Universidad de Valladolid

Escuela de Doctorado Universidad de Valladolid

**PROGRAMA DE DOCTORADO EN CONSERVACIÓN Y USO SOSTENIBLE DE LOS SISTEMAS FORESTALES**

TESIS DOCTORAL:

# ECOLOGY AND PUBLIC HEALTH: RODENTS AS RESERVOIRS OF ZOONOSES IN THE FARMLAND OF NORTHWESTERN SPAIN

Presentada por Silvia Herrero Cófreces para optar al grado de Doctor/a por la Universidad de Valladolid

Dirigida por:

Dr. Juan José Luque Larena
Dr. François R. Mougeot

– 2022 –

UVa iap

Instituto Universitario de Investigación iuFOR GESTIÓN FORESTAL SOSTENIBLE

# INDEX

# ABSTRACT

Zoonoses are a concerning issue for public health and the last pandemic event caused by covid-19 is a good example of it. Human activity is enhancing the transmission, intensity and emergence of practically all zoonoses. Synantropic qualities of some rodent species provide them with exceptional features as amplifiers of emerging zoonoses. Vectors are also important elements transmitting the pathogen between hosts and make it more likely for a disease to cross the species barrier and become zoonotic. Circulation of pathogens involves several reservoirs and hosts, each one with a different level of competence for vectors and transmission of pathogens. The overlap of infected hosts, competent vectors, and humans in the same habitats and at the same time increase considerably the zoonotic risk. The disease ecology based on the One-Health considers pathogens as elements interconnected with the natural environment, wildlife, domestic animals and humans. An effective monitoring, comprehensive understanding of the system functioning, and determination of the spatial-temporal patterns provide us with crucial information for disease prevention.

In this thesis, I studied the zoonotic relevance of wild populations of a sympatric small mammal community (*Microtus arvalis*, *Apodemus sylvaticus*, *Mus spretus* and *Crocidura russula*) that inhabit intensive farming in NW Spain. Here, *M. arvalis* is considered a host and amplifier of *Francisella tularensis* (the etiological agent causing tularemia) but little is known about the circulation of this and other zoonotic pathogens in the system. I focused on improving the scientific knowledge of the dynamic of zoonotic pathogens and vectors of the sympatric small mammal community inhabiting those intensive agricultural landscapes. In the first chapter, I reviewed current knowledge on the role of common vole in tularemia epidemiology and identified relevant knowledge gaps in the "*Francisella tularensis*–*M. arvalis*" system. In the second chapter, I characterized the most common arthropod vectors (fleas and ticks) parasitizing the small mammal host community. In the third and four chapters, I screened the host community for some zoonotic micropathogens and macroparasites: bacteria (*F. tularensis* and *Bartonella*), viruses (hantaviruses, arenaviruses and orthopoxviruses) and gastrointestinal helminths. Transversely, I examined variations in the parasitological parameters (prevalence, intensity and abundance) according to host species and sex, habitat (crop type), seasonality and the population dynamics of host species, with particular emphasis on the vole population cycles.

I have detected *F. tularensis*, nine *Bartonella* species, three types of viruses and eight different helminth taxa. Half of those pathogens are considered zoonotic. Results showed that the small mammals surveyed that lives in sympatry with *M. arvalis* seem to have no relevant role in the

circulation and maintenance of *F. tularensis*. Fluctuating population dynamics of *M. arvalis* and seasonality can affect the dynamic of vectors and pathogens. High-density periods of *M. arvalis* (outbreak years and summer) favored the circulation of viruses and bacteria, and increased the abundance of fleas, potentially also increasing the zoonotic risk for human populations. The infestation levels by ticks and gastrointestinal helminths were higher during the crash phase of the vole cycle. These and other pathogens could contribute to the maintenance of a low vole population phase, by limiting and delaying the recovery of the vole population after a crash.

This thesis contributed new knowledge of the circulation of zoonotic pathogens and vectors in this farming system, with public health implications. Of particular importance are the roles that vole outbreaks play as an amplifier and spill-over agent of zoonotic diseases; the need to consider new viruses (in particular, hantavirus) in public health surveillance; and the usefulness of community-based monitoring of pathogen circulation, maintenance and transmission to improve prevention.

# RESUMEN

Las zoonosis son un tema preocupante para la salud pública y el último evento pandémico causado por la covid-19 es un buen ejemplo de ello. La actividad humana está potenciando la transmisión, intensidad y emergencia de prácticamente todas las zoonosis. Las cualidades sinantrópicas de algunas especies de roedores les confieren características excepcionales como amplificadores de zoonosis emergentes. Los vectores también son elementos importantes que transmiten patógenos entre hospedadores y hacen más probable que una enfermedad salte la barrera de especie y se convierta en zoonótica. En la circulación de patógenos intervienen varios reservorios y hospedadores, cada uno con diferente nivel de idoneidad para la supervivencia de los vectores y de transmisión de cada patógeno. El solapamiento en lugar y tiempo de huéspedes infectados, vectores competentes y seres humanos aumenta considerablemente el riesgo zoonótico. La ecología de las enfermedades basada en el concepto de One-Health considera a los patógenos como elementos interconectados con el entorno natural, la fauna salvaje, los animales domésticos y los seres humanos. Un seguimiento eficaz, la comprensión exhaustiva del funcionamiento del sistema y la determinación de los patrones espacio-temporales proporcionan información crucial para la prevención de enfermedades.

En esta tesis, he estudiado la relevancia zoonótica de las poblaciones silvestres de una comunidad de pequeños mamíferos simpátricos (*Microtus arvalis*, *Apodemus sylvaticus*, *Mus spretus* y *Crocidura russula*) que habitan zonas agrarias intensificadas del noroeste de España. En este caso, *M. arvalis* se considera un hospedador y amplificador de *Francisella tularensis* (el agente etiológico causante de la tularemia), pero poco se conoce sobre la circulación de éste y otros patógenos zoonóticos en el sistema. Me centré en mejorar el conocimiento científico de la dinámica de los patógenos zoonóticos y los vectores en la comunidad de pequeños mamíferos simpátricos que habitan esos paisajes agrícolas intensivos. En el primer capítulo, revisé los conocimientos actuales sobre el papel del topillo común en la epidemiología de la tularemia e identifiqué algunas lagunas de conocimiento relevantes en el sistema "*Francisella tularensis*-*M. arvalis*". En el segundo capítulo, caractericé los vectores artrópodos más comunes (pulgas y garrapatas) que parasitan a la comunidad de pequeños mamíferos. En los capítulos tercero y cuarto, examiné la comunidad de hospedadores en busca de algunos micropatógenos y macroparásitos zoonóticos: bacterias (*F. tularensis* y *Bartonella*), virus (hantavirus, arenavirus y ortopoxvirus) y helmintos gastrointestinales. De forma transversal, he examinado las variaciones de los parámetros parasitológicos (prevalencia, intensidad y abundancia) en función de la especie y el sexo del hospedador, el hábitat (tipo de cultivo), la

estacionalidad y la dinámica poblacional de las especies hospedadoras, con especial atención a los ciclos poblacionales de los topillos.

He detectado *F. tularensis*, nueve especies de *Bartonella*, tres tipos de virus y ocho taxones de helmintos diferentes. La mitad de esos patógenos se consideran zoonóticos. Los resultados mostraron que los pequeños mamíferos estudiados que co-habitan con *M. arvalis* no parecen tener un papel relevante en la circulación y el mantenimiento de *F. tularensis*. La dinámica poblacional fluctuante de *M. arvalis* y la estacionalidad pueden afectar a la dinámica de vectores y patógenos. Los periodos de alta densidad de *M. arvalis* (años de brotes y verano) favorecieron la circulación de virus y bacterias, y aumentaron la abundancia de pulgas, incrementando también potencialmente el riesgo zoonótico para las poblaciones humanas. Los niveles de infestación por garrapatas y helmintos gastrointestinales fueron mayores durante la fase de colapso poblacional del topillo campesino. Estos y otros patógenos podrían contribuir al mantenimiento de la población de topillos en una fase de baja densidad, limitando y retrasando la recuperación de la población de topillos después del colapso poblacional.

Esta tesis aportó nuevos conocimientos sobre la circulación de patógenos y vectores zoonóticos en este sistema agrícola, con implicaciones para la salud pública. Son especialmente relevantes el papel de los brotes de topillos como amplificadores y propagadores de enfermedades zoonóticas; la necesidad de tener en cuenta nuevos virus que pueden estar circulando en el sistema (en particular, hantavirus) de cara a la vigilancia con motivos de la salud pública; y la utilidad de la monitorización de zoonoses basada en la comunidad para conocer la circulación, persistencia y transmisión de patógenos de cara a mejorar las estrategias de prevención.

# GENERAL INTRODUCTION

## General context: emerging infectious diseases

An emerging infectious disease (EID) is an illness that affects a human population for the first time or a pre-existing one with a rapid increase in the number of new cases or quick geographically spread to new areas (1). Newly emerging infections are usually caused by a microbial genetic mutation, viral genetic recombination, pathogen-host switch, changes in the ecology of reservoirs, hosts or vectors, modification of human behavior, or environmental alterations. Re-emerging diseases are usually associated with microbial evolutionary vigor, increased zoonotic encounters, habitat encroachment of wildlife, and cyclic climate-related events (2).

The EIDs are a major threatening and challenging issue for public health, food security and global economies. An estimated 15 million deaths (>25% of overall worldwide deceases) are related directly to infectious diseases each year (2). In fact, they are the main cause of death in the world (3). A study by the World Bank about economic losses due to the six major outbreaks of EIDs between 1997 and 2009 (Nipah virus, 1998; West Nile fever, 1999; SARS, 2002; avian influenza, 2004; bovine spongiform encephalopathy, 2004; and Rift Valley fever, 2006) estimated that losses amounted to at least $80 billion (4). Other estimations for the cost of losses ranged from $374 billion for a mild pandemic to $7.3 trillion for a severe one (5). However, in light of what happened during the current covid-19 pandemic, the costs of EIDs may be underestimated. According to 2020 provisional data, this zoonotic pandemic alone could be directly responsible for 12-22% of all deaths in the European Union (6,7). Estimations quantify the overall economical and personal losses worldwide from $16 trillion (8) to $21 trillion (9). In view of the above, the covid-19 is a good example of the huge impacts of EIDs, stressing that effective surveillance and a rapid detection strategy are essential to minimize illness, fatalities and economic costs (3).

## Zoonoses

### Basic concepts

A zoonosis is an animal disease that can be transmitted to humans (10), which means that the infectious agent has crossed the species barrier to infect people (1). Wildlife, as opposed to domestic animals, is the origin of more than 70% of all known EIDs and of almost half (43%) of the epidemic outbreaks recorded between 1940 and 2004, which are increasingly frequent (11). Wildlife zoonotic agents that infect humans include 80% of viruses, 50% of bacteria, 40% of fungi, 70% of protozoa, and 95% of helminths (12).

There are four basic concepts to understand zoonoses, related to the pathogen, host, reservoir and vector (1,13). The World Health Organization defines a pathogen as an infectious agent able to cause a specific disease in a susceptible organism; they can be viruses, bacteria, protozoa, helminths, fungi and prions. Prions are proteinaceous infectious particles, helminths are macroscopic endoparasites (called hereafter macroparasites), and the other groups are microscopic organisms (called hereafter micropathogens). A host is a suitable organism for the growth and multiplication of a pathogen. A reservoir is an organism or environmental source able to maintain the pathogen alive. Reservoir organisms are hosts that do not suffer a serious illness despite harboring the pathogen. Last, a vector is an organism (usually, insects) that carries the pathogen between hosts or from an environmental reservoir to a host. Thus, the movement of the pathogen between hosts is necessary to understand the disease dynamics. Pathogen spreading is a phenomenon that involves transmission between individuals of a natural competent host community via a reservoir, host or vector that harbored the pathogen (13). An invasive transmission where a pathogen crosses from a natural host species to a new sympatric one is called spillover (14). Note that spillover differs from spread events since the first implies the infection of a new host species. This step is essential to the emergence of zoonoses since most of these diseases are naturally hosted by wildlife (11), as already explained.

**Implications in human health risk**

The emergence of zoonoses, both recent and historical, is a logical consequence of the ecology and evolution of any living species that tend to exploit new niches, in this case, new hosts (15). The emergence of zoonotic diseases can have very diverse natural and human-induced causes (Figure 1), but the common feature is the change in the ecology of the host, pathogen, or both (14,16). At some point, human domination of the Earth's ecosystem is influencing or facilitating the transmission, distribution, intensity, and/or emergence of practically all zoonoses (2,17,18). Land-use changes (e.g. deforestation, agricultural intensification) have been associated with the emergence of zoonotic diseases (19,20). Agricultural drivers were associated with nearly 50% of zoonoses in humans that have emerged since the mid-20th century (20). Agricultural intensification and the subsequent ecosystem alterations facilitate disease transmission by the expansion of ecotones, encroachment of wildlife habitats, loss of biodiversity, replacement of natural vegetation by crops, increasing of intensive livestock with low genetic diversity and high-density populations, use of agrochemicals and drugs, and movement of species and goods (19,20). The aggregation of humans in densely populated urban areas is also having an impact on the emergence of zoonoses, favoring the opportunity for transmission of zoonotic infections. Most of the zoonoses in temperate regions are indeed called 'crowd epidemic diseases' (21). Other host ecological traits (such as life-history characteristics,

seasonality, colonialism and sympatry) and environmental fluctuations (weather patterns and climate change) also play a role in pathogen transmission and persistence (14,22). We should not underestimate that small changes in the pathogen system can result in disproportionate consequences in terms of spread risk (22).

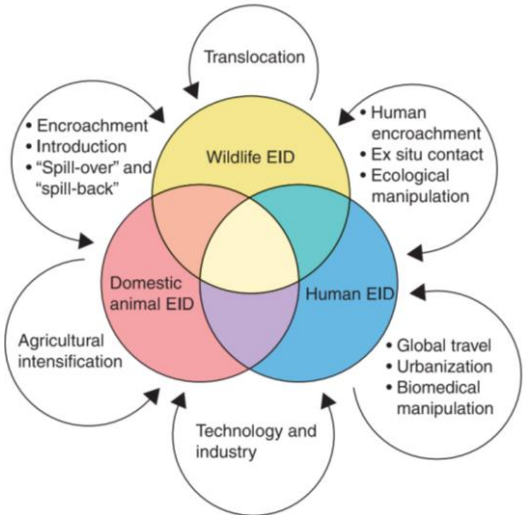


Figure 1. Some key factors driving disease emergence between wildlife, domestic animals and human populations (14).

The viability of parasite infection is the possibility of a successful infection, which depends on both the host and the pathogen characteristics and determines the competence of the host (23). Regarding the host, the risk of zoonotic infection depends on the hazard (i.e. the availability of a pathogen to infect a host), exposure (i.e. the probability of contact between the pathogen and the host) and susceptibility (i.e. the probability of an exposed host of being infected and affected by a pathogen) (24,25). Hazard is determined by the pathogen according to its resource requirements, specificity, virulence (severity of the disease) and genetic variability as well as infectious dose and route of transmission. On the other hand, exposure and susceptibility are defined by the host. Exposure is influenced by behavior, population density, sex ratio, mobility, proximity to a reservoir/vector, and the number of other competent hosts, as well as abiotic factors such as climatic and habitat conditions. Susceptibility depends on the structural barriers, condition and immune system of the host (24,26,27). Thus, transmission risk depends on a large number of intrinsic and extrinsic factors affecting both host and pathogen but also the vectors and state of other sympatric individuals, leading to non-linear relationships within the community that can result in a complex epidemic evolution (28). Hence, the problem of predicting where the next epidemic will occur or which pathogen will break out. Predictions suggest that the risk of zoonotic emergence is high in tropical forests, areas with elevated mammal biodiversity, agro-ecosystems suffering drastic anthropogenic land-use changes, and regions outside

the tropics with a high density of humans and livestock (29). And regarding zoonotic agents, RNA viruses and multihost pathogens have the potential to cross the species barrier, spillover, and emerge (16). It seems unlikely that most emerging zoonoses will be eliminated (30), based on their ecologically natural origin, their high adaptability and spillover unpredictability. The coronavirus and Ebola epidemics are good examples showing us how interconnected are nature and human health, and how a zoonotic spillover event can become a concerning environmental issue, a global public health emergency and a socioeconomic challenge (4,31,32). In this context, surveillance has arisen as one of the best preventive tools against zoonotic disease emergencies (33).

**Episystems: towards an ecological perspective of diseases**

The traditional view of human zoonotic diseases used to focus on simple host-pathogen systems (34,35), usually studied from a parasitological and epidemiological point of view, with a clear aim of disease surveillance and control (13). However, co-infections and multi-host pathogens are common situations in nature (36,37). On the one hand, co-occurrent pathogens coexist in the same host at the same time, having an impact on the host, other pathogens or both. Thus, direct or indirectly, they influence other pathogens and affect the burden of each pathogen species within the host (36,38). Pathogen interactions can likewise affect host susceptibility, infection duration, transmission risks or symptomatology (39). On the other hand, multi-host pathogens have two or more reservoir hosts in which they persist, but also other susceptible hosts in which they occur, causing sporadic outbreaks (37). Multi-host pathogens cause 60% of human zoonoses and 80% of diseases from a zoonotic origin in domestic animals (37). The traditional study of diseases fails to take into account the diversity of relationships and factors that govern disease dynamics so a new comprehensive vision should be implemented. The dynamics of multi-host pathogens in multispecies host communities remain a methodological challenge for disease ecology but is essential to unveil the complexity behind the emergence of diseases (39,40).

The One-Health approach was born to support this interconnected eco-systemic context of zoonoses and human health. The One Health Commission defined it as “an integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals and ecosystems” (41). These ecosystems focus on communities of pathogens, hosts, vectors, the environment where they live, and their relationships are also called “episystems” (Figure 2) (25). The transmission and spread of a pathogen depend on multiple interconnected biological, ecological and anthropic factors. The acceptance of this new perspective conditions the emergence of different complementary study scales of the same system, with different factors and variables that rule the structure and the dynamic of each one (23,25–27,42). There is one first scale of pathogen community, determined by

requirements, composition and relationships between pathogen species that share simultaneously or sequentially the same host. The second level is the host-pathogen system, where individual host traits such as age, size, sex, breeding status, host condition, immune response and trade-offs rule the infection. The third scale, the population scale, is controlled by the host and pathogen sex ratio, host to host transmission, behavior, demography and dynamic of the host species, and density dependence factors. The community scale is the following step, where host diversity and density, abundance fluctuations, migration, competition, predation, food availability, indirect relationships and introductions of hosts and/or parasites determine the interactions and feedback loops within the community. Finally, the ecosystem scale is regulated by large-scale features and patterns such as climate, abiotic environment, habitat characteristics, spatial structure, food webs, keystone species, dispersal movements, stochastic events, human policies, and globalization, with different regional and biogeographical scales. This new scenario includes pathogens as an element that is part of the system, interconnected with the rest of the elements and factors, and not merely opportunistic fastidious organisms causing occasional disease problems.

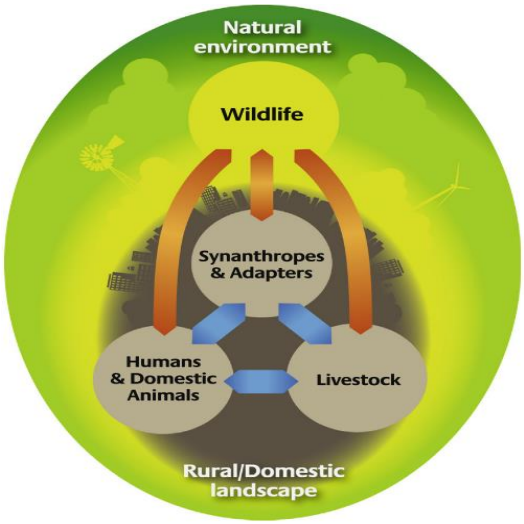


Figure 2. Possible flow of parasite transmission between humans, domestic animals and wildlife in different host ecosystems (43).

Actions based on the simple host-pathogen paradigm can trigger cascading effects, if the attempt of controlling one pathogen favors another pathogen with which it has an antagonist relationship (38), altering the whole system equilibrium in an unintended direction. Furthermore, similar communities may be ruled by different processes if they are under different ecological contexts (44), so a holistic view is essential to understand the peculiarities of each episystem. Pathogen management should hence be adapted to each situation.

Zoonoses have natural life cycles where the pathogens are perpetuated, with spillover events to people and livestock as a result of human activities (43). If we want to carry out an efficient investigation in order to prevent future epidemic events, correct preventive surveillance should be carried out. Basic knowledge of the pathogen pool in the wildlife fauna is necessary to determine potential zoonotic species and future EIDs (43). The establishment of intra- e interspecific relationships between pathogens and hosts is essential to understand the dynamics of generalist pathogens (40) as well as the identification of "samplers" (high susceptible hosts of acquiring novel infections), "spreaders" (hosts with high potential for disseminating a novel infection) and "sentinels" (elements, individuals or locations providing information on the state and evolution of a disease) (16). The effective monitoring of these elements in wildlife, human and domestic ecosystems would provide us with a useful tool for disease prevention (21,45). Nonetheless, we are still far to achieve that goal and current actions are still focused on local and regional detection, and control of post-emergence outbreaks (4). The insufficient prevention measures and the lack of global strategies considering urbanization and connectivity (4,29) will likely result in a large mortality and morbidity rate due to zoonoses, as was brought to light with the recent covid-19 outbreak (29). Some initiatives have been launched in the last few years, such as The Global Virome Project (46), which aims to detect new viruses and systematically sample competent hosts to provide updated data for potential public health interventions. Another initiative is the PREDICT Project of the Emerging Pandemic Threats program, which is a predictive modeling procedure used to identify regions, wildlife hosts, and human-animal interfaces with a high probability of an emerging zoonotic event (47). To achieve the ambitious objective of understanding episystems, the collaboration between multidisciplinary experts (Figure 3) will be necessary across local and regional scales, incorporating human behavior into models (25,48).

