



Clinical gamasoidosis and antibody response in two patients infested with *Ornithonyssus bursa* (Acari: Gamasida: Macronyssidae)

José Francisco Lima-Barbero^{1,2} · Marta Sánchez Sánchez¹ · Alejandro Cabezas-Cruz³ · Lourdes Mateos-Hernández³ · Marinela Contreras¹ · Isabel G. Fernández de Mera¹ · Margarita Villar¹ · José de la Fuente^{1,4} 

Received: 29 March 2019 / Accepted: 24 July 2019 / Published online: 31 July 2019
© Springer Nature Switzerland AG 2019

Abstract

Blood-feeding ectoparasites constitute a growing burden for human and animal health, and animal production worldwide. In particular, mites (Acari: Gamasida) of the genera *Dermanyssus* (Dermanyssidae) and *Ornithonyssus* (Macronyssidae) infest birds and cause gamasoidosis in humans. The tropical fowl mite, *Ornithonyssus bursa*, is commonly found in tropical and subtropical countries but rarely reported in Europe. In this research we characterized the first two cases in Spain of clinical gamasoidosis diagnosed in patients infested with *O. bursa*, and investigated the IgE, IgM and IgG antibody response to mite proteins and the carbohydrate Gal α 1-3Gal β 1-(3)4GlcNAc-R (α -Gal) involved in the tick-bite associated alpha-Gal syndrome (AGS). The results suggested that *O. bursa* is establishing across Mediterranean countries, and may increase the risk for gamasoidosis. The immune antibody response to mite proteins was higher for IgM and similar for IgE and IgG antibodies between patients and non-allergic control individuals exposed to mite or tick bites. The anti- α -Gal antibody levels were similar between patients and controls, a result supported by the absence of this carbohydrate in mites. These results suggested that mite bites do not correlate with antibody response to acarine proteins or α -Gal, and are not associated with the AGS.

Keywords Mite · Humoral immune response · α -Gal · Acariasis · Dermatitis · Gamasoidosis · Alpha-Gal syndrome

✉ José de la Fuente
jose_delafuente@yahoo.com

¹ SaBio, Instituto de Investigación en Recursos Cinegéticos IREC-CSIC-UCLM-JCCM, Ronda de Toledo 12, 13071 Ciudad Real, Spain

² Sabiotec, S.A. Ed. Polivalente UCLM, Camino de Moledores, 13005 Ciudad Real, Spain

³ UMR BIPAR, INRA, ANSES, Ecole Nationale Vétérinaire d'Alfort, Université Paris-Est, 94700 Maisons-Alfort, France

⁴ Department of Veterinary Pathobiology, Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, OK 74078, USA

Introduction

The main hosts of avian mites (Acari: Gamasida) are various species of wild and domestic birds, and mammals are only accidental hosts (Sikes and Chamberlain 1954; Roy et al. 2010). Differences exist in the ecology and biology of the various mite species, but most of the cases of avian mites feeding on humans are associated to the disappearing of their avian host which forces them to seek new meal sources (Regan et al. 1987; Orton et al. 2000). The poultry red mite, *Dermanyssus gallinae* (De Geer) (Dermanyssidae), is the most commonly reported avian mite which bites humans (George et al. 2015), followed by *Ornithonyssus* species (Macronyssidae) including the northern fowl mite, *Ornithonyssus sylviarum* (G. Canestrini & Fanzago) and the tropical fowl mite, *Ornithonyssus bursa* (Berlese) (Orton et al. 2000).

The tropical fowl mite is a common blood-sucking ectoparasite of birds that is mostly distributed across tropical and subtropical countries (Denmark and Cromroy 2003; Oliveira et al., 2012). It has been reported that *O. bursa* are unable to feed on humans under laboratory conditions (Sikes and Chamberlain 1954). In Europe, its presence has been only described on swallows in Denmark (Gjelstrup and Møller 1986) and on humans in Italy (Castelli et al. 2015). In tropical and sub-tropical countries, *O. bursa* is involved in the development of human gamasoidosis (Fox 1957; Semenas and Rocha 1998; Mentz et al. 2015; Bassini-Silva et al. 2019). Gamasoidosis, also known as avian-mite dermatitis and acariasis, is a frequently unrecognised ectoparasitosis in human medicine (Orton et al. 2000). Lesions often range from papules to grouped papular urticarial or papulovesicular eruption (Orton et al. 2000).

The alpha-Gal syndrome (AGS) is an allergic reaction triggered by IgE antibodies against the carbohydrate Gal α 1-3Gal β 1-(3)4GlcNAc-R (α -Gal) (van Nunen et al. 2007; Commins et al. 2009; recently reviewed by de la Fuente et al. 2019). The AGS is associated to tick bites and the α -Gal modification is present in glycoproteins from ticks and non-carnivorous mammals (van Nunen et al. 2007; Commins et al. 2009; Cabezas-Cruz et al. 2014, 2018; Steinke et al. 2015; Platts-Mills et al. 2015; Mateos-Hernández et al. 2017; Galili 2018; Hilger et al. 2019). Tick bites induce high levels of anti- α -Gal IgE antibodies in humans that mediate delayed anaphylaxis to red meat consumption, and immediate anaphylaxis to tick bites, xenotransplantation and certain drugs such as cetuximab (Mateos-Hernández et al. 2017; Hilger et al. 2019). However, the possible involvement of other blood sucking acarines in the AGS has not been investigated.

