mange is mainly based on the administration of acaricides. The use of these chemical compounds has serious drawbacks; i.e. the development of drug resistance (Curie et al., 2004), adverse environmental effects (Sanderson et al., 2007), residues in animal products (Imperiale et al., 2004) and health hazards to humans (Bradberry et al., 2005). As a consequence, the development of 'non-chemical' methods, such as vaccination, is desirable. The achievement of a vaccine against sarcoptic mange is thought to be feasible as animals having recovered from a previous infection show resistance to re-infection (Arlian et al., 1994, 1996b; Tarigan, 2002). Furthermore, it has been suggested that the mechanism involved in the development of acquired resistance against *S. scabiei* is related to IgE responses (Tarigan, 2003; Tarigan and Huntley, 2005).

The aim of the present study was to monitor sheep clinically after primary and secondary experimental challenges with the mange mite *S. scabiei* var. *ovis*, and to characterize the specific serum IgG and IgE antibody responses.

2. Materials and methods

2.1. Experimental animals

First, ten *S. scabiei*-naïve adult sheep (>1 year) were challenged with *S. scabiei* var. *ovis* mites (primary infection group). During week 10 post-challenge (PC), five randomly selected sheep were treated with ivermectin (Ivomec®, Merial, two injections one week apart s.c., at 200 $\mu g\ kg^{-1}$ of body weight) and the other five removed from the study. One of the ivermectin-treated sheep died two weeks after treatment from causes not related to the experiment. The remaining four treated sheep were kept until eight weeks after treatment, by which time the mange lesions had disappeared. After recovering from the primary infection, the four sheep were challenged again with the same *S. scabiei* var. *ovis* strain (secondary infection group) and monitored for another eight weeks. As a positive control for this infection three *S. scabiei*-naïve adult sheep were also challenged at the same time (secondary infection control group).

2.2. Preparation of the S. scabiei var. ovis challenge-inocula and experimental challenge

Experimental challenges were performed using crusts with *S. scabiei* var. *ovis* mites collected from a severely affected ewe. Briefly, the approximate total number of *S. scabiei* mites on the donor sheep was determined on the day before challenge from the total lesion area and the mean number of mites per 1 cm^2 of lesion. The latter measure was estimated by scraping the donor sheep at several 1 cm^2 points in the lesion, and counting the *S. scabiei* larvae, nymphs and adults under the microscope after 10% KOH digestion of the crusts and concentration of the mites by floating with saturated sucrose solution. Thereafter, on the day of challenge the donor sheep were euthanized (T-61®, Intervet International B.V.), all the crusts from the lesion removed and chopped to produce particles of around 5 mm diameter, and aliquots with approximately 2000 mites were prepared. The sheep were challenged with an aliquot each, by maintaining the crusts in contact with the convex surface of one ear for 48 h by means of a dressing.

Blood samples were taken by jugular puncture at weekly intervals and after centrifugation at $900 \times g$ for 15 min the sera were removed and stored at −20 °C.

2.3. Clinical monitoring

The mange lesions which developed after challenge in the convex surface of the ear were recorded in a diagram and subsequently graded by comparing with reference lesion pictures which had been scored in accordance with the affected area. The scale scores were as follows: score 0 if no lesion was visible on the ear; score 1 when <10% of the area was affected; score 2 when 10–25% of the area was affected; score 3 when 25–50% of the area was affected; score 4, when 50–75% of the area was affected; and score 5 when >75% of the area was affected.

Skin scrapings were collected from the lesion and examined for live *S. scabiei* mites after incubation at 30 °C for 30 min, and in addition they were also examined using the digestion–concentration technique (see Section 2.2).

2.4. Mite extract

The mite extract used in both ELISA and Western-blotting (WB) techniques was a crude saline extract kindly supplied by Dr. John F. Huntley (MRI, Penicuik, Scotland). This extract had been prepared from *S. scabiei* var. *caprae* mites collected from a mangy goat as described by Tarigan and Huntley (2005) and stored frozen at −20 °C until being processed as it is described next. After defrosting, they had been washed once in ice-cold PBS, followed by another wash in PBS-1% (w/v) SDS at room temperature (RT) and 10 further washes in ice-cold PBS to remove the SDS. The mites had been then transferred to a ribolyser tube (Lysing Matrix C, Q-biogene) and homogenised in PBS with a shaker machine (FastPrep® FP120, Q-biogene) for four 30-s cycles with cooling between each. After centrifugation at $5500 \times g$ for 5 min the supernatant (mite extract) had been removed, its protein concentration measured by the

Bradford method (Bradford Reagent, Sigma) with BSA as standard and stored frozen at −20°C until use.

2.5. ELISA

High-binding microtiter plates (Costar®, Corning Incorporated, USA) were coated overnight at 4 °C with 50 μl per well of a solution of the mite extract adjusted to 5 μg ml^{-1} of protein with carbonate–bicarbonate buffer (70 mM $NaHCO_3$, 30 mM Na_2CO_3, 0.2 g l^{-1} NaN_3, pH 9.6). After washing three times with PBS-T20 (PBS, 0.5 ml l^{-1} Tween 20) the plates were incubated for 1 h at 37 °C with 50 μl per well of sheep sera appropriately diluted (1/200 for IgG or 1/10 for IgE) in PBS-T80-NaCl (PBS, 5 ml l^{-1} Tween 80, 0.5 M NaCl) and added in duplicate. The plates were washed again, then incubated for 1 h at 37 °C with 50 μl per well of an appropriate mAb diluted in PBS-T80-NaCl: clone VPM6 (Bird et al., 1995) diluted 1/20 for IgG detection or clone 2F1 (Bendixsen et al., 2004) diluted 1/200 for IgE detection. After a further washing, the plates were incubated for 1 h at 37 °C with 50 μl per well of biotin-labelled goat anti-mouse IgG antibodies (Sigma®, USA) diluted 1/5000 in PBS-T80-NaCl. The plates were washed once more, and then incubated for 30 min at 37 °C with 50 μl per well of streptavidin-HRP (GE Healthcare, UK) diluted 1/40,000 in PBS-T80-NaCl and washed again. Peroxidase activity was then visualized using the chromogen substrate OPD (OPD Tablets, Dako, Denmark) in accordance with the manufacturer's instructions. The reaction was stopped with 50 μl per well of 0.5 M H_2SO_4, then the Optical Density (OD) was measured at 492 nm using a spectrophotometer and the OD for each serum calculated (mean OD of the duplicate test wells). ELISA studies were carried out at two-week intervals throughout the study. Positive and negative control sera were tested on each plate and the ODs of the samples were adjusted relative to them to obtain comparable results between plates.

2.6. SDS-PAGE/Western-blotting

Aliquots of the mite extract with 100 μg of protein were mixed 1:1 with Laemmli reducing sample buffer (Bio-Rad Laboratories) and boiled for 5 min. Each mixture was then loaded into Tris/HCl 12% acrylamide/bisacrylamide gel, together with 10 μl of broad-range molecular weigh markers (prestained SDS-PAGE Standards, Bio-Rad Laboratories). The gels were subjected to electrophoresis at 200 V for 45 min using Tris/Glycine/SDS running buffer (25 mM Tris, 192 mM glycine, 5 g l^{-1} SDS). After this, the separated proteins from the gels were transferred onto nitrocellulose membranes (Trans-Blot® Transfer Medium, Bio-Rad Laboratories) at 100 V for 1.5 h using Tris/Glycine transfer buffer (25 mM Tris, 0.192 mM glycine, 200 ml l^{-1} methanol). Protein transfer was checked by staining the membrane with Ponceau S solution (0.1% Ponceau S pure, 50 ml l^{-1} acetic acid). Electrophoresis and transfers were done using the Mini Polyacrilamide Gel System (Bio-Rad Laboratories) and the PowerPac™ Basic Power Supply (Bio-Rad Laboratories).

The transferred membranes were then blocked for 1 h at RT with PBS-T80-NaCl, and after drying were cut into strips 3 mm wide. Each strip was incubated for 1 h at RT with 1 ml of sheep sera diluted 1/200 for IgG detection or 1/10 for IgE detection, in PBS-T80-NaCl. The strips were then washed three times, for 5 min on each occasion, with TBS (20 mM Tris, 0.5 mM NaCl, pH 7.6) and incubated for 1 h at RT with 1 ml of the appropriate mAb diluted in PBS-T80-NaCl: clone VPM6 diluted 1/20 for IgG detection or clone 2F1 diluted 1/1000 for IgE detection. After another washing step, the strips were incubated for 1 h at RT with 1 ml of biotin-labelled goat anti-mouse IgG antibodies (Sigma) diluted 1/5000 in PBS-T80-NaCl. The strips were washed again, and then incubated for 30 min at RT with 1 ml of streptavidin-HRP (GE Healthcare) diluted 1/20,000 in PBS-T80-NaCl. After another washing the peroxidase activity was visualized using a chemiluminescent substrate (ECL™, GE Healthcare, UK) in accordance with the manufacturer's instructions. WB studies were performed on sera collected prior to challenge (week 0), and at weeks 4 and 8 PC. Positive and negative control sera were tested during each run of the assay. Further controls were ensured by replacing the serum or the mAb with dilution buffer.

The software package Quantity One® 4.5.0 (Bio-Rad Laboratories) was used to capture the images from the scanner (GS 800 Calibrated Densitometer, Bio-Rad Laboratories) and to determine the molecular weight of the reactive bands, using as reference the strip with the molecular weight markers. The antibody binding intensity of each serum to bands of the mite extract was scored (separately for IgG and IgE) as 0, 1, 2, 3, 4 or 5. The score was 0 when there was no binding, 1 for the weakest binding and so on up to 5 for the strongest. Thereafter, the mean binding intensity of the positive sera (score ≥ 1) to the band was graded as follows: weak (mean score 1–1.9), medium (2–2.9), strong (3–3.9) or very strong (4–5).

2.7. Statistical analysis

The one-sample Kolmogorov–Smirnov test was used to determine whether the paired-difference variables of ODs were normally distributed, and because this was always the case, the paired-samples *t*-test was used to compare antibody levels at different time points within each group, and to compare significantly elevated antibody levels at the same time point between groups. When the intention was to do the latter, in order to prevent specific antibody levels being carried over from the primary infection to the secondary infection, the pre-challenge antibody level was previously subtracted for each sheep and thus only the antibody level elicited by the relevant challenge was used for comparison. All statistical tests were performed with SPSS 15.0 for Windows®, and the alpha value was set at 0.05.

3. Results

3.1. Clinical monitoring

The results of clinical-lesion examination in weeks 1, 4 and 8 PC are shown in Table 1. After the primary challenge

Table 1
Mean mange lesion scores at different times in the course of primary and secondary experimental infections of sheep with *S. scabiei* var. *ovis*.

	Prior to challenge	Week 1 after challenge	Week 4 after challenge	Week 8 after challenge
Primary infection group ($n = 10/n = 4$)[a]	0/0	1.9/2.25	2.7/2.75	3.5/3.75
Secondary infection group ($n = 4$)	0	2	1.75	1.25
Secondary infection control group ($n = 3$)	0	2	2.67	4

[a] $n = 10$ includes the ten sheep, while $n = 4$ includes only the four sheep which took part in both primary and secondary infection groups.

all sheep exhibited mange lesions at the site of challenge, these consisting of abundant exudates which later developed to form alopoecia and crust formations, and there was a progressive growth in the mange lesion area as indicated by an increase in the mean mange lesion score. Live *S. scabiei* mites were detected in skin scrapings from seven sheep (70%) in week 10 PC, while a large number of dead mites (using the digestion–concentration technique) were detected in all of them.

Following ivermectin treatment in week 10 PC, the mange lesions progressively disappeared and there were no visible lesions of mange in any sheep by eight weeks later, when the secondary challenge was performed.

After the secondary challenge, the four sheep exhibited mange lesions at the site of challenge which were visually similar to those observed in the primary infection group. However, after week 1 PC the lesions progressively disappeared, as indicated by a decrease in the mean mange lesion score, to form alopoecic areas. Live *S. scabiei* mites were not detected in any of the four skin scrapings collected at week 8 PC, while a few dead mites were detected in one (25%). The mean mange lesion scores of the secondary infection control group approximated to those of the primary infection group.

3.2. ELISA

The results of ELISA are shown in Fig. 1. Before primary challenge the ELISA OD values were low (<0.15 for IgG and <0.1 for IgE) in all sheep. After the primary challenge, most of the sheep developed specific serum IgG and IgE antibodies. A significant increase in the IgG levels were first detected two weeks PC ($p < 0.05$, one-tailed), which were strongly increased at four weeks PC, after which the OD values levelled to a plateau. IgG seroconversion (cut-off value 0.197; defined as the mean plus 3 SD of ODs of sera at week 0) was demonstrated in nine sheep (90%) in week 4 PC, and they remained seropositive in week 8 PC. A significant increase in the IgE level was also first detected in week 2 PC ($p < 0.01$, one-tailed) and then progressively increased. IgE seroconversion (cut-off value 0.093) was found in five animals (50%) at week 4 PC and in an additional one (60%) at week 8 PC.

After ivermectin treatment a decrease was detected in the specific IgG and IgE levels. Prior to the secondary challenge the IgG antibody level was still significantly higher when compared to pre-primary-challenge ($p < 0.05$, one-tailed), but the IgE antibody level was similar ($p > 0.05$, one-tailed).