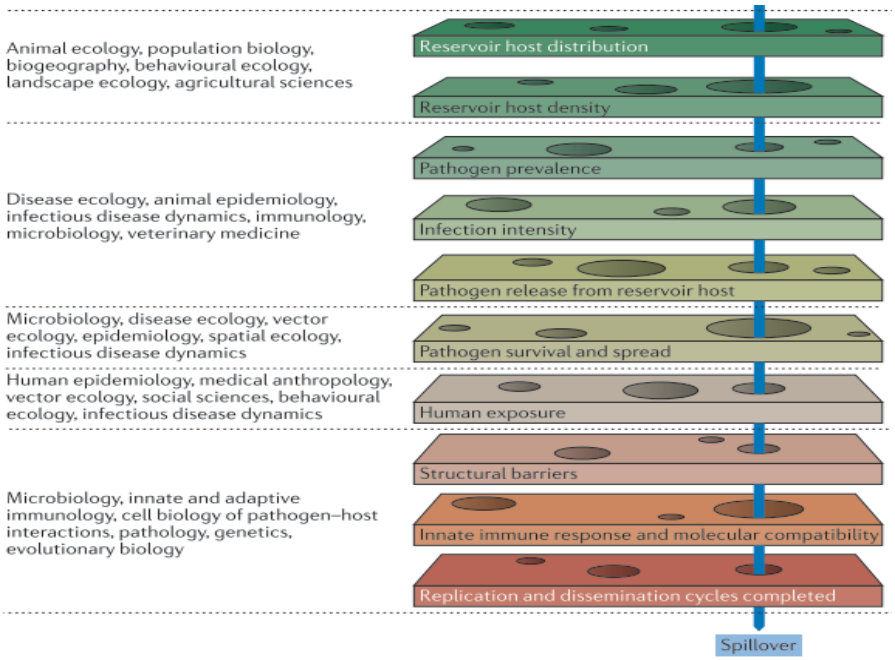


Figure 3. Barriers to spillover and suitable disciplines for the study of each determinant. Opportunities to go through each barrier are depicted as holes; a successful spillover is represented by the blue arrow (24).

## Rodents and public health

### The ecological role of rodents

Rodents (order Rodentia) are the most abundant, diversified and widely distributed order of living mammals in the world (49). They are key ecosystem engineers, affecting water flows at micro- and macro-scales, soil characteristics, nutrient cycles, plant community structure and succession, and habitat characteristics of other animal species (50). They also have a key functional role in many ecosystems, as prey of avian, mammalian or reptilian predators, including endangered species and highly specialized predators like small mustelids (51,52). Rodents have also been linked to higher vertebrate, invertebrate and plant species richness in the ecosystems they inhabit (51). Additionally, some herbivorous rodents are essential pioneer species facilitating the recovery of abandoned and degraded habitats and post-fire ecosystems through their seed dispersal activity (53–55).

Rodents also stand as important competitors globally with humans for food and they are concerning reservoirs of diseases of veterinary and public health interest (49,56,57). Wildlife provides a pool of pathogens that play a crucial role in the emergence of zoonoses (14). In fact, most zoonoses have spilled over from warm-blooded vertebrates, predominantly mammals (21). Specifically, predictions point to rodents as the main mammalian reservoir groups of zoonotic pathogens (58), since they are considered the taxa with the greatest pathogen diversity (59). Around 90 different diseases

are linked to rodents, including more than thirty viral zoonoses (and rising), more than twenty bacterial illnesses, twenty helminthiases, close to a dozen of protozoa, and four fungal diseases (a number that is considered underestimated) (49,59). These zoonotic pathogens can reach humans through direct contact (rodent bite, contact with material contaminated (water, food, surfaces) by infected rodent urine and feces) or inhalation of infectious aerosols. Indirect transmission routes are more difficult to establish and may occur by transference to food products through livestock infection or infection through ectoparasitic arthropod vectors (49,60)

**What makes rodents so relevant in episystems?**

The fast-paced life strategy is a common characteristic of all rodent species, which provides them with a high capacity for evolutionary adaptation. This makes rodents the most diverse mammal group in terms of species number (59). Generalist rodents are more permissive species than specialists and, consequently, tend to have a greater geographic range size (59). Furthermore, some rodent species are tightly linked to human settlements and activities (57,61,62). The result of a fast-paced life strategy, generalist requirements and a commensal lifestyle is greater resilience against perturbations and human activities (63). Consequently, generalist rodents can host a wider range of pathogenic species than specialists (64), making rodents the group with more species harboring zoonoses (10% of all rodents are hosts) (59). An ultimate and extreme concern arises from the fact that rodents are a key reservoir group for zoonotic diseases, and could be intentionally or unintentionally used as carriers of biological weapons during a bio-attack against humans, livestock, soil or water sources (65).

Four are the main factors influencing the transmission of rodent-borne zoonoses in the disease model (Figure 4): the pool of pathogens, the pathogen prevalence and intensity, the rodent population density, and the intra- and interspecific contact network of those rodents (64,66). Pathogen prevalence and intensity among rodent populations would increase with abundance as a result of horizontal transmission among rodents (67). The zoonotic risk is thus related to rodent population density, which increases the probability of contact between people and infected rodents (60). Accordingly, the most concerning scenario involve rodents with fluctuating population dynamics (cyclic, outbreaking species characterized by boom-bust dynamics) and a wide variety and burden of pathogens, which would increase the efficiency of spreading and, subsequently, trigger the spillover risk (64).

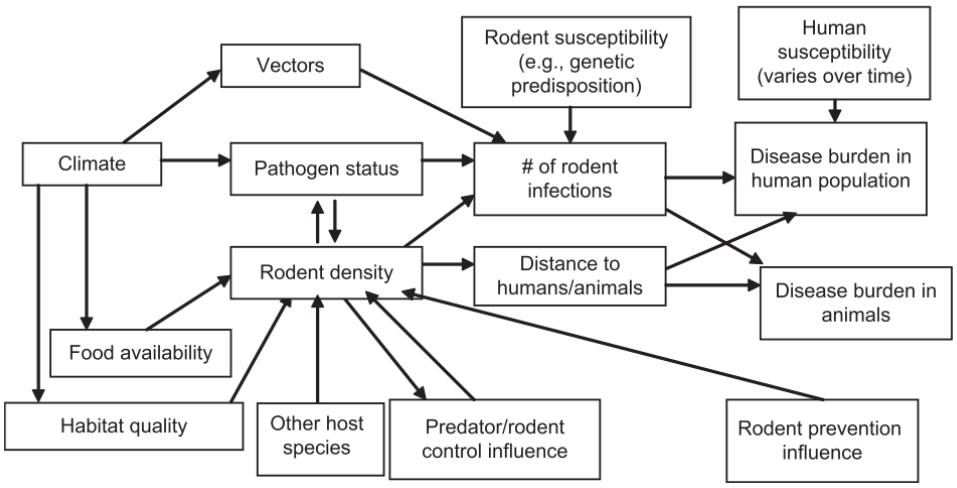


Figure 4. A simplified rodent-borne disease model (49).

The areas at high risk of disease emergence are concentrated in tropical forests and human-modified environments, where animal reservoirs are more frequent (21,30). In these habitats, rodents are the dominant vertebrate wildlife fauna, worsening the zoonotic risk (63,64). Opportunistic qualities of some rodents can enhance even more their role in emerging zoonoses, taking advantage of their resilient traits in a world in constant and profound transformation (63,66,68). Synanthropic species are a current real problem in rural, urban and suburban areas worldwide. They increase the human-host interface and bring some pathogens into close contact with high-density livestock and human populations (22,62,64). Rodents act as silent reservoirs and amplifying hosts that help to maintain and favor the transmission of zoonotic pathogens within the system (49,64). Another two features are rising as challenging factors that can increase the emerging zoonotic risk of rodents: the increase in cropping intensity and climate change (69). The first would provide a constant source of food for rodents, allowing them to breed uninterruptedly; the second would alter the distribution range of rodent hosts and hence the pathogens too. Rodents could be carriers of diseases to newly colonized and invaded areas, with unpredictable consequences (70,71).

Recommendations for rodent-borne disease investigation highlight the importance of focusing on pathogen distribution across rodent species, pathogens shared between rodents hosts and the contact routes between rodents and humans (64). Current rodent management and control programs are designed and applied to prevent economic losses when the spread and spillover of pathogens have already occurred (72). Programs should focus more on providing people with information about the relevance of emerging zoonotic diseases and the importance of prevention policies to avoid the introduction of pathogens into anthropic environments.

# Vectors and public health

### Vectors and zoonoses

As mentioned above, a vector is an organism that carries a pathogen from a reservoir or an infected host to another host, which confers the vector-borne diseases a greater likelihood of becoming zoonotic (37). Even though, their impact on the incidence of vector-borne diseases (VBDs) has been underestimated (73). Since 1940, 131 VBDs have emerged, almost half of them in the last 15 years (74). The principal vector animals are ticks, mosquitoes, fleas, flies and sandflies (10), with the two former accounting for 40% and 36% of the VBDs respectively (74). Most pathogens causing emerging VBDs can be classified as bacteria, viruses or protozoa (18,74,75). Bacteria of the family Rickettsiaceae are the most abundant, causing spotted fever and several types of tick typhus. We cannot forget other bacteria such as *Borrelia* spp. (causing Lyme disease and borreliosis), *Francisella tularensis* (causing tularemia), *Anaplasma phagocytophilum* (causing granulocytic anaplasmosis) or *Bartonella* spp. (causing bartonellosis). Many viruses belong to the family Flaviviridae, including the yellow fever virus, West Nile virus, dengue virus, Zika virus, tick-borne encephalitis or hepatitis virus. Other concerning zoonotic vector-borne viruses are the chikungunya virus or Crimean Congo hemorrhagic fever virus. Protozoa gather etiological agents of some persistent and re-emerging diseases such as *Plasmodium* sp. (causing malaria) and *Leishmania* sp.

All arthropod vectors have two common characteristics: they are ectotherm (i.e. they are not able to regulate their body temperature) and hematophagous (i.e. they are obligated or facultative blood feeders). Based on these two features, the emergence of VBDs is tightly linked to climatic changes that determine the environmental temperature, and variations in host behavior that condition the presence of suitable animals to feed on. Humans, therefore, have a decisive responsibility for the zoonotic transmission risk, since their way of life is contributing to global change, and some occupations and outdoor activities especially increase the chances of a host-vector-pathogen encounter (76,77). Changes in weather features can affect the incubation period, feeding activity, behaviors, body size and age-specific mortality of vectors, affecting the vector fitness and hence the dynamics of both vector and disease too (78). Thus, wetter and warmer climatic predictions would favor suitable conditions for cold-blooded animals like arthropod vectors (79,80) and more immediately, for highly mobile mosquito-borne populations (81). Climatic changes will also provoke modifications in the distribution of host, reservoirs or both, influencing the zoonotic transmission area (18). Despite the fact that the movement of species, goods and people are sometimes behind the dispersion of vectors and their pathogens into non-endemic regions (82), suitable conditions are necessary for the persistence of the disease (80). Land-use changes have been associated with 26%

of all emerging BVDs, international trade and commerce with 11%, and climate with another 10% (74). When infected hosts, competent vectors and people overlap in the same habitats and at the same time, the zoonotic risk increases considerably (77).

**Vectors and transmission risk**

Zoonotic risk is highly influenced by the implication of vectors in the pathogen transmission routes. Indeed, alterations in either the life cycle of a vector or the ecosystem characteristics can have unpredictable and undesirable consequences, as illustrated in some of the following examples.

Lyme disease is an emerging tick-borne zoonosis in North America and Europe. It is caused by a group of *Borrelia* species (named *Borrelia burgdorferi* sensu lato) and transmitted by four species of hard ticks (the *Ixodes ricinus* complex). This bacterium has a generalist feeding behavior and an important ability to evade the defenses of its reservoir, which explains its widespread distribution (83,84). The abandonment of agricultural land into wooded surfaces has favored the increase of deer (preferred host for the ticks) and mouse populations (main reservoir of the bacterium). The use of those areas by the practice of outdoor activities has triggered the increase in Lyme disease incidence via tick bites (81).

The bubonic plague is a historic and well-known VBD, caused by the bacterium *Yersinia pestis*. The main transmission route is a flea bite through a rat-flea-human pathway. The decrease in the synanthropic rat populations forces the fleas to feed on alternative hosts, that is, humans (24). This disease is usually linked with past epidemics since it caused three major pandemics in history during the 6$^{th}$, 14$^{th}$ and 19$^{th}$ centuries. However, the plague represents nowadays a persistent threat in Africa and a re-emerging zoonosis in North America, Central Asia and the Middle East, through alternative transmission routes such as eating infected animals or handling infected cats (85). Additionally, global warming can trigger ecological cascading effects that lead to an increase in plague prevalence of more than 50% (86).

West Nile virus is another emerging VBD that is transmitted by different species of mosquitoes. It is endemic to tropical regions of Africa, southern Asia, and northern Australia. However, it was introduced in North America in 1999 by still unknown causes (87). Since then, 25000 people have been diagnosed with neuroinvasive disease, which includes severe symptoms such as meningitis or encephalitis (88). These cases represent just 1% of all infections (89) and hence, more than 2.5 million people have become infected by this zoonosis. Hosts in endemic areas are birds such as crows, sparrows, waterbirds and passerines of the *Ploceidae* family. In the invaded areas, the virus rapidly adapted to local mosquito species that parasitize an unexpected competent host that permit the persistence of the disease: robins. The populations of these birds have increased due to urbanization

and agricultural expansion, habitats where the highest transmission of the pathogen to humans is found (87).

These three examples show the intricate sylvatic cycles of vector-borne pathogens in nature. Circulation involves several wild reservoirs and hosts, each one with a different level of competence for the vector and the pathogen transmission (27,83). Moreover, co-infections of vectors are very common (90) and a non-systemic transmission route via co-feeding of vectors on non-infected hosts is also possible (91). All these features increase the complexity of the pathogen circulation even more if vectors are implicated.

## The study case of zoonoses in rodents inhabiting the farmland of NW Spain

Castilla-y-León is an autonomous region located in NW Spain, with a surface of 94226 km2. The farming sector is one of the main economic activities of the region, with 69% of the total surface dedicated to agricultural uses, more than 2 million livestock units, and 100000 farms (92). The center of the region is a highly intensified farming landscape. A significant percentage of the surface is considered arable land, which can exceed 85% in some areas (Figure 5). The main study area for the works conducted in this thesis is a region called “Tierra de Campos”, covering the Northeast of Zamora, the North of Valladolid, and the center of Palencia provinces.

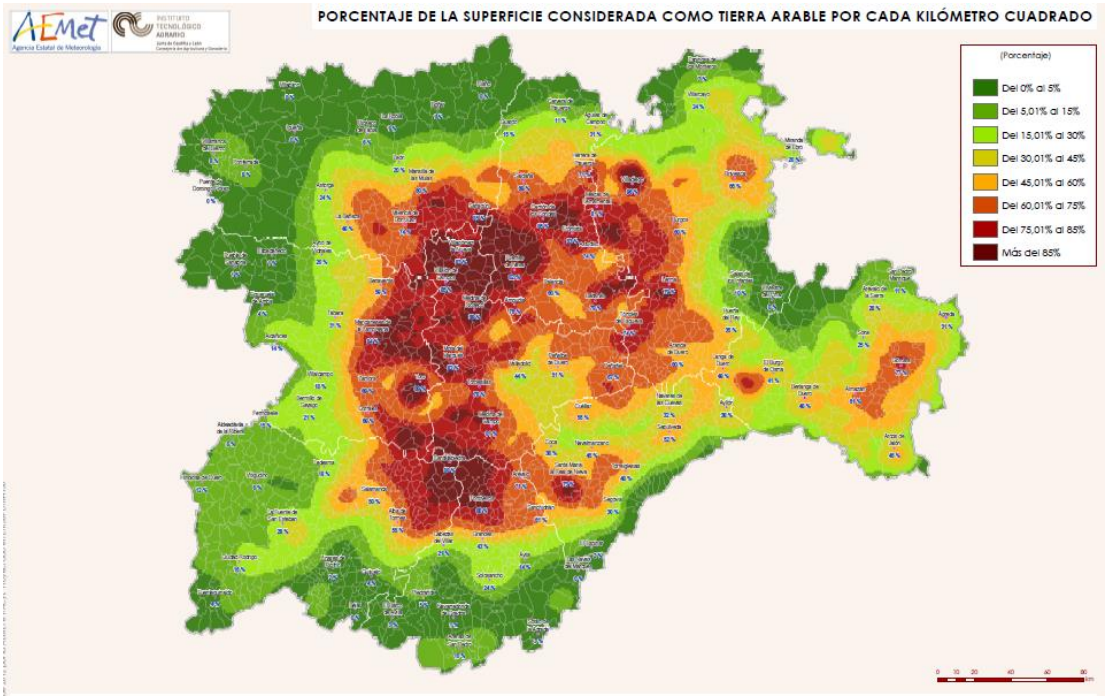


Figure 5. Map of the Castilla y Léon region showing the percentage of surface considered as arable land per square kilometer (93).

In these agricultural habitats, *Microtus arvalis* (common vole) is the most abundant small mammal where it cohabits with other two mice species (*Apodemus sylvaticus* [long-tailed field mouse] and *Mus spretus* [western Mediterranean mouse]) and an insectivore (*Crocidura russula* [great white-toothed shrew]) (94). *Microtus arvalis* is a fossorial rodent characterized by cyclic population outbreaks (95), which colonized the study area between 1970 and 1990. During vole outbreaks in NW Spain, there have been claims of crop damage (95) and they have been linked to tularemia epidemics (96). This rodent is considered a host and amplifier of several zoonotic pathogens in Spain (97–100) and elsewhere in Europe (101–105). Its populations often greatly fluctuate in abundance, and these "boom-bust" population dynamics of *M. arvalis* are a key feature of the species to understand the circulation, spread and spillover of certain pathogens, such as *Francisella tularensis* (the etiological agent of tularemia) (97,98). In NW Spain, the common vole populations have been characterized as cyclic, with a 3-year period (106,107).

The study of rodents from a zoonotic point of view should focus on the prevalence and distribution of pathogens within hosts, subpopulations of the host (e.g. individual traits such as sex, age or condition), habitat types, and temporal patterns (60). A long temporal series of data achieved by seasonal monitoring of these small mammals provided a unique opportunity to investigate which pathogens are hosted by the small mammal guild, to explore variation in the parasitological parameters

(prevalence, intensity, abundance and aggregation), with particular attention to the effect of *M. arvalis* abundance fluctuations on the pathogen circulation and zoonotic risk.

## Objectives of the thesis

The general objectives of this thesis were (Figure 6): i) to improve the scientific knowledge of the dynamics of zoonotic pathogens and vectors in the small mammal guild (i.e., *M. arvalis*, *A. sylvaticus*, *M. spretus* and *C. russula*) inhabiting the highly intensified farmland of the Castilla-y-León region; and ii) to determine spatial-temporal patterns according to some relevant intrinsic (host species, vector species, host sex) and extrinsic traits (phase of the vole population cycle, density of each sympatric small mammal host, season, habitat).

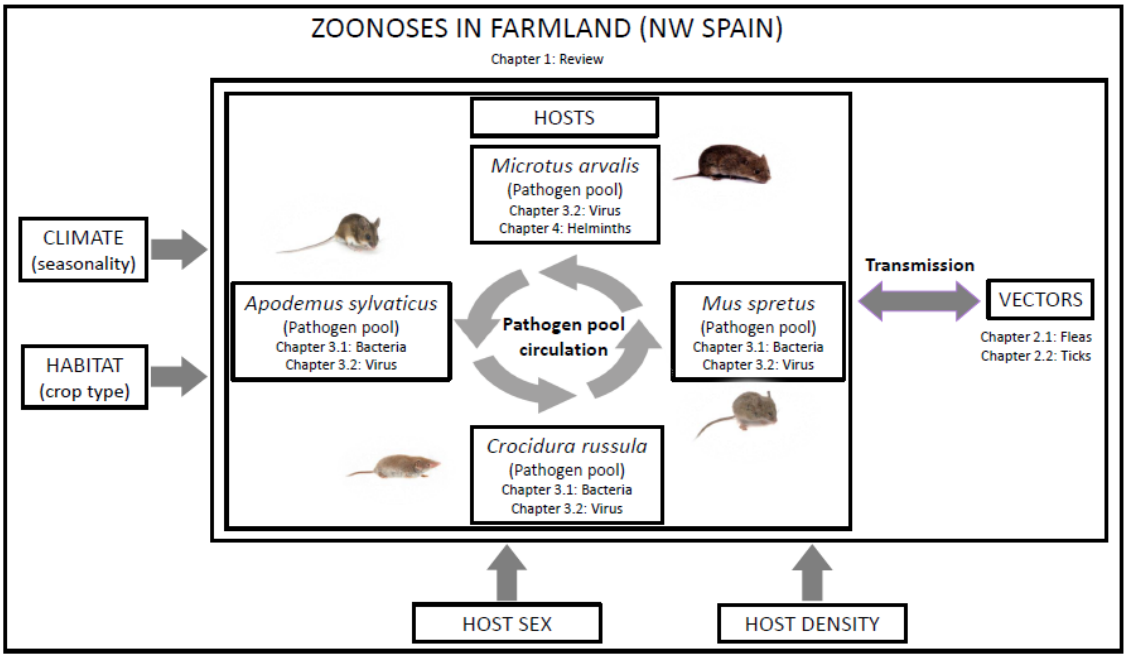


Figure 6. Graphical summary of the chapters of this thesis and their relationships.