In this work we present the first report of gamasoidosis due to *O. bursa* in two patients in Spain, and investigated the possible implication of humoral immune response against mite proteins and α -Gal in clinical gamasoidosis.

Materials and methods

Patients with clinical gamasoidosis and collection of serum samples

An adult male (76 years old) (patient 1) and an adult female (71 years old) (patient 2), members of the same family, were reported with clinical gamasoidosis in June 2018 in the city of Jafre (Girona, Spain). Blood samples were collected from patient 1, patient 2, an individual exposed to *D. gallinae* bites (control 1), and a non-allergic individual

with record of tick bites (control 2). Serum was collected and conserved at -80°C until analyzed. The blood extraction with research purposes was done with the individuals' informed consent at their respective primary healthcare centres.

Mite collection and identification

Mites were recovered by patients with adhesive tape mostly from head and arms. Nine of these mites were preserved in 96% ethanol. Specimens were cleared in Nesbitt's fluid and slide-mounted with Hoyer's medium according to Walter and Krantz (2009). Morphological identification of four adult female mites was carried out under a light microscope. Every studied specimen was classified as *O. bursa* according to Denmark and Cromroy (2003), Radovsky (2007) and Ueckermann et al. (2016).

Preparation of mite soluble protein extracts

Poultry red mite, a mite closely related to *O. bursa*, was used for preparation of soluble protein extracts. One hundred starved adult mites were snap frozen in liquid nitrogen and disgregated with pestle and mortar. The disgregated material was resuspended in ice-cold PBS supplemented with complete Protease Inhibitor Cocktail (Roche Diagnostics, Mannheim, Germany). After centrifugation at 5000 rpm for 20 min at 4°C , insoluble material and debris were removed and soluble material was decanted and centrifuged for a second time. The resulting soluble proteins were immediately snap frozen and stored at -80°C until used.

Determination of IgE, IgM and IgG antibody titres against mite proteins

Soluble protein extracts from *D. gallinae* were used as mite antigen to coat the ELISA plates. Plates were coated with 50 ng of mite proteins per well in carbonate/bicarbonate buffer and incubated overnight at 4°C . Following four washes with 200 μl of PBS containing 0.05% Tween 20 (PBST), blocking was carried out using 100 μl per well of 1% human serum albumin (HSA) in PBS and incubated for 1 h at room temperature. Following four washes with PBST, patient and control sera were added at 1:50 dilution in PBS, incubated for 1 h at 37°C and rewashed $4\times$ with PBST. Then, 100 μl per well of goat anti-human immunoglobulin-peroxidase IgG (FC specific), IgM (μ -chain specific), and IgE (ϵ -chain specific) (Sigma-Aldrich, Madrid, Spain) were added at 1:1000 dilution in blocking buffer (1% HSA in phosphate buffered saline, PBS supplemented with 0.05% Tween 20) (Sigma-Aldrich). The plates were incubated for 1 h at 37°C and then washed $4\times$ with PBST. The reaction was visualised by adding 100 μl of 3,3',5,5'-tetramethylbenzidine (TMB) (Promega, Madison, WI, USA) and incubated for 20 min in the dark at room temperature. After stopping the reaction with 50 μl sulfuric acid, the optical density (O.D.) was measured at 450 nm with a Multiskan FC ELISA reader (Thermo Fisher, Waltham, MA, USA). The average value of the blanks (wells without mite proteins coating; $N=4$) was subtracted from all reads, and the average of three technical replicates for each sample was used for analysis. Normalized values were compared between patients and controls by Student's *t* test with unequal variance ($\alpha=0.05$; $N=2$ biological replicates).

Determination of IgE, IgM and IgG antibody titres against α -Gal

Anti- α -Gal IgG, IgM and IgE antibody titers were determined in serum samples from patients and control individuals following Mateos-Hernández et al. (2017) with some minor modifications. ELISA plates were coated with 100 μ l/well (50 ng) of Gal1-3Gal β 1-4GlcNAc-Human serum albumin (HSA) (Carbosynth, Compton, UK) in carbonate/bicarbonate buffer and incubated overnight at 4 °C. Then, 100 μ l of blocking buffer (Sigma-Aldrich) was added to each well and incubated for 1 h at room temperature followed by four washes with PBST. Sera were added to plates at 1:500 dilutions in blocking buffer and incubated for 1 h at 37 °C, followed by four washes with PBST. Plates were incubated with goat anti-human immunoglobulins-peroxidase IgG, IgM, or IgE as described above for mite proteins. The average value of the blanks (wells without Gal α 1-3Gal β 1-4GlcNAc-HSA coating; N = 4) was subtracted from all reads, and the average of four technical replicates for each sample was used for analysis. Normalized values were compared between patients and controls by Student's t-test with unequal variance (α = 0.05; N = 2 biological replicates).