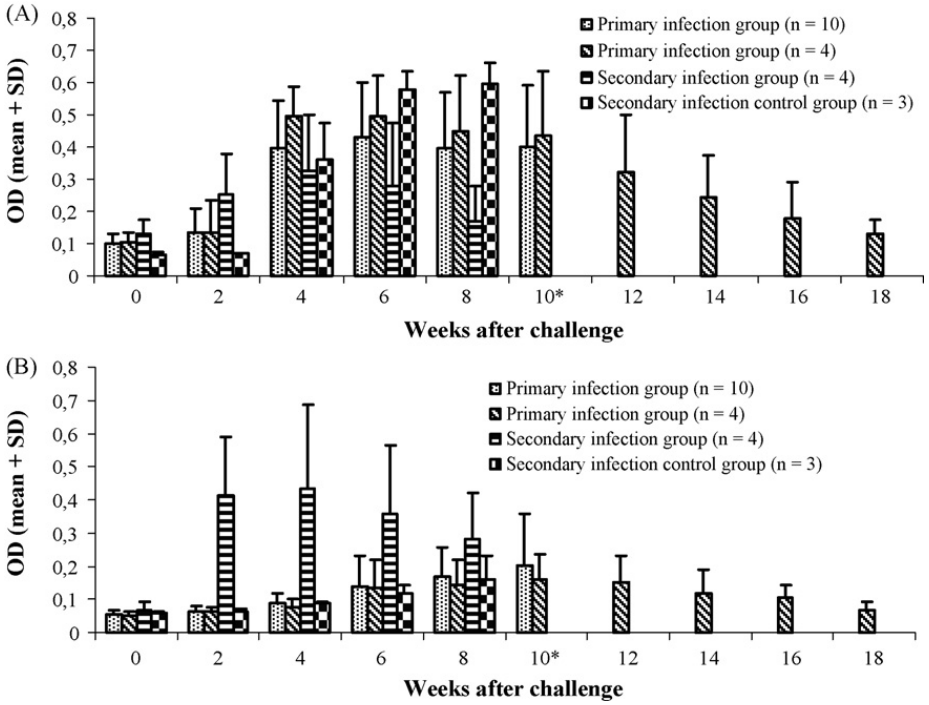


Fig. 1. Specific serum IgG (A) and IgE (B) antibodies measured by ELISA in the course of primary and secondary experimental infections of sheep with *S. scabiei* var. *ovis*. * Indicates treatment with ivermectin of the primary infection group ($n = 4$), which corresponded with the four sheep which took part in both primary and secondary infections.

Table 2
Frequencies of labelling (%) and mean binding intensities[a] of IgG in sheep sera collected at different stages in the course of primary and secondary experimental infections to *S. scabiei* var. *caprae* proteins.

Molecular weight (kDa)	Primary infection ($n = 10$)			Secondary infection ($n = 4$)		
	Prior to challenge	Week 4 after challenge	Week 8 after challenge	Prior to challenge	Week 4 after challenge	Week 8 after challenge
180	0%	0%	0%	0%	0%	25%/s
120	30%/w	80%/s	90%/s	0%	100%/m	50%/m
100	0%	80%/m	90%/m	0%	75%/w	50%/w
85	0%	80%/m	80%/m	0%	75%/w	50%/w
52	90%/w	100%/s	100%/s	100%/m	100%/m	75%/w
47	0%	80%/w	80%/w	0%	50%/w	75%/w
46	30%/w	100%/m	100%/m	0%	50%/m	75%/w
44	70%/w	100%/s	100%/s	75%/w	100%/m	100%/m
39	0%	90%/w	100%/w	0%	25%/w	25%/w
38	90%/w	100%/m	100%/m	50%/w	75%/w	75%/w
36	100%/w	100%/m	100%/m	50%/w	75%/w	75%/w

[a] It was estimated from only the sera positive to the band: w: weak; m: medium; s: strong; vs: very strong.

Table 3
Frequencies of labelling (%) and mean binding intensities[a] of IgE in sheep sera collected at different stages in the course of primary and secondary experimental infections to *S. scabiei* var. *caprae* proteins.

Molecular weight (kDa)	Primary infection ($n = 10$)			Secondary infection ($n = 4$)		
	Prior to challenge	Week 4 after challenge	Week 8 after challenge	Prior to challenge	Week 4 after challenge	Week 8 after challenge
180	0%	0%	40%/m	0%	75%/m	75%/w
120	10%/w	60%/s	70%/vs	50%/m	100%/vs	75%/s
100	0%	0%	10%/w	0%	25%/w	25%/w
90	10%/w	10%/w	50%/w	0%	100%/w	75%/w

[a] It was estimated from only the sera positive to the band: w: weak; m: medium; s: strong; vs: very strong.

After the secondary challenge, a significant increase in the specific IgG level was detected in week 4 PC ($p < 0.05$, one-tailed), but in weeks 6 and 8 PC the IgG level was similar to pre-secondary-challenge ($p > 0.05$, one-tailed). However, the elicited IgG level in week 4 PC was similar to that found in the same sheep during the primary infection ($p > 0.05$, two-tailed). The specific IgE level showed a rapid and strong increase in the first two weeks ($p < 0.05$, one-tailed) and reached the highest value at week 4 PC, while after that the level started to decrease but still remained significantly elevated at week 8 PC ($p < 0.05$, one-tailed). The elicited IgE level in week 2 PC was significantly higher than that detected in the same sheep during the primary infection ($p < 0.05$, two-tailed), but later was similar ($p > 0.05$, two-tailed). The IgG and IgE antibody levels in the secondary infection control group were similar to those of the primary infection group.

3.3. WB

The results of WB analysis are shown in Tables 2 and 3 and Fig. 2. Prior to the primary challenge IgG-reactive bands of 120, 52, 46, 44, 38 and 36 kDa, and IgE-reactive bands of 120 and 90 kDa were detected in some sheep, albeit weakly. Following primary infection, additional IgG and IgE-reactive bands were demonstrated. The IgG analysis at week 4 PC showed that there was reaction to four additional bands of 100, 85, 47, and 39 kDa in most of the ewes, and the binding intensities and/or frequencies to the reactive bands detected before challenge also increased. A similar profile was demonstrated in week 8 PC, with only a small increase in binding intensities and frequencies. IgE reactivity in week 4 PC was also increased, with six sheep sera showing strong labelling intensity to the 120 kDa band, although reactivity to the 90 kDa band remained unchanged. In week 8 PC, weak IgE-reactions to two additional bands of 180 and 100 kDa were detected. The binding intensities and frequencies for the 120 and 90 kDa IgE-reactive bands were also increased. The immunodominant bands were defined as bands reacting with at least 50% sheep sera and with a very strong or strong mean binding intensity. Based on this definition, the immunodominant IgG-reactive bands in weeks 4 and 8 PC were 120, 52 and 44 kDa, while in contrast there was only one immunodominant IgE-reactive band in weeks 4 and 8 PC, which had 120 kDa.

The antibody profile prior to the secondary challenge was similar to that observed prior to the primary challenge. Following the secondary challenge, one additional IgG-reactive band (180 kDa) was detected, but in general there were lower binding intensities than those demonstrated following primary challenge. The secondary IgE-reaction was also mainly confined to the 120 kDa band, with peak binding intensity observed in week 4 PC. This 120 kDa band appeared to be immunodominant for IgE antibodies following secondary challenge.

4. Discussion

In this study it was shown that primary and secondary experimental challenges of sheep with *S. scabiei* var. *ovis* induce the development of mange lesions and the

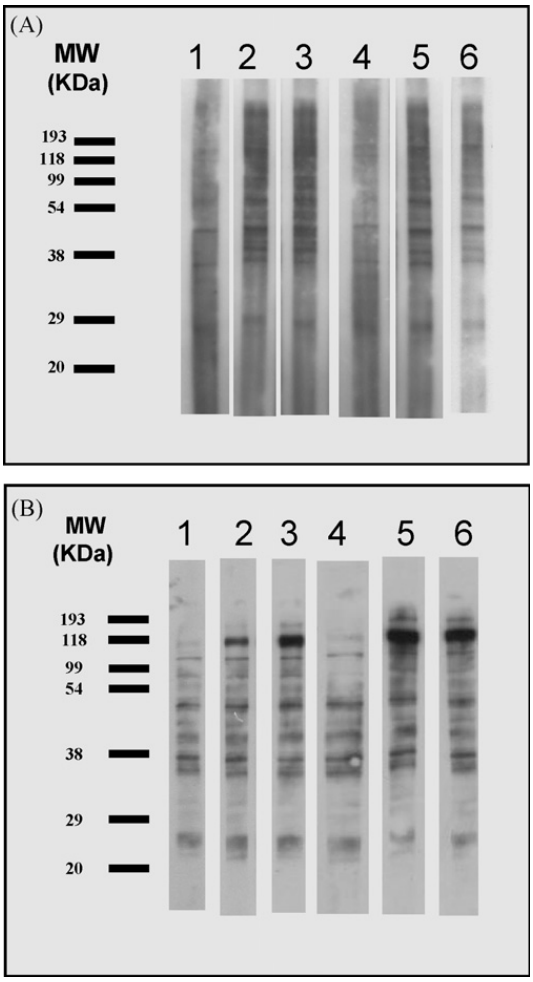


Fig. 2. WB strips showing recognition of *S. scabiei* var. *caprae* proteins by serum IgG (A) and IgE (B) antibodies in the course of primary and secondary experimental infections of sheep number 7 with *S. scabiei* var. *ovis*. Lane 1: prior to primary challenge; lane 2: week 4 after primary challenge; lane 3: week 8 after primary challenge; lane 4: prior to secondary challenge; lane 5: week 4 after secondary challenge; and lane 6: week 8 after secondary challenge.

elicitation of serum IgG and IgE antibodies directed to proteins/peptides of a wide range of molecular weights. Interestingly, the secondary infection corresponded with reduced mange lesion area, absence of detectable live *S. scabiei* mites in skin scrapings collected in week 8 PC and a concomitant increase in specific serum IgE antibodies.

This study was based on the detection of antibodies elicited by *S. scabiei* var. *ovis* using a mite extract prepared from *S. scabiei* var. *caprae*. It is known that each variety of *S. scabiei* may produce a range of specific proteins (Arlian et al., 1996a). However, the level of cross-antigenicity between them is usually high and a heterologous variety can be successfully used to detect specific antibodies. For instance, ELISAs based on extracts from *S. scabiei* var. *vulpes* have been used for diagnosing sarcoptic mange in pigs, dogs and chamois (Bornstein et al., 1996; Bornstein and Wallgren, 1997; Lower et al., 2001; Rambozzi et al., 2004). In the present study, the specific antibody responses detected by using the *S. scabiei* var. *caprae*-based ELISA to detect antibodies in sheep after challenge were significant and quite pronounced, suggesting that this heterologous variety can be used with high performance to detect antibodies elicited by *S. scabiei* var. *ovis*. Nevertheless, it is possible that *S. scabiei* var. *ovis* has specific antibody-reactive proteins, and as a result they would not have been identified.

Prior to primary challenge and despite ELISA OD values being low, serum reactivity to several bands of the mite extract was detected by WB analysis. This may have been due to the presence of antibodies elicited by free-living mites such as *Dermatophagoides pteronyssinus* or *Acarus siro* which cross-react with *S. scabiei* proteins (Arlian et al., 1991; Van der Heijden et al., 2000). In the primary infection, live *S. scabiei* mites were detected in only 70% skin scrapings in week 10 PC, but all sheep developed mange lesions that spread over the ear and in addition a large number of dead mites were also detected. This would indicate that the technique used to detect live mites had relatively low sensitivity, a finding in agreement with Gutiérrez et al. (1996) who studied sarcoptic mange in pigs. The kinetics of specific serum IgG and IgE antibodies were similar to the findings of other authors studying sarcoptic mange in rabbits, foxes and goats (Arlian et al., 1994; Bornstein et al., 1995; Tarigan, 2004). As the infection progressed, the average IgG and IgE values tended to rise which is consistent with the higher number of reactive bands, and the increase in the labelling intensities and frequencies detected by WB. However, despite the development of clinical-lesions in all sheep, IgG and IgE seroconversion was not found in 10% and 40% of sheep, respectively, in week 8 PC. The absence of detectable levels of IgE antibodies in a significant proportion of sera from *S. scabiei*-infected humans, dogs and goats has previously been reported (Arlian and Morgan, 2000; Morgan et al., 1997; Tarigan, 2004).

In the present study, the antibody profile was studied by WB and reactions were demonstrated to bands of a wide range of molecular weights. However, heterogeneity of reaction within each of these bands was not determined and they may represent two or more different antibody-reactive proteins with the same or similar molecular weights. As a consequence, the present studies cannot confirm either that bands which reacted with both IgG and IgE antibodies were due to the same antigens being detected by both isotypes. The immunodominant bands include those proteins/peptides more intensively and frequently recognized by the immune system, which are more likely to be the best candidates for a sensitive immunodiagnostic test based on the detection of induced antibodies. The immunodominant IgG-reactive bands detected during the primary infection (120, 52 and 44 kDa) were reactive with most of the sheep sera collected in weeks 4 and 8 PC, and therefore include antigens which are candidates for developing a highly sensitive immunodiagnostic test for sarcoptic mange in sheep. In contrast, the 120 kDa allergen seems to have limited immunodiagnostic value, as it was reactive with only 70% of sheep sera taken in week 8 PC. Of note at this point was the finding that also some pre-challenge sera produced weak reaction to the immunodominant bands. The origin of these cross-reactive antibodies is unclear, as stated before. Nevertheless, antibodies to these bands were markedly

increased in a time-dependent manner following primary challenge, indicating that these proteins may still be relatively specific. Furthermore, previous studies have demonstrated little cross-antigenicity between some closely related mite proteins (Cheong et al., 2003; Kuo et al., 2003).