As an initial point (**Chapter 1**), I carried out a review of a decade of research conducted on the biological interaction between the zoonotic bacterium *Francisella tularensis* and the colonizing rodent *Microtus arvalis.* I highlighted the importance of considering the vole's cyclic population dynamics, and some knowledge gaps in the "*Francisella–Microtus*" interactions, determining some future research guidelines in this system, from a zoonotic point of view. The rest of the thesis was organized into three general chapters: ectoparasite vectors (**Chapter 2**), micropathogens (**Chapter 3**) and macroparasites (**Chapter 4**) (Figure 6). The objectives of the **Chapter 2** were: i) to identify and quantify the main ectoparasite vectors of the small mammal community (specifically, fleas (**Chapter 2.1**) and ticks (**Chapter 2.2**)), and to evaluate variables and factors that may influence the

parasitological parameters (prevalence, intensity and abundance). I focused on those two vector types because fleas are the most frequent ectoparasites of *M. arvalis*, in which *F. tularensis* and *Bartonella* have been detected (108); and because ticks are amongst the most relevant vector in terms of zoonotic transmission risk (74). I expected: i) some vector species to be shared between *M. arvalis* and other sympatric small mammals; ii) a higher prevalence of vectors when vectors and hosts are more active; iii) ectoparasites to switch between hosts and therefore to show varying levels of infestation depending on host density fluctuations. The **Chapter 3** is divided into two parts regarding the two most concerning emerging zoonotic micropathogens: bacteria (**Chapter 3.1**) and viruses (**Chapter 3.2**). The specific objective of the **Chapter 3.1** was to determine the prevalence of the zoonotic bacteria *F. tularensis* and *Bartonella* species in the small mammal guild that co-habit with a *M. arvalis* population where these two bacteria have been previously detected (in the voles and in their fleas (97,108)). I expected: i) a high diversity of *Bartonella* in the guild due to the host specificity of this bacterium; ii) the prevalence and abundance of *Bartonella* species shared with *M. arvalis* to vary in co-habiting hosts (mice and shrew) depending on the phase of the vole population cycle, because of potential spill-over; iii) the prevalence and abundance of *Bartonella* species shared among murid rodents (i.e. *A. sylvaticus* and *M. spretus*) to vary seasonally (murid populations have seasonal dynamics); iv) a positive association between the flea species (as potential vectors) and *Bartonella* species that are shared in the guild. The specific objective of the **Chapter 3.2** was to carry out a preliminary screening of three zoonotic viruses that are widespread throughout European rodent populations but have not yet been looked for in our study area recurrently affected by vole outbreaks: hantaviruses, arenaviruses and orthopoxviruses (102). According to the bibliography (49,102), I expected: i) hantaviruses to potentially occur in *M. arvalis*; ii) arenaviruses to potentially occur in *M. spretus*; iii) orthopoxviruses to potentially occur in *M. arvalis* and *A. sylvaticus*. In the last chapter of the thesis (**Chapter 4**) my aim was to identify and quantify the presence of gastrointestinal helminths in *M. arvalis*, to look for potential zoonotic species, and to investigate a potential regulatory role of helminths in the host population dynamics. I evaluated several key conditions for gastrointestinal helminths to have a regulatory role, specifically: parasite aggregation, a delayed response to changes in host density and a negative influence on vole fecundity or condition. Each chapter has its own discussion of the results and findings. They are followed by a general discussion which is organized in four parts: i) the contribution of this thesis to the knowledge of the zoonoses circulating in the agricultural system and public health implications; ii) the role of host population dynamics in the circulation of pathogens; iii) the role of pathogens on *M. arvalis* population dynamics; iv) an introduction to co-infection patterns and future lines of investigation.

# CHAPTER 1

# COLONIZING RODENTS AND TULAREMIA EMERGENCE

# Linking zoonosis emergence to farmland invasion by fluctuating herbivores: common vole populations and tularemia outbreaks in NW Spain

Silvia Herrero-Cófreces[1,2], François Mougeot[3], Xavier Lambin[4] and Juan José Luque-Larena[1,2]

[1] Dpto. Ciencias Agroforestales, Escuela Técnica Superior de Ingenierías Agrarias, Universidad de Valladolid, Palencia, Spain,

[2] Instituto Universitario de Investigación en Gestión Forestal Sostenible, Universidad de Valladolid, Palencia, Spain,

[3] Grupo de Gestión de Recursos Cinegéticos y Fauna Silvestre, Instituto de Investigación en Recursos Cinegéticos (IREC, CSIC-UCLM-JCCM), Ciudad Real, Spain,

[4] School of Biological Sciences, University of Aberdeen, Aberdeen, United Kingdom

This chapter has been published as:

Herrero-Cófreces Silvia, Mougeot François, Lambin Xavier, Luque-Larena Juan José. Linking Zoonosis Emergence to Farmland Invasion by Fluctuating Herbivores: Common Vole Populations and Tularemia Outbreaks in NW Spain. *Front Vet Sci*. 2021, 8:1–13. doi:10.3389/fvets.2021.698454

# CHAPTER 2

# ECTOPARASITE VECTORS

# CHAPTER 2.1

# FLEAS

# Patterns of flea infestation in rodents and insectivores from intensified agro-ecosystems, Northwest Spain

Silvia Herrero-Cófreces[1,2], Manuel Fabio Flechoso[1,3], Ruth Rodríguez-Pastor[1,2], Juan José Luque-Larena[1,2], Francois Mougeot[4]

[1] Dpto. Ciencias Agroforestales, ETSIIAA, Universidad de Valladolid, Avda. de Madrid 44, 34004 Palencia, Spain

[2] Instituto Universitario de Investigación en Gestión Forestal Sostenible, Palencia, Spain

[3] Dpto. Biología Animal (Zoología). Universidad de Salamanca. Campus Unamuno S/N 37007 Salamanca, Spain

[4] Instituto de Investigación en Recursos Cinegéticos, IREC (CSIC-UCLM-JCCM), Ronda de Toledo s/n, 13071, Ciudad Real, Spain

This chapter has been published as:

Herrero-Cófreces Silvia, Flechoso Manuel Fabio, Rodríguez-Pastor Ruth, Luque-Larena Juan José, Mougeot François. Patterns of flea infestation in rodents and insectivores from intensified agro-ecosystems, Northwest Spain. *Parasites&Vectors*. 2021, 14:16. doi:10.1186/s13071-020-04492-6

# CHAPTER 2.2

# TICKS

# Patterns of tick infestation in rodents and insectivores from intensified agro-ecosystems, NW Spain

*Silvia Herrero-Cófreces [1,2*], Juan José Luque-Larena [1,2], Francois Mougeot [3]

[1] Dpto. Ciencias Agroforestales, ETSIIAA, Universidad de Valladolid, Avda. de Madrid 44, 34004 Palencia, Spain

[2] Instituto Universitario de Investigación en Gestión Forestal Sostenible, Palencia, Spain

[3] Instituto de Investigación en Recursos Cinegéticos, IREC (CSIC-UCLM-JCCM), Ronda de Toledo s/n, 13071, Ciudad Real, Spain

*Provisional authors of this draft

## Abstract

Ticks are the arthropod vector group that more efficiently transmit zoonotic agents from wildlife to humans and domesticated animals. Rodents are key hosts for ticks and many synanthropic species benefit from human-altered environments. Changes in habitat characteristics and climatic conditions due to global change and human activities can affect directly the distribution and phenology of ticks and hosts, altering the zoonotic risk in large areas. We carried out a long-term survey of ticks parasitizing small mammals in intensive farmland, including *Microtus arvalis*, *Apodemus sylvaticus*, *Mus spretus* and *Crocidura russula*. We stratified small mammal surveillance by season and habitat. Here we report on the prevalence, mean intensity, mean abundance and aggregation patterns (variance-to-mean ratio and Discrepancy index) of the main tick species. Generalized Linear Mixed Models were used to study the variation of parasitological parameters according to host species, host sex, trapping month, phase (increase, peak, crash years) of the host population cycle and habitat. The tick community parasitizing small mammals was dominated (94% of infested hosts and 93% of ticks identified) by *Rhipicephalus turanicus*. All ticks were collected in July, presenting a mean prevalence of 9.4%. *Microtus arvalis* showed lower tick prevalence but higher tick infestation range among the small mammal hosts. We found differences in tick prevalence between species, being higher in *C. russula* (19.4%). Results showed a negative correlation with sympatric mouse density and a significant female-biased prevalence among voles. Ticks were only collected from *M. spretus* or *C. russula* in years with increasing vole population density. However, the tick prevalence among voles was higher in the crash phase than in the increase phase. Crop type was not relevant in the variation of the prevalence patterns analyzed. *Rhipicephalus turanicus* should be further studied regarding its potential tick-borne role in zoonotic diseases and the cyclic population dynamics of *M. arvalis* in the study area that may affect the circulation of tick-borne pathogens.


**Keywords:** aggregation, *Apodemus sylvaticus*, *Crocidura russula*, cyclic vole population, ectoparasites, host sex effects, Ixodida, *Microtus arvalis*, *Mus spretus*, rodents

## Introduction

Ticks are an arthropod group vectoring zoonotic agents from wildlife to humans and domesticated animals (1,2), being responsible for the majority of the vector-borne diseases in temperate regions of the northern hemisphere (3). Although humans are accidental hosts for many ticks, the diversity of ticks that can potentially infest humans is relatively high (4). Ticks transmit and maintain the circulation of a broad variety of pathogens. They include viruses (e.g., Kysanur virus, Crimean-Congo virus, Powassan virus, tick-borne encephalitis virus), protozoa (e.g., *Theileria* spp., *Hepatozoon* spp., *Babesia* spp.) and bacteria (e.g., *Borrelia* spp., *Anaplasma* spp., *Ehrlichia* spp. *Rickettsia* spp., *Coxiella burnetii* and *Francisella tularensis*) (5).

The presence, behavior and length of the active period of ticks are closely determined by habitat characteristics, climatic conditions and microclimate in the vegetation level (6). Changes in these parameters modulate the risk of contact between ticks and hosts and hence, the transmission risk of tick-borne diseases. Agriculture intensification is a major source of ecosystem changes, causing habitat fragmentation and an increase in water availability. Land-use changes can alter the distribution range of hosts (7) and hence, may affect the distribution of the parasites that they can harbor. Habitat changes that create more humid, temperate and buffered microhabitats favor the population growth of ticks (6,8). Ecotones can also promote host-tick-pathogen interactions (9). International travel and trade favor the mobility of people, domestic animals and goods, and the translocation of wild species, including their parasites (2). All these changes modify the host-tick-pathogen dynamics and the result is a rising emergence of tick-borne diseases (2,8,10).

Ticks go through three stages in their life cycle (larva, nymph and adult), feeding the immature stages usually on small mammals, mostly rodents (3). However, rodents are not only key hosts of many concerning zoonoses (11) but also very efficient vectors in the transmission and amplification of disease (12). Commensal rodents have been clearly favored in the current situation of global change caused by anthropogenic activities, although other wild rodents have also increased their abundance and/or distribution range in response to it (13,14). Rodents may carry zoonotic pathogens and vectors into human-modified areas (2), into farms (15) and directly indoors (16). Additionally, some occupations and changes in people's behavior (urbanization, leisure activities) are leading humans into tick and wild rodent habitats (1,2).

In NW Spain, the common vole (*Microtus arvalis*) is the most abundant rodent inhabiting the intensified agricultural landscapes (17). This rodent invaded large farmland areas, due to habitat changes caused by agriculture (14,18,19), becoming the most abundant small mammal in the colonized area (17). When screening for pathogens in the colonizing vole population, positive

results have been found for *Francisella tularensis* (20), *Bartonella* spp. (21), *Coxiella burnetii* (22) and *Leishmania* sp. (23), some of them tick-borne zoonoses. This vole population shows irruptive population dynamics every three years (24), with the subsequent potential of amplification of zoonotic diseases (20) and a significant increase in the number of competent hosts for immature questing ticks (12,25). It has been proved that rodent densities and climate indices can be used to predict the dynamic of some tick-borne diseases (25). But previously, a baseline basic knowledge of host-tick ecology must be acquired to understand the possible role of ticks in the circulation of rodent-borne diseases in the ecosystem. Here, we report on i) the tick community harbored by common vole and other coexisting small mammals; ii) the prevalence, intensity and aggregation pattern of the main tick species; and iii) the variation of tick prevalence according to host sex, trapping month, phase of the host population cycle and habitat (i.e. crop type).

## Methods

### Study area

The study was conducted in an intensive agricultural region of Castilla-y-León (called "Tierra de Campos"), NW Spain. Six study areas (40 $km^2$ each) are located in the provinces of Palencia (42º01´N, 4º42´W), Valladolid (41º34´N, 5º14´W) and Zamora (41º50´N, 5º36´W) (two sites per province) were selected for the study. The landscape is dominated by cereal fields and interspersed by irrigated crops (mainly alfalfa), with scattered fallows and remnant semi-natural vegetation (17). The climatic conditions are characterized by wide seasonal temperature oscillations, typical of continental-Mediterranean areas: hot and dry summers with persistent drought periods, cold and long winters with frequent frost events, and precipitation mostly concentrated during autumn and spring (26).

### Study small mammals

Four species are the most abundant small mammals (>95%) in the studied habitats: common vole (*Microtus arvalis*), long-tailed field mouse (*Apodemus sylvaticus*), western Mediterranean mouse (*Mus spretus*) and greater white-toothed shrew (*Crocidura russula*) (17). Local common vole populations show multiannual fluctuations while the two mouse species mainly display seasonal fluctuations (24).

### Small mammal trappings

Fieldwork consisted of seasonal live trappings three times a year (March, July and November) between July 2009 and November 2016. In each study area, we randomly sampled the three most

relevant habitats (i.e., cereals, alfalfa and fallows), following the spatially stratified methodology explained in (17). We used live traps (8 × 9 × 23 cm; LFAHD Sherman©) baited with carrot and apple that were set open for 24 h. Each animal was individually coded; date, site and crop field were noted when trapped. See (17) and (24) for more details. For this study, we screened 2660 small mammals: 1597 *M. arvalis* (60.0%), 604 *A. sylvaticus* (22.7%), 383 *M. spretus* (14.4%) and 76 *C. russula* (2.9%). See the Additional table for more detailed information about caught animals per trapping session.

**Tick collection from trapped animals and identification**

Each live-trapped animal was sexed and euthanized with $CO_2$. Manipulation, transportation and euthanasia followed a humane protocol ethically approved by the University of Valladolid Ethical Committee (CEEBA code: 4801646). Immediately after euthanasia, we carefully collected the ectoparasites from each animal by blowing the fur and combing it with a louse comb, while holding the animal over a white plastic pan (520 × 420 × 95 mm) half-filled with water. Animal carcasses in sealed plastic bags were placed one hour in the fridge before checking again, to ensure that no ticks were missed. Ticks from each animal were separately collected and individually stored in labeled tubes filled with 70% ethanol. We identified ticks at the species level whenever possible, using a stereomicroscope (Nikon SMZ25) and identification keys (27–29). We collected a total of 567 ticks from 143 small mammal hosts: 342 ticks from *M. arvalis* (n= 89), 133 from *A. sylvaticus* (n = 34), 29 from *M. spretus* (n = 8) and 63 from *C. russula* (n = 12).

**Data analysis**

We obtained information about mean prevalence (infected hosts divided by hosts examined), summarized as prevalence ± 95% traditional Clopper-Pearson confidence limits (CI); and mean intensity (number of ticks divided by infected hosts), as of intensity ± standard deviation (SE). We also determined the level of aggregation of the tick distribution on hosts using two complementary indices: the variance-to-mean ratio (VMR), and the Discrepancy index (*D*)(30). These descriptive statistics were obtained using the Quantitative Parasitology (QPweb) software version 1.0.15 (31), with default bootstrap values.

Regarding the prevalence of ticks, we checked for patterns and differences between and within host species. We then explored variations of the tick community. We used Generalized Linear Mixed-Effects Models (GLMM) to explore the variation of prevalence. The explanatory variables included were host sex (male, female), crop type (alfalfa, cereal, fallow), trapping month (March, July, November; hereafter spring, summer and autumn, respectively), phase of the vole population cycle (increase, peak, crash; see the Additional table for more details), the mean density

of the host species (other than *M. arvalis*). Mean densities were calculated as the average number of captures per 100 traps per 24 h for a given seasonal sampling period and trapping site. Trapping site (Palencia, Valladolid, Zamora) and year of sampling were included as a combined random term (to account for possible unintended temporal or spatial variations), due to the big differences in the sample size between years and sites. We followed a forward procedure in the model selection, testing first single variables and sequentially adding significant terms (Anova $P < 0.05$ level), avoiding correlated variables (i.e. mean density of mouse hosts and mean density of the host species when referring to *A. sylvaticus* and *M. spretus*). Differences between levels of the significant categorical factors were tested using post-hoc Tukey tests. Statistical models were carried out using the "lme4" (32) packages of the R4.1.2 software (33).

## Results

### Tick community

The tick community included up to three different genera and several species: *Rhipicephalus turanicus* (n = 425), *Hyalomma* sp. (n = 13), *Rhipicephalus* sp. (n = 12), *Dermacentor* sp. (n = 4) and *Rhipicephalus pusillus* (n = 3); 108 specimens remained unidentified (including those not identified and those noted but not collected). Regarding tick stages, 45 of the specimens (7.9% of ticks) were larvae collected from 18 hosts (0.7% of hosts); the rest were nymphs. *Rhipicephalus turanicus* was the dominant tick in all the host species, representing 93% of all ticks identified. The rest of the tick species found were almost anecdotical: three *R. pusillus* were hosted by one individual of *C. russula*, four *A. sylvaticus* harbored four *Dermacentor* sp., and up to twelve *Hyalomma* sp. were found in three *A. sylvaticus* and one *M. arvalis* (Table 1).

Since all ticks were collected during summer, the subsequent calculations were limited to the animals collected in this trapping season. Overall, tick prevalence on small mammal hosts averaged 4% (8.0-11.0). Overall tick infestation rate differed between host species ($X^2 = 11.199$, $df = 3$, $P = 0.011$). Tick prevalence was higher in *C. russula* (19.4%), lower in *M. arvalis* (7.8%) and intermediate in *M. spretus* and *A. sylvaticus* (11.9% and 13.5%, respectively). Tukey test showed differences between *C. russula* and any of the other rodent hosts. If focused on *R. turanicus*, this interspecific difference was also significant ($X^2 = 11.585$, $df = 3$, $P = 0.009$) among hosts, although the Tukey test revealed weaker differences between *C. russula* and *M. arvalis* ($P = 0.060$) than with the other mouse species.

Table 1. Parasitological parameters of the tick community collected from the small mammal hosts studied from NW Spain (2009-2016).

| **Host** [n total][1] | **Tick** | **n. identified fleas** [n. hosts[2]] | **Fleas intensity range** | **Tick prevalence all year** Mean % (CI)[3] | **Tick prevalence summer** Mean % (CI)[3] | **Tick intensity summer** Mean (± SE) | **Variance / mean ratio summer** | **Discrepancy index July** (CI)[4] |
|---|---|---|---|---|---|---|---|---|
| *A. sylvaticus* | *Rhipicephalus turanicus* | 120 [28] | 1-21 | 4.6 (3.1-6.6) | 11.2 (7.5-15.7) | 4.3 (± 0.8) | 8.5 | 0.936 (0.912-0.958) |
| [604/251] | *Dermacentor* sp. | 4 [1] | 4 | 0.2 (0.0-0.9) | 0.4 (0.0-2.2) | 4.0 (± 0.1) | 4.0 | 0.992 (0.972-0.992) |
| | *Hyalomma* sp. | 3 [3] | 1 | 0.5 (0.1-1.4) | 1.2 (0.2-3.5) | 1.0 (± 0.5) | 1.0 | 0.984 (0.952-0.992) |
| *C. russula* | *Rhipicephalus turanicus* | 57 [10] | 1-21 | 13.2 (6.5-22.9) | 16.1 (8.0-27.7) | 5.7 (± 1.8) | 11.2 | 0.908 (0.856-0.950) |
| [76/62] | *Rhipicephalus pusillus* | 3 [1] | 3 | 1.3 (0.0-7.1) | 1.6 (0.0-8.7) | 3.0 (± 0.3) | 3.0 | 0.968 (0.873-0.968) |
| | *Rhipicephalus* sp. | 2 [2] | 1 | 2.6 (0.3-9.2) | 3.2 (0.4-11.2) | 1.0 (± 0.1) | 1.0 | 0.952 (0.873-0.968) |
| *M. spretus* | *Rhipicephalus turanicus* | 22 [5] | 1-14 | 1.3 (0.4-3.0) | 7.5 (2.5-16.6) | 4.4 (± 0.7) | 9.6 | 0.949 (0.914-0.971) |
| [383/67] | *Rhipicephalus* sp. | 1 [1] | 1 | 0.3 (0.0-1.4) | 1.5 (0.0-8.0) | 1.0 (± 0.1) | 1.0 | 0.971 (0.912-0.971) |
| *M. arvalis* | *Rhipicephalus turanicus* | 226 [74] | 1-46 | 4.6 (3.7-5.8) | 0.4 (0.1-0.9) | 3.1 (± 0.7) | 17.5 | 0.972 (0.956-0.980)* |
| [1597/1135] | *Rhipicephalus* sp. | 9 [4] | 1-6 | 0.3 (0.1-0.6) | 0.1 (0.0-0.5) | 2.3 (± 0.1) | 4.3 | 0.997 (0.994-0.998)* |
| | *Hyalomma* sp. | 10 [1] | 10 | 0.1 (0.0-0.3) | 7.8 (6.3-9.5) | 10.0 (± 0.1) | 10.0 | 0.998 (0.996-0.998)* |

CI: Confidence interval; SE: Standard error; [1] Number of total captured hosts all year/summer; [2] Number of infested hosts; [3] 95% Confidence interval by Clopper-Pearson; [4] 95% Confidence interval by bootstrap method; * Sample too big for bootstrap confident limits; the percentile method was used instead

D-Index values were close to 1 in all cases. The value of the variance-to-mean ratios (20.0) was five times higher than the mean intensity (4.0 ± 0.7), with a maximum aggregation pattern in *M. arvalis* (27.0), seven times higher than their mean intensity (3.8 ± 1.0). The lower number of ticks per host was harbored by *Mus spretus* [1-14], followed by *C. russula* and *A. sylvaticus* ([1-21] in both cases). *Microtus arvalis* showed a lower tick prevalence but a higher tick infestation range [range 1-46]. Co-infections with two different tick species were very unusual among the infested small mammals (5.6%; 8/143), representing 0.5% of the screened animals in summer (8/1515). However, the percentage could be even lower because ticks from six of these eight hosts (they had unidentified ticks or ticks identified at genus level *Rhipicephalus*) could be most likely *R. turanicus*. See Table 1 for more detailed information on prevalence, intensity and aggregation indices.

**Variation of tick prevalence according to crop type, host sex and vole phase**

The variation pattern on tick prevalence in *M. arvalis* was explained by sex ($X^2$ = 5.735, *df* = 1, *P* = 0.017) and the density of coexisting mouse hosts ($X^2$ = 4.468, *df* = 1, *P* = 0.035). Female voles (9.6%) were more parasitized than males (5.8%), and the presence of sympatric mice was negatively correlated with the tick prevalence in voles. Variations in *R. turanicus* infestation rate was significantly explained by sex ($X^2$ = 6.717, *df* = 1, *P* = 0.010) and phase of the vole cycle ($X^2$ = 6.831, *df* = 2, *P* = 0.033). Post-hoc tests indicated that the prevalence was higher in females (8.4%) than males (4.5%), and voles were more infested by this tick in the crash phase (7.9%) than during the increase phase (3.2%).