Determination of α -Gal levels in *Dermanyssus gallinae*

For protein extraction, 100 mg of *D. gallinae* tissue was homogenized in 500 μ l PBS with 1% Triton X-100 (Sigma-Aldrich) using steel balls and the homogenizer Precellys 24 Dual (Bertin, France) at 6000 rpm for 3 $\times$ 30 s each. Proteins were quantified using the bicinchoninic acid assay (BCA) (Thermo Fisher) with BSA as standard. To determine α -Gal levels, an inhibition ELISA was performed as previously reported by Lu et al. (2018). Briefly, increasing concentrations (5, 10, 15 and 20 ng/ μ l) of total proteins were incubated with the anti- α -Gal monoclonal antibody M86 mAb (Enzo Life Sciences, Farmingdale, NY, USA). The relative amount of unbound M86 mAb was then measured by ELISA using Gal- α 1-3Gal-BSA (α -Gal-BSA) (Dextra Laboratories, Bristol, UK) as coating antigen (200 ng/well). The goat anti-mouse IgM-HRP (Sigma-Aldrich) was used as secondary antibody and the O.D. was measured at 450 nm with a Multiskan FC ELISA reader (Thermo Fisher). Three replicates were used for each sample. The average value of the blanks was subtracted from all reads before further analysis. The inhibition (%) was calculated as $[(\text{O.D. M86 mAb alone}) - (\text{O.D. M86 mAb incubated with X ng}/\mu\text{l proteins})] \times 100 / (\text{O.D. M86 mAb alone})$, where X corresponds to the various concentrations of total protein extracts used for incubation with M86 mAb in the ELISA. Total proteins extracted from *Sus scrofa* kidney samples and α -Gal-BSA were used as positive controls. Total proteins extracted from a kidney sample from α -Gal-deficient (α -Gal KO) *S. scrofa* were used as negative control.

Sequence analysis in Dermanyssoidea species

Taxonomy for Dermanyssoidea species was investigated using the NCBI Taxonomy database (Federhen 2012). Analysis of protein sequence identity among Dermanyssoidea and the systematic search of the *D. gallinae* genome (Burgess et al. 2018) with galactosyltransferase sequences previously identified in arthropods (Cabezas-Cruz et al. 2018) were conducted using the Blastp tool from BLAST (Altschul et al. 1990; Madden et al. 1996).

Results and discussion

Case report of clinical gamasoidosis caused by *Ornithonyssus bursa*

During June 2018, two cases of gamasoidosis were reported in the city of Jafre (Girona, Spain). An adult male (76 years old) (patient 1) and an adult female (71 years old) (patient 2), members of the same family, suffered from severe itching. Both patients attended their primary health centre and were initially diagnosed with unspecific bug bites. The lesions were mostly distributed through arms, torso and head (Fig. 1A). Patients suffered of papulous dermatitis due to the presence of erythematous and urticarial papules, and patient 1 showed a more severe dermatitis with over 100 papules (Fig. 1A). Patients associated the itching with working in the garden. The house garden included several pine trees where a group of approximately 40 Eurasian collared doves (*Streptopelia decaocto*) were found roosting and nesting. The feeling of being bitten allowed the patients to identify and collect the mites. Collected mites were classified as *O. bursa* based on (i) article II of the chelicerae elongated with no basal bottleneck, (ii) sternal shield broader than long, (iii) three pairs of setae on the sternal plate, (iv) dorsal shield tapers posteriorly, and (v) anal shield pear-shaped and anal opening in the anterior half of the plate (Fig. 1B). Although a topical