The clinical and antibody response of sheep after a secondary challenge with *S. scabiei* var. *ovis* was also studied. A positive control for this infection was obtained by applying aliquots of challenge-inocula to three *S. scabiei*-naïve ewes at the same time. The mange lesion area and the ELISA antibody levels of these positive control animals were similar to those observed in the primary infection group, which suggests that the pathogenicity of the mite doses used in the primary and secondary challenges was similar, so the two infections can validly be compared. The secondary infection group had a marked reduction in the mean mange lesion score compared with that of the same sheep in the primary infection group and no live *S. scabiei* mites were detected in any skin scraping collected in week 8 PC. These results suggest that the sheep had developed some immunity during the primary infection which was able to ameliorate the secondary infection, thus confirming previous reports concerning dogs, goats and rabbits (Arlian et al., 1994, 1996b; Tarigan, 2002). This secondary infection was characterized by an anamnestic IgE response, resulting in an elicitation of specific serum IgE levels significantly higher than those detected during the primary infection. The mechanism involved in the development of resistance to re-infection by *S. scabiei* remains unclear but it is believed that the early immune response occurring in the local skin plays a major role and may be associated with IgE-mediated responses (Tarigan, 2003). This conclusion is also tentatively supported by the absence of specific serum IgE antibodies in a vaccination trial which failed to provide protection against infection in goats, despite the presence of high levels of specific serum IgG antibodies (Tarigan and Huntley, 2005). This lack of correlation between specific serum IgG levels and resistance agrees with the findings of Arlian et al. (1994), and the results from the present study support the view that the development of resistance to *S. scabiei* is more closely correlated with the presence of specific serum IgE antibodies.

In summary, this study demonstrates that primary and secondary challenges of sheep with *S. scabiei* var. *ovis* result in both IgG and IgE antibody responses, and that secondary infections are ameliorated. The IgG antibody responses react to a number of antigens which would represent good targets for developing an immunodiagnostic test to detect *S. scabiei* var. *ovis* infections; conversely antigenic epitopes for IgE antibodies were mainly restricted to a 120 kDa band which would include a vaccine candidate.

Acknowledgements

The present study was partially financed by a grant (LE010A05) from the Education Bureau of the Castile and Leon Regional Government (Consejería de Educación de la Junta de Castilla y León). FRC and MTCG hold a fellowship from the Education Bureau of the Castile and Leon Regional Government, co-financed by the European Social Fund. The authors are very grateful to Dr. J.F. Huntley (Moredun Research Institute, Penicuik, Scotland) for reading the manuscript with a critical eye and for kindly supplying the VPM6 and 2F1 mAbs, and the mite extract used in this work.

References

Arlian, L.G., Morgan, M.S., 2000. Serum antibody to *Sarcoptes scabiei* and house dust mite prior to and during infestation with *S. scabiei*. Vet. Parasitol. 90, 315–326.

Arlian, L.G., Morgan, M.S., Arends, L.L., 1996a. Immunologic cross-reactivity among various strains of *Sarcoptes scabiei*. J. Parasitol. 82, 66–72.

Arlian, L.G., Morgan, M.S., Rapp, C.M., Vyszenski-Moher, D.L., 1996b. The development of protective immunity in canine scabies. Vet. Parasitol. 62, 133–142.

Arlian, L.G., Morgan, M.S., Vyszenski-Moher, D.L., Stemmer, B.L., 1994. *Sarcoptes scabiei*: the circulating antibody response and induced immunity to scabies. Exp. Parasitol. 78, 37–50.

Arlian, L.G., Vyszenski-Moher, D.L., Ahmed, S.G., Estes, S.A., 1991. Cross-antigenicity between the scabies mite, *Sarcoptes scabiei*, and the house dust mite, *Dermatophagoides pteronyssinus*. J. Invest. Dermatol. 96, 349–354.

Bendixsen, T., Windon, R.G., Huntley, J.F., Mackellar, A., Davey, R.J., McClure, S.J., Emery, D.L., 2004. Development of a new monoclonal antibody to ovine chimeric IgE and its detection of systemic and local IgE antibody responses to the intestinal nematode *Trichostrongylus colubriformis*. Vet. Immunol. Immunopathol. 97, 11–24.

Bird, P., Jones, P., Allen, D., Donachie, W., Huntley, J., McConnell, I., Hopkins, J., 1995. Analysis of the expression and secretion of isotypes of sheep B cell immunoglobulins with a panel of isotype-specific monoclonal antibodies. Res. Vet. Sci. 59, 189–194.

Bornstein, S., Thebo, P., Zakrisson, G., 1996. Evaluation of an enzyme-linked immunosorbent assay (ELISA) for the serological diagnosis of canine sarcoptic mange. Vet. Dermatol. 7, 21–27.

Bornstein, S., Wallgren, P., 1997. Serodiagnosis of sarcoptic mange in pigs. Vet. Rec. 141, 8–12.

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 Vet. Scand. 36, 509–519.

Bradberry, S.M., Cage, S.A., Proudfoot, A.T., Vale, J.A., 2005. Poisoning due to pyrethroids. Toxicol. Rev. 24, 93–106.

Cheong, N., Soon, S.C., Ramos, J.D.A., Kuo, I.C., Kolortkar, P.R., Lee, B.W., Chua, K.Y., 2003. Lack of human IgE cross-reactivity between mite allergens Blo t 1 and Der p 1. Allergy 58, 912–920.

Cordero del Campillo, M., Rojo-Vázquez, F.A., 1999. Parasitología Veterinaria. McGraw-Hill Interamericana, Madrid, Spain.

Curie, B.J., Harumal, P., McKinnon, M., Walton, S.F., 2004. First documentation of *in vivo* and *in vitro* ivermectin resistance in *Sarcoptes scabiei*. Clin. Infect. Dis. 39, 8–12.

Curtis, C.F., 2001. Evaluation of a commercially available enzyme-linked immunosorbent assay for diagnosis of canine sarcoptic mange. Vet. Rec. 148, 238–239.

Fthenakis, G.C., Karagiannidis, A., Alexopoulos, C., Brozos, C., Papadopoulos, E., 2001. Effects of sarcoptic mange on the reproductive performance of ewes and transmission of *Sarcoptes scabiei* to newborn lambs. Vet. Parasitol. 95, 63–71.

Fthenakis, G.C., Papadopoulos, E., Himonas, C., Leontides, L., Kritas, S., Papatsas, J., 2000. Efficacy of moxidectin against sarcoptic mange and effects on milk yield of ewes and growth of lambs. Vet. Parasitol. 87, 207–216.

Gutiérrez, J.F., Méndez de Vigo, J., Castellá, J., Muñoz, E., Ferrer, D., 1996. Prevalence of sarcoptic mange in fattening pigs sacrificed in a slaughterhouse of northeastern Spain. Vet. Parasitol. 61, 145–149.

Imperiale, F.A., Busetti, M.R., Suárez, V.H., Lanusse, C.E., 2004. Milk excretion of ivermectin and moxidectin in dairy sheep: assessment of drug residues during cheese elaboration and ripening period. J. Agric. Food Chem. 52, 6205–6211.

Jacobson, M., Bornstein, S., Wallgren, P., 1999. The efficacy of simplified eradication strategies against sarcoptic mange mite infections in swine herds monitored by an ELISA. Vet. Parasitol. 81, 249–258.

Kuhn, C., Lucius, R., Matthes, H.F., Meusel, G., Reich, B., Kalinna, B.H., 2008. Characterisation of recombinant immunoreactive antigens of the scab mite *Sarcoptes scabiei*. Vet. Parasitol. 153, 329–337.

Kuo, I.C., Cheong, N., Trakultivakorn, M., Lee, B.W., Chua, K.Y., 2003. An extensive study of human IgE cross-reactivity of Blo t 5 and Der p 5. J. Allergy Clin. Immunol. 111, 603–609.

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

Morgan, M.S., Arlian, L.G., Estes, S.A., 1997. Skin test and radioallergosorbent test characteristics of scabietic patients. Am. J. Trop. Med. Hyg. 57, 190–196.

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

Rodríguez-Cadenas, F., Carbajal-González, M.T., Fregeneda-Grandes, J.M., Aller-Gancedo, J.M., Huntley, J.F., Rojo-Vázquez, F.A. Development and evaluation of an antibody ELISA for sarcoptic mange in sheep and a comparison with the skin-scraping method. Submitted to Prev. Vet. Med.

Rueda-López, M., 2006. Elimination of mange due to *Sarcoptes scabiei* var. *suis* from a 1800-sow farrow-to-finish farm. Vet. Rec. 159, 595–597.

Sanderson, H., Laird, B., Pope, L., Brain, R., Wilson, C., Johnson, D., Bryning, G., Peregrine, A.S., Boxall, A., Solomon, K., 2007. Assessment of the environmental fate and effects of ivermectin in aquatic mesocosms. Aquat. Toxicol. 85, 229–240.

Tarigan, S., 2002. Dermatopathology of caprine scabies and protective immunity in sensitised goats against *Sarcoptes scabiei*. Jurnal Ilmu Ternak dan Veteriner 7, 265–271.

Tarigan, S., 2003. Histopathological changes in naïve and sensitised goats caused by *Sarcoptes scabiei*. Jurnal Ilmu Ternak dan Veteriner 8, 114–121.

Tarigan, S., 2004. Antibody responses in naïve and sensitised goats infested by *Sarcoptes scabiei*. Jurnal Ilmu Ternak dan Veteriner 9, 258–265.

Tarigan, S., Huntley, J.F., 2005. Failure to protect goats following vaccination with soluble proteins of *Sarcoptes scabiei*: evidence for a role for IgE antibody in protection. Vet. Parasitol. 133, 101–109.

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. Vet. Parasitol. 89, 95–107.

Capítulo III

Resumen de los trabajos publicados

Summary of the published articles

Resumen de los trabajos publicados

En el primer trabajo, titulado **"Development and evaluation of an antibody ELISA for sarcoptic mange in sheep and a comparison with the skin scraping method"**, se compara la exactitud de dos técnicas para el diagnóstico de la sarna sarcóptica ovina (la detección de anticuerpos específicos mediante ELISA y la visualización de ácaros en raspados cutáneos) utilizando tanto el análisis ROC, que se basa en la comparación de la exactitud de la técnica con un método de referencia (Greiner y col., 2000), como el análisis de clases latentes para técnicas independientes, basado en la aplicación de las pruebas de diagnóstico a dos poblaciones y la asunción de que la sensibilidad y especificidad de las pruebas son iguales en ambas poblaciones; que la prevalencia es diferente en ambas poblaciones; y que las dos pruebas son condicionalmente independientes (Hui y Walter, 1980).

El estudio epidemiológico, el examen clínico y el análisis de las muestras cutáneas de de los 42 rebaños muestreados en el oeste de la Comunidad Autónoma de Castilla y León (León, Zamora y Salamanca) pusieron de manifiesto que: 11 no estaban afectados por procesos cutáneos ni producidos por artrópodos (Grupo NE); 18 estaban afectados por enfermedades cutáneas o producidas por artrópodos diferentes de *S. scabiei* (Grupo NN), como ectima contagioso, peloderosis, pediculosis, miasis, sarna psoróptica, dermatofitosis, estafilocias y enfermedades de etiología desconocida o producidas por otros ácaros; y, finalmente, 13 rebaños estaban afectados de sarna sarcóptica (Grupo SM). Los animales de este último grupo se clasificaron en tres subgrupos según la extensión de las lesiones de sarna sarcóptica.

Previamente a la evaluación de la exactitud del ELISA, se valoró su repetibilidad y se demostró que la variación de los resultados dentro de la misma placa, entre placas y entre diferentes lotes de antígeno era baja, lo cual demuestra que el protocolo de la técnica ELISA utilizado está suficientemente estandarizado.

Al comparar ambas técnicas con el método de referencia (Figura 1 de la primera publicación) se obtuvo que el área bajo la curva ROC (AUC) era de 0,967 (95% CI: 0,949–0,985) para la técnica ELISA y de 0,915 (95% CI: 0,863–0,968) para el método de raspado cutáneo, por lo que ambas técnicas tiene elevada capacidad de discriminación entre los animales infectados por *S. scabiei* y los no infectados (Greiner y col., 2000).

También se identificaron cinco factores biológicos que determinan el resultado a la prueba ELISA. En los animales infectados: la presencia/ausencia de *S. scabiei* en el análisis de un raspado recogido de una zona lesionada; y en los animales no infectados el status de infección por *Trichophyton verrucosum*, el status de infección por Tyroglyphidae, la presencia en el mismo rebaño de animales con lesiones cutáneas similares a la sarna sarcóptica, y el status de infección del rebaño por *Oestrus ovis*. Estos resultados permiten reajustar los parámetros de sensibilidad y especificidad de la técnica ELISA cuando se utilice para el diagnóstico de esta enfermedad en otra población.