When focusing on mouse hosts, only their overall density showed a negative correlation with tick prevalence, but the relationship was marginally significant ($X^2$ = 2.875, *df* = 1, *P* = 0.090). Regarding the overall tick infestation rate in *A. sylvaticus*, only the density of their own species was near significant ($X^2$ = 2.866, *df* = 1, *P* = 0.090), with a negative correlation tendency. In *M. spretus*, most ticks (27/29) and infested animals (7/8 positive animals) were trapped during the increase phase. The same tendency was detected in *C. russula*, with 62/63 collected ticks and 11/12 infested hosts from animals caught during the increase phase. Both *M. spretus* and *C. russula* samples were not large enough to perform further analyses.

No significant result was found according to crop type in the variation of the prevalence patterns analyzed.

Table 2. Results of generalized linear models (best models) explaining tick prevalence of the most abundant small mammal hosts trapped in July from NW Spain (2009-2016).

| Host | Tick | Predictor | Estimate ± SE | Z-value | *P* |
|---|---|---|---|---|---|
| All hosts | All ticks | Intercept | -2.104 ± 0.470 | -4.469 | < 0.001 |
| | | Species (*C. russula*) | 1.293 ± 0.469 | 2.755 | 0.006 |
| | | Species (*M. arvalis*) | 0.028 ± 0.330 | 0.084 | 0.933 |
| | | Species (*M. spretus*) | -0.454 ± 0.474 | -0.958 | 0.338 |
| | | Mouse density | -0.178 ± 0.085 | -2.098 | 0.036 |
| | *R. turanicus* | Intercept | -3.284 ± 0.605 | -5.432 | < 0.001 |
| | | Species (*C. russula*) | 1.445 ± 0.547 | 2.639 | 0.008 |
| | | Species (*M. arvalis*) | 0.027 ± 0.383 | 0.071 | 0.943 |
| | | Species (*M. spretus*) | -0.871 ± 0.567 | -1.536 | 0.125 |
| *M. arvalis* | All ticks | Intercept | -1.843 ± 0.459 | -4.013 | < 0.001 |
| | | Sex (male) | -0.587 ± 0.245 | -2.395 | 0.017 |
| | | Mouse density | -0.216 ± 0.102 | -2.114 | 0.035 |
| | *R. turanicus* | Intercept | -1.604 ± 0.637 | -2.516 | 0.012 |
| | | Sex (male) | -0.700 ± 0.270 | -2.592 | 0.010 |
| | | Phase (increase) | -3.537 ± 1.374 | -2.574 | 0.010 |
| | | Phase (peak) | -1.134 ± 0.897 | -1.265 | 0.206 |

# Discussion

### Tick community

One tick species, *R.turanicus*, dominated parasitation (93%) of the small mammal community. The climatic conditions can favor the predominance of *R. turanicus*, species that is well adapted to arid conditions in Mediterranean countries such as steppe and semi-desert habitats (28). This also explains the differences in the tick community and the almost absence of this tick from all surrounding regions (34). This tick species is a generalist ectoparasite with three-host stages over the life cycle that feeds on a wide range of mammals. Immature stages (i.e. larvae and nymphs) mainly feed on small mammals and hares while adults prefer cattle, sheep, dogs, wild canids, felids and mustelids, but also large ground-feeding birds and lizards (27,28). Mustelids (American mink), canids (red fox), large ground-feeding birds (great bustard), lagomorphs (Iberian hare and European rabbit) and small rodents (voles, mice and shrews) are common species in the study area (35). The other occasional tick identified at the species level was the nidicolous *R. pusillus*, which feeds on lagomorphs, preferably on European rabbit (28), another frequent species (35). All ticks identified were larva and nymph stages. Since most immature tick species feed often on

rodents (6), it is not surprising that other tick species such as *Dermacentor* sp. and *Hyalomma* sp. were occasionally collected.

It is known that *R. turanicus* can infect and transmit zoonotic pathogens to humans (36). This tick harbors several pathogens (*Anaplasma* sp., *Coxiella* sp., *Rickettsia* sp. *Hepatozoon* sp., *Theileria sp.*), not only as a host but also as a reservoir (37–40). In the study area, at least one of these bacteria (*Coxiella* sp.) has been detected in the *M. arvalis* population (23). Despite being a tick currently restricted to arid areas, climate change can favor their expansion. Scenarios involving predictions of rising temperature and decreasing precipitation can expand its distribution range over large areas of Spain and southern Europe (41). A well-documented example of *R. turanicus* colonization and its potential zoonotic risk is the case of the Cyprus island (38,42). Its wide host range, high reproductive rate, fast life cycle and tolerance to live in human-altered habitats are suggested to be behind this colonizing behavior (42). Its phenology is another factor to consider in determining the risk via tick bite. All ticks were collected only from small mammals trapped in summer, in accordance with the active period of this tick, whose questing activity is restricted to warmer seasons (spring and summer) (28). This fact can increase the risk for humans in the region since it is the period with the higher activity outdoors.

Regarding prevalence results, the average overall tick infestation rate in the small mammal community (9.4%) is similar to those obtained in other tick surveys on small mammals, with values varying between 3.7 and 42.3% (43–45), although sample sizes were small in most cases. However, the prevalence of *R. turanicus* would be higher than in the few surveys where this tick was detected, mainly in foxes, with a prevalence lower than 8% (42,44).

D-Index values were close to 1, indicating that ticks were highly aggregated in the host community. In addition, the values of the variance-to-mean ratios higher than the mean intensity were indicative of a marked parasite aggregation between the individual infected animals. Our results show the typical aggregation pattern of parasites (46), important in the establishment and persistence of vector-borne pathogens (47,48).

**Variation of tick prevalence according to crop type, host sex and vole phase**

The overall tick prevalence in voles was negatively correlated with the presence of other sympatric mouse species. This could be the result of a dilution effect from voles to other hosts available or that mice are more competent hosts for ticks. The second hypothesis would be more plausible because this tendency also occurred in the peak phase when voles were between seven and ten times more abundant for ticks than mice. This could be also partially supported by the pattern of tick prevalence in the mouse hosts, where the tick prevalence showed a marginally negative

correlation with mouse density but no relationship was found with the vole phase. Regarding *R. turanicus* in voles, a gathering pattern may happen in the crash phase, since the higher prevalence of this tick occurred when the vole density dropped down to minimum values. At this moment, contact between the remaining voles could be more likely to occur (while defending their territory or looking for females) than with other non-competitor mice or shrews.

Post-hoc tests indicated that the tick prevalence (both overall and *R. turanicus* prevalences) was higher in females than males. Colonial animals were reported to have a higher level of infestation due to the closer and more frequent contact among individuals with social behavior (49). This pattern was also found relating to flea prevalence in the same population, where voles do not have the male-biased tendency shown in other sympatric small mammal hosts (50).

Ticks in *M. spretus* and *C. russula* were only collected in years with an increasing tendency in the vole population density. This could be explained by a spillover effect during the recolonization process from the expanding common vole population, favoring the contact with other cohabiting hosts. However, the vole phase is not significant in the case of *A. sylvaticus*, the second most common small mammal (17), where only density on their own species had marginally negative significant relevance. This refuted the spillover hypothesis because it would be more probable to infect an *A. sylvaticus* than *M. spretus* or *C. russula*, which are less abundant. Other reasons that could explain this pattern would be differences in behavior, interspecific interaction, use of resources or parasitic competence between these host species, but further research is needed.

Habitat characteristics and microclimate at the vegetation level are characteristics that determine the activity of ticks (6,51). The crop types also affect the abundance of small mammals (17) and the risk of tick bites in farmers (52). However, the crop type was not relevant in the variation of the prevalence patterns analyzed. In other tick surveys, vegetation factors such as tree cover, the proximity of shrubby vegetation and the presence of natural or semi-natural habitats have been found as significant for ticks (51,53,54). However, in these highly intensified agro-ecosystems, those habitats are anecdotal. It would have been more appropriate to additionally test for differences between the inside and the edge of crops, given their influence on the distribution and abundance of small mammal hosts (17) and ticks (53).

## Conclusion

*Rhipicephalus turanicus* was by far the most prevalent tick parasitizing the four species that dominate the small mammal guild in arid ecosystems of NW Spain. We found strong sex-biased differences in vole hosts, and the density of sympatric mice and the phase of the vole cycle could explain the variation patterns in tick prevalence. A better understanding of interspecific relationships

among hosts would help to know in more detail the life cycle of *R. turanicus* in the area and the possible role in the circulation of tick-borne zoonotic pathogens. This tick is a species restricted to arid Mediterranean areas (28) and well adapted to tolerate several months of drought (41). Climate swifts to moderate autumns and winters would broaden the active period of ticks and hence, their transmission risk of zoonoses while feeding (6,41). This tick should be further studied and surveilled regarding its potential tick-borne role of emerging zoonotic diseases in the area which, combined with the effects of climate change and the cyclic population dynamic of *M. arvalis*, may have unpredictable consequences on the circulation of zoonotic pathogens in the future.

## Declarations

### Ethics approval and consent to participate

The trapping methods applied in this study were approved by the ethics committee of our Institution (CEEBA, Universidad de Valladolid; authorization code: 4801646) and we counted with the official trapping permits from DGMN (Junta de Castilla-y-León), as well as compulsory national certificates (B and C categories) to manipulate living animals for research.

### Competing interests

The authors declare that they have no competing interests.

### Funding

This work was funded by the projects ECOCYCLES (Partner 5–EUI2008-03658), ECOVOLE (CGL2012-35348), ECOTULA (CGL2015-66962-C2-1-R), BOOMRAT (PID2019-109327RB-I00) and GESINTTOP (co-funded by Instituto Tecnológico Agrario de Castilla y León - Junta de Castilla y León (ITACYL-JCYL), Diputación Provincial de Palencia and Diputación Provincial de Valladolid). SHC was supported by a PhD studentship from Junta de Castilla-y-León and the European Social Fund (Orden 10/11/2016) and the project GESINTTOP.

### Authors' contributions

JJLL and FM obtained all the funding and designed the monitoring and study. JJLL and SHC collected the small mammal data. SHC identified tick species. SHC and FM performed the statistical analysis. SHC drafted the manuscript. All authors critically revised the paper and approved the final manuscript.

**Acknowledgments**

We are grateful to all the personnel that contributed to the vole monitoring and the tick collection (Leticia Arroyo, Daniel Jareño, José-Luis Guzmán, Ruth Rodríguez-Pastor and Juan Romairone). We thank Félix Valcárcel, Sonia Olmeda and Dolors Vidal for helping with tick identification.

# References

1. Jongejan F, Uilenberg G. The global importance of ticks. *Parasitology* (2004) **129**:S3–S14. doi:10.1017/S0031182004005967
2. Pfäffle M, Littwin N, Muders S V., Petney TN. The ecology of tick-borne diseases. *Int J Parasitol* (2013) **43**:1059–1077. doi:10.1016/j.ijpara.2013.06.009
3. Rochlin I, Toledo A. Emerging tick-borne pathogens of public health importance: a mini-review. *J Med Microbiol* (2020) **69**:781–791. doi:10.1099/jmm.0.001206
4. Dantas-Torres F, Chomel BB, Otranto D. Ticks and tick-borne diseases: a One Health perspective. *Trends Parasitol* (2012) **28**:437–446. doi:10.1016/j.pt.2012.07.003
5. Madison-Antenucci S, Kramer LD, Gebhardt LL, Kauffman E. Emerging tick-borne diseases. *Clin Microbiol Rev* (2020) **33**:e00083-18. doi:10.1128/CMR.00083-18
6. Estrada-Peña A. Ticks as vectors: taxonomy, biology and ecology. *Rev Sci Tech l'OIE* (2015) **34**:53–65. doi:10.20506/rst.34.1.2345
7. Herrero-Cófreces S, Mougeot F, Lambin X, Luque-Larena JJ. Linking zoonosis emergence to farmland invasion by fluctuating herbivores: common vole populations and tularemia outbreaks in NW Spain. *Front Vet Sci* (2021) **8**:900. doi:10.3389/fvets.2021.698454
8. McMahon BJ, Morand S, Gray JS. Ecosystem change and zoonoses in the Anthropocene. *Zoonoses Public Health* (2018) **65**:755–765. doi:10.1111/zph.12489
9. Jones BA, Grace D, Kock R, Alonso S, Rushton J, Said MY, McKeever D, Mutua F, Young J, McDermott J, et al. Zoonosis emergence linked to agricultural intensification and environmental change. *Proc Natl Acad Sci* (2013) **110**:8399–8404. doi:10.1073/pnas.1208059110
10. Colwell DD, Dantas-Torres F, Otranto D. Vector-borne parasitic zoonoses: Emerging scenarios and new perspectives. *Vet Parasitol* (2011) **182**:14–21. doi:10.1016/j.vetpar.2011.07.012
11. Han BA, Schmidt JP, Bowden SE, Drake JM. Rodent reservoirs of future zoonotic diseases. *Proc Natl Acad Sci* (2015) **112**:7039–7044. doi:10.1073/pnas.1501598112
12. Bordes F, Blasdell K, Morand S. Transmission ecology of rodent-borne diseases: New frontiers. *Integr Zool* (2015) **10**:424–435. doi:10.1111/1749-4877.12149
13. Mendoza H, Rubio A V., García-Peña GE, Suzán G, Simonetti JA. Does land-use change increase the abundance of zoonotic reservoirs? Rodents say yes. *Eur J Wildl Res* (2020) **66**:6. doi:10.1007/s10344-019-1344-9
14. Jareño D, Viñuela J, Luque-Larena JJ, Arroyo L, Arroyo B, Mougeot F. Factors associated with the colonization of agricultural areas by common voles *Microtus arvalis* in NW Spain.

*Biol Invasions* (2015) **17**:2315–2327. doi:10.1007/s10530-015-0877-4

15. Meerburg BG. Rodents are a risk factor for the spreading of pathogens on farms. *Vet Microbiol* (2010) **142**:464–465. doi:10.1016/j.vetmic.2009.06.038

16. Hornok S, Földvári G, Rigó K, Meli ML, Gönczi E, Répási A, Farkas R, Papp I, Kontschán J, Hofmann-Lehmann R. Synanthropic rodents and their ectoparasites as carriers of a novel haemoplasma and vector-borne, zoonotic pathogens indoors. *Parasites and Vectors* (2015) **8**:1–6. doi:10.1186/s13071-014-0630-3

17. Rodríguez-Pastor R, Luque-Larena JJ, Lambin X, Mougeot F. "Living on the edge": The role of field margins for common vole (*Microtus arvalis*) populations in recently colonised Mediterranean farmland. *Agric Ecosyst Environ* (2016) **231**:206–217. doi:10.1016/j.agee.2016.06.041

18. Luque-Larena JJ, Mougeot F, Viñuela J, Jareño D, Arroyo L, Lambin X, Arroyo B. Recent large-scale range expansion and outbreaks of the common vole (*Microtus arvalis*) in NW Spain. *Basic Appl Ecol* (2013) **14**:432–441. doi:10.1016/j.baae.2013.04.006

19. Luque-Larena JJ, Mougeot F, Arroyo B, Lambin X. " Got rats ?" Global environmental costs of thirst for milk include acute biodiversity impacts linked to dairy feed production. *Glob Chang Biol* (2018) **24**:2752–2754. doi:10.1111/gcb.14170

20. Rodríguez-Pastor R, Escudero R, Vidal MD, Mougeot F, Arroyo B, Lambin X, Vila-Coro AM, Rodríguez-Moreno I, Anda P, Luque-Larena JJ. Density-dependent prevalence of *Francisella tularensis* in fluctuating vole populations, northwestern Spain. *Emerg Infect Dis* (2017) **23**:1377–1379. doi:10.3201/eid2308.161194

21. Rodríguez-Pastor R, Escudero R, Lambin X, Vidal MD, Gil H, Jado I, Rodríguez-Vargas M, Luque-Larena JJ, Mougeot F. Zoonotic pathogens in fluctuating common vole (*Microtus arvalis*) populations: occurrence and dynamics. *Parasitology* (2019) **146**:389–398. doi:10.1017/S0031182018001543

22. González-Barrio D, Jado I, Viñuela J, García JT, Olea PP, Arce F, Ruiz-Fons F. Investigating the role of micromammals in the ecology of *Coxiella burnetii* in Spain. *Animals* (2021) **11**:654. doi:10.3390/ani11030654

23. González-Barrio D, Viñuela J, Santamaría AE, Olea PP, García JT, Ruiz-Fons F. Prevalence of zoonotic pathogens in common vole (*Microtus arvalis*) during a population outbreak. in *Proceedings of the 15th Rodens et Spatium* (Olomouc), 129.

24. Mougeot F, Lambin X, Rodríguez-Pastor R, Romairone J, Luque-Larena JJ. Numerical response of a mammalian specialist predator to multiple prey dynamics in Mediterranean farmlands. *Ecology* (2019) **100**:e02776. doi:10.1002/ecy.2776

25. Tkadlec E, Václavík T, Široký P. Rodent host abundance and climate variability as predictors of tickborne disease risk 1 year in advance. *Emerg Infect Dis* (2019) **25**:1738–1741. doi:10.3201/eid2509.190684

26. Rivas-Martínez S, Penas Á, del Río S, Díaz González TE, Rivas-Sáenz S. "Bioclimatology of the Iberian Peninsula and the Balearic Islands," in *The Vegetation of the Iberian Peninsula*, ed. J. Loidi Cham: Springer International Publishing (2017) 29–80. doi:10.1007/978-3-319-54784-8_2

27. Estrada-Peña A, Bouattour A, Camicas JL, Walker AR. *Ticks of domestic animals in the Mediterranean region. A guide to identification of species*. Zaragoza: University of Zaragoza (2004).

28. Estrada-Peña A, Mihalca AD, Petney TN. *Ticks of Europe and North Africa*. Cham: Springer International Publishing (2017). doi:10.1007/978-3-319-63760-0

29. Manilla G. *Fauna d'Italia, Acari Ixodida*. Bologna: Edizioni Calderini (1998).

30. Poulin R. The disparity between observed and uniform distributions: A new look at parasite aggregation. *Int J Parasitol* (1993) **23**:937–944. doi:10.1016/0020-7519(93)90060-C

31. Reiczigel J, Marozzi M, Fábián I, Rózsa L. Biostatistics for parasitologists – A primer to quantitative parasitology. *Trends Parasitol* (2019) **35**:277–281. doi:10.1016/j.pt.2019.01.003

32. Bates D, Mächler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. *J Stat Softw* (2015) **67**. doi:10.18637/jss.v067.i01

33. R Core Team. R: The R project for statistical computing. (2019)

34. Barandika JF, Olmeda SA, Casado-Nistal MA, Hurtado A, Juste RA, Valcárcel F, Anda P, García-Pérez AL. Differences in questing tick species distribution between atlantic and continental climate regions in Spain. *J Med Entomol* (2011) **48**:13–19. doi:10.1603/ME10079

35. Palomo LJ, Gisbert J, Blanco JC. *Atlas y libro rojo de los mamíferos terrestres de España*. Madrid: Dirección General para la Biodiversidad-SECEM-SECEMU (2007).

36. Fuente J de la, Estrada-Peña A, Venzal JM, Kocan KM, Sonenshine DE. Overview: Ticks as vectors of pathogens that cause disease in humans and animals. *Front Biosci* (2008) **1**:6938–6946. doi:10.2741/3200

37. Lalzar I, Harrus S, Mumcuoglu KY, Gottlieb Y. Composition and seasonal variation of *Rhipicephalus turanicus* and *Rhipicephalus sanguineus* bacterial communities. *Appl Environ Microbiol* (2012) **78**:4110–4116. doi:10.1128/AEM.00323-12

38. Chochlakis D, Ioannou I, Papadopoulos B, Tselentis Y, Psaroulaki A. *Rhipicephalus turanicus*: from low numbers to complete establishment in Cyprus. Its possible role as a bridge-vector. *Parasit Vectors* (2014) **7**:P11. doi:10.1186/1756-3305-7-S1-P11

39. Giannelli A, Lia RP, Annoscia G, Buonavoglia C, Lorusso E, Dantas-Torres F, Baneth G, Otranto D. *Rhipicephalus turanicus*, a new vector of *Hepatozoon canis*. *Parasitology* (2017) **144**:730–737. doi:10.1017/S003118201600250X

40. Azmi K, Ereqat S, Nasereddin A, Al-Jawabreh A, Baneth G, Abdeen Z. Molecular detection of *Theileria*, *Babesia*, and *Hepatozoon* spp. in ixodid ticks from Palestine. *Ticks Tick Borne Dis* (2016) **7**:734–741. doi:10.1016/j.ttbdis.2016.03.003

41. Estrada-Peña A, Venzal JM. Climate niches of tick species in the Mediterranean region: modeling of occurrence data, distributional constraints, and impact of climate change. *J Med Entomol* (2007) **44**:1130–1138. doi:10.1093/jmedent/44.6.1130

42. Tsatsaris A, Chochlakis D, Papadopoulos B, Petsa A, Georgalis L, Angelakis E, Ioannou I, Tselentis Y, Psaroulaki A. Species composition, distribution, ecological preference and host association of ticks in Cyprus. *Exp Appl Acarol* (2016) **70**:523–542. doi:10.1007/s10493-016-0091-9

43. Barandika JF, Hurtado A, García-Esteban C, Gil H, Escudero R, Barral M, Jado I, Juste RA, Anda P, Garcia-Perez AL. Tick-borne zoonotic bacteria in wild and domestic small mammals in Northern Spain. *Appl Environ Microbiol* (2007) **73**:6166–6171. doi:10.1128/AEM.00590-07

44. Domínguez-Peñafiel G, Giménez-Pardo C, Gegúndez MI, Lledó L. Prevalence of ectoparasitic arthropods on wild animals and cattle in the Las Merindades area (Burgos, Spain). *Parasite* (2011) **18**:251–260. doi:10.1051/parasite/2011183251

45. Cevidanes A, Proboste T, Chirife AD, Millán J. Differences in the ectoparasite fauna between micromammals captured in natural and adjacent residential areas are better explained by sex and season than by type of habitat. *Parasitol Res* (2016) **115**:2203–2211. doi:10.1007/s00436-016-4962-0

46. Krasnov BR, Poulin R, Morand S. "Patterns of macroparasite diversity in small mammals," in *Micromammals and macroparasites: from evolutionary ecology to management*, eds. S. Morand, B. R. Krasnov, R. Poulin Tokyo: Springer Japan (2006) 197–231.