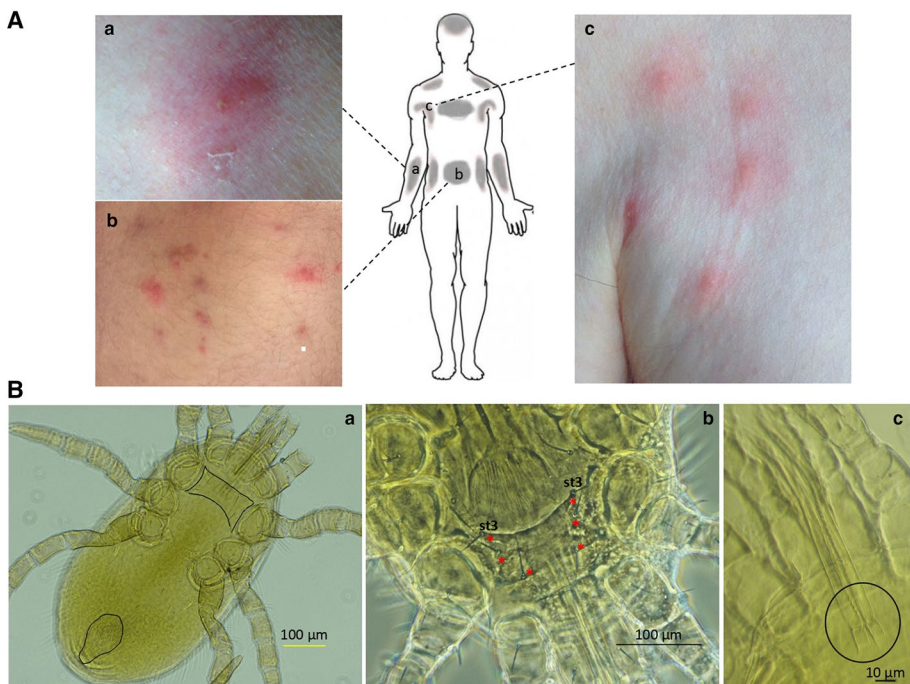


Fig. 1 Clinical gamasoidosis caused by *Ornithonyssus bursa*. **A** Distribution of papular dermatitis lesions associated with clinical gamasoidosis in Spanish patients included (a) excoriations on the arms, (b) papular lesions on the abdomen, and (c) dermatitis on the arms. **B** *Ornithonyssus bursa* taxonomic characteristics identified in female adult specimens (10× under the light microscope) included (a) broader than longer sternal shield and a pear-shaped anal shield and anal opening in the anterior half of the plate, (b) with a third pair of setae (st3) in the posterior corner of the shield (20×), and (c) an article II of the chelicerae elongated with no basal bottleneck (40×)

treatment with a corticosteroid cream was established for both patients, the lesions lasted for approximately 1 month. Lesions did not disappear until the doves were displaced, the nests were removed, and the external walls of the house were sprayed once a week with alphacypermethrin (Camaleón Plus, Bioplagen, Bollullos de la Mitación, Seville, Spain).

Gamasoidosis is difficult to diagnose in its initial phases, and it is often interpreted by patients and medical staff as undetermined bug bites, unless a clinical suspicion as identified in the current case is present (Krinsky 1983). Furthermore, some of the avian mites do not stay on the host for long periods of time as they usually leave after the blood meal, which causes the lack of determination of the etiological agents unless the mites are found on the patient. Finally, the identification of mite species requires specific training and knowledge to be reliable on the diagnosis. These limitations in the diagnosis of gamasoidosis affect the efficacy of the prescribed treatment. As shown here, topical corticosteroid ointment was not useful for resolving the dermatitis signs, probably due to a continued mite reinfestation. In the present case, the effective treatment measures were those related to the elimination of the causative mites, such as the removal of the bird nests and the disinfestation of the environment.

Humoral immune response to mite proteins and α -Gal

The response to ectoparasite bites varies from no reaction to a local reaction at the bite site or a generalized anaphylactic reaction (Castelli et al. 2008; Okahashi et al. 2015; Cabezas-Cruz et al. 2015; Mateos-Hernández et al. 2017; Jackson, 2018; Schlosser et al. 2019). Tick bites trigger a humoral immune response in infested individuals, but allergic reactions to tick bites involve, among others, symptoms of the AGS that are associated with anti- α -Gal IgE levels (Mateos-Hernández et al. 2017; Hodžić et al. 2019; Mateo-Borrega et al. 2019). Furthermore, these findings correlate with the capacity of ticks to synthesize α -Gal attached to glycoproteins differentially recognized by patients with AGS (Mateos-Hernández et al. 2017; Cabezas-Cruz et al. 2018). In contrast, anti- α -Gal IgE/IgG levels have been correlated with protection against infectious diseases such as malaria, tuberculosis and leishmaniosis caused by pathogens with this modification on their surface (Yilmaz et al. 2014; Cabezas-Cruz et al. 2017; Iniguez et al. 2017). However, the α -Gal content in other blood-feeding acarines and the role of humoral immune response to this carbohydrate and mite proteins in allergic reactions has not been investigated.

To address this question, IgG, IgM and IgE antibody titers against mite proteins and α -Gal were investigated in the patients with clinical gamasoidosis and control individuals exposed to mites and ticks. Proteins from *D. gallinae* were used as a proxy for *O. bursa* because these organisms are closely related Dermanyssoidea species (<https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Undefined&id=41438&lvl=3&keep=1&srchmode=1&unlock>). For example, although little information is available for *Ornithonyssus* spp. protein sequences, a 83.82–85.55% amino acid sequence identity (E-value $8e-96$ to $3e-104$) exists between *O. sylvarum* cytochrome oxidase subunit I (CBI75315.1) and *Dermanyssus* spp. including *D. gallinae* (85.55% identity, E-value = $1e-101$; BAU09261.1).