El hecho de que la sensibilidad diagnóstica de la técnica ELISA depende del resultado a la prueba de análisis de raspado cutáneo, es también importante en caso de que ambas técnicas se utilicen en combinación para establecer un diagnóstico. Si el ELISA se utiliza con un punto de corte en el que la sensibilidad diagnóstica de ambas técnicas es condicionalmente dependiente (por ejemplo 15 ó 35 %DO), la sensibilidad combinada será menor que en caso

de independencia si se utilizan combinadas en serie, pero mayor si se utilizan combinadas en paralelo (Gardner y col., 2000).

No obstante, en la estimación de la exactitud de una prueba diagnóstica mediante la comparación con un método de referencia hay que tener en cuenta los posibles sesgos. En el presente trabajo se identificó que el método de referencia introducía sesgos de selección y de información en las estimaciones de la sensibilidad y especificidad diagnósticas del ELISA, pero en sentidos contrarios, por lo que el sesgo resultante dependerá de cuánto se contrarrestan.

Finalmente, se estimó la exactitud de ambas técnicas mediante un análisis de clases latentes para pruebas independientes (Hui and Walter, 1980). Para ello, en primer lugar se redistribuyeron los animales utilizados en el análisis ROC en dos nuevas poblaciones, para cumplir con los requisitos de igualdad de sensibilidad y especificidad diagnósticas en las dos poblaciones que se requiere para utilizar esta metodología. Asimismo, también se introdujeron en el análisis los animales clínicamente sanos de rebaños afectados por sarna sarcóptica (subgrupo CH) para conseguir que la sensibilidad y especificidad en las dos nuevas poblaciones fuera similar a la población diana de los test. Como resultado más relevante se detectó que la sensibilidad diagnóstica de la prueba de análisis de raspado cutáneo (62,8%) es mucho menor que la del ELISA utilizado con un punto de corte de de 17,8 %DO (87,6%), pero se detectó una mayor similitud en el caso de la especificidad (100% para la técnica de análisis de raspado cutáneo y 95,9% para el ELISA).

Consideramos que se cumplía el requisito de diferencia de prevalencia real e igual sensibilidad y especificidad diagnósticas de ambas técnicas en las dos poblaciones. También consideramos que no existe dependencia entre la especificidad de ambas técnicas, pero la

sensibilidad podría ser dependiente, ya que mediante el análisis ROC se obtuvo que la covarianza de la sensibilidad era de 0,03, que equivale al 28% de la máxima posible, lo que demuestra que hay una baja dependencia entre la sensibilidad diagnóstica de ambas técnicas.

El segundo trabajo, titulado **"Cross-sectional sero-epidemiological survey of sarcoptic mange in sheep of Western Castile and León, Spain"**, utiliza la técnica ELISA anteriormente validada para estimar la prevalencia de la infección por *S. scabiei* en el ganado ovino de la zona oeste de Castilla y León (provincias de León, Zamora y Salamanca), y para estudiar la asociación ente diversas variables y el status serológico.

Se obtuvo una prevalencia verdadera de rebaño del 22,6% (95% CI: 17,8–27,4), una prevalencia verdadera individual del 7,2% (95% CI: 6,1–8,3) y una prevalencia verdadera individual en los rebaños seropositivos del 31,3% (95% CI: 27,2–35,4). La prevalencia de rebaño de la sarna sarcóptica detectada es mayor que la prevalencia de sarna psoróptica en Gran Bretaña (9%; Bisdorff y col., 2006) y Suiza (12%; Falconi y col., 2002).

Se comprobó que, a pesar de haber seguido un muestreo de doble etapa, el error en las estimaciones de las prevalencias individuales no necesitaba correccion respecto al error correspondiente a un muestreo aleatorio simple, ya que el coeficiente de correlación intraclase para seropositividad no era significativo. Sin embargo, no se propagó el error en las estimaciones de los parámetros de exactitud de la técnica ELISA, y por lo tanto, el error en las prevalencias verdaderas se habría subestimado (Greiner y Gardner, 2000; Christensen y Gardner, 2000).

Asimismo, se corrigieron las prevalencias aparentes a las prevalencias verdaderas para evitar el sesgo de información debido a la utilización de una técnica de diagnóstico

imperfecta. También se demostró que, a pesar de haber muestrado el mismo número de animales en todos los rebaños independientemente de su tamaño, que era muy variable en la población, no se había introducido sesgo de selección en las estimaciones de la prevalencia individual, ya que no había correlación significativa entre en nivel de seropositividad dentro del rebaño y el tamaño del mismo.

En el análisis de factores de riesgo se detectó que la prevalencia aparente individual de sarna sarcóptica es mayor en las provincias de Zamora y Salamanca (OR = 1,7, 95% CI: 1,2–2,6; OR= 1,9, 95% CI: 1,3–2,8, respectivamente) que en la provincia de León (OR=1). Aunque se necesitan más estudios para conocer qué factores están implicados en la existencia de diferencias en la prevalencia entre las provincias, es muy posible que estén relacionados con el manejo. Por ejemplo, en la infección por *S. scabiei* en cerdos, la utilización de cama de paja es un factor que favorece el contagio, mientras que la limpieza con agua a alta presión y la desinfección son factores que reducen el riesgo de infección por piojos (Damriyasa y col. 2004).

El tercero de los trabajos se titula **"Clinical evaluation and antibody responses in sheep after primary and secondary experimental challenges with the mange mite *Sarcoptes scabiei* var. *ovis*"**. En este trabajo se describe la realización de una infección experimental en ovejas con *S. scabiei* var. *ovis*, en la cual se estudió la evolución clínica, así como la respuesta inmune humoral de IgG e IgE, tanto a una infección primaria (n = 10) como a una infección secundaria (n=4).

Se observó que, en la infección primaria, las lesiones de sarna sarcóptica aumentaban progresivamente de tamaño y se detectaron ácaros vivos en el 70% de los animales a las diez semanas postinfección. Por su parte, en la infección secundaria las lesiones eran de menor

tamaño y no se detectaron ácaros vivos en ningún animal. Estos resultados demuestran que las ovejas, durante la infección primaria, desarrollan inmunidad que es capaz de conferir resistencia frente a infecciones posteriores. Nuestros hallazgos son similares a los de otros autores que han estudiado la respuesta a pruebas desafío en perros, conejos y cabras (Arlian y col., 1994b, 1996b; Tarigan, 2002).

En el estudio de la respuesta inmune humoral a la infección primaria mediante la técnica ELISA, se detectó respuesta de IgG e IgE específicas a las dos semanas de la infección. Los niveles de IgG alcanzaban una meseta a las cuatro semanas postinfección, mientras que los niveles de la IgE aumentaban progresivamente. Tras la infección secundaria, se observó respuesta de IgG a las cuatro semanas postinfección, pero a las seis semanas los niveles ya eran similares a los observados antes de la infección secundaria. Sin embargo, los niveles de IgE alcanzaban niveles muy altos a las dos semanas postinfección y posteriormente descendían pero todavía permanecían elevados a las ocho semanas postinfección. Estos resultados son también similares a los que han obtenido otros autores en conejos, zorros y cabras (Arlian y col., 1994b; Bornstein y col., 1995; Tarigan, 2004).

El mecanismo por el que se desarrolla resistencia a la reinfección por *S. scabiei* es desconocido, pero podría estar relacionado con la respuesta inmune local, seguramente mediada por IgE (Tarigan, 2003). También llegaron a esta conclusión Tarigan y Huntley (2005) en un estudio de desafío en cabras inmunizadas con extractos de *S. scabiei* var. *caprae* en el que no se logró proteger a los animales de la infección, ya que se detectó que los niveles de IgG que se habían inducido durante la inmunización eran altos, pero, en contra, no se había conseguido inducir la producción de IgE.

En el estudio de la respuesta inmune humoral por Western blot, se detectó respuesta inmune de IgG frente a 11 bandas del antígeno crudo y respuesta de IgE a cuatro bandas. Las bandas reconocidas con mayor frecuencia y mayor intensidad por las IgG eran las de 120, 52 y 44 kDa, y puesto que eran reactivas con la mayoría de los sueros a las cuatro y ocho semanas de la infección primaria incluyen antígenos que son buenos candidatos para el desarrollo de un test inmunodiagnóstico de alta sensibilidad. Además, como dicha reacción había experimentado un fuerte aumento tras la infección en comparación a niveles previos a la misma, el test de inmunodiagnóstico podría tener también alta especificidad. En el caso de la respuesta de IgE a la infección secundaria, estaba dirigida fundamentalmente a una banda de 120 kDa que incluye una proteína que puede ser un buen candidato para el desarrollo de una vacuna frente a la sarna sarcóptica de las ovejas.

Una limitación de este estudio es que se utilizaron antígenos de *S. scabiei* var. *caprae* para detectar anticuerpos producidos durante infecciones en ovinos con su variedad específica. Se sabe que cada variedad puede producir un conjunto de proteínas específicas (Arlian y col., 1996a), pero normalmente el nivel de reacción antigénica cruzada es elevado. En este estudio, este hecho se sugiere por la elevada respuesta de anticuerpos que se detectó mediante la técnica ELISA. No obstante, puede que determinados antígenos específicos de *S. scabiei* var. *ovis* no hayan sido identificados.

Summary of the published articles

In the first paper, entitled **"Development and evaluation of an antibody ELISA for sarcoptic mange in sheep and a comparison with the skin scraping method"**, we estimate the accuracy of the two techniques for diagnosing sarcoptic mange in sheep by ROC analysis, which is based on the comparison of the technique against a defined reference method (Greiner et al., 2000), and by latent-class analysis for independent tests, which is based on the application of the two techniques to two populations under the assumptions that the diagnostic sensitivity and specificity of the tests are the same in both populations, the prevalence in the two populations is different and the two test are conditionally independent given the true status (Hui and Walter, 1980).

The epidemiological survey, the clinical examination and the analysis of the skin samples collected from 42 sheep flocks in Western Castile and Leon (León, Zamora and Salamanca provinces) allowed us to conclude that 11 were free from cutaneous diseases or caused by arthropods (Group NE), 18 were affected by cutaneous diseases or caused by Arthropoda other than *S. scabiei* (Group NN), which included contagious ecthyma, peloderosis, pediculosis, myasis, sheep scab, dermathophytosis, hypodermosis and diseases with unknown aetiology or caused by other mites, and finally 13 flocks were affected by sarcoptic mange (Group SM). The sheep in the latter group were graded in accordance with the extent of the mange lesion.

Previously to estimating the accuracy of the ELISA, it was evaluated its repeatability and it was demonstrated that the outcomes are stable within plate, between plates and between

the different batches of antigen, indicating that the protocol of ELISA used was sufficiently standardized.

The comparison of the two techniques against the reference method (Figure 1 of the first publication) yielded and area under the ROC curve (AUC) of 0.967 (95% CI: 0.949–0.985) for the ELISA and 0.915 (95% CI: 0.863–0.968) for the skin-scraping method, and thus both techniques have high ability to discriminate between the sheep infected by *S. scabiei* and those uninfected, as defined by the reference method (Greiner et al., 2000).

We also identified five biological factors which determine the ELISA outcome. In infected animals the presence/absence of *S. scabiei* mites in the analysis of a skin scraping collected from the lesion, and in uninfected the *Trichophyton verrucosum* status, the Tyroglyphidae status, presence/absence a cutaneous condition resembling sarcoptic mange in the adjacent sheep and the flock-level *Oestrus ovis* status. These results allow readjusting the sensitivity and specificity of the ELISA when it is used for diagnosing sarcoptic mange in a different population.

The finding that the diagnostic sensitivity of the ELISA depends on the result to the skin-scraping method is also important when both techniques are used in combination. In the case the ELISA is used at an OD% cut-off value in which the sensitivity of the techniques are conditionally dependent (for instance 15 or 35 OD%), then the combined sensitivity will be less than that assuming conditional independence if the test are used in serial interpretation scheme, but higher if they are used in a parallel (Gardner et al., 2000).

However, during the estimation of the accuracy of a diagnostic test by comparison with a reference method it should be taken into account the possibility of introducing bias. In the

present study, we identified that the reference method introduced selection and information biases in the estimation of the sensitivity and specificity of the ELISA, but in opposite senses, and therefore the resulting bias depends on how much they counteract.

Finally, it was estimated the accuracy of the two techniques by latent class analysis for independent tests (Hui and Walter, 1980). For this purpose, the animals used in the ROC analysis were firstly redistributed into two new populations in order to achieve the requirement of same diagnostic sensitivity and specificity of the two techniques in the two populations, which is needed to use this methodology. In addition, we also introduced the healthy animals from sarcoptic mange infected flocks (subgrupo CH) in the two new populations, in order to achieve the sensitivity and specificity of the techniques in the two populations being similar to that in the intended population for the test. As relevant result, it was detected that the diagnostic sensitivity of the skin-scraping method (62.8%) is lower than that of the ELISA when used at 17.8 OD% cut-off value (87.6%), but there was a better agreement in the diagnostic specificity (100% for the skin-scraping analysis and 95.9% for the ELISA).