47. Randolph SE, Gern L, Nuttall PA. Co-feeding ticks: Epidemiological significance for tick-borne pathogen transmission. *Parasitol Today* (1996) **12**:472–479. doi:10.1016/S0169-4758(96)10072-7

48. Hawlena H, Khokhlova IS, Abramsky Z, Krasnov BR. Age, intensity of infestation by flea parasites and body mass loss in a rodent host. *Parasitology* (2006) **133**:187. doi:10.1017/S0031182006000308

49. Krasnov BR, Bordes F, Khokhlova IS, Morand S. Gender-biased parasitism in small mammals: patterns, mechanisms, consequences. *Mammalia* (2012) **76**:1–13. doi:10.1515/mammalia-2011-0108

50. Herrero-Cófreces S, Flechoso MF, Rodríguez-Pastor R, Luque-Larena JJ, Mougeot F. Patterns of flea infestation in rodents and insectivores from intensified agro-ecosystems, Northwest Spain. *Parasit Vectors* (2021) **14**:16. doi:10.1186/s13071-020-04492-6

51. Richardson EA, Taylor CE, Jabot B, Martin E, Keiser CN. The effects of habitat type and pathogen infection on tick host-seeking behaviour. *Parasitology* (2022) **149**:59–64. doi:10.1017/S0031182021001554

52. Li S, Juhász-Horváth L, Trájer A, Pintér L, Rounsevell MDA, Harrison PA. Lifestyle, habitat and farmers' risk of exposure to tick bites in an endemic area of tick-borne diseases in Hungary. *Zoonoses Public Health* (2018) **65**:e248–e253. doi:10.1111/zph.12413

53. Medlock JM, Vaux AGC, Hansford KM, Pietzsch ME, Gillingham EL. Ticks in the ecotone: the impact of agri-environment field margins on the presence and intensity of *Ixodes ricinus* ticks (Acari: Ixodidae) in farmland in southern England. *Med Vet Entomol* (2020) **34**:175–183. doi:10.1111/mve.12428

54. Lindström A, Jaenson TGT. Distribution of the common tick, *Ixodes ricinus* (Acari: Ixodidae), in different vegetation types in Southern Sweden. *J Med Entomol* (2003) **40**:375–378. doi:10.1603/0022-2585-40.4.375

# Additional file

Additional table. Number of samples analyzed from each trapping session in “Tierra de Campos” region, Castilla-y-León, NW Spain, 2009–2016.

| Year | Month | Phase | **Host** (sample size) | | | | |
|---|---|---|---|---|---|---|---|
| | | | ***A. sylvaticus*** | ***C. russula*** | ***M. spretus*** | ***M. arvalis*** | **All species** |
| 2009 | All months | Crash | 44 | 2 | 42 | 200 | 288 |
| | July | | 1 | 0 | 0 | 182 | 183 |
| | November | | 43 | 2 | 42 | 18 | 105 |
| 2010 | All months | Increase | 90 | 7 | 55 | 59 | 211 |
| | March | | 10 | 0 | 0 | 9 | 19 |
| | July | | 38 | 7 | 24 | 21 | 90 |
| | November | | 42 | 0 | 31 | 29 | 102 |
| 2011 | All months | Peak | 65 | 4 | 44 | 200 | 313 |
| | March | | 31 | 3 | 19 | 24 | 77 |
| | July | | 4 | 0 | 1 | 129 | 134 |
| | November | | 30 | 1 | 24 | 47 | 102 |
| 2012 | All months | Crash | 53 | 1 | 12 | 78 | 144 |
| | March | | 9 | 0 | 3 | 8 | 20 |
| | July | | 29 | 1 | 4 | 61 | 95 |
| | November | | 15 | 0 | 5 | 9 | 29 |
| 2013 | All months | Increase | 112 | 18 | 51 | 103 | 284 |
| | March | | 6 | 1 | 2 | 3 | 12 |
| | July | | 76 | 17 | 12 | 42 | 147 |
| | November | | 30 | 0 | 37 | 58 | 125 |
| 2014 | All months | Peak | 100 | 9 | 93 | 706 | 908 |
| | March | | 38 | 3 | 31 | 103 | 175 |
| | July | | 26 | 3 | 10 | 535 | 574 |
| | November | | 36 | 3 | 52 | 68 | 159 |
| 2015 | All months | Crash | 65 | 1 | 9 | 22 | 97 |
| | March | | 20 | 1 | 2 | 18 | 41 |
| | July | | 38 | 0 | 4 | 3 | 45 |
| | November | | 7 | 0 | 3 | 1 | 11 |
| 2016 | All months | Peak | 75 | 34 | 77 | 229 | 415 |
| | March | | 7 | 0 | 4 | 2 | 13 |
| | July | | 39 | 34 | 12 | 162 | 247 |
| | November | | 29 | 0 | 61 | 65 | 155 |
| All years | All months | | 604 | 76 | 383 | 1597 | 2660 |

# CHAPTER 3

# MICROPATHOGENS

# CHAPTER 3.1

# BACTERIA

# Zoonotic pathogen spillover in small mammals co-habiting with fluctuating common voles (*Microtus arvalis*)

*Silvia Herrero-Cófreces [1,2*], Juan José Luque-Larena [1,2], Francois Mougeot [3]

[1] Dpto. Ciencias Agroforestales, ETSIIAA, Universidad de Valladolid, Avda. de Madrid 44, 34004 Palencia, Spain

[2] Instituto Universitario de Investigación en Gestión Forestal Sostenible, Palencia, Spain

[3] Instituto de Investigación en Recursos Cinegéticos, IREC (CSIC-UCLM-JCCM), Ronda de Toledo s/n, 13071, Ciudad Real, Spain

*Provisional authors of this draft

## Abstract

Small mammals are wild reservoirs of multiple pathogens and the cyclic population dynamic of some rodents has been linked to the growing risk of zoonoses worldwide. Rodents act as spreaders of many diseases and play key roles in disease spillover to other sympatric competent host species including human populations. In intensive farmland of NW Spain, *Microtus arvalis* (common vole) exhibit cyclic population dynamics and recurrently causes regional outbreaks. *Microtus arvalis* harbors *Francisella tularensis* and five *Bartonella* spp. The former varies seasonally; the latter is density-dependent. This study aimed to investigate *F. tularensis* and *Bartonella* diversity and prevalence in the sympatric small mammal guild (*Apodemus sylvaticus*, *Mus spretus* and *Crocidura russula*), and test for a potential spillover of pathogens from *M. arvalis* to other hosts during vole abundance fluctuations. We studied how pathogen prevalence varied with host sex, season, the phase of the *M. arvalis* cycle (increase, peak and crash), and the abundance of alternative small mammal host species. Because *Bartonella* is vectored by fleas, we also related pathogen prevalence to the most abundant flea species, shared amongst small mammal hosts. We extracted DNA from a mix of liver and spleen collected from hosts and used molecular analysis to screen for *Bartonella* species and *F. tularensis*. *Francisella tularensis* was only detected in one *A. sylvaticus*, but not in other hosts, and nine *Bartonella* genotypes were identified. The small mammal guild shared the two commonest *Bartonella* species (*B. grahamii* and *B. elisabethae*). *Bartonella grahamii* prevalence in cohabiting hosts (mice and shrew) varied with the *M. arvalis* cycle phase, with greater prevalence at peak density. *Bartonella* prevalence also peaked during autumn in *A. sylvaticus*. Mixed infections occurred in 85.9% of all *Bartonella*-positive hosts and were more frequent during the peak phase of *M. arvalis* cycle. *Bartonella* prevalence was higher in *M. spretus* and decreased with increasing mouse abundance. Fleas were more prevalent and abundant in the guild during the peak phase of the *M. arvalis* cycle. However, we found no association between *Bartonella* and the flea species that are common with *M. arvalis*. Only the flea *Leptopsylla taschenbergi* seemed to have some role in the *B. elisabethae* circulation. We conclude that *Bartonella* should receive more attention concerning its zoonotic potential because five out of the nine species detected were zoonotic, including the two most frequent ones. Rodents, their fluctuation patterns, and seasonality should be taken into account in the understanding of *Bartonella* circulation. The small mammal guild surveyed that lives in sympatry with *Microtus arvalis* seem to have no role in the circulation and maintenance of *F. tularensis*.


**Keywords:** *Apodemus sylvaticus*, *Bartonella*, co-infection patterns, *Crocidura russula*, effects of cyclic hosts, *Francisella tularensis*, *Mus spretus*

## Introduction

Mammals are important reservoir hosts of zoonoses and among them, rodents include the highest number of zoonotic host species (1) (2). Moreover, rodents often act as reservoirs of zoonotic diseases including viruses, bacteria, protozoa, fungi and helminths (3,4). Nowadays, most emerging infectious diseases (54,3%) are caused by bacteria (5). Coinfections are a frequent phenomenon (6) and play key roles in the ecology of pathogens (7). The presence of one pathogen can have a negative, positive o no effect on a second pathogen (8). These interactions between pathogens can be direct via competition or facilitation, or indirect via the host immune response (6). The result is an interconnected web of interactions (8) that determines the variability of pathogens, host susceptibility, infection length, transmission risk and symptomatology (6). Host density and abundance are also key variables in the circulation of rodent-borne pathogens (2). The irruptive, boom-bust population dynamics of some rodents have been involved in the disease transmission, amplification and spillover of many diseases affecting humans worldwide (9–13). The alteration of ecosystems intensifies the emergence of rodent-borne diseases, especially in opportunistic, generalist and/or synanthropic taxa (2,14).

*Microtus arvalis* (common vole) is one of the most widespread small mammals in Europe and is a main agricultural pest during population outbreaks (Jacob and Tkadlec, 2010). In the intensive agroecosystems of NW Spain, *M. arvalis* have become the most abundant small mammal species (15) following a recent colonization event (16). *Microtus arvalis* harbors *Francisella tularensis* (the etiological agent of tularemia (17) and five species of *Bartonella* (18). These bacteria are facultative intracellular pathogens causing zoonotic diseases (19,20). Epidemics of tularemia have been linked to *M. arvalis* outbreaks (21), a rodent host that acts as an amplifier and spreader of this bacterium (13,18,22). The main transmission routes are by inhalation or by contact with infected wild animals (19). *Bartonella* is a flea-borne pathogen that is common in rodents. *Bartonella* species have undergone a close process of evolutionary adaptation to their host (20) and more than 35 different species have been described so far (23). At least 28 *Bartonella* species can be hosted by rodents (20,23) and 12 of them are known to be zoonotic (23,24). Co-occurrence of *F. tularensis* and *Bartonella* has been described in *M. arvalis* (25), and both bacteria have been detected in fleas collected from this host (25). In NW Spain, the most abundant fleas infesting *M. arvalis* and sympatric small mammals are *Ctenophthalmus apertus*, *Leptopsylla taschenbergi* and *Nosopsyllus fasciatus* (26). Seasonality and host abundance shape this pathogen's ecology (18), but further investigation would be necessary to understand the role of sympatric hosts. This study aimed to determine the prevalence of *F. tularensis* and *Bartonella* species in three small mammal species (*Apodemus sylvaticus*, *Mus spretus* and *Crocidura russula*) that cohabit with *M. arvalis*. We further tested if pathogen prevalence among these

hosts varied with host sex, season, phase of *M. arvalis* cycle, the density of each host, and the prevalence of the main flea species. We hypothesized that *M. arvalis* would contribute to the emplification of pathogens during periods of high abundance, which could spill-over to other cohabiting small hosts. We further predicted that *Bartonella* species linked to the flea species that are shared amongst rodent host would be those spilling-over during the *M. arvalis* population increases.

## Materials and methods

### Study area

The study was carried out in an 80 $km^2$ area located in Palencia province (Castilla-y-León region, NW Spain). The area is an intensive agricultural landscape with a mosaic structure dominated by cereal crops with scattered alfalfa fields (15). The climate is continental-Mediterranean with a marked seasonality (27).

### Small mammal guild

*Microtus arvalis* (common vole) is the most abundant small mammal species in the study area (15) and is characterized by cyclic population dynamics (interannual fluctuations in abundance, with a 3-year period (28,29). *Microtus arvalis* lives in sympatry with other small mammals, especially *Apodemus sylvaticus* (long-tailed field mouse), *Mus spretus* (western Mediterranean mouse) and *Crocidura russula* (great white-toothed shrews), which, unlike *M. arvalis*, show only seasonal fluctuations (28,30). These four species represent the vast majority (> 95%) of the sympatric small mammal guild in the area (15).

### Small mammal trapping and sample collection

Fieldwork consisted of seasonal live-trapping events conducted every 4 months, during March, July and November. Populations were monitored from March 2013 to March 2015, following the methodology detailed in (15). This period covered an entire *M. arvalis* population cycle, with an increase phase during 2013, a population peak in 2014 followed by a crash in 2015 (18,28). To trap small mammals, we used Sherman© traps (8 cm × 9 cm × 23 cm; LFAHD Sherman©) baited with carrots, which were set open in the morning and retrieved 24h later. Each captured individual was given a unique ID code. The density of small mammal species was estimated as the number of captured animals per 100 traps per 24 h (28). We held all the necessary ethical and legal permits for manipulation and scientific capture. Throughout the study period, we sampled 341 small mammals (*A. sylvaticus* = 225, *M. spretus* = 65, *C. russula* = 51) which were screened for pathogens and fleas (see Table S1 in the Appendix for more details).

Once in the laboratory, trapped animals were euthanized through $CO_2$ inhalation, following a humane protocol approved by the ethics committee of our institution (CEEBA, Universidad de Valladolid; authorization code: 4801646). Immediately after death, fleas were quantified, collected and identified from those animals that arrived alive at the laboratory (see (26) for more details). Fleas could be checked from 159 live small mammals (*A, sylvaticus* = 113, *M. spretus* = 37 and *C. russula* = 9) and collected from 55 of those individuals (*A. sylvaticus* = 47, *M. spretus* = 4 and *C. russula* = 3). We stored animal carcasses at -23˚C until dissection. Following standard aseptic protocols, we collected the spleen and liver from each animal, separately labeled in tubes and stored them at -23˚C until molecular analysis.

**Molecular screening**

Previous works have characterized *Francisella tularensis* and *Bartonella* infections in *M. arvalis* from the same populations and study period (18) and we used a similar screeing here to assess the prevalence of these bacteria in the cohabiting small mammals (*A. sylvaticus*, *M. spretus* and *C. russula*).

DNA was extracted from a mix of liver and spleen using QIAamp DNA Mini Kit® (QIAamp® DNA Mini Kit, Qiagen, Hilden, Germany) and following the manufacturer's instructions. The quantity of DNA was measured with a Nanodrop ND-1000. Milli-Q water was used as a negative control for DNA extraction. Samples were tested using a real-time polymerase chain reaction (PCR), followed by a reverse line blotting (RLB) for species-level identification of positive samples.

For *F. tularensis* detection, we use two probes in the real-time PCR: ISFtu2 gene (insertion sequence highly sensitive to detect *Francisella* genus) and tul4 (a gene that encodes outer membrane proteins specific for *F. tularensis*) (31). *Francisella*-positive samples were analyzed by conventional PCR and further specific hybridization with RLB, using the 233-bp fragment on a variable region of lpnA gene. A sample of *Francisella tularensis* type A was used as a positive control since type A strain is restricted to North America (32); Milli-Q water was used as a negative control. More details in (33).

For *Bartonella* detection, we use a multiplex PCR targeting a conservative sequence from the 16S rRNA in the real-time PCR. *Bartonella*-positive samples were screened with an RLB for the identification of 36 different species and genotypes, using the variable intergenic transcribed spacer 16S-23S rRNA. We used an internal amplification control and Milli-Q water as a negative control. More details in (34) and (35).

**Data analysis**

We used generalized linear models (GLMs) to explore variations in the probability of being infected (prevalence) by each pathogen. We first tested for differences between host species (*A. sylvaticus*, *M.*

*spretus*, *C. russula*), and according to host sex (male, female), season (March, July, November; hereafter spring, summer and autumn, respectively), *M. arvalis* cycle phase (increasing, peak, crash year; for more details, see Table S1 in the Appendix and (18)), and the density of study hosts (*A. sylvaticus*, *M. spretus*, *C. russula*). Two-way interactions between sex and both season and *M. arvalis* cycle phase were tested whenever possible, but the small sample size did not allow to include these for all host species. The *M. arvalis* cycle phase effect was only tested to study the prevalence variation of *Bartonella* species that were shared between *M. arvalis* and other hosts (see Table S2 in the Appendix and (18)). We investigated flea-*Bartonella* specificity by testing for association between the prevalence of the commonest *Bartonella* species (dependent variable) and flea species (prevalence of *Ctenophthalmus apertus*, *Nosopsyllus fasciatus* and *Leptopsylla taschenbergi* as explanatory variables). Additionally, we tested whether flea prevalence in cohabiting hosts varied according to the vole cycle phase. Because *Bartonella* are vectored by fleas, and fleas can infest switch hosts, we tested whether the prevalence and abundance of fleas (dependent variables) on mice (*A. sylvaticus*, *M. spretus*) varied according to the vole cycle phase, expecting greater flea burdens on mice during the vole population crash. Model selection followed a backward-selection procedure (using the "drop1" function in R), sequentially removing non-significant terms, starting with interactions. We considered a $P = 0.05$ threshold as significant, and a $P = 0.10$ threshold as marginally significant. Post-hoc Tukey tests were calculated to examine differences between levels of the significant factors. Statistical analyses were performed using the "R2admb" (36) package of the R4.1.2 software (37).

# RESULTS

### Pathogen prevalence

*Francisella tularensis* was detected in one *A. sylvaticus* (0.4%; 1/225) but was not detected in the other small mammals screened (*M. spretus*, n=65; *C. russula*; n=51). In the positive individual, no co-infection with *Bartonella* was identified.

*Bartonella* was detected in 45.7% (156/341) of screened animals. The overall prevalence was differed between host species ($X^2 = 19.451$, $df = 2$, $P < 0.001$). *Bartonella* prevalence was higher in the two mouse hosts (*A. sylvaticus*, *M. spretus*) than in the shrew (*C. russula;* Table 1). Nine *Bartonella* genotypes were identified: *B. chomelii*, *B. elisabethae*, *B. grahamii*, *B. rochalimae*, *B. taylorii*, *B. tribocorum*, *B. birtlesii*, *B. cooperplainsense* and *B. vinsonii berkhoffii*. The two most prevalent species in the guild were *B. grahamii* (44.0%; 150/341) and *B. elisabethae* (39.0%; 133/341) and were shared by the three host species. Other *Bartonella* species were shared by two of the hosts (Table 1) such as *B. taylorii*, detected in both mouse species, and *B. chomelii*, which was found in *A. sylvaticus* and *C.*

*russula*. Some genotypes were found in a single host species (Table 1). See Appendix for more detailed information.

Table 1. Sample size, prevalence and frequency of *Bartonella* species occurrence among the small mammal guild studied, NW Spain, 2013–2015.

| | ***A. sylvaticus*** | | | ***C. russula*** | | | ***M. spretus*** | | |
|---|---|---|---|---|---|---|---|---|---|
| | **N** | **P (%)** | **F (%)** | **N** | **P (%)** | **F (%)** | **N** | **P (%)** | **F (%)** |
| Total hosts | 225 | - | - | 51 | - | - | 65 | - | - |
| All *Bartonella* spp. | 106 | 47.1 | 100 | 9 | 17.6 | 00 | 41 | 63.1 | 100 |
| *B. elisabethae* | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1.5 | 2.4 |
| with *B. grahamii* | 82 | 36.4 | 77.4 | 0 | 0 | 0 | 35 | 53.8 | 85.4 |
| with *B. grahamii* and *B. rochalimae* | 1 | 0.4 | 0.9 | 0 | 0 | 0 | 0 | 0 | 0 |
| with *B. grahamii* and *B. taylorii* | 1 | 0.4 | 0.9 | 0 | 0 | 0 | 2 | 3.9 | 4.9 |
| with *B. grahamii* and *B. tribocorum* | 0 | 0 | 0 | 8 | 15.7 | 88.9 | 0 | 0 | 0 |
| with *B. grahamii, B. tribocorum* and *B. chomelii* | 0 | 0 | 0 | 1 | 2.0 | 11.1 | 0 | 0 | 0 |
| with *B. grahamii*, *B. birtlesii*, *B. cooperplainsense* and *B. vinsonii berkhoffii* | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3.1 | 4.9 |
| *B. grahamii* | 16 | 7.1 | 15.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| with *B. chomelii* | 1 | 0.4 | 0. | 0 | 0 | 0 | 0 | 0 | 0 |
| with *B. rochalimae* | 1 | 0.4 | 0.9 | 0 | 0 | 0 | 0 | 0 | 0 |
| *B. taylorii* | 4 | 1.8 | 3.8 | 0 | 0 | 0 | 1 | 1.5 | 2.4 |

N, number of infected hosts; P, *Bartonella* prevalence; F, *Bartonella* frequency

### *Bartonella* prevalence variation according to host sex, season and host abundance

We first analyzed variation in overall *Bartonella* prevalence (all *Bartonella* species and hosts combined) and then analyzed variation in the two most abundant *Bartonella* species *B. elisabethae* and *B. grahamii* separately (Table 2). For *C. russula,* which was infected with *B. elisabethae* and *B. grahamii*, the small sample size, did not allow us to analyze the result by species and we only examined overall *Bartonella* spp. (Table 2).

The overall model took into account the abovementioned differences in prevalence between the three study hosts (Table 2), and further revealed differences in *Bartonella* prevalence between seasons ($X^2 = 8.519$, $df = 2$, $P = 0.014$), and according to the vole cycle phase ($X^2 = 25.215$, $df = 2$, $P < 0.001$; Table 2). Overall *Bartonella* prevalence was highest during the peak phase (57.8%) as compared with the increase (38.3%) or crash phase of the vole cycle (9.1%). Prevalence was also higher in autumn (53.0%) than in spring (42.1%) or summer (36.1%). We further analyzed variation in

the prevalence of the two commonest *Bartonella* species (*B. grahamii* and *B. elisabethae)* in each mouse species separately.