The analysis of serum samples from patients and control individuals revealed IgG, IgM and IgE antibody titers against mite proteins (Fig. 2a). Patient 1 with a more severe dermatitis than patient 2 showed higher IgG but not IgM or IgE antibody levels than other individuals. A lower level of IgM antibody titers was observed in the patients when compared to the control samples ($p=0.025$) (Fig. 2a). The same tendency was observed with IgE

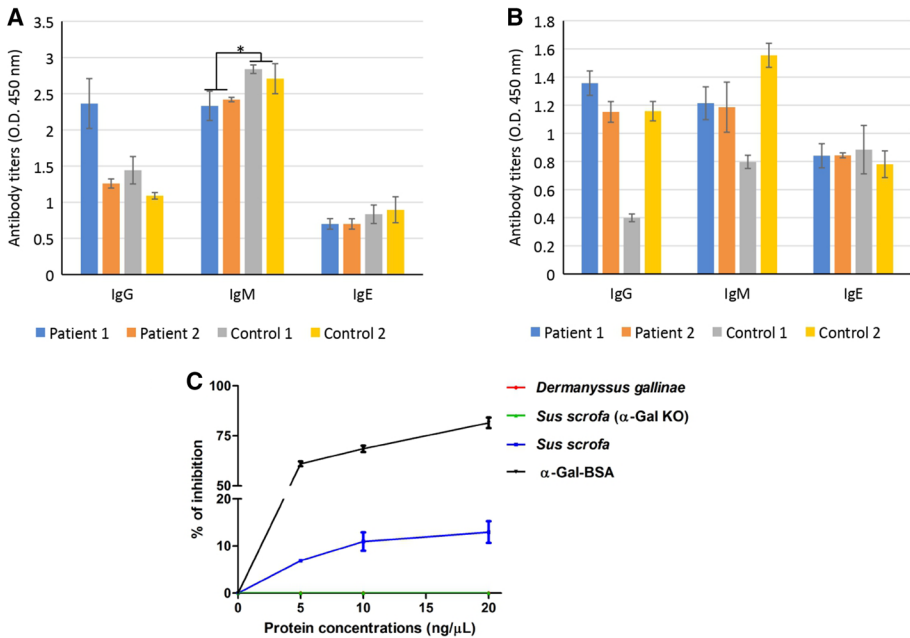


Fig. 2 Immune antibody response to mite proteins and α -Gal. **a** Mean ($\pm$ SD; $N=3$ technical replicates) IgG, IgM and IgE antibody titers to **(a)** *Dermanyssus gallinae* proteins and **b** α -Gal, determined by ELISA in serum samples from patients and control individuals. Antibody titres were determined as O.D. at 450 nm. Antibody titers from patient and control sera were compared by Student's t-test with unequal variance (**a**— $p=0.025$; **b**— $p>0.05$; both $N=2$ biological replicates). **c** Absence of α -Gal in *D. gallinae* mites. The inhibition (%) of the binding capacity of the M86 mAb produced by increasing concentrations of total proteins extracted from *D. gallinae* was determined by ELISA. Total proteins extracted from *Sus scrofa* kidneys and α -Gal-BSA were used as positive controls. Total proteins extracted from α -Gal-deficient *S. scrofa* (α -Gal KO) were used as negative control. Increasing concentrations of total protein extracts of *D. gallinae* or α -Gal-deficient *S. scrofa* did not inhibit M86 mAb binding to α -Gal-BSA. In contrast, increasing concentrations of *S. scrofa* kidney protein extract and α -Gal-BSA inhibited M86 mAb binding to α -Gal-BSA in a dose-dependent manner

antibody titers but without statistical significance ($p=0.059$) (Fig. 2a). However, although control 1 individual showed lower levels of IgG and IgM antibodies against α -Gal, no differences were observed in the IgG, IgM and IgE antibody levels between patients and control individuals (Fig. 2b). As expected, higher IgM/IgG than IgE anti- α -Gal antibody levels were observed in most individuals as these antibodies are now recognized as among the most abundant antibodies produced in response to gut microbiota (Galili 1984). The limited number of individuals included in the study and the influence of other variables (e.g., individual's age) may affect the results of the antibody against mite proteins and α -Gal. Nevertheless, these results suggested that no correlation exists between clinical gamasoidosis and the antibody response to mite proteins and α -Gal.

Additionally, these results together with the fact that control 1 individual exposed to *D. gallinae* bites showed the lowest IgG and IgM antibody levels to α -Gal posed the question about the α -Gal content in mites. The results of the ELISA inhibition with *D. gallinae* protein extracts and in comparison with positive and negative control samples indicated that mites do not contain α -Gal or the levels are very low (Fig. 2c). This result was further supported by the absence of galactosyltransferase sequences previously identified in

arthropods (Cabezas-Cruz et al. 2018) in the *D. gallinae* genome (Burgess et al. 2018). Nevertheless, ticks inhibit host immune response by different mechanisms (Mans and Neitz 2004; de la Fuente et al. 2016, 2017) that may affect individual responses to ectoparasite bites (e.g., lower IgM antibody levels to mite proteins in patients when compared to controls; Fig. 2a) (Waitayakul et al. 2006; Mateos-Hernández et al. 2017; Mateo-Borrega et al. 2019), but these mechanisms are not known in mites.