We consider that the requirement of different actual prevalence and same sensitivity and specificity of the two techniques in the two populations was fulfilled. We also think that there is no dependency between the specificity, but the sensitivity may be dependent, as by ROC analysis it was observed that the sensitivity covariance was 0.03, which equals to 28% of the maximum possible value, indicating that there is a low dependency between the diagnostic sensitivities.

In the second paper, entitled **"Cross-sectional sero-epidemiological survey of sarcoptic mange in sheep of Western Castile and León, Spain"**, it is used the ELISA

previously validated to estimate the prevalence of infection by *S. scabiei* of the ovine livestock in Western Castile and Leon (León, Zamora y Salamanca provinces), Spain, and to study the association between different variables and the serological status.

It was found that the flock-level true prevalence was 22.6% (95% CI: 17.8–27.4), the individual-level true prevalence 7.2% (95% CI: 6.1–8.3) and the individual-level true prevalence within the seropositive flocks 31.3% (95% CI: 27.2–35.4). The flock-level prevalence is higher than the prevalence of sheep scab in Great Britain (9%; Bisdorff et al., 2006) and Switzerland (12%; Falconi et al., 2002).

It was found that, despite the two-stage sampling procedure used at individual level, the error on the individual-level prevalence estimates did not need correction respect to the error corresponding to a simple random sampling, as it was found that the intra-class correlation coefficiente was not significant. However, the error on the estimates of the accuracy parameters of the ELISA was not propagated and therefore the error on the true prevalence estimates would have been underestimated (Greiner and Gardner, 2000; Christensen and Gardner, 2000).

In this study, the apparent prevalences were corrected to the true prevalences in order to avoid information bias due to the analyses of sera with an imperfect test. It was also found that, although we sampled the same number of animals within the flocks irrespective of the flock size, which were highly variable in the population, it did not produce selection bias in the individual-level prevalence estimates, as there was not correlation between the seropositivity level within the flock and the flock size.

In the risk factors analysis, it was detected that the individual-level apparent prevalence of sarcoptic mange in the Zamora and Salamanca provinces (OR = 1.7, 95% CI: 1.2–2.6; OR= 1.9, 95% CI: 1.3–2.8, respectively) is higher than in the Leon province (OR=1). Although more studies are needed to know the factors that determine the difference of prevalence between the provinces, it is possible that they are related with husbandry measures. For instance, the use of straw bedding is a factor which favours the transmission of *S. scabiei* in pigs, whereas the cleaning with high pressure water and the disinfection reduce the risk of infection by lice (Damriyasa et al., 2004).

The third paper is entitled **"Clinical evaluation and antibody responses in sheep after primary and secondary experimental challenges with the mange mite *Sarcoptes scabiei* var. *ovis*"**. In this work, it is described an experimental infection of sheep with *S. scabiei* var. *ovis*, in which it was studied the clinical evolution and the IgG and IgE antibody responses, both to primary (n = 10) and secondary (n=4) experimental challenges.

It was detected that, in the primary infection, the area of sarcoptic mange lesions increased progressively and *S. scabiei* alive mites were detected in 70% of sheep in week ten post-infection. However, in the secondary challenge, the area of mange lesion was reduced and no alive mites were detected in any sheep. These results demonstrate that the sheep had developed immunity during the primary infection, which was able to ameliorate a subsequent challenge. These findings are in agreement with other studies carried out in dogs, rabbits and goats (Arlian et al., 1994b and 1996b; Tarigan, 2002).

The humoral immune response of sheep was studied by ELISA, and in the primary infection it was detected IgG and IgE antibody responses in week two post-infection. The IgG level reached a plateau in week four post-infection, whereas the IgE levels increased

progressively. In the secondary infection, it was detected IgG antibody response in week four post-infection, but in week six post-infection the level was similar to those observed prior to secondary challenge. However, the IgE levels rose to a high level in week two post-infection, and then decreased progressively but were still significantly elevated in week eight post-infection. These results are similar to those obtained by other authors in rabbit, fox and goat (Arlian et al., 1994b; Bornstein et al., 1995; Tarigan, 2004).

The mechanism underlying the development of resistance against a reinfection by *S. scabiei* is not known, but it is believed to be related with local immune response and mediated by IgE (Tarigan, 2003). This conclusion is also tentatively supported by Tarigan and Huntley (2005) as, after performing a challenge in goats immunized with extracts of *S. scabiei* which induced high level of specific IgG but no IgE, they detected that the animals had not been conferred protection against infection.

The humoral immune response was also studied by Western blot, and it was detected IgG reaction to 11 bands of the crude antigen and IgE reaction to four bands. The bands more frequently and intensively recognized by IgG were the 120, 52 and 44 kDa, and as these were reactive with most of the animals in week four and eight post primary challenge, they are good candidates to develop an immunodiagnostic test with high sensitivity. In addition, as this reaction markedly increased after challenge, the test may also be highly specific. The IgE antibody response during the secondary challenge targets mainly to a band of 120 kDa, which would include a protein that is a candidate for the development of a vaccine against sarcoptic mange of sheep.

A limitation of the present study is the use of antigens from *S. scabiei* var. *caprae* to detect antibodies elicited in sheep by its specific variety. It is known that each variety may

produce a range of specific proteins (Arlian y col., 1996a), but usually the level of cross-antigenicity is high. In the present study, that is suggested from the high antibody response detected by ELISA. Nevertheless, it may be possible that some antigens specific of *S. scabiei* var. *ovis* were not identified.

Capítulo IV

Conclusiones

Conclusions

Conclusiones

Las conclusiones a las que hemos llegado tras los resultados obtenidos en nuestro estudio son las siguientes:

I. "Development and evaluation of an antibody ELISA for sarcoptic mange in sheep and a comparison with the skin-scraping method".

1. Mediante análisis ROC se obtuvo que la prueba ELISA desarrollada y el método de análisis de raspados cutáneos tienen alta exactitud para determinar la presencia/ausencia de infección por *S. scabiei* en los ovinos de la región oeste de Castilla y León, España.
2. El análisis de clases latente demostró que el ELISA, utilizado con un punto de corte de 17,8 %DO, tiene una sensibilidad diagnóstica mucho mayor que el método de análisis de raspados cutáneo. Sin embargo, la especificidad diagnóstica de ambas técnicas es similar.
3. Se observó que el factor biológico presencia/ausencia de *S. scabiei* en el análisis de un raspado de la zona lesionada, está relacionado con la sensibilidad diagnóstica del ELISA, y que los factores biológicos presencia/ausencia de infección por Tyroglyphidae, presencia/ausencia de infección por *Trichophyton verrucosum*, presencia/ausencia de infección del rebaño por *Oestrus ovis* y presencia/ausencia de una enfermedad relacionada con la sarna sarcóptica en los animales adyacentes están relacionados con la especificidad diagnóstica del ELISA, pero esto depende del punto de corte que se utilice. Estos resultados permiten una extrapolación adecuada de la técnica a otras poblaciones/subpoblaciones ovinas.

4. La prueba ELISA desarrollada es útil para confirmar la presencia o ausencia de sarna sarcóptica, para estimar su prevalencia, para realizar estudios de análisis de factores de riesgo y para monitorizar la eficacia de programas de control y erradicación.

II. "Cross-sectional sero-epidemiological survey of sarcoptic mange in sheep of Western Castile and Leon, Spain".

1. Las prevalencias individual y de rebaño de sarna sarcóptica en los ovinos de la zona oeste de Castilla y León son mayores de las que requieren unas buenas condiciones sanitarias, poniendo de manifiesto la necesidad de identificar y aplicar medidas de control eficaces.
2. El tamaño del rebaño y su orientación productiva no están relacionados con el estado serológico de los rebaños o de los animales individuales. Sin embargo, las ovejas de las provincias de Zamora y Salamanca tienen una mayor probabilidad de ser seropositivas que las de la provincia de León.

III. "Clinical evaluation and antibody responses in sheep after primary and secondary experimental challenges with the mange mite *Sarcoptes scabiei* var. *ovis*".

1. La infección secundaria de los ovinos por *S. scabiei* var. *ovis* mejora espontáneamente, al contrario que la infección primaria dónde las lesiones aumentan progresivamente.
2. La infección primaria y secundaria de las ovejas por *S. scabiei* var. *ovis* induce la producción de anticuerpos IgG e IgE. La respuesta IgG es más pronunciada durante la infección primaria y la respuesta IgE durante la secundaria.
3. La respuesta de anticuerpos IgG se dirige frente a once antígenos, de los cuales tres, de pesos moleculares 120, 52 y 44 kDa, son buenos candidatos para el desarrollo de un test

de inmunodiagnóstico de la infección por *S. scabiei* var. *ovis*. De modo contrario, los epitopos para las IgE se localizan sobre todo en un antígeno de 120 kDa que representa un buen candidato para desarrollar una vacuna.

Conclusions

The conclusions we have reached from the results of our study are the following:

I. "Development and evaluation of an antibody ELISA for sarcoptic mange in sheep and a comparison with the skin-scraping method".

1. ROC analysis determines that the ELISA developed and the skin-scraping method can be conceived as highly accurate tests for determining the status of sheep of the entire ovine population from Western Castilla y León region in Spain in relation to *S. scabiei* infection.
2. The latent-class analysis shows that the ELISA, at 17.8 OD% cut-off value, is markedly more sensitive than the skin-scraping method. However, they have a better agreement in their diagnostic specificity.
3. It was detected that the biological factor presence/absence of *S. scabiei* in the analysis of a skin scraping collected from the lesion is related with the diagnostic sensitivity of the ELISA, and the biological factors Tyroglyphidae individual status, *Trichophyton verrucosum* individual status, *Oestrus ovis* flock status and presence/absence of a clinical disease neighbour to *S. scabiei* infection in the adjacent animals are related with the diagnostic specificity of the ELISA, but this depends on the OD% cut-off value used. These results allow an adequate extrapolation of this technique to other populations/subpopulations of sheep.
4. The developed ELISA can be used to support confirmation of presence or absence of sarcoptic mange, to estimate the prevalence, to carry out risk factors analysis surveys, and to monitor the efficacy of control and eradication programmes.

II. "Cross-sectional sero-epidemiological survey of sarcoptic mange in sheep of Western Castile and Leon, Spain".

1. The flock-level and individual-level true prevalences of *S. scabiei*-infection in ovine livestock of Western Castilla y León region in Spain are higher that those required for good sanitary conditions, evidencing the need to search and implement effective control measures.
2. The size and the primary production purpose of the flock are not related with the *S. scabiei* serological status of the flock and the individual sheep, but sheep maintained in the provinces of Zamora and Salamanca had greater odds of being seropositive than those located in Leon province.

III. "Clinical evaluation and antibody responses in sheep after primary and secondary experimental challenges with the mange mite *Sarcoptes scabiei* var. *ovis*".

1. Secondary challenge of sheep with *S. scabiei* var. *ovis* is ameliorated, in contrast to primary infection, where the lesions spread over.
2. Primary and secondary challenges of sheep with *S. scabiei* var. *ovis* result in the elicitation of both IgG and IgE antibody responses. The IgG antibody responses were more pronounced during the primary challenge and the IgE antibody responses during the secondary challenge.
3. The IgG antibody responses react to eleven antigens, of which three, with molecular weights of 120, 52 and 44 kDa, would represent good targets for developing an immunodiagnostic test to detect *S. scabiei* var. *ovis* infections. Conversely, antigenic epitopes for IgE antibodies are mainly restricted to a 120 kDa band which would include a vaccine candidate.

Capítulo V

Bibliografía

– Abu-Samra, M.T., Hago, B.E., Aziz, M.A., Awad, F.W., 1981. Sarcoptic mange in sheep in the Sudan. Ann. Trop. Med. Parasitol. 75, 639-645.

– Abu-Samra, M.T., Ibrahim, K.E., Aziz, M.A., 1984. Experimental infection of goats with *Sarcoptes scabiei* var. *ovis*. Ann. Trop. Med. Parasitol. 78, 55-61.

– Abdel-Ghaffar, F., Al-Quraishy, S., Sobhy, H., Semmler, M., 2008. Neem seed extract shampoo, Wash Away Louse, an effective plant agent against *Sarcoptes scabiei* mites infesting dogs in Egypt. Parasitol. Res. 104, 145-148.

– Alasaad, S., Soglia, D., Spalenza, V., Maione, S., Soriguer, R.C., Pérez, J.M., Rasero, R., Degiorgis, M.P., Nimmervoll, H., Zhu, X.Q., Rossi, L., 2009. Is ITS-2 rDNA suitable marker for genetic characterization of *Sarcoptes* mites from different wild animals in different geographic areas? Vet. Parasitol. 159, 181-185.

– Al-Rawashdeh, O.F., Al-Ani, F.K., Sharrif, L.A., Al-Qudah, K.M., Al-Hami, Y., Frank, N., 2000. A survey of camel (*Camelus dromedarius*) diseases in Jordan. J. Zoo Wildl. Med. 31, 335-338.

– 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. J. Med. Entomol. 44, 1054-1063.

– Arlian, L.G., Morgan, M.S., 2000. Serum antibody to *Sarcoptes scabiei* and house dust mite prior to and during infestation with *S. scabiei*. Vet. Parasitol. 90, 315-326.

– Arlian, L.G., Morgan, M.S., Arends, J.J., 1996a. Immunologic cross-reactivity among various strains of *Sarcoptes scabiei*. J. Parasitol. 82, 66-72.