Table 2. Results of selected generalized linear models (GLMs) explaining *Bartonella* prevalence in study small mammal hosts, NW Spain, 2013–2015.

| Host | *Bartonella* | Predictor | Estimate ± SE | Z-value | *P* |
|---|---|---|---|---|---|
| All hosts | *Bartonella* spp. | Intercept | -2.102 ± 1.111 | -1.893 | <0.001 |
| | | Species (CR) | -1.449 ± 0.406 | -3.574 | <0.001 |
| | | Species (MS) | 0.405 ± 0.303 | 1.336 | 0.182 |
| | | Season (spring) | -0.201 ± 0.366 | 1.336 | 0.182 |
| | | Season (autumn) | 0.692 ± 0.299 | 2.313 | 0.021 |
| | | MA phase (increasing) | 1.252 ± 1.111 | 1.127 | 0.260 |
| | | MA phase (peak) | 2.413 ± 1.083 | 2.227 | 0.026 |
| *A. sylvaticus* | *B. elisabethae* | Intercept | -0.692 ± 0.295 | -2.348 | 0.019 |
| | | Season (spring) | -0.144 ± 0.429 | -0.336 | 0.737 |
| | | Season (autumn) | 1.901 ± 0.596 | 3.187 | 0.001 |
| | | MS density | -0.307 ± 0.142 | -2.160 | 0.031 |
| | *B. grahamii* | Intercept | -2.245 ± 1.144 | -1.963 | 0.049 |
| | | Season (spring) | -0.057 ± 0.458 | -0.125 | 0.900 |
| | | Season (autumn) | 2.061 ± 0.692 | 2.981 | 0.003 |
| | | MA phase (increasing) | 1.619 ± 1.179 | 1.374 | 0.169 |
| | | MA phase (peak) | 2.462 ± 1.120 | 2.197 | 0.028 |
| | | MS density | -0.335 ± 0.171 | -1.957 | 0.050 |
| *C. russula* | *Bartonella* spp. | Intercept | -19.570 ± 2404.670 | -0.008 | 0.994 |
| | | Sex (male) | 18.670 ± 2404.670 | 0.008 | 0.994 |
| *M. spretus* | *B. elisabethae* | Intercept | 1.401 ± 0.599 | 2.337 | 0.019 |
| | | MS density | -0.290 ± 0.164 | -1.775 | 0.076 |
| | *B. grahamii* | Intercept | -0.251 ± 0.356 | -0.705 | 0.481 |
| | | MA phase (peak) | 1.391 ± 0.540 | 2.574 | 0.010 |

SE, standard error

For *B. grahamii* prevalence in *A. sylvaticus*, we found a significant effect of season ($X^2$ = 14.796, *df* = 2, $P < 0.001$), of vole cycle phase ($X^2$ = 13.258, *df* = 2, $P$ = 0.001) and a negatively association with *M. spretus* density ($X^2$ = 3.989, *df* = 1, $P$ = 0.046). The infection rate was higher values in autumn (55.6%) than in spring (32.7%) or summer (38.7%). *Bartonella grahamii* prevalence was greater during the increasing phase (41.3%) and peak phase (54.8%) of the vole cycle than during the crash phase (9.1%).

For *B. grahamii* prevalence in *M. spretus,* we only found a marginally significant effect of the vole cycle phase ($X^2$ = 2.831, *df* = 1, *P* = 0.092), with a higher prevalence during the peak (75.8%) than during the increase phase (43.8%; no data were available during the crash phase).

For *B. elisabethae* prevalence in *A. sylvaticus*, we found an effect of season ($X^2$ = 14.744, *df* = 2, *P* < 0.001) and a negative correlation with *M. spretus* density ($X^2$ = 4.836, *df* = 1, *P* = 0.028). We found more infected *A. sylvaticus* in autumn (49.1%) than in spring (23.6%) and summer (29.0%).

For *B. elisabethae* prevalence in *M. spretus,* we only found a marginally significant effect of *M. spretus* by density ($X^2$ = 3.282, *df* = 1, *P* = 0.070), showing a negative association with this bacterium.

*Bartonella* prevalence in *C. russula* differed between sexes ($X^2$ = 4.783, *df* = 2, *P* < 0.091); all the infected animals were males. The sample size for this species was too small to analyze further variations.

***Bartonella* and fleas**

Almost half of the animals parasitized by fleas were *Bartonella*-positive (Table 3). In the overall mouse sample studied, the prevalence and abundance of *N. fasciatus* (prevalence: $X^2$ = 5.963, *df* = 2, *P* = 0.051; abundance: $X^2$ = 8.088, *df* = 2, *P* = 0.018) and *C. apertus* (prevalence: $X^2$ = 9.714, *df* = 2, *P* = 0.008; abundance: $X^2$ = 17.979, *df* = 2, *P* < 0.001) differed between phases of *M. arvalis* cycle. The prevalence and abundance of both flea species were higher in the cohabiting mouse hosts during the peak phase of the vole cycle as compared with the increase phase. We found no differences between phases in the prevalence of *L. taschenbergi* ($X^2$ = 3.678, *df* = 2, *P* = 0.159). Despite the significant result of this variable in the model ($X^2$ = 6.099, *df* = 2, *P* = 0.047), the Tukey test showed no differences in the abundance of *L. taschenbergi* between the increase (0.31) and peak phases (0.38); no trapped mice were infested with this flea during the crash period.

Regarding the association between fleas and *Bartonella* spp., we found a positive correlation between the prevalence of *L. taschenbergi* and the prevalence of *B. elisabethae* ($X^2$ = 4.767, *df* = 1, *P* = 0.029) in *A. sylvaticus*. The model also included prevalence of *N. fasciatus* ($X^2$ = 3.315, *df* = 1, *P* = 0.069), with an almost significant negative correlation. The number of *M. spretus* and *C. russula* harboring fleas (5/65 and 3/51, respectively) was too low to test the relationship between their fleas and *Bartonella* species.

Table 3. *Bartonella* infection in small mammal hosts parasitized by fleas, NW Spain, 2013–2015.

| Host spp. | Flea spp. | Hosts with fleas N | *Bartonella* spp. N (%) | *B. elisabethae* N (%) | *B. grahamii* N (%) |
|---|---|---|---|---|---|
| *A. sylvaticus* | NF | 17 | 8 (47.1) | 4 (23.5) | 7 (41.2) |
| | CA | 23 | 11 (47.8) | 7 (30.4) | 11 (47.8) |
| | LT | 24 | 14 (58.3) | 12 (50.0) | 13 (54.2) |
| | Other | 1 | 1 (100) | 1 (100) | 1 (100) |
| | All | 47 | 23 (48.9) | 17 (36.2) | 22 (46.8) |
| *C. russula* | NF | 1 | 0 | 0 | 0 |
| | CA | 1 | 0 | 0 | 0 |
| | Other | 1 | 0 | 0 | 0 |
| | All | 3 | 0 | 0 | 0 |
| *M. spretus* | NF | 3 | 2 (66.7) | 2 (66.7) | 2 (66.7) |
| | CA | 3 | 3 (100) | 3 (100) | 2 (66.7) |
| | LT | 1 | 0 | 0 | 0 |
| | All | 5 | 4 (80.0) | 4 (80.0) | 4 (80.0) |
| All hosts | NF | 21 | 10 (47.6) | 6 (28.6) | 9 (42.9) |
| | CA | 27 | 14 (51.9) | 10 (37.0) | 13 (48.1) |
| | LT | 25 | 14 (56.0) | 12 (48.0) | 13 (52.0) |
| | All | 55 | 27 (49.1) | 21 (38.2) | 26 (47.3) |

N, number of positive hosts; NF, *Nosopsyllus fasciatus*; CA, *Ctenophthalmus apertus*; LT, *Leptopsylla taschenbergi*

### *Bartonella* mixed infections

Most infected hosts had mixed infections (85.9%; 134/156). The occurrence of co-infections varied with the *M. arvalis* phase cycle both in *A. sylvaticus* ($X^2$ = 15.690, *df* = 2, *P* < 0.001) and *M. spretus* ($X^2$ = 10.457, *df* = 2, *P* = 0.001). Mixed infections in *A. sylvaticus* were significantly more frequent during the peak than during the crash phase. In *M. spretus*, mixed infections were also higher in the peak phase as compared with the increase phase (Table 4). Co-infection occurrence also differed between seasons in *A. sylvaticus* ($X^2$ = 12.479, *df* = 2, *P* = 0.002), with a greater occurrence in autumn (Table 4). For *C. russula*, the sample size was too small to study coinfection variation.

Table 4. *Bartonella* mixed infections on the small mammal guild studied, NW Spain, 2013–2015.

| Host species | *Bartonella* spp. | Infected hosts N (%) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | All hosts | Season | | | Phase of *M. arvalis* cycle | | |
| | | | Spring | Summer | Autumn | Increase | Peak | Crash |
| *A. sylvaticus* | 1 species | 20 (8.9) | 4 (7.3) | 9 (14.5) | 7 (6.5) | 10 (8.3) | 10 (10.8) | 0 (0) |
| | Any mixed infection | 86 (38.2) | 14 (25.5) | 18 (29.0) | 54 (50.0) | 42 (34.7) | 43 (46.2) | 1 (9.1) |
| | ≤ 2 species | 84 (37.3) | 13 (23.6) | 18 (29.0) | 53 (49.1) | 41 (33.9) | 42 (45.2) | 1 (9.1) |
| | ≤ 3 species | 2 (0.9) | 1 (1.8) | 0 (0) | 1 (0.9) | 1 (0.8) | 1 (1.1) | 0 (0) |
| | ≤ 4 species | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| | ≤ 5 species | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| *C. russula* | 1 species | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| | Any mixed infection | 9 (17.7) | 3 (37.5) | 3 (11.1) | 3 (18.8) | 4 (13.3) | 5 (23.8) | 0 (0) |
| | ≤ 2 species | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| | ≤ 3 species | 8 (15.7) | 2 (25.0) | 3 (11.1) | 3 (18.8) | 4 (13.3) | 4 (19.1) | 0 (0) |
| | ≤ 4 species | 1 (2) | 1 (12.5) | 0 (0) | 0 (0) | 0 (0) | 1 (4.8) | 0 (0) |
| | ≤ 5 species | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| *M. spretus* | 1 species | 2 (3.1) | 1 (7.7) | 0 (0) | 1 (2.3) | 0 (0) | 2 (6.1) | 0 (0) |
| | Any mixed infection | 39 (60.0) | 10 (76.9) | 5 (62.5) | 24 (54.6) | 14 (43.8) | 25 (75.8) | 0 (0) |
| | ≤ 2 species | 35 (53.9) | 8 (61.5) | 5 (62.5) | 22 (50.0) | 14 (43.8) | 21 (63.6) | 0 (0) |
| | ≤ 3 species | 2 (3.1) | 2 (15.4) | 0 (0) | 0 (0) | 0 (0) | 2 (6.1) | 0 (0) |
| | ≤ 4 species | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| | ≤ 5 species | 2 (3.1) | 0 (0) | 0 (0) | 2 (4.6) | 0 (0) | 2 (6.1) | 0 (0) |

N, number of infected hosts; %, percentage of total animals

# DISCUSSION

### Pathogen prevalence

We screened 341 hosts for *Francisella tularensis* but only detected the bacterium in one *A. sylvaticus* (0.4%). This result contrasts with previous findings that revealed a 20% prevalence in sympatric *M. arvalis* and a maximum prevalence of 34% during the peak phase of the vole cycle. *Francisella tularensis* was also detected in 6% of the tested fleas collected from *M. arvalis* (18). Altogether, our results suggest that, unlike *M. arvalis*, *Apodemus sylvaticus*, *M. spretus* and *C. russula* do not play any relevant role in the circulation of *F. tularensis* in the system.

By contrast, we found a high *Bartonella* prevalence in study hosts (45.7%), similar to the infection rates found in sympatric *M. arvalis* (47%) (18) and in small mammal guild from peri-urban areas in Spain (48.7%) (38). This prevalence was double that those reported in other Spanish regions (35,39). The low infection rate among *C. russula* and the prevalence values among *A. sylvaticus* were

similar to other surveys (35,38). Noteworthy discrepancies were found in the *M. spretus*, whose prevalence was between two and twenty times higher than those reported in other surveys in Spain (35,38,39). The *Bartonella* prevalence increases in *M. spretus* to 81.8% as the time density and *Bartonella* prevalence in *M. arvalis* occurs (18). However, the prevalence in *M. spretus* during low-density periods of *M. arvalis* (43.8%) was higher than the value found in voles (less than 20%) (18). *Microtus arvalis* may have an amplification role during peak periods. Nevertheless, other variables not considered in this study may explain the high prevalence during the low-density periods of *M. arvalis*. Congenital infections via transplacental transmission is a successful route of some *Bartonella* species (40), so this possibility should be further investigated. Among zoonotic *Bartonella* species (24), we detected five genotypes: *B. elisabethae*, *B. grahamii*, *B. rochalimae, B. tribocorum* and *B. vinsonii berkhoffii*. The detection of one case of *B. chomelii* in *A. sylvaticus* and one in *C. russula* is unusual since this bacteria is a well-known pathogen of domestic cattle (23).

The most common *Bartonella* species, *B. grahamii*, was shared by the three species studied and the fluctuating *M. arvalis* (18). The other most abundant *Bartonella* species, *B. elisabethae*, was shared by the three small mammals but was not detected in *M. arvalis* (18). *Bartonella taylorii* was rarely found in the guild, despite being the most abundant *Bartonella* species among sympatric *M. arvalis* (18). In similar surveys carried out in Spain (18,35,38,39), the most frequent *Bartonella* species in *A. sylvaticus* and *M. arvalis* was *B. taylorii*, with a low prevalence of *B. elisabethae* in any host and *B. grahamii* virtually absent in small mammals. Those dissimilarities in *Bartonella* distribution patterns may be due to the presence of *M. arvalis* in the guild or differences in reservoirs and/or competent vectors that modify transmission routes, favoring the infection of *B. grahamii* and *B. elisabethae* detrimental to *B. taylorii*. Other possible hypotheses could be differences in the behavior or immune response between different host populations, known its effects on the pathogen community at a host scale (41,42).

**_Bartonella_ prevalence variation according to host sex, season and host abundance**

Intrinsic and extrinsic variables can modify the circulation of pathogens. Host sex was only significant in *C. russula*, for which *Bartonella* infections were only detected in males. We expected differences between females and males in the two mouse species since *Bartonella* is a flea-borne bacterium and there is a male bias pattern regarding the most abundant flea species (i.e., *L. taschenbergi*) (26). However, we detected similar *Bartonella* prevalences in both sexes.

We also found a significant seasonal pattern only in *A. sylvaticus*, with more *Bartonella*-positive animals in autumn. This is a general pattern found in other studies, where the peak of *Bartonella* prevalence was detected in this season (38,43,44), although the reasons remain unclear.

The number of *Bartonella*-positive hosts and, specifically, the prevalence of *B. grahamii* (a common pathogen in *M. arvalis* and the other three small mammal hosts) showed significant variations related to the cyclic population dynamic of *M. arvalis*. The prevalence was higher when the abundance of *M. arvalis* peaked. The density of *M. arvalis* could favor the *Bartonella* circulation among the guild, increasing the zoonotic risk in the area. This could be explained by an amplification and spillover effect from *M. arvalis* to other competent hosts while the abundance of *M. arvalis* increased. Regarding the abundance of other small mammals, there was a negative association between the density of *M. spretus* and *Bartonella* in *A. sylvaticus*, and with the prevalence of *B. elisabethae* in *M. spretus*. It is known that some *Bartonella* spp. are strongly influenced by host density (44) and that *Bartonella* is a very diversified group evolutionarily adapted to their host (20). It induces a high host-specificity of some *Bartonella* species and variants (45). A possible dilution effect towards *M. spretus* could cause a decrease in the prevalence in sympatric hosts. It may be possible that *M. spretus* is a more competent host, but further studies would be necessary to determine the specificity of *Bartonella* species in this host.

***Bartonella* and fleas**

Fleas can define and alter pathways of pathogen circulation. *Nosopsyllus fasciatus* and *C. apertus* (the commonest flea species in *M. arvalis*) (26) rise in prevalence and abundance among the guild studied during high-density periods of *M. arvalis*. The prevalence of the overall *Bartonella* spp. and *B. grahamii* (the only genotype detected in the four small mammal species) increased directly with *M. arvalis* abundance in the small mammal guild studied. The same tendency was found in a sympatric vole population (18). However, we found no clear association between the prevalence of *B. grahamii* and the presence of flea species on hosts, as could be expected if *N. fasciatus* and *C. apertus* had a direct role in the transmission of this *Bartonella* from *M. arvalis* to sympatric host species. We however found a significant positive association between the prevalence of *B. elisabethae* and the presence of the flea *L. taschenbergi*, which is consistent with vector specificity. Nevertheless, we do not know whether the fleas collected from hosts were infected or not; which *Bartonella* species harbor the positive fleas; whether the infection was caused by fleas or not (44); and what role plays the vertical transmission, an important source of *Bartonella* infection in mice (40). Furthermore, it has been suggested that different variants of the same bacteria can separately circulate in host and fleas, decreasing the probabilities of transmission between sympatric hosts (45). Many aspects of the *Bartonella* transmission remain unknown, so results should be interpreted with caution.

### *Bartonella* mixed infections

Most positive animals analyzed had mixed infections and many *Bartonella* spp. infested multiple hosts, as reported elsewhere (18,46). Parasites with multiple hosts and hosts with multiple pathogens are general patterns in host-pathogen communities (47,48). The presence of mixed infections in *A. sylvaticus* and *M. spretus* were more frequent with high vole density, a period when the prevalence and abundance of common flea species were higher. This could be explained by a greater probability that several fleas feed sequentially on the same host, transmitting different *Bartonella* species. This would need to be confirmed with the experimental investigation.

## Conclusions

Our *F. tularensis* screenings suggest that *A. sylvaticus*, *M. spretus* and *C. russula* do not play a relevant role in the circulation of *F. tularensis* in our system, unlike *M. arvalis* which have been previously shown to play a key role in tularemia epidemiology (13,22). Nevertheless, the present study has revealed patterns in the prevalence of several *Bartonella* species in terms of host species, season and host density of sympatric small mammals. The commonest genotypes are *B. elisabethae* and *B. grahamii*. We found significant variations in the prevalence of *B. grahamii* (common between *M. arvalis* and the other sympatric small mammals analyzed) in mouse hosts between phases of the vole cycle, which reinforce the importance of considering abundance variations in the host and a key role for boom-bust species in the amplification and spillover transmission of diseases. The cyclic population dynamic of *M. arvalis* could affect the spread of *Bartonella* species and the increase of coinfections in the host community. Further investigation is necessary to understand the possible role of *L. taschenbergi* as a vector of *B. elisabethae*. Extrapolation of results to other populations should be done with caution due to the important dissimilarities between patterns found in other similar communities.