In summary, this report together with the recent human infestation in Italy (Castelli et al. 2015) suggested that *O. bursa* is establishing across Mediterranean countries. Warmer winters due to the global warming are probably facilitating the survival of the mites that reach these countries by migratory birds or by international trade of live birds. Although the risk for gamasoidosis may increase with *O. bursa* expansion, the results reported here suggested that mite bites do not correlate with antibody titers to acarine proteins or α -Gal, and are not associated with the AGS.

Acknowledgements We thank Angelika Schnieke and Konrad Fischer (Livestock Biotechnology, School of Life Sciences Weihenstephan, Technische Universität München, Freising, Germany) for providing kidney samples from α -Gal-deficient (α -Gal KO) *S. scrofa*. This research was supported by the Consejería de Educación, Cultura y Deportes, JCCM, Spain, project CCM17-PIC-036 (SBPLY/17/180501/000185). JFLB was supported by Doctorado Industrial from Ministerio de Ciencia, Innovación y Universidades, Spain (contract DI-14-06917) and Sabioteq S.A., Spain. MV was funded by the Universidad de Castilla La Mancha, Spain.

References

- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. *J Mol Biol* 215:403–410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2)
- Bassini-Silva R, Jacinavicius FDC, Hernandez FA, Ochoa R, Bauchan GR, Dowling AP, Barros-Battesti DM (2019) Dermatitis in humans caused by *Ornithonyssus bursa* (Berlese 1888) (Mesostigmata: Macronyssidae) and new records from Brazil. *Rev Bras Parasitol Vet* 1:4. <https://doi.org/10.1590/s1984-296120180097>
- Burgess STG, Bartley K, Nunn F, Wright HW, Hughes M, Gemmell M, Haldenby S, Paterson S, Rombauts S, Tomley FM, Blake DP, Pritchard J, Schicht S, Strube C, Øines Ø, Van Leeuwen T, Van de Peer Y, Nisbet AJ (2018) Draft genome assembly of the poultry red mite, *Dermanyssus gallinae*. *Microbiol Resour Announc* 7(18):e01221-18. <https://doi.org/10.1128/mra.01221-18>
- Cabezas-Cruz A, Valdés J, de la Fuente J (2014) Cancer research meets tick vectors for infectious diseases. *Lancet Infect Dis* 10:916–917. [https://doi.org/10.1016/S1473-3099\(14\)70902-8](https://doi.org/10.1016/S1473-3099(14)70902-8)
- Cabezas-Cruz A, Mateos-Hernández L, Pérez-Cruz M, Valdés J, Fernández de Mera IG, Villar M, de la Fuente J (2015) Regulation of the immune response to α -Gal and vector-borne diseases. *Trends Parasitol* 31:470–476. <https://doi.org/10.1016/j.pt.2015.06.016>
- Cabezas-Cruz A, Mateos-Hernández L, Alberdi P, Villar M, Riveau G, Hermann E, Schacht A, Khalife J, Correia-Neves M, Gortazar C, de la Fuente J (2017) Effect of blood type on anti- α -Gal immunity and the incidence of infectious diseases. *Exp Mol Med* 49:e301. <https://doi.org/10.1038/emmm.2016.164>
- Cabezas-Cruz A, Espinosa PJ, Alberdi P, Símio L, Valdés JJ, Mateos-Hernández L, Contreras M, Villar M, de la Fuente J (2018) Tick galactosyltransferases are involved in α -Gal synthesis and play a role during *Anaplasma phagocytophilum* infection and *Ixodes scapularis* tick vector development. *Sci Rep* 8:14224. <https://doi.org/10.1038/s41598-018-32664-z>
- Castelli E, Caputo V, Morello V, Tomasino RM (2008) Local reactions to tick bites. *Am J Dermatopathol* 30:241–248. <https://doi.org/10.1097/DAD.0b013e3181676b60>
- Castelli E, Viviano E, Torina A, Caputo V, Bongiorno MR (2015) Avian mite dermatitis: an Italian case indicating the establishment and spread of *Ornithonyssus bursa* (Acari: Gamasida: Macronyssidae) (Berlese, 1888) in Europe. *Int J Dermatol* 54:795–799. <https://doi.org/10.1111/ijd.12739>
- Commins SP, Satinover SM, Hosen J, Mozena J, Borish L, Lewis BD, Woodfolk JA, Platts-Mills TAE (2009) Delayed anaphylaxis, angioedema, or urticaria after consumption of red meat in patients with IgE antibodies specific for galactose- α -1,3-galactose. *J Allergy Clin Immunol* 123:426–433. <https://doi.org/10.1016/j.jaci.2008.10.052>