– Arlian, L.G., Morgan, M.S., Estes, S.A., Walton, S.F., Kemp, D.J., Currie, B.J., 2004b. Circulating IgE in patients with ordinary and crusted scabies. J. Med. Entomol. 41, 74-77.

– Arlian, L.G., Morgan, M.S., Neal, J.S., 2003. Modulation of cytokine expression in human keratinocytes and fibroblasts by extracts of scabies mites. Am. J. Trop. Med. Hyg. 69, 652-656.

– Arlian, L.G., Morgan, M.S., Neal, J.S., 2004a. Extracts of scabies mites (Sarcoptidae: *Sarcoptes scabiei*) modulate cytokine expression by human peripheral blood mononuclear cells and dendritic cells. J. Med. Entomol. 41, 69-73.

– 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. J. Med. Entomol. 43, 283-287.

– Arlian, L.G., Morgan, M.S., Rapp, C.M., Vyszenski-Moher, D.L., 1996b. The development of protective immunity in canine scabies. Vet. Parasitol. 62, 133-142.

– Arlian, L.G., Morgan, M.S., Vyszenski-Moher, D.L., Stemmer, B.L., 1994b. *Sarcoptes scabiei*: the circulating antibody response and induced immunity to scabies. Exp. Parasitol. 78, 37-50.

– Arlian, L.G., Rapp, C.M., Morgan, M.S., 1995. Resistance and immune response in scabies-infested hosts immunized with *Dermatophagoides* mites. Am. J. Trop. Med. Hyg. 52, 539-545.

– Arlian, L.G., Rapp, C.M., Stemmer, B.L., Morgan, M.S., Moore, P.F., 1997. Characterization of lymphocyte subtypes in scabietic skin lesions of naive and sensitized dogs. Vet. Parasitol. 68, 347-358.

– Arlian, L.G., Rapp, C.M., Vyszenski-Moher, D.L., Morgan, M.S., 1994a. *Sarcoptes scabiei*: histopathological changes associated with acquisition and expression of host immunity to scabies. Exp. Parasitol. 78, 51-63.

– Arlian, L.G., Runyan, R.A., Achar, S., Estes, S.A., 1984a. Survival and infectivity of *Sarcoptes scabiei* var. *canis* and var. *hominis*. J. Am. Acad. Dermatol. 11, 210-215.

– Arlian, L.G., Runyan, R.A., Estes, S.A., 1984c. Cross infestivity of *Sarcoptes scabiei*. J. Am. Acad. Dermatol. 10, 979-986.

– Arlian, L.G., Runyan, R.A., Sorlie, L.B., Estes, S.A., 1984b. Host-seeking behavior of *Sarcoptes scabiei*. J. Am. Acad. Dermatol. 11, 594-598.

– Arlian, L.G., Vyszenski-Moher, D.L., 1988. Life cycle of *Sarcoptes scabiei* var. *canis*. J. Parasitol. 74, 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. Exp. Appl. Acarol. 6, 181-187.

– Astiz, S., Legaz-Huidobro, E., Mottier, L., 2011. Efficacy of long-acting moxidectin against sarcoptic mange in naturally infested sheep. Vet. Rec. 169, 637.

– Bates, P., 2003. Sarcoptic mange (*Sarcoptes scabiei* var. *vulpes*) in a red fox (*Vulpes vulpes*) population in north-west Surrey. Vet. Rec. 152, 112-114.

– Bergström, F.C., Reynolds, S., Johnstone, M., Pike, R.N., Buckle, A.M., Kemp, D.J., Fischer, K., Blom, A.M., 2009. Scabies mite inactivated serine protease paralogs inhibit the human complement system. J. Immunol. 82, 7809-7817.

– 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*). J. Wildl. Dis. 47, 829-835.

– Berrilli, F., D'Amelio, S., Rossi, L., 2002. Ribosomal and mitochondrial DNA sequence variation in *Sarcoptes* mites from different hosts and geographical regions. Parasitol. Res. 88, 772-777.

– Bezold, G., Lange, M., Schiener, R., Palmedo, G., Sander, C.A., Kerscher, M., Peter, R.U., 2001. Hidden scabies: diagnosis by polymerase chain reaction. Br. J. Dermatol. 144, 614-618.

– Bisdorff, B., Milnes, A., Wall R., 2006. Prevalence and regional distribution of scab, lice and blowfly strike in Great Britain. Vet. Rec. 158, 749-752.

– Bornstein, S., Frossling, J., Naslund, K., Zakrisson, G., Morner, T., 2006. Evaluation of a serological test (indirect ELISA) for the diagnosis of sarcoptic mange in red foxes (*Vulpes vulpes*). Vet. Dermatol. 17, 411-416.

– Bornstein, S., Mörner T., Samuel, W.M., 2001. *Sarcoptes scabiei* and sarcoptic mange. In: Samuel W.M., Pybus M.J., Kocan A.A. (Ed.), Parasitic Diseases of Wild Mammals, Second Edition, Iowa State University Press, Ames, Iowa, USA. Pág. 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. Vet. Dermatol. 7, 21-28.

– Bornstein, S., Wallgren, P., 1997. Serodiagnosis of sarcoptic mange in pigs. Vet. Rec. 141, 8-12.

– 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 Vet. Scand. 36, 509-519.

– Brimer, L., Bak, H., Henriksen, S.A., 2004. Rapid quantitative assay for acaricidal effects on *Sarcoptes scabiei* var. *suis* and *Otodectes cynotis*. Exp. Appl. Acarol. 33, 81-91.

– Brook, I., 1995. Microbiology of secondary bacterial infection in scabies lesions. J. Clin. Microbiol. 33, 2139-2140.

– Burgess, I., 1994. *Sarcoptes scabiei* and scabies. Adv. Parasitol. 33, 235-292.

– Casáis, R., Prieto, M., Balseiro, A., Solano, P., Parra, F., Martín Alonso, J.M., 2007. Identification and heterologous expression of a *Sarcoptes scabiei* cDNAencoding a structural antigen with immunodiagnostic potential. Vet. Res. 38, 435-450.

– Chakrabarti, A., 1990. Pig handler's itch. Int. J. Dermatol. 29, 205-206.

– Chakrabarti, A., Chatterjee, A., Chakrabarti, K., Sengupta, D.N., 1981. Human scabies from contact with water buffaloes infested with *Sarcoptes scabiei* var. *bubalis*. Ann. Trop. Med. Parasitol. 75, 353-357.

– Christensen, J., Gardner, I.A., 2000. Herd-level interpretation of test results for epidemiologic studies of animal diseases. Prev. Vet. Med. 45, 83-106.

– Čorba, J., Várady, M., Praslička, J., Tomašovičová, O., 1995. Efficacy of moxidectin against mixed (*Psoroptes ovis* and *Sarcoptes scabiei* var. *ovis*) mange infestation in sheep. Vet. Parasitol. 56, 339-344.

– Cordero del Campillo, M., Rojo-Vázquez, F.A. (Coordinadores), 1999. Parasitología Veterinaria. McGraw-Hill Interamericana, Madrid, 968 pp.

– Corke, M.J., Broom, D.M., 1999. The behaviour of sheep with sheep scab, *Psoroptes ovis* infestation. Vet. Parasitol. 83, 291-300.

– Currie, B.J., Harumal, P., McKinnon, M., Walton, S.F., 2004. First documentation of *in vivo* and *in vitro* ivermectin resistance in *Sarcoptes scabiei*. Clin. Infect. Dis. 39, 8-12.

– Damriyasa, I.M., Failing, K., Volmer, R., Zahner, H., Bauer, C., 2004. Prevalence, risk factors and economic importance of infestations with *Sarcoptes scabiei* and *Haematopinus suis* in sows of pig breeding farms in Hesse, Germany. Med. Vet. Entomol. 18, 361-367.

– Davis, D.P., Moon, R.D., 1987. Survival of *Sarcoptes scabiei* (De Geer) stored in three media at three temperatures. J. Parasitol. 73, 661-662.

– De, U.K., Dey, S., 2010. Evaluation of organ function and oxidant/antioxidant status in goats with sarcoptic mange. Trop. Anim. Health Prod. 42, 1663-1668.

– De Pennington, N., Colles, K.M., 2011. *Sarcoptes scabiei* infestation of a donkey in the UK. Equine Vet. J. 23, 19-23.

– Dia, M.L., Diop, C., 2005. Etude comparative de l'efficacité clinique et parasitologique de trois formulations injectables à base d'ivermectine dans le traitment de la gale sarcoptique du mouton. Revue Med. Vét. 2, 87-90.

– Dimri, U., Sharma, M.C., 2004a. Effects of sarcoptic mange and its control with oil of *Cedrus deodara*, *Pongamia glabra*, *Jatropha curcas* and benzyl benzoate, both with and without ascorbic acid on growing sheep: epidemiology; assessment of clinical, haematological, cell-mediated humoral immune responses and pathology. J. Vet. Med. A Physiol. Pathol. Clin. Med. 51, 71-78.

– Dimri, U., Sharma, M.C., 2004b. Effects of sarcoptic mange and its control with oil of *Cedrus deodara*, *Pongamia glabra*, *Jatropha curcas* and benzyl benzoate, both with and without ascorbic acid on growing sheep: assessment of weight gain, liver function, nutrient digestibility, wool production and meat quality. J. Vet. Med. A Physiol. Pathol. Clin. Med. 51, 79-84.

– Domínguez, G., Espí, A., Prieto, J.M., de la Torre, J.A., 2008. Sarcoptic mange in Iberian wolves (*Canis lupus signatus*) in northern Spain. Vet. Rec. 162, 754-755.

– Domínguez-Peñafiel, G., Giménez-Pardo, C., Gegúndez MI, Lledó, L., 2011. Prevalence of ectoparasitic arthropods on wild animals and cattle in the Las Merindades area (Burgos, Spain). Parasite 18, 251-260.

– Dougall, A., Holt, D.C., Fischer, K., Currie, B.J., Kemp, D.J., Walton, S.F., 2005. Identification and characterization of *Sarcoptes scabiei* and *Dermatophagoides pteronyssinus*

glutathione S-transferases: implication as a potential major allergen in crusted scabies. Am. J. Trop. Med. Hyg. 73, 977-984.

– Du, Y.H., Jia, R.Y., Yin, Z.Q., Pu, Z.H., Chen, J., Yang, F., Zhang, Y.Q., Lu, Y., 2008. Acaricidal activity of extracts of neem (*Azadirachta indica*) oil against the larvae of the rabbit mite *Sarcoptes scabiei* var. *cuniculi in vitro*. Vet. Parasitol. 157, 144-148.

– Du, Y.H., Li, J.L., Jia, R.Y., Yin, Z.Q., Li, X.T., Lv, C., Ye, G., Zhang, L., Zhang, Y.Q., 2009. Acaricidal activity of four fractions and octadecanoic acid-tetrahydrofuran-3,4-diyl ester isolated from chloroform extracts of neem (*Azadirachta indica*) oil against *Sarcoptes scabiei* var. *cuniculi* larvae *in vitro*. Vet. Parasitol. 163, 175-178.

– Dupuy, A., Dehen, L., Bourrat, E., Lacroix, C., Benderdouche, M., Dubertret, L., Morel, P., Feuilhade de Chauvin, M., Petit, A., 2007. Accuracy of standard dermoscopy for diagnosing scabies. J. Am. Acad. Dermatol. 56, 53-62.

– Elder, B.L., Arlian, L.G., Morgan, M.S., 2006. *Sarcoptes scabiei* (Acari: Sarcoptidae) mite extract modulates expression of cytokines and adhesion molecules by human dermal microvascular endothelial cells. J. Med. Entomol. 43, 910-915.

– Elder, B.L., Arlian, L.G., Morgan, M.S., 2009. Modulation of human dermal microvascular endothelial cells by *Sarcoptes scabiei* in combination with proinflammatory cytokines, histamine, and lipid-derived biologic mediators. Cytokine 47, 103-111.

– Estes, S.A., Kummel, B., Arlian, L., 1983. Experimental canine scabies in humans. J. Am. Acad. Dermatol. 9, 397-401.

– Fain, A., 1968. Etude de la variabilite de *Sarcoptes scabiei* avec une revision des Sarcoptidae. Acta Zool. Pathol. Antverp. 47, 1-196.

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

– Falconi, F., Ochs, H., Deplazes, P., 2002. Serological cross-sectional survey of psoroptic sheep scab in Switzerland. Vet. Parasitol. 109, 119-127.

– Feather, L., Gough, K., Flynn, R.J., Elsheikha H.M., 2010. A retrospective investigation into risk factors of sarcoptic mange in dogs. Parasitol. Res. 107, 279-283.

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

– Fischer, K., Holt, D.C., Harumal, P., Currie, B.J., Walton, S.F., Kemp, D.J, 2003a. Generation and characterization of cDNA clones from *Sarcoptes scabiei* var. *hominis* for an expressed sequence tag library: identification of homologues of house dust mite allergens. Am. J. Trop. Med. Hyg. 68, 61-64.

– Fischer, K., Holt, D.C., Wilson, P., Davis, J., Hewitt, V., Johnson, M., McGrath, A., Currie, B.J., Walton, S.F., Kemp, D.J., 2003b. Normalization of a cDNA library cloned in lambda ZAP by a long PCR and cDNA reassociation procedure. BioTechniques 34, 250-254.