## Acknowledgements

We thank Fabio Flechoso for helping with flea identification, and Raquel Escudero and Nerea Castro from the Instituto de Salud Carlos III for collaborating with DNA extraction and bacterium identification. This work was funded by the projects ECOCYCLES (Partner 5-EUI2008-03658), ECOVOLE (CGL2012-35348), ECOTULA (CGL2015-66962-C2-1-R) and RESERTULA (CLG2015-66962-C2-2-R). SHC was supported by a Ph.D. studentship from Junta de Castilla-y-León (co-funded by the European Social Fund, Orden 10/11/2016). RRP was supported by a Ph.D. studentship from the University of Valladolid (co-funded by Banco Santander, RR 30/04/2014)

## References


1. Han BA, Kramer AM, Drake JM. Global patterns of zoonotic disease in mammals. *Trends Parasitol* (2016) **32**:565–577. doi:10.1016/j.pt.2016.04.007
2. Bordes F, Blasdell K, Morand S. Transmission ecology of rodent-borne diseases: New frontiers. *Integr Zool* (2015) **10**:424–435. doi:10.1111/1749-4877.12149
3. Han BA, Schmidt JP, Bowden SE, Drake JM. Rodent reservoirs of future zoonotic diseases. *Proc Natl Acad Sci* (2015) **112**:7039–7044. doi:10.1073/pnas.1501598112
4. Meerburg BG, Singleton GR, Kijlstra A. Rodent-borne diseases and their risks for public health. *Crit Rev Microbiol* (2009) **35**:221–270. doi:10.1080/10408410902989837
5. Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, Daszak P. Global trends in emerging infectious diseases. *Nature* (2008) **451**:990–993. doi:10.1038/nature06536
6. Vaumourin E, Vourc'h G, Gasqui P, Vayssier-Taussat M. The importance of multiparasitism: examining the consequences of co-infections for human and animal health. *Parasit Vectors* (2015) **8**:545. doi:10.1186/s13071-015-1167-9
7. Seabloom EW, Borer ET, Gross K, Kendig AE, Lacroix C, Mitchell CE, Mordecai EA, Power AG. The community ecology of pathogens: coinfection, coexistence and community composition. *Ecol Lett* (2015) **18**:401–415. doi:10.1111/ele.12418
8. Telfer S, Lambin X, Birtles RJ, Beldomenico PM, Burthe SJ, Paterson S, Begon M. Species interactions in a parasite community drive infection risk in a wildlife population. *Science* (2010) **330**:243–246. doi:10.1126/science.1190333
9. Kallio ER, Begon M, Henttonen H, Koskela E, Mappes T, Vaheri A, Vapalahti O. Cyclic hantavirus epidemics in humans — Predicted by rodent host dynamics. *Epidemics* (2009) **1**:101–107. doi:10.1016/j.epidem.2009.03.002
10. Madhav NK, Wagoner KD, Douglass RJ, Mills JN. Delayed density-dependent prevalence of Sin Nombre virus antibody in Montana deer mice (*Peromyscus maniculatus*) and implications for human disease risk. *Vector-Borne Zoonotic Dis* (2007) **7**:353–364. doi:10.1089/vbz.2006.0605
11. Tkadlec E, Václavík T, Široký P. Rodent host abundance and climate variability as predictors of tickborne disease risk 1 year in advance. *Emerg Infect Dis* (2019) **25**:1738–1741. doi:10.3201/eid2509.190684
12. Olayemi A, Fichet-Calvet E. Systematics, Ecology, and Host Switching: Attributes affecting emergence of the Lassa virus in rodents across western Africa. *Viruses* (2020) **12**:312. doi:10.3390/v12030312
13. Herrero-Cófreces S, Mougeot F, Lambin X, Luque-Larena JJ. Linking zoonosis emergence to farmland invasion by fluctuating herbivores: common vole populations and tularemia outbreaks in NW Spain. *Front Vet Sci* (2021) **8**:900. doi:10.3389/fvets.2021.698454
14. Hornok S, Földvári G, Rigó K, Meli ML, Gönczi E, Répási A, Farkas R, Papp I, Kontschán J, Hofmann-Lehmann R. Synanthropic rodents and their ectoparasites as carriers of a novel haemoplasma and vector-borne, zoonotic pathogens indoors. *Parasites and Vectors* (2015) **8**:1–6. doi:10.1186/S13071-014-0630-3
15. Rodríguez-Pastor R, Luque-Larena JJ, Lambin X, Mougeot F. "Living on the edge": The role of field margins for common vole (*Microtus arvalis*) populations in recently colonised Mediterranean farmland. *Agric Ecosyst Environ* (2016) **231**:206–217.

doi:10.1016/j.agee.2016.06.041

16. Luque-Larena JJ, Mougeot F, Viñuela J, Jareño D, Arroyo L, Lambin X, Arroyo B. Recent large-scale range expansion and outbreaks of the common vole (*Microtus arvalis*) in NW Spain. *Basic Appl Ecol* (2013) **14**:432–441. doi:10.1016/j.baae.2013.04.006

17. Rodríguez-Pastor R, Escudero R, Vidal MD, Mougeot F, Arroyo B, Lambin X, Vila-Coro AM, Rodríguez-Moreno I, Anda P, Luque-Larena JJ. Density-dependent prevalence of *Francisella tularensis* in fluctuating vole populations, northwestern Spain. *Emerg Infect Dis* (2017) **23**:1377–1379. doi:10.3201/eid2308.161194

18. Rodríguez-Pastor R, Escudero R, Lambin X, Vidal MD, Gil H, Jado I, Rodríguez-Vargas M, Luque-Larena JJ, Mougeot F. Zoonotic pathogens in fluctuating common vole (*Microtus arvalis*) populations: occurrence and dynamics. *Parasitology* (2019) **146**:389–398. doi:10.1017/S0031182018001543

19. Carvalho CL, Lopes de Carvalho I, Zé-Zé L, Núncio MS, Duarte EL. Tularaemia: A challenging zoonosis. *Comp Immunol Microbiol Infect Dis* (2014) **37**:85–96. doi:10.1016/j.cimid.2014.01.002

20. Gutiérrez R, Krasnov BR, Morick D, Gottlieb Y, Khokhlova IS, Harrus S. *Bartonella* infection inrodents and their flea ectoparasites: an overview. *Vector-Borne Zoonotic Dis* (2015) **15**:27–39. doi:10.1089/vbz.2014.1606

21. Luque-Larena JJ, Mougeot F, Vidal Roig D, Lambin X, Rodríguez-Pastor R, Rodríguez-Valín E, Anda P, Escudero R. Tularemia outbreaks and common vole (*Microtus arvalis*) irruptive population dynamics in Northwestern Spain, 1997–2014. *Vector-Borne Zoonotic Dis* (2015) **15**:568–570. doi:10.1089/vbz.2015.1770

22. Luque-Larena JJ, Mougeot F, Arroyo B, Vidal MD, Rodríguez-Pastor R, Escudero R, Anda P, Lambin X. Irruptive mammal host populations shape tularemia epidemiology. *PLOS Pathog* (2017) **13**:e1006622. doi:10.1371/journal.ppat.1006622

23. Breitschwerdt EB. Bartonellosis, One Health and all creatures great and small. *Vet Dermatol* (2017) **28**:96-e21. doi:10.1111/vde.12413

24. Buffet J-P, Kosoy M, Vayssier-Taussat M. Natural history of *Bartonella* -infecting rodents in light of new knowledge on genomics, diversity and evolution. *Future Microbiol* (2013) **8**:1117–1128. doi:10.2217/fmb.13.77

25. Rodríguez-Pastor R, Mougeot F, Vidal MD, Jado I, González-Martín-Niño RM, Escudero R, Luque-Larena JJ. Zoonotic bacteria in fleas parasitizing common voles, Northwestern Spain. *Emerg Infect Dis* (2019) **25**:1423–1425. doi:10.3201/eid2507.181646

26. Herrero-Cófreces S, Flechoso MF, Rodríguez-Pastor R, Luque-Larena JJ, Mougeot F. Patterns of flea infestation in rodents and insectivores from intensified agro-ecosystems, Northwest Spain. *Parasit Vectors* (2021) **14**:16. doi:10.1186/s13071-020-04492-6

27. Rivas-Martínez S, Penas Á, del Río S, Díaz González TE, Rivas-Sáenz S. "Bioclimatology of the Iberian Peninsula and the Balearic Islands," in *The Vegetation of the Iberian Peninsula*, ed. J. Loidi Cham: Springer International Publishing (2017) 29–80. doi:10.1007/978-3-319-54784-8_2

28. Mougeot F, Lambin X, Rodríguez-Pastor R, Romairone J, Luque-Larena JJ. Numerical response of a mammalian specialist predator to multiple prey dynamics in Mediterranean farmlands. *Ecology* (2019) **100**:e02776. doi:10.1002/ecy.2776

29. Roos D, Caminero-Saldaña C, Elston D, Mougeot F, García-Ariza M, Arroyo B, Luque-Larena

JJ, Revilla FR, Lambin X. From pattern to process? Dual travelling waves, with contrasting propagation speeds, best describe a self-organised spatio-temporal pattern in population growth of a cyclic rodent. *Ecol Lett* (under revision) doi:10.22541/AU.164302552.25540752/V1

30. Palomo LJ, Gisbert J, Blanco JC. Atlas y libro rojo de los mamíferos terrestres de España. *Atlas y Libr rojo los mamíferos Terr España* (2007).

31. Versage JL, Severin DDM, Chu MC, Petersen JM. Development of a multitarget real-time TaqMan PCR assay for enhanced detection of *Francisella tularensis* in complex specimens. *J Clin Microbiol* (2003) **41**:5492–5499. doi:10.1128/JCM.41.12.5492-5499.2003

32. World Health Organization. *WHO guidelines on tularaemia*. Geneva: World Health Organization (2007). Available at: https://apps.who.int/iris/handle/10665/4379 [Accessed May 25, 2020]

33. Escudero R, Toledo Á, Gil H, Kovácsová K, Rodríguez-Vargas M, Jado I, García-Amil C, Lobo B, Bhide M, Anda P, et al. Molecular method for discrimination between *Francisella tularensis* and *Francisella*-like endosymbionts. *J Clin Microbiol* (2008) **46**:3139–3143. doi:10.1128/JCM.00275-08

34. Garcia-Esteban C, Gil H, Rodríguez-Vargas M, Gerrikagoitia X, Barandika JF, Escudero R, Jado I, Garcia-Amil C, Barral M, Garcia-Perez AL, et al. Molecular method for *Bartonella* species identification in clinical and environmental samples. *J Clin Microbiol* (2008) **46**:776–779. doi:10.1128/JCM.01720-07

35. Gil H, García-Esteban C, Barandika JF, Peig J, Toledo A, Escudero R, Jado I, Rodríguez-Vargas M, García-Amil C, Lobo B, et al. Variability of *Bartonella* genotypes among small mammals in Spain. *Appl Environ Microbiol* (2010) **76**:8062–8070. doi:10.1128/AEM.01963-10

36. Bolker B, Skaug H, Maintainer JL. R2admb: "ADMB" to R interface functions. (2017).

37. R Core Team. R: The R project for statistical computing. (2019).

38. Cevidanes A, Altet L, Chirife AD, Proboste T, Millán J. Drivers of Bartonella infection in micromammals and their fleas in a Mediterranean peri-urban area. *Vet Microbiol* (2017) **203**:181–188. doi:10.1016/j.vetmic.2017.03.023

39. Márquez FJ, Rodríguez-Liébana JÁ, Pachón-Ibáñez ME, Docobo-Pérez F, Hidalgo-Fontiveros A, Bernabeu-Wittel M, Muniain MA, Pachón J. Molecular screening of *Bartonella* species in rodents from South Western Spain. *Vector-Borne Zoonotic Dis* (2008) **8**:695–700. doi:10.1089/vbz.2007.0257

40. Tołkacz K, Alsarraf M, Kowalec M, Dwuznik D, Grzybek M, Behnke JM, Bajer A. *Bartonella* infections in three species of *Microtus*: Prevalence and genetic diversity, vertical transmission and the effect of concurrent *Babesia microti* infection on its success. *Parasites and Vectors* (2018) **11**:1–15. doi: 10.1186/s13071-014-0630-3

41. Cox FEG. Concomitant infections, parasites and immune responses. *Parasitology* (2001) **122**:S23–S38. doi:10.1017/S003118200001698X

42. Barnard CJ, Behnke JM. "Behaviour, life history strategies and parasite infection in rodents", in *Micromammals and Macroparasites: from evolutionary ecology to management*, eds. S. Morand, B. Krasnov, R. Poulin. Tokyo: Springer Japan (2006), 475–511. doi:10.1007/978-4-431-36025-4_22

43. Kosoy MY. Ecological associations between bacteria of the genus *Bartonella* and mammals. *Biol Bull* (2010) **37**:716–724. doi:10.1134/S1062359010070071

44. Telfer S, Begon M, Bennett M, Bown KJ, Burthe SJ, Lambin X, Telford G, Birtles RJ.

Contrasting dynamics of *Bartonella* spp. in cyclic field vole populations: the impact of vector and host dynamics. *Parasitology* (2007) **134**:413. doi:10.1017/S0031182006001624

45. Withenshaw SM, Devevey G, Pedersen AB, Fenton A. Multihost *Bartonella* parasites display covert host specificity even when transmitted by generalist vectors. *J Anim Ecol* (2016) **85**:1442–1452. doi:10.1111/1365-2656.12568

46. Gutiérrez R, Morick D, Cohen C, Hawlena H, Harrus S. The effect of ecological and temporal factors on the composition of *Bartonella* infection in rodents and their fleas. *ISME J* (2014) **8**:1598–1608. doi:10.1038/ismej.2014.22

47. Woolhouse MEJ. Population biology of multihost pathogens. *Science* (2001) **292**:1109–1112. doi:10.1126/science.1059026

48. Taylor LH, Latham SM, Woolhouse MEJ. Risk factors for human disease emergence. *Philos Trans R Soc London Ser B Biol Sci* (2001) **356**:983–989. doi:10.1098/rstb.2001.0888

# Appendix

Table S1. Sample size and population density of small mammal species analyzed from each trapping session, NW Spain, 2013–2015.

| Year | Season | *M. arvalis* phase | *A. sylvaticus* | | | *C.russula* | | | *M. spretus* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | F (n) | M (n) | D | F (n) | M (n) | D | F (n) | M (n) | D |
| 2013 | all | increasing | 48 | 73 | 10.6 | 11 | 19 | 2.4 | 8 | 24 | 3.3 |
| | spring | | 2 | 5 | 0.8 | 0 | 1 | 0.1 | 0 | 0 | 0.0 |
| | summer | | 12 | 22 | 4.7 | 7 | 9 | 2.1 | 0 | 3 | 0.3 |
| | autumn | | 34 | 46 | 13.8 | 4 | 9 | 2.7 | 8 | 21 | 4.8 |
| 2014 | all | peak | 37 | 56 | 4.9 | 9 | 12 | 1.2 | 11 | 22 | 1.7 |
| | spring | | 12 | 25 | 4.9 | 2 | 5 | 1.1 | 3 | 10 | 1.9 |
| | summer | | 14 | 14 | 6.0 | 4 | 7 | 2.3 | 1 | 4 | 1.1 |
| | autumn | | 11 | 17 | 3.8 | 3 | 0 | 0.4 | 7 | 8 | 2.0 |
| 2015 | all | crash | 6 | 5 | 0.3 | 0 | 0 | 0.0 | 0 | 0 | 0.0 |
| | spring | | 6 | 5 | 0.3 | 0 | 0 | 0.0 | 0 | 0 | 0.0 |

F, female; M, male; D, density; n, number of trapped and screened animals

Table S2. Prevalence (%) of *Bartonella* genotypes occurrence among the small mammal guild studied and *Microtus arvalis*, Castilla-y-León, NW Spain, 2013–2015.

| ***Bartonella genotypes*** | ***A. sylvaticus*** | ***C.russula*** | ***M. spretus*** | ***M. arvalis* *** |
|---|---|---|---|---|
| *B. chomelii* | 0.4 | 2.0 | 0.0 | 0.0 |
| *B. elisabethae* | 37.3 | 17.6 | 61.5 | 0.0 |
| *B. grahamii* | 45.3 | 17.6 | 60.0 | 21.3 |
| *B. rochalimae* | 0.9 | 0.0 | 0.0 | 19.2 |
| *B. taylorii* | 2.2 | 0.0 | 4.6 | 30.0 |
| *B. tribocorum* | 0.0 | 17.6 | 0.0 | 0.0 |
| *B. birtlesii* | 0.0 | 0.0 | 3.1 | 0.0 |
| *B. cooperplainsense* | 0.0 | 0.0 | 3.1 | 0.0 |
| *B. vinsonii* | 0.0 | 0.0 | 3.1 | 0.0 |
| *B. doshiae* | 0.0 | 0.0 | 0.0 | 5.8 |
| *B. clarridgeae* | 0.0 | 0.0 | 0.0 | 2.5 |
| All *Bartonella* spp. | 47.1 | 17.7 | 63.1 | 46.7 |

* Adapted from Rodríguez-Pastor et al. (2019). Zoonotic pathogens in fluctuating common vole (*Microtus arvalis*) populations: occurrence and dynamics. *Parasitology*, *146*(3), 389–398. doi:10.1017/S0031182018001543

# CHAPTER 3.2

# VIRUSES

# Viral zoonoses in small wild mammals and detection of hantavirus, Spain

Silvia Herrero-Cófreces[1,2], François Mougeot[3], Tarja Sironen[4], Hermann Meyer[5], Ruth Rodríguez-Pastor[1,2], Juan José Luque-Larena[1,2]

[1] Dpto. Ciencias Agroforestales, ETSIIAA, Universidad de Valladolid, Avda. de Madrid 44, 34004 Palencia, Spain

[2] Instituto Universitario de Investigación en Gestión Forestal Sostenible, Palencia, Spain

[3] Instituto de Investigación en Recursos Cinegéticos, IREC (CSIC-UCLM-JCCM), Ronda de Toledo s/n, 13071, Ciudad Real, Spain

[4] Department of Virology, Haartman Institute, University of Helsinki, Helsinki, Finland

[5] Bundeswehr Institute of Microbiology, Munich, Germany

This chapter has been published as:

Silvia Herrero-Cófreces, François Mougeot, Tarja Sironen, Hermann Meyer, Ruth Rodríguez-Pastor, Juan José Luque-Larena. Viral zoonoses in small wild mammals and detection of hantavirus, Spain. *Emerging Infectious Diseases*. 2022, 28(6):1294-1296. doi:10.3201/eid2806.212508.

# CHAPTER 4

# MACROPARASITES

# Helminth parasites of the common vole *Microtus arvalis* in intensive farming landscapes of NW Spain

*Silvia Herrero-Cófreces [1,2], Juan José Luque-Larena [1,2], Francois Mougeot [3]

[1] Dpto. Ciencias Agroforestales, ETSIIAA, Universidad de Valladolid, Avda. de Madrid 44, 34004 Palencia, Spain

[2] Instituto Universitario de Investigación en Gestión Forestal Sostenible, Palencia, Spain

[3] Instituto de Investigación en Recursos Cinegéticos, IREC (CSIC-UCLM-JCCM), Ronda de Toledo s/n, 13071, Ciudad Real, Spain

*Provisional authors of this draft

## Abstract

Helminths are a heterogeneous group of worm-like internal macroparasites that can reduce host fitness. The specific life cycle of each helminth species determines the route and risk of infection and the host-parasite traits. Host-parasite interactions may induce cyclic population dynamics when three main conditions are fulfilled: parasite aggregation among hosts, the time lag in parasite recruitment behind host abundance (delayed density-dependence), and depression of host fecundity (litter size) and/or survival. In Spain, common vole (*Microtus arvalis*) populations periodically fluctuate in abundance every 3 years. The roles that helminths may play in driving or influencing these cycles are still largely unknown. Here we report on the gastrointestinal helminth community of common voles. We first document which helminth species infest voles, their prevalence, intensity, abundance and aggregation patterns. Second, for the main helminth species, we study the variation of parasitological parameters (prevalence, intensity, abundance) according to host sex, season and the phase of the vole population cycle (increase, peak or crash). Finally, we explored associations between helminth parasitological parameters and host condition (body-mass condition, organ hypertrophy) and fecundity (litter size). We found that overall helminth prevalence averaged 24.9%, and showed a high aggregation level. *Syphacia* sp. was the commonest species (82.5% of all the collected helminths), followed by *Anoplocephaloides dentata* (8%). Season (autumn) and vole cycle phase significantly explained the intensity and abundance of both helminth species. Male-biased differences were also detected regarding *Syphacia* prevalence. We found no association between helminth abundance and body-mass condition or organ hypertrophy, but we found a negative correlation between the abundance of helminths and the litter size in vole hosts in summer, consistent with a negative influence on fecundity. Based on these findings, we discuss the regulatory roles that helminth parasites could play in cyclic populations of common voles.


**Keywords:** gastrointestinal endoparasites; host fecundity; host population cycles; regulation; rodent; *Syphacia*

## Introduction

Helminths are a heterogeneous group of worm-like internal macroparasites than include trematodes, cestodes and nematodes. They have at least three stages: egg, larva and adult; some species have several larval stages each parasitizing different hosts. Trematodes are non-segmented flat helminths with an indirect life cycle, that is, they need an intermediate host for the survival of the larval stage and a different definitive host for the adult stage. Cestodes are segmented flat helminth with an indirect life cycle. And nematodes are cylindrical helminths with direct or indirect cycles (1). Eggs of helminths are free in the habitat, so eggs or larvae must be eaten or penetrate the host. The intermediate host harboring a larval stage is usually eaten by the definitive host, where the next stage emerges (2). Thus, the specific life cycle of each helminth species determines the route and risk of infection of hosts and the host-parasite traits.

Parasites normally cause damage to their hosts through a reduction of individual fitness. Negative effects include increases in host mortality or morbidity, a decrease in host fecundity and a decrease in energy available (due to an increase in the immune system activity or decrease of nutrients and food intake) (2), ultimately affecting the population dynamic of the host. A central question of population ecology is to understand what factors determine the rate of population change and the dynamics of natural populations. Birth, death and migration rates are key elements, together with the mechanisms that can affect them: predation, parasitism, food availability and territoriality (3,4). Predation and food shortage has been considered as significant extrinsic factors affecting host fluctuations (5). However, the effect of parasitism was long underestimated, although some authors had suggested the relevance that this factor could have (6–8). Parasites can deeply affect their hosts, the community structure and even the functioning of the ecosystem in which they are embedded (2,9–11). According to theoretical models, investigation of parasite-induced regulation has highlighted under which conditions parasites can modulate host populations and cause cyclic fluctuations in abundance, depending on the type of density-dependence in parasite recruitment, the degree of parasite aggregation among hosts, and the level of parasitic effects on host fecundity and survival (7,12). For parasites to destabilize host populations, aggregation levels should be high and there should be delayed density-dependence, that is, parasite abundance should vary with host abundance, but with a time delay (6,7). Macroparasite-induced regulation examples reviewed by Tompkins et al. (2002) indicate that parasites that reduce host survival rather than fecundity have a regulating but stabilizing effect on host dynamic; but if they deplete host fecundity, they tend to destabilize host populations.

Investigations focusing on the effect of helminths on host population dynamics are diverse. Several studies find no clear relationship between certain helminths and their host (14–16), whereas others have demonstrated negative effects on hosts population parameters (Deter, Charbonnel, Cosson, & Morand, 2008; Pedersen & Greives, 2008; Winternitz, Yabsley, & Altizer, 2012). Helminth infections can cause physiological pathologies and have been associated with hypertrophy of organs involved in immune response, such as the spleen (21–23), adrenal glands (21,24) or liver (25,26). High infestation intensity of helminths has been associated with host population crashes (some examples on D.M. Tompkins & M. Begon, 1999), consistent with delayed density-dependence patterns. Notwithstanding, other authors support the hypothesis that parasitism usually plays a secondary rather than a main driving role, acting synergistically with other factors (19,27–31). This shows the high variability in the ecological response of different hosts against each helminth species (32).

Population regulation has a special interest in hosts with cyclic population dynamics and, particularly, in those highly fluctuating such as some rodents (7,13,33,34). Population cycles in rodents display large-scale multiannual fluctuations (4,35) with a marked low phase (5,36). Differences in body mass, social behavior, age structure, age at sexual maturation, survival and reproductive rates are patent and opposite in each phase (36). In Spain, the common vole (*Microtus arvalis*) is the most abundant rodent inhabiting the intensified farming landscapes in the Northwest (37). In this habitat, vole populations show a cyclic dynamic with abundance outbreaks every 3 years (38,39). Nevertheless, the influence of helminths on shaping vole fluctuations is still unknown. In this study, our aims were two-fold. First, we explored the gastrointestinal helminth community composition in wild fluctuating common vole populations. Second, we determined the main parasite parameters: prevalence, intensity, abundance and aggregation pattern, of the main helminth species, evaluating the variation of these parameters according to host sex, season and the phase of the host population cycle (increase, peak, crash). If helminths have a destabilizing influence on their common vole host populations, we expected a high parasite aggregation level, and higher prevalence or abundance during the crash phase of the cycle, as compared with the higher density phases (20,31). Finally, we evaluated correlatively the potential effects of helminths on vole fecundity (litter size) and condition (body weight, and weight of immunological-related organs such as spleen, adrenal glands and liver).