- de la Fuente J, Villar M, Cabezas-Cruz A, Estrada-Peña A, Ayllón N, Alberdi P (2016) Tick-host-pathogen interactions: conflict and cooperation. *PLoS Pathog* 12:e1005488. <https://doi.org/10.1371/journal.ppat.1005488>
- de la Fuente J, Antunes S, Bonnet S, Cabezas-Cruz A, Domingos A, Estrada-Peña A, Johnson N, Kocan KM, Mansfield KL, Nijhof A, Papa A, Rudenko N, Villar M, Alberdi P, Torina A, Ayllón N, Vancová M, Golovchenko M, Grubhoffer L, Caracappa S, Fooks AR, Gortazar C, Rego ROM (2017) Tick-pathogen interactions and vector competence: identification of molecular drivers for tick-borne diseases. *Front Cell Infect Microbiol* 7:114. <https://doi.org/10.3389/fcimb.2017.00114>
- de la Fuente J, Pacheco I, Villar M, Cabezas-Cruz A (2019) The alpha-Gal syndrome: new insights into the tick-host conflict and cooperation. *Parasit Vectors* 12(1):154
- Denmark HA, Cromroy HL (2003) Tropical Fowl Mite, *Ornithonyssus bursa* (Berlese) (Arachnida: Acari: Macronyssidae). Bulletin EENY-297. Gainesville, University of Florida. <http://edis.ifas.ufl.edu/pdffiles/IN/IN57500.pdf>
- Federhen S (2012) The NCBI taxonomy database. *Nucleic Acids Res* 40:D136–D143. <https://doi.org/10.1093/nar/gkr1178>
- Fox I (1957) *Ornithonyssus bursa* (Berlese) attacking Man, in Puerto Rico. *J Econ Entomol* 50:838. <https://doi.org/10.1093/jee/50.6.838>
- Galili U (1984) A unique natural human IgG antibody with anti-alpha-galactosyl specificity. *J Exp Med* 160:1519–1531. <https://doi.org/10.1084/jem.160.5.1519>
- Galili U (2018) Evolution in primates by “Catastrophic-selection” interplay between enveloped virus epidemics, mutated genes of enzymes synthesizing carbohydrate antigens, and natural anticarbohydrate antibodies. *Am J Phys Anthropol* 168:352–363. <https://doi.org/10.1002/ajpa.23745>
- George DR, Finn RD, Graham KM, Mul MF, Maurer V, Moro CV, Sparagano OA (2015) Should the poultry red mite *Dermatophysson gallinae* be of wider concern for veterinary and medical science? *Parasites Vectors* 8:178. <https://doi.org/10.1186/s13071-015-0768-7>
- Gjelstrup P, Møller AP (1986) A tropical mite, *Ornithonyssus bursa* (Berlese, 1888) (Macronyssidae, Gamasida) in Danish swallow (*Hirundo rustica*) nests with a review of mites and ticks from Danish birds. *Entomol Medd* 5:119–122
- Hilger C, Fischer J, Wölbing F, Biedermann T (2019) Role and mechanism of galactose-alpha-1,3-galactose in the elicitation of delayed anaphylactic reactions to red meat. *Curr Allergy Asthma Rep* 19:3. <https://doi.org/10.1007/s11882-019-0835-9>
- Hodžić A, Mateos-Hernández L, de la Fuente J, Cabezas-Cruz A (2019) Delayed hypersensitivity reaction to mammalian galactose- α -1,3-galactose (α -Gal) after repeated tick bites in a patient from France. *Ticks Tick Borne Dis* 10:1057–1059. <https://doi.org/10.1016/j.ttbdis.2019.05.017>
- Iniguez E, Schocker NS, Subramaniam K, Portillo S, Montoya AL, Al-Salem WS, Torres CL, Rodriguez F, Moreira OC, Acosta-Serrano A, Michael K, Almeida IC, Maldonado RA (2017) An α -Gal-containing neoglycoprotein-based vaccine partially protects against murine cutaneous leishmaniasis caused by *Leishmania major*. *PLoS Negl Trop Dis* 11:e0006039. <https://doi.org/10.1371/journal.pntd.0006039>
- Jackson WL (2018) Mammalian meat allergy following a tick bite: a case report. *Oxf Med Case Rep* 2:58–60. <https://doi.org/10.1093/omcr/omx098>
- Krinsky WL (1983) Dermatoses associated with the bites of mites and ticks (Arthropoda: Acari). *Int J Dermatol* 22:75–91
- Lu Y, Shao A, Shan Y, Zhao H, Leiguo M, Zhang Y, Tang Y, Zhang W, Jin Y, Xu L (2018) A standardized quantitative method for detecting remnant alpha-Gal antigen in animal tissues or animal tissue-derived biomaterials and its application. *Sci Rep* 8:15424. <https://doi.org/10.1038/s41598-018-32959-1>
- Madden TL, Tatusov RL, Zhang J (1996) Applications of network BLAST server. *Methods Enzymol* 266:131–141. [https://doi.org/10.1016/S0076-6879\(96\)66011-X](https://doi.org/10.1016/S0076-6879(96)66011-X)
- Mans BJ, Neitz AW (2004) Adaptation of ticks to a blood-feeding environment: evolution from a functional perspective. *Insect Biochem Mol Biol* 34:1–17. <https://doi.org/10.1016/j.ibmb.2003.09.002>
- Mateo-Borrega MB, Garcia B, Hernando Larramendi C, Azofra J, González-Mancebo E, Alvarado MI, Alonso Díaz de Durana MD, Núñez-Orjales R, Diéguez MC, Guilarte M, Soriano-Galarraga AM, Sosa G, Ferrer A, García-Moral A, Beristain AM, Bartra J (2019) IgE-mediated sensitization to galactose-alpha-1,3-galactose (α -gal) in urticaria and anaphylaxis in Spain: geographical variations and risk factors. *J Investig Allergol Clin Immunol*. <https://doi.org/10.18176/jiaci.0373>
- Mateos-Hernández L, Villar M, Moral A, García-Rodríguez C, Arias TA, de la Osa V, Feo F, Fernández de Mera IG, Alberdi P, Ruiz-Fons F, Cabezas-Cruz A, Estrada-Peña A, de la Fuente J (2017) Tick-host conflict: immunoglobulin E antibodies to tick proteins in patients with anaphylaxis to tick bite. *Oncotarget* 8:20630–20644. <https://doi.org/10.18632/oncotarget.15243>