– Fitzgerald, S.D., Cooley, T.M., Murphy, A., Cosgrove, M.K., King, B.A., 2004. Sarcoptic mange in raccoons in Michigan. J. Wildl. Dis. 40, 347-350.

– Folz, S.D., Kratzer, D.D., Kakuk, T.J., Rector, D.L., 1984. Evaluation of a sponge-on therapy for canine scabies. J. Vet. Pharmacol. Ther. 7, 29-34.

– Fthenakis, G.C., Karagiannidis, A., Alexopoulos, C., Brozos, C., Papadopoulos, E., 2001. Effects of sarcoptic mange on the reproductive performance of ewes and transmission of *Sarcoptes scabiei* to newborn lambs. Vet. Parasitol. 95, 63-71.

– Fthenakis, G.C., Papadopoulos, E., Himonas, C., Leontides, L., Kritas, S., Papatsas, J., 2000. Efficacy of moxidectin against sarcoptic mange and effects on milk yield of ewes and growth of lambs. Vet. Parasitol. 87, 207-216.

– 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. Br. J. Dermatol. 163, 892-894.

– Gardner, I.A., Stryhn, H., Lind, P., Collins, M.T., 2000. Conditional dependence between tests affects the diagnostic and surveillance of animal diseases. Prev. Vet. Med. 45, 107-122.

– González-Candela, M., Cubero-Pablo, M.J., Martín-Atance, P., León-Vizcaíno, L., 2006. Potential pathogens carried by Spanish ibex (*Capra pyrenaica hispanica*) in southern Spain. J. Wildl. Dis. 42, 325-334.

– 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. J. Wildl. Dis. 40, 456-465.

– Greiner, M. Gardner, I.A., 2000. Application of diagnostic tests in veterinary epidemiologic studies. Prev. Vet. Med. 45, 43-59.

– Greiner, M., Pfeiffer, D., Smith, R.D., 2000. Principles and practical application of the receiver-operating characteristic analysis for diagnostic tests. Prev. Vet. Med. 45, 23-41.

– Gutiérrez, J.F., Méndez de Vigo, J., Castellá, J., Muñoz, E., Ferrer, D., 1996. Prevalence of sarcoptic mange in fattening pigs sacrificed in a slaughterhouse of northeastern Spain. Vet. Parasitol. 61, 145-149.

– Haas, N., Wagemann, B., Hermes, B., Henz, B.M., Heile, C., Schein, E., 2005. Crossreacting IgG antibodies against fox mite antigens in human scabies. Arch. Dermatol. Res. 296, 327-331.

– Harumal, P., Morgan, M., Walton, S.F., Holt, D.C., Rode, J., Arlian, L.G., Currie, B.J., Kemp, D.J., 2003. Identification of a homologue of a house dust mite allergen in a cDNA

library from *Sarcoptes scabiei* var *hominis* and evaluation of its vaccine potential in a rabbit *S. scabiei* var. *canis* model. Am. J. Trop. Med. Hyg. 68, 54-60.

– Heilesen, B.M., 1946. Studies on *Acarus scabiei* and scabies. Acta Derm. Venereol. 26, 1-370.

– Hidalgo-Argüello, M.R., Díez-Baños, N., Martínez-González, B., Rojo-Vázquez, F.A., 2001. Efficacy of moxidectin 1% injectable against natural infection of *Sarcoptes scabiei* in sheep. Vet. Parasitol. 102, 143-150.

– Hollanders, W., Harbers, A.H., Huige, J.C., Monster, P., Rambags, P.G., Hendrikx, W.M., 1995. Control of *Sarcoptes scabiei* var. *suis* with ivermectin: influence on scratching behaviour of fattening pigs and occurrence of dermatitis at slaughter. Vet. Parasitol. 58, 117-127.

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

– Holt, D.C., Fischer, K., Allen, G.E., Wilson, D., Wilson, P., Slade, R., Currie, B.J., Walton, S.F., Kemp, D.J., 2003. Mechanisms for a novel immune evasion strategy in the scabies mite *Sarcoptes scabiei*: a multigene family of inactivated serine proteases. J. Invest. Dermatol. 121, 1419-1424.

– Huffam, S.E., Currie, B.J., 1998. Ivermectin for *Sarcoptes scabiei* hyperinfestation. Int. J. Infect. Dis. 2, 152-154.

– Hui, S.L., Walter, S.D., 1980. Estimating the error rates of diagnostic tests. Biometrics 36, 167-171.

– Ibrahim, K.E., Abu-Samra, M.T., 1985. A severe outbreak of sarcoptic mange among goats naturally infected with a sheep strain of *Sarcoptes scabiei*. Rev. Elev. Med. Vet. Pays Trop. 38, 258-265.

– Jacober, P., Ochs, H., Torgerson, P.R., Schnyder, M., Deplazes, P., 2006. A method for sheep scab control by applying selective treatment based on flock serology. Vet. Parasitol. 136, 373-378.

– Jacobson, M., Bornstein, S., Wallgren, P., 1999. The efficacy of simplified eradication strategies against sarcoptic mange mite infections in swine herds monitored by an ELISA. Vet. Parasitol. 81, 249-258.

– Jayaraj, R., Hales, B., Viberg, L., Pizzuto, S., Holt, D., Rolland, J.M., O'Hehir, R.E., Currie, B.J., Walton, S.F., 2011. A diagnostic test for scabies: IgE specificity for a recombinant allergen of *Sarcoptes scabiei*. Diagn. Microbiol. Infect. Dis. 71, 403-407.

– 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. Intern. Med. 45, 857-859.

– Kaya, D., Inceboz, T., Kolatan, E., Güneli, E., Yilmaz, O., 2011. Comparison of efficacy of ivermectin and doramectin against mange mite (*Sarcoptes scabiei*) in naturally infested rabbits in Turkey. Vet. Ital. 46, 51-56.

– Kristjansson, A.K., Smith, M.K., Gould, J.W., Gilliam, A.C., 2007. Pink pigtails are a clue for the diagnosis of scabies. J. Am. Acad. Dermatol. 57, 174-175.

– Kuhn, C., Lucius, R., Matthes, H.F., Meusel, G., Reich, B., Kalinna, B.H., 2008. Characterisation of recombinant immunoreactive antigens of the scab mite *Sarcoptes scabiei*. Vet. Parasitol. 153, 329-337.

– Kutzer, E., Grunberg, W., 1969. Transmission of animal sarcoptic mange to human beings. Berliner und Munchener Tierarztliche Wochenschrift 82, 311-314.

– Lavín, S., Ruiz-Bascaran, M., Marco, I., Fondevila, M.D., Ramis, A.J., 2000. Experimental infection of chamois (*Rupicapra pyrenaica parva*) with *Sarcoptes scabiei* derived from naturally infected goats. J. Vet. Med. B Infect. Dis. Vet. Public Health 47, 693-499.

– Ljunggren, E.L., Bergstrom, K., Morrison, D.A., Mattsson, J.G., 2006. Characterization of an atypical antigen from *Sarcoptes scabiei* containing an MADF domain. Parasitology 132, 117-126.

– Ljunggren, E.L., Nilsson, D., Mattsson, J.G., 2003. Expressed sequence tag analysis of *Sarcoptes scabiei*. Parasitology 127, 139-145.

– Loewenstein M, Ludin A, Schuh M. 2006. Comparison of scratching behaviour of growing pigs with sarcoptic mange before and after treatment, employing two distinct approaches. Vet. Parasitol. 140, 334-343.

– Malik, R., McKellar Stewart, K., Sousa, C.A., Krockenberger, M.B., Pope, S., Ihrke, P., Beatty, J., Barrs, V.R., Walton, S., 2006. Crusted scabies (sarcoptic mange) in four cats due to *Sarcoptes scabiei* infestation. J. Feline Med. Surg. 8, 327-339.

– Martin, R.W., Handasyde, K.A., Skerratt, L.F., 1998. Current distribution of sarcoptic mange in wombats. Aust. Vet. J. 76, 411-414.

– Mattsson, J.G., Ljunggren, E.L., Bergström, K., 2001. Paramyosin from the parasitic mite *Sarcoptes scabiei*: cDNA cloning and heterologous expression. Parasitology 122, 555-562.

– McDonald, M., Currie, B.J., Carapetis, J.R., 2004. Acute rheumatic fever: a chink in the chain that links the heart to the throat? Lancet Infect. Dis. 4, 240-245.

– Meana, A., Aller, J.M. (Coordinadores), 2006. Patología cutánea de origen infeccioso y parasitario en ovino y caprino. Ovis, 102.

– Meijer, P., van Voorst Vader, P.C., 1990. Canine scabies in man. Ned. Tijdschr. Geneeskd. 134, 2491-2493.

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

– Mellanby, K., 1972. Scabies. Second Edition. Hampton (UK), E W Classey.

– Menzano, A., Rambozzi, L., Rossi, L., 2004. Outbreak of scabies in human beings, acquired from chamois (*Rupicapra rupicapra*). Vet. Rec. 155, 568.

– Menzano, A., Rambozzi, L., Rossi, L., 2007. A severe episode of wildlife-derived scabies in domestic goats in Italy. Small Rumin. Res. 70, 154-158.

– Mika, A., Goh, P., Holt, D.C., Kemp, D.J., Fischer, K., 2011. Scabies mite peritrophins are potential targets of human host innate immunity. PLoS Negl. Trop. Dis. 5, e1331.

– Millán, J., Casáis, R., Delibes-Mateos, M., Calvete, C., Rouco, C., Castro, F., Colomar, V., Casas-Díaz, E., Ramírez, E., Moreno, S., Prieto, J.M., Villafuerte, R., 2012. Widespread exposure to *Sarcoptes scabiei* in wild European rabbits (*Oryctolagus cuniculus*) in Spain. Vet. Parasitol. 183, 323-329.

– Ministerio de Medio Ambiente y Medio Rural y Marino, 2010. Anuario de Estadística 2010, 1219 pp.

– Morgan, M.S., Arlian, L.G., 2006. Enzymatic activity in extracts of allergy-causing astigmatid mites. J. Med. Entomol. 43, 1200-1207.

– Morgan, M.S., Arlian, L.G., 2010. Response of human skin equivalents to *Sarcoptes scabiei*. J. Med. Entomol. 47, 877-883.

– Moriello, K.A., 2003. Zoonotic skin diseases of dogs and cats. Anim. Health Res. Rev. 4, 157-168.

– Morris, D.O., Dunstan, R.W., 1996. A histomorphological study of sarcoptic acariasis in the dog: 19 cases. J. Am. Anim. Hosp. Assoc. 32, 119-124.

– Morsy, T.A., Rahem, M.A., el-Sharkawy, E.M., Shatat, M.A., 2003. *Eucalyptus globulus* (camphor oil) against the zoonotic scabies, *Sarcoptes scabiei*. J. Egypt. Soc. Parasitol. 33, 47-53.

– Mounsey, K., Ho, M.F., Kelly, A., Willis, C., Pasay, C., Kemp, D.J., McCarthy, J.S., Fischer, K., 2010a. A tractable experimental model for study of human and animal scabies. PLoS Negl. Trop. Dis. 4, e756.

– Mounsey, K.E., Dent, J.A., Holt, D.C., McCarthy, J., Currie, B.J., Walton, S.F., 2007. Molecular characterisation of a pH-gated chloride channel from *Sarcoptes scabiei*. Invert. Neurosci. 7, 149-156.

– Mounsey, K.E., Holt, D.C., McCarthy, J., Walton, S.F., 2006. Identification of ABC transporters in *Sarcoptes scabiei*. Parasitology 132, 883-892.

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

– Mounsey, K.E., Pasay, C.J., Arlian, L.G., Morgan, M.S., Holt, D.C., Currie, B.J., Walton, S.F., McCarthy, J.S., 2010b. Increased transcription of Glutathione S-transferases in acaricide exposed scabies mites. Parasit. Vectors 3, 43.

– Mounsey, K.E., Willis, C., Burgess, S.T., Holt, D.C., McCarthy, J., Fischer, K., 2012. Quantitative PCR-based genome size estimation of the astigmatid mites *Sarcoptes scabiei*, *Psoroptes ovis* and *Dermatophagoides pteronyssinus*. Parasit. Vectors 5, 3.

– Mueller, R.S., Bettenay, S.V., Shipstone, M., 2001. Value of the pinnal-pedal reflex in the diagnosis of canine scabies.Vet. Rec. 148, 621-623.

– Mullins, J.S., Arlian, L.G., Morgan, M.S., 2009. Extracts of *Sarcoptes scabiei* De Geer downmodulate secretion of IL-8 by skin keratinocytes and fibroblasts and of GM-CSF by fibroblasts in the presence of proinflammatory cytokines. J. Med. Entomol. 46, 845-851.

– Mulugeta, Y., Yacob, H.T., Ashenafi, H., 2010. Ectoparasites of small ruminants in three selected agro-ecological sites of Tigray Region, Ethiopia. Trop. Anim. Health Prod. 42, 1219-1224.

– Neynaber, S., Wolff, H., 2008. Diagnosis of scabies with dermoscopy. CMAJ 178, 1540-1541.