# Materials and methods

### Helminths in voles

The common vole is a small fossorial herbivore rodent widespread throughout Europe (40). Like many other rodents, the common vole follows an r–strategy, showing litter sizes between 1 to 13 after 21 days of gestation (40,41). Females are highly social under favorable conditions (42). The focal vole populations in this study are characterized by multiannual cyclic fluctuations (38) whose density can change from 5-10 individuals per hectare in low phases, to more than 200 during peaks (41).

The most common gastrointestinal (GI thereafter) helminths harbored by *Microtus* spp. are cestodes and nematodes (43–46), although some digenean trematodes may also use rodents as definitive hosts (2). Cestodes harbored by small mammals have an indirect life cycle, participating rodents as intermediate/paratenic or definitive hosts (with an invertebrate intermediate host). The parasite enters the host by eating either worm eggs or an infested intermediate host. Nematodes have a direct life cycle and the main routes of infection in small mammals are via skin penetration and oral ingestion of eggs; autoinfection, arthropod-borne injection or ingestion of infective tissues can also occur (2).

### Study area and trapping design

We studied the helminth community of common voles in NW Spain. The study area consisted of 80 $Km^2$ located in the province of Palencia (42°01´N, 4°42´), in Northwest Spain, a region mostly influenced by continental-Mediterranean conditions (47). Fieldwork (live trapping) was carried out three times a year (March, July and November, hereafter referred to as “spring”, “summer” and “autumn”, respectively) between July 2010 and March 2015. Our time series (2010-2015) of sample collection included two population peaks (2011, 2014), two years of population increase (2010, 2013) and two years of population crash (2012, 2015; see (38,39)). Sampling included 15 trapping sessions with a total of 12,600 traps per night (840 traps/night per trapping session) following the same methodology as Rodríguez-Pastor, Luque-Larena, Lambin, & Mougeot (2016). Traps (8 × 9 × 23 cm; LFAHD Sherman©) were set open for 24 h, with carrot and apple used as bait. Each animal captured was individually identified with a unique code; date and location were noted when trapped. Immediately after trapping, voles were placed in individual cages (29 x 22 x 14 cm; Panlab®) provided with food, water and bedding material and transported to the laboratory.

**Laboratory procedure**

Every vole was sexed, weighed (with an electronic balance, to the nearest 0.1g), measured with a ruler (total length without tail, nearest 1 mm), and euthanized with $CO_2$. Animals were stored at -23 ºC until dissection, which followed standard protocols: spleen, liver and adrenal glands were separately weighed; reproductive system of females was checked for embryos, detecting pregnant females and counting the total number of embryos; GI tract was removed and kept frozen at -23 ºC until helminth survey. Once defrosted, GI tracts were individually placed in a petri dish with a thin layer of tap water, cutting the esophagus before the stomach and rectum close to the end. We cut ligaments and straighten the small intestine by cutting the mesenteries; we separated the small intestine, large intestine, and caecum. Independently, we processed every section, cutting it longitudinally (starting at the posterior end) and stirring carefully to spread the content. Then, we looked for helminths by naked eye first and screened them under a magnifying glass later. Cestodes found were placed in a separate petri dish with a thin layer of tap water in order to relax and fixed flat the individuals. Specimens were preserved in 70% ethanol at -23 ºC afterward. Helminths were identified based on morphological characters (48–50). A total of 380 common voles were surveyed (see Table S1 in the Appendix for more detailed information).

**Data analysis**

We studied variation in the following parameters: (i) mean prevalence (number of infected hosts divided by the number of hosts examined), (ii) mean abundance (total number of parasites divided by the number of hosts examined) and (iii) mean intensity (total number of parasites divided by the number of infected hosts) for all helminths found in common voles. Data were summarized as prevalence ± 95% confidence intervals (CI; traditional Clopper-Pearson confidence limits) and mean intensity or abundance ± standard error (SE). We quantified the level of aggregation of helminths on voles using two complementary indices: (i) the Variance-to-mean ratio (VMR) and (ii) the Discrepancy index (*D*) following Poulin (1993). These descriptive statistics were obtained using the Quantitative Parasitology (QPweb) software version 1.0.15 (52).

For the main (most prevalent) helminth species, we used Generalized linear models (GLMs) to study variation according to host sex (male, female), season (spring, summer, autumn), and the phase of the host population cycle (increase, peak or crash). The cycle phase was determined using a vole abundance index (number of voles trapped per 100 traps per 24 h; see Herrero-Cófreces et al., 2021; Mougeot et al., 2019). Depending on sample size we also tested for two-way interactions between sex and the other two factors when possible. Prevalence data were fitted to models using a binomial distribution, and abundance and intensity data using a negative

binomial distribution. We studied the variation of the overall helminth community (pooling all species, including rare ones and non-identified) and of the commonest helminth species. We tested for associations between vole condition (body mass) and helminth abundance for male and female voles separately. The body mass models included vole size (body length) as a covariate (to analyze variation in mass corrected for size). We tested for an association between the number of embryos (number of embryos per female, including zero for non-pregnant individuals) and helminth abundance among female voles. Explanatory variables included body size, season, the phase of the host population cycle and helminth abundance (log-transformed) as independent variables. Interactions between helminth abundance and season were also tested. Finally, we tested for associations between the weight of spleen, liver and adrenal glands and helminth abundance, including in the models host weight, sex, and one-way interactions between sex and the abundance of helminths. We checked the normality of the residuals with the Lilliefors test in body-mass conditions and organ models. When necessary, dependent variables were transformed (log-transformation for the weight of host, spleen, liver and adrenal glands). The model selection followed a backward-selection procedure (using the "drop1" function in R), removing non-significant terms ($p = 0.10$ level) sequentially, starting with interactions. We tested differences between levels of significant variables using post-hoc Tukey test. These statistical analyses were carried out using the "lme4" (54) and "R2admb" (55) packages, and the R software version 3.6.1 (56).

# Results

### Helminths infecting common voles

Among the 380 common voles screened for intestinal parasites we collected 641 helminth individuals belonging to eight different taxa (Figure 1), namely: *Anoplocephaloides dentata*, *Heligmosomoides laevis*, *Heligmosomoides* sp., *Heligmosomum* sp., *Paranoplocephaloides gracilis*, *Paranoplocephaloides omphaloides*, *Syphacia* sp. and *Trichuris* sp. Up to twelve specimens (1.9 % of the total) could not be identified. Considering the overall helminth community, prevalence averaged 24.9% (20.7-29.6), intensity 6.75 (±2.08), and abundance 1.68 (±0.54). The helminth sample showed a high aggregation pattern, with VMR = 65.77 and $D = 0.92$ (0.89-0.96). More details on the helminth parasites parameters are provided in Table 1. The commonest helminth was *Syphacia* sp., accounting for 84.2% of all the identified helminths collected from half of the parasitized voles. The second most prevalent species was *A. dentata*, occurring in one out of four parasitized voles, accounting for 8.1% of the identified helminths. *Syphacia* sp. showed the highest prevalence, mean intensity and mean abundance of all the helminth species, and the

highest value for the VMR. *Syphacia* sp. also presented the widest infection range per host [intensity ranged from 1 to 185], while the intensity of other species varied between one to six individuals per host. The second most abundant helminth (i.e., *A. dentata*) showed half the prevalence value, five times less intensity and ten times less abundance than the most frequent parasite among the vole sample.

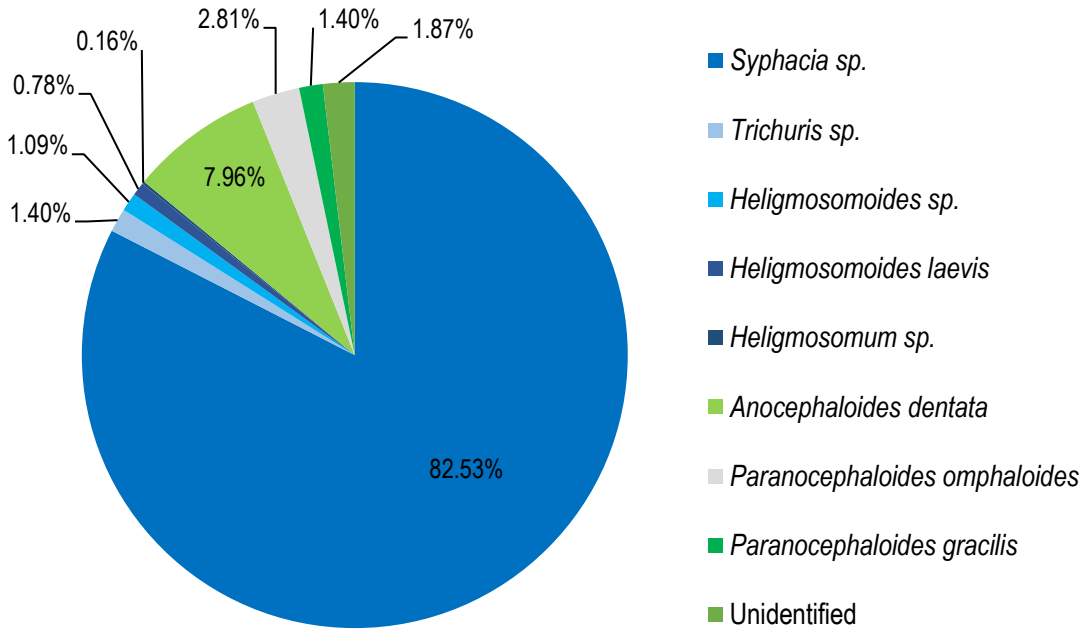


Figure 1. Occurrence frequency of gastrointestinal helminth species collected from wild common voles in NW Spain (2010-2015). Nematodes in blue colors and cestodes in green colors.

Table 1. Parasitological parameters of the gastrointestinal helminth community collected from a common vole population in NW Spain (2010-2015).

| **Helminths** | **Identified helminths** (% helminths collected) | **Range** | **Location in host**[1] | **Hosts infected** | **Prevalence %** (CI)[1] | **Mean intensity** (±SE)[3] | **Mean abundance** (±SE)[3] | **Variance / mean ratio** | **Discrepancy index** (CI)[4] |
|---|---|---|---|---|---|---|---|---|---|
| Total number of helminths | 628 (98.0) | 1-185 | ST, SI, C | 95 | 25.0 (20.7-29.7) | 6.75 (±2.08) | 1.69 (±0.54) | 65.47 | 0.92 (0.89-0.96) |
| *Syphacia* sp. | 529 (82.5) | 1-185 | ST, SI, C | 47 | 12.4 (9.2-16.1) | 11.26 (±2.11) | 1.39 (±0.54) | 79.30 | 0.96 (0.94-0.98) |
| *Anocephaloides dentata* | 51 (8.0) | 1-5 | C | 24 | 6.3 (4.1-9.3) | 2.12 (±0.11) | 0.13 (±0.03) | 2.76 | 0.95 (0.94-0.97) |
| *Paranocephaloides omphaloides* | 18 (2.8) | 1-3 | ST, SI | 14 | 3.7 (0.2-6.1) | 1.29 (±0.05) | 0.05 (±0.01) | 1.43 | 0.97 (0.95-0.98) |
| *Paranocephaloides gracilis* | 9 (1.4) | 1-2 | SI | 6 | 1.6 (0.6-3.4) | 1.50 (±0.04) | 0.02 (±0.01) | 1.95 | 0.98 (0.97-0.99) |
| *Trichuris* sp. | 9 (1.4) | 1-6 | C | 3 | 0.8 (0.2-2.3) | 2.67 (±0.06) | 0.02 (±0.02) | 4.98 | 0.99 (0.99-1.00) |
| *Heligmosomoides* sp. | 7 (1.1) | 1-5 | ST, SI | 3 | 0.8 (0.2-2.3) | 2.33 (±0.01) | 0.02 (±0.05) | 3.54 | 0.99 (0.99-1.00) |
| *Heligmosomoides laevis* | 5 (0.8) | 1-4 | SI | 2 | 0.5 (0.1-1.9) | 2.50 (±0.04) | 0.01 (±0.01) | 4.46 | 0.99 (0.98-1.00) |
| *Heligmosomum* sp. | 1 (0.2) | 1 | ST | 1 | 0.3 (0.0-1.5) | 1.0 (NA) | < 0.01 (±0.01) | NA | NA |

[1] ST, stomach; SI, small intestine; C, caecum; [2] 95% Confidence interval by Clopper-Pearson; [3] Standard error; [4] 95% Confidence interval by the bootstrap method

**Variation according to host sex, season and host population cycle**

Variation in overall helminth abundance (all species combined) was explained by the interaction between season and cycle phase ($X^2$ = 21.237, df = 4, P < 0.001), with higher abundance during the summer of crash years (Figure 2). For overall helminth prevalence, we found significant differences between sexes ($X^2$ = 7.702, df = 1, P = 0.006; higher prevalence in males than in females) and seasons ($X^2$ = 8.595, df = 2, P = 0.014; higher in autumn; Table 2). Regarding helminth intensity, we found a marginally significant effect of month ($X^2$ = 5.147, df = 2, P = 0.076) and sex in interaction with cycle phase ($X^2$ = 5.147, df = 2, P = 0.076). Intensity tended to be lower during autumn, compared to spring and summer results, and was higher in female voles during crash years (Table 2).

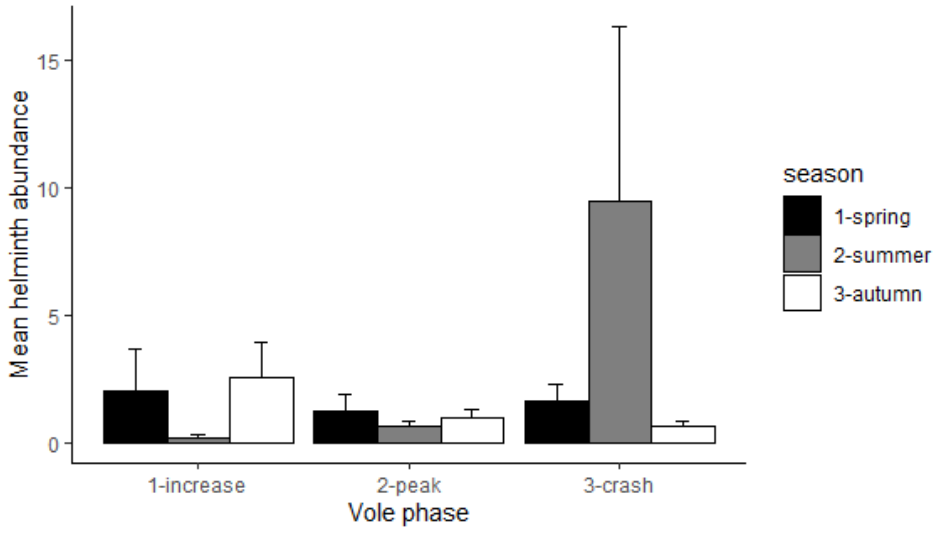


Figure 2. Mean abundance of helminths during different seasons and phases of the population cycle of common vole population in NW Spain (2010-2015). Error bars represent standard error.

We further explored variation in the prevalence, abundance and intensity of *Syphacia* sp. and *A. dentata*., the two commonest helminths found among voles. For *P. omphaloides*, we were only able to study prevalence variation, but sample size limitations prevented us from analyzing variation in the rest of the detected helminths.

We found an almost significant male-biased difference in prevalence of *Syphacia* sp. ($X^2$ = 3.372, df = 1, P = 0.066). Abundance varied with vole phase (Figure 3), with higher values occurring during crash years ($X^2$ = 6.687, df = 2, P = 0.035). Intensity was also higher during crash years ($X^2$ = 7.639, df = 2, P = 0.022) and among females during spring and summer, but not in autumn when was lower than males ($X^2$ = 6.018, df = 2, P = 0.049).

The cestode *A. dentata* was absent from summer samples, but there were significant differences between the other two seasons for all parameters. Prevalence of *A. dentata* was higher in

autumn than in spring, but only among males ($X^2$ = 5.736, df = 2, P = 0.057), and higher values were found during the crash than during the increase or peak phases ($X^2$ = 9.498, df = 2, P = 0.009). Abundance varied with season ($X^2$ = 31.859, df = 2, P < 0.01) and vole phase ($X^2$ = 9.079, df = 2, P = 0.011; Figure 3). Results were higher in autumn and in crash years. Intensity was also higher in autumn ($X^2$ = 4.584, df = 1, P = 0.032). The prevalence of *P. omphaloides* was also higher during in autumn but only among females ($X^2$ = 6.418, df = 2, P = 0.040).

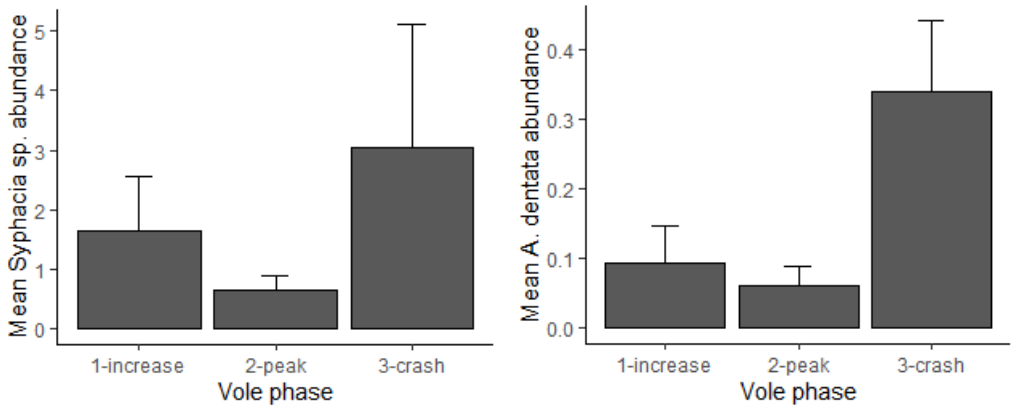


Figure 3. Abundance of the commonest helminth species in different phases of the population cycle of common voles in NW Spain (2010-2015). Error bars represent standard error.

Table 2. Results of generalized linear models explaining parasitic parameters of common voles in NW Spain (2010-2015).

| Parameter | Helminths | Predictor | Estimate ± SE | Z-value | *P* |
|---|---|---|---|---|---|
| Prevalence | All helminths | Intercept | -1.381 ± 0.269 | -5.141 | <0.001 |
| | | Sex (male) | 0.673 ± 0.245 | 2.743 | 0.006 |
| | | Month (summer) | -0.484 ± 0.302 | -1.602 | 0.109 |
| | | Month (autumn) | 0.349 ± 0.303 | 1.151 | 0.259 |
| | *Syphacia* sp. | Intercept | -2.274 ± 0.248 | -9.190 | <0.001 |
| | | Sex (male) | 0.579 ± 0.320 | 1.813 | 0.070 |
| | *Anocephaloides dentata* | Intercept | -1.674 ± 0.783 | -2.139 | 0.032 |
| | | Sex (male) | -0.392 ± 0.684 | -0.529 | 0.597 |
| | | Month (summer) | -18.475 ± 1884.361 | -0.010 | 0.992 |
| | | Month (autumn) | -1.856 ± 0.929 | -1.997 | 0.046 |
| | | Phase (peak) | -0.907 ± 0.761 | -1.192 | 0.233 |
| | | Phase (crash) | 0.756 ± 0.652 | 1.160 | 0.246 |
| | | Sex (male) x Month (summer) | 0.234 ± 2682.090 | 0.000 | 0.999 |
| | | Sex (male) x Month (autumn) | 2.407 ± 1.057 | 2.278 | 0.023 |
| | *Paranocephaloides omphaloides* | Intercept | -3.871 ± 1.010 | -3.831 | <0.001 |
| | | Sex (male) | 0.594 ± 1.241 | 0.479 | 0.632 |
| | | Month (summer) | -15.695 ± 1180.407 | -0.013 | 0.989 |

| Parameter | Helminths | Predictor | Estimate ± SE | Z-value | *P* |
|---|---|---|---|---|---|
| | | Month (autumn) | 1.455 ± 1.113 | 1.308 | 0.191 |
| | | Sex (male) x Month (summer) | 16.0145 ± 1180.407 | 0.014 | 0.989 |
| | | Sex (male) x Month (autumn) | -1.377 ± 1.509 | -0.912 | 0.362 |
| Mean intensity | All helminths | Intercept | 2.291 ± 0.465 | 4.928 | <0.001 |
| | | Sex (male) | 0.042 ± 0.536 | 0.079 | 0.937 |
| | | Month (summer) | 0.262 ± 0.314 | 0.833 | 0.405 |
| | | Month (autumn) | -0.514 ± 0.324 | -1.588 | 0.112 |
| | | Phase (peak) | -0.609 ± 0.479 | -1.272 | 0.204 |
| | | Phase (crash) | 0.965 ± 0.619 | 1.559 | 0.119 |
| | | Sex (male) x Phase (peak) | -0.513 ± 0.634 | -0.809 | 0.419 |
| | | Sex (male) x Phase (crash) | -1.627 ± 0.764 | -2.128 | 0.033 |
| | *Syphacia* sp. | Intercept | 3.150 ± 0.588 | 5.359 | <0.001 |
| | | Sex (male) | -1.610 ± 0.627 | -2.568 | 0.010 |
| | | Month (summer) | 0.541 ± 0.689 | 0.786 | 0.432 |
| | | Month (autumn) | -0.881 ± 0.638 | -1.380 | 0.167 |
| | | Phase (peak) | -0.837 ± 0.474 | -1.765 | 0.078 |
| | | Phase (crash) | 0.251 ± 0.564 | 0.445 | 0.657 |
| | | Sex (male) x Month (summer) | 0.142 ± 0.851 | 0.167 | 0.868 |
| | | Sex (male) x Month (autumn) | 1.926 ± 0.887 | 2.173 | 0.030 |
| | *Anocephaloides dentata* | Intercept | 1.036 ± 0.180 | 5.769 | <0.001 |
| | | Month (autumn) | -0.605 ± 0.287 | -2.111 | 0.035 |
| Abundance | All helminths | Intercept | 0.693 ± 1.463 | 0.474 | 0.636 |
| | | Month (summer) | -2.428 ± 1.717 | -1.414 | 0.158 |
| | | Month (autumn) | 0.246 ± 1.529 | 0.161 | 0.872 |
| | | Phase (peak) | -0.478 ± 1.503 | -0.318 | 0.751 |
| | | Phase (crash) | -0.199 ± 1.578 | -0.126 | 0.900 |
| | | Month (summer) x Phase (peak) | 1.743 ± 1.774 | 0.982 | 0.326 |
| | | Month (autumn