- Mentz MB, Silva GLD, Silva CE (2015) Dermatitis caused by the tropical fowl mite *Ornithonyssus bursa* (Berlese)(Acari: Macronyssidae): a case report in humans. Rev Soc Bras Med Trop 48:786–788. <https://doi.org/10.1590/0037-8682-0170-2015>
- Okahashi K, Oiso N, Yano Y, Kawada A (2015) Tick attachment cement with a feeding cavity in the deep dermis of the penis. Acta Derm Venereol 95:741–742. <https://doi.org/10.2340/00015555-2021>
- Oliveira CB, Tonin AA, Monteiro SG (2012) Parasitismo do ácaro *Ornithonyssus bursa* em humanos no Sul do Brasil. Acta Sci Vet 40:1–3
- Orton DI, Warren LJ, Wilkinson JD (2000) Avian mite dermatitis. Clin Exp Dermatol 25:129–131. <https://doi.org/10.1046/j.1365-2230.2000.00594.x>
- Platts-Mills TA, Schuyler AJ, Tripathi A, Commins SP (2015) Anaphylaxis to the carbohydrate side chain alpha-gal. Immunol Allergy Clin North Am 35:247–260. <https://doi.org/10.1016/j.iac.2015.01.009>
- Radovsky FJ (2007) A new species of *Ornithonyssus* (Acari: Macronyssidae) in western North America and a review of nearctic species. Int J Acarol 33:223–229. <https://doi.org/10.1080/01647950708684526>
- Regan AM, Metersky ML, Craven DE (1987) Nosocomial dermatitis and pruritus caused by pigeon mite infestation. Arch Intern Med 147:2185–2187. <https://doi.org/10.1001/archinte.1987.00370120121021>
- Roy L, Chauve CM, Buronfosse T (2010) Contrasted ecological repartition of the northern fowl mite *Ornithonyssus sylviarum* (Mesostigmata: Macronyssidae) and the chicken red mite *Dermanyssus gallinae* (Mesostigmata: Dermanyssidae). Acarologia 50:207–219. <https://doi.org/10.1051/acarologia/20101958>
- Schlosser R, Meissner M, Kaufmann R, Valesky EM (2019) Recurrent anaphylaxis due to pigeon tick bites. J Dtsch Dermatol Ges 17:195–196. <https://doi.org/10.1111/ddg.13739>
- Semenas L, Rocha JA (1998) Un motivo poco común de crisis de llanto en un recién nacido. Arch Arg Pediatr 96:131–133
- Sikes RK, Chamberlain RW (1954) Laboratory observations on three species of bird mites. J Parasitol 40:691–697. <https://doi.org/10.2307/3273713>
- Steinke JW, Platts-Mills TA, Commins SP (2015) The alpha-gal story: lessons learned from connecting the dots. J Allergy Clin Immunol 135:589–596
- Ueckermann EA, Moraza ML, Roy L, Palevsky E (2016) Identifying potential predatory mites for the control of the red poultry mite. FA1404 COREMI Training School, Rehovot
- van Nunen S, O'Connor KS, Clarke LR, Boyle RX, Fernando SL (2007) The association between *Ixodes holocyclus* tick bite reactions and red meat allergy. Intern Med J 39:A132
- Waitayakul A, Somsri S, Sattabongkot J, Looareesuwan S, Cui L, Udomsangpetch R (2006) Natural human humoral response to salivary gland proteins of *Anopheles* mosquitoes in Thailand. Acta Trop 98:66–73. <https://doi.org/10.1016/j.actatropica.2006.02.004>
- Walter DE, Krantz GW (2009) Collecting, rearing, and preparing specimens. In: Krantz G, Walter D (eds) A manual of acarology, 3rd edn. Texas Tech University Press, Lubbock, pp 83–96
- Yilmaz B, Portugal S, Tran TM, Gozzelino R, Ramos S, Gomes J, Regalado A, Cowan PJ, d'Apice AJ, Chong AS, Doumbo OK, Traore B, Crompton PD, Silveira H, Soares MP (2014) Gut microbiota elicits a protective immune response against malaria transmission. Cell 159:1277–1289. <https://doi.org/10.1016/j.cell.2014.10.053>