– Nong, X., Fang, C.L., Wang, J.H., Gu, X.B., Yang, D.Y., Liu, T.F., Fu, Y., Zhang, R.H., Zheng, W.P., Peng, X.R., Wang, S.X., Yang, G.Y., 2012. Acaricidal activity of extract from *Eupatorium adenophorum* against the *Psoroptes cuniculi* and *Sarcoptes scabiei in vitro*. Vet. Parasitol. en prensa (doi:10.1016/j.vetpar.2011.12.015).

– O.I.E., 2011. Manual of diagnostic tests and vaccines for terrestrial animals. Chapter 2.9.8.: Mange. http://www.oie.int

– Oleaga, A., Balseiro, A., Gortázar, C., 2008a. Sarcoptic mange in two roe deer (*Capreolus capreolus*) from northern Spain. Eur. J. Wildl. Res. 54, 134-137.

– Oleaga, A., Casáis, R., Balseiro, A., Espí, A., Llaneza, L., Hartasánchez, A., Gortázar, C., 2011. New techniques for an old disease: sarcoptic mange in the Iberian wolf. Vet. Parasitol. 181, 255-266.

– Oleaga, A., Casáis, R., González-Quirós, P., Prieto, M., Gortázar, C., 2008b. Sarcoptic mange in red deer from Spain: improved surveillance or disease emergence?. Vet. Parasitol. 154, 103-113.

– Orkin, M., Maibach, H.I., 1985. Modern aspects of scabies. Curr. Probl. Dermatol.;13, 109-127.

– Osman, S.A., Hanafy, A., Amer, S.E., 2006. Clinical and therapeutic studies on mange in horses. Vet. Parasitol. 141, 191-195.

– Papadopoulos, E., Fthenakis, G.C., 1999. Administration of moxidectin for treatment of sarcoptic mange in a flock of sheep. Small Rumin. Res. 31, 165-168.

– Papadopoulos, E., Fthenakis, G.C., Himonas, C., Tzora, A., Leontides, L., 2000. Persistent efficacy of moxidectin against *Sarcoptes scabiei* in sheep. J. Vet. Pharmacol. Ther. 23, 111-112.

– Pasay, C., Arlian, L., Morgan, M., Gunning, R., Rossiter, L., Holt, D., Walton, S., Beckham, S., McCarthy, J., 2009. The effect of insecticide synergists on the response of scabies mites to pyrethroid acaricides. PLoS Negl. Trop. Dis. 3, e354.

– Pasay, C., Arlian, L., Morgan, M., Vyszenski-Moher, D., Rose, A., Holt, D., Walton, S., McCarthy, J., 2008. High-resolution melt analysis for the detection of a mutation associated with permethrin resistance in a population of scabies mites. Med. Vet. Entomol. 22, 82-88.

– Pasay, C., Mounsey, K., Stevenson, G., Davis, R., Arlian, L., Morgan, M., Vyszenski-Moher, D., Andrews, K., McCarthy, J., 2010. Acaricidal activity of eugenol based compounds against scabies mites. PLoS One 5, e12079.

– Pasay, C., Walton, S., Fischer, K., Holt, D., McCarthy, J., 2006. PCR-based assay to survey for knockdown resistance to pyrethroid acaricides in human scabies mites (*Sarcoptes scabiei* var. *hominis*). Am. J. Trop. Med. Hyg. 74, 649-657.

– Pence, D.B., Ueckermann, E., 2002. Sarcoptic mange in wildlife. Rev. Sci. Tech. 21, 385-398.

– 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 Rev. 32, 26-39.

– Petersen, H.H., Nielsen, J.P., Heegaard, P.M., 2004. Application of acute phase protein measurements in veterinary clinical chemistry. Vet. Res. 35, 163-187.

– Prins, C., Stucki, L., French, L., Saurat, J.H., Braun, R.P., 2004. Dermoscopy for the *in vivo* detection of *Sarcoptes scabiei*. Dermatology 208, 241-243.

– Rahbari, S., Nabian, S., Bahonar, A.R., 2009. Some observations on sheep sarcoptic mange in Tehran province, Iran. Trop. Anim. Health Prod. 41, 397-401.

– Rahman, M.M., Lecchi, C., Fraquelli, C., Sartorelli, P., Ceciliani, F., 2010. Acute phase protein response in Alpine ibex with sarcoptic mange. Vet. Parasitol. 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.). Vet. Res. 35, 701-708.

– Rambozzi, L., Menzano, A., Molinar Min A.R., Rossi, L., 2007. Immunoblot analysis of IgG antibody response to *Sarcoptes scabiei* in swine. Vet. Immunol. Immunopathol. 115, 179-183.

– Ramos-e-Silva, M., 1998. Giovan Cosimo Bonomo (1663-1696): discoverer of the etiology of scabies. Int. J. Dermatol. 37, 625-630.

– Rapp, C.M., Morgan, M.S., Arlian, L.G., 2006. Presence of host immunoglobulin in the gut of *Sarcoptes scabiei* (Acari: Sarcoptidae). J. Med. Entomol. 43, 539-542.

– Reinig, E.F., Albertson, D., Sarma, D.P., 2011. Pink pigtail in a skin biopsy: What is your diagnosis? Dermatol. Online J. 17, 12.

– 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. J. Infect. 50, 375-381.

– Roncalli, R.A., 1987. The history of scabies in veterinary and human medicine from biblical to modern times. Vet. Parasitol. 25, 193-198.

– Roth, W., 1991. Scabies resistant to lindane 1% lotion and crotamiton 10% cream. J. Am. Acad. Dermatol. 24, 502-503.

– Rueda-López, M., 2006. Elimination of mange due to *Sarcoptes scabiei* var. *suis* from a 1800-sow farrow-to-finish farm. Vet. Rec. 159, 595-597.

– Ryser-Degiorgis, M.P., Ryser, A., Bacciarini, L.N., Angst, C., Gottstein, B., Janovsky, M, Breitenmoser, U., 2002. Notoedric and sarcoptic mange in free-ranging lynx from Switzerland. J. Wildl. Dis. 38, 228-232.

– Sargison, N.D., 1995. Differential diagnosis and treatment of sheep scab. In Practice 17, 3-9.

– Sharma, D.K., Saxena, V.K., Sanil, N.K., Singh, N., 1997. Evaluation of oil of *Cedrus deodara* and benzyl benzoate in sarcoptic mange in sheep. Small Rumin. Res. 26, 81-85.

– Skerratt, L.F., Campbell, N.J., Murrell, A., Walton, S., Kemp, D., Barker, S.C., 2002. The mitochondrial 12S gene is a suitable marker of populations of *Sarcoptes scabiei* from wombats, dogs and humans in Australia. Parasitol. Res. 88, 376-379.

– Smith, M.E., Claypole, C.T., 1967. Canine scabies in dogs and humans. J. Am. Med. Assoc. 199, 59-64.

– Soglia, D., Rasero, R., Rossi, L., Sartore, S., Sacchi, P., Maione, S., 2007. Microsatellites as markers for comparison among different populations of *Sarcoptes scabiei*. Ital. J. Anim. Sci. 6, 214-216.

– Speare, R., Mcconnell, A., 1997. *Sarcoptes scabiei* resistant to permethrin. Sixth Annual Scientific Meeting of the Australasian College of Tropical Medicine, 19–20 Julio de 1997, Townsville, Australia, p. 28.

– Sréter, T., Széll, Z., Varga, I., 2003. Ectoparasite infestations of red foxes (*Vulpes vulpes*) in Hungary. Vet. Parasitol. 115, 349-354.

– Steer, A.C., Jenney, A.W., Kado, J., Batzloff, M.R., La Vincente, S., Waqatakirewa, L., Mulholland, E.K., Carapetis, J.R., 2009. High burden of impetigo and scabies in a tropical country. PLoS Negl. Trop. Dis. 23, e467.

– 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. Vet. Parasitol. 67, 247-258.

– Tabassam, S.M., Iqbal, Z., Jabbar, A., Sindhu, Z.U., Chattha, A.I., 2008. Efficacy of crude neem seed kernel extracts against natural infestation of *Sarcoptes scabiei* var. *ovis*. J. Ethnopharmacol. 115, 284-287.

– Tarigan S., 2002. Dermatopathology of caprine scabies and protective immunity in sensitised goats against *Sarcoptes scabiei*. Jurnal Ilmu Ternak dan Veteriner 7, 265-271.

– Tarigan S., 2003. Histopathological changes in naïve and sensitised goats caused by *Sarcoptes scabiei*. Jurnal Ilmu Ternak dan Veteriner 8, 114-121.

– Tarigan S., 2004. Antibody responses in naïve and sensitised goats infested by *Sarcoptes scabiei*. Jurnal Ilmu Ternak dan Veteriner 9, 258-265.

– Tarigan, S., 2005. Ingestion of host inmunoglobulins by *Sarcoptes scabiei*. Jurnal Ilmu Ternak dan Veteriner 10, 35-40.

– Tarigan S., Huntley J.F., 2005. Failure to protect goats following vaccination with soluble proteins of *Sarcoptes scabiei*: evidence for a role for IgE antibody in protection. Vet. Parasitol. 133, 101-109.

– Terada, Y., Murayama, N., Ikemura, H., Morita, T., Nagata, M., 2010. *Sarcoptes scabiei* var. *canis* refractory to ivermectin treatment in two dogs. Vet. Dermatol. 21, 608-612.

– Thrusfield, M., 2005. Veterinary Epidemiology (Thrid Edition). Blackwell Science, pp 584.

– Turan, E., Erdemir, A.T., Gurel, M.S., Basaran, Y.K., 2011. The detection of *Sarcoptes scabiei* in human skin by *in vivo* confocal microscopy. Eur. J. Dermatol. 21, 1004-1005.

– Urquhart, G.M., Armour, J., Duncan, J.L., Dunn, A.M., Jennings, F.W., 1988. Sarcoptic mange of cattle. In: Veterinary Parasitology. Longman Science and Technology, Harlow, pp. 186.

– Vercruysse, J., Rehbein, S., Holdsworth, P.A., Letonja, T., Peter, R.J., 2006. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) guidelines for evaluating the efficacy of acaricides against (mange and itch) mites on ruminants. Vet. Parasitol. 136, 55-66.

– Wallgren, P., Bornstein, S., 1997. The spread of porcine sarcoptic mange during the fattening period revealed by development of antibodies to *Sarcoptes scabiei*. Vet. Parasitol. 73, 315-324.

– Walton, S.F., Choy, J.L., Bonson, A., Valle, A., McBroom, J., Taplin, D., Arlian, L., Mathews, J.D., Currie, B., Kemp, D.J., 1999. Genetically distinct dog-derived and human-derived *Sarcoptes scabiei* in scabies-endemic communities in northern Australia. Am. J. Trop. Med. Hyg. 61, 542-547.

– Walton, S.F., Currie, B.J., Kemp, D.J., 1997. A DNA fingerprinting system for the ectoparasite *Sarcoptes scabiei*. Mol. Biochem. Parasitol. 85, 187-196.

– Walton, S.F., Dougall, A., Pizzutto, S., Holt, D., Taplin, D., Arlian, L.G., Morgan, M., Currie, B.J., Kemp, D.J., 2004a. Genetic epidemiology of *Sarcoptes scabiei* (Acari: Sarcoptidae) in northern Australia. Int. J. Parasitol. 34, 839-849.

– Walton, S.F., McKinnon, M., Pizzutto, S., Dougall, A., Williams, E., Currie, B.J., 2004b. Acaricidal activity of *Melaleuca alternifolia* (tea tree) oil: *in vitro* sensitivity of *Sarcoptes scabiei* var. *hominis* to terpinen-4-ol. Arch. Dermatol. 140, 563-566.

– Walton, S.F., Myerscough, M.R., Currie, B.J., 2000. Studies *in vitro* on the relative efficacy of current acaricides for *Sarcoptes scabiei* var. *hominis*. Trans. R. Soc. Trop. Med. Hyg. 94, 92-96.

– Wells, B., Burgess, S.T., McNeilly, T.N., Huntley, J.F., Nisbet, A.J., 2012. Recent developments in the diagnosis of ectoparasite infections and disease through a better understanding of parasite biology and host responses. Mol. Cell. Probes 26, 47-53.

– Willadsen, P., 1997. Novel vaccines for ectoparasites. Vet. Parasitol. 71, 209-222.

– Willis, C., Fischer, K., Walton, S.F., Currie, B.J., Kemp, D.J., 2006. Scabies mite inactivated serine protease paralogues are present both internally in the mite gut and externally in feces. Am. J. Trop. Med. Hyg. 75, 683-687.

– Woodley, D., Saurat, J.H., 1981. The burrow ink test and the scabies mite. J. Am. Acad. Dermatol. 4, 715-722.

– Xu, J., Fan, Q.J., Yin, Z.Q., Li, X.T., Du, Y.H., Jia, R.Y., Wang, K.Y., Lv, C., Ye, G., Geng, Y., Su, G., Zhao, L., Hu, T.X., Shi, F., Zhang, L., Wu, C.L., Tao, C., Zhang, Y.X., Shi, D.X., 2010. The preparation of neem oil microemulsion (*Azadirachta indica*) and the

comparison of acaricidal time between neem oil microemulsion and other formulations *in vitro*. Vet. Parasitol. 169, 399-403.

– Zahler, M., Essig, A., Gothe, R., Rinder, H., 1999. Molecular analyses suggest monospecificity of the genus *Sarcoptes* (Acari: Sarcoptidae). Int. J. Parasitol. 29, 759-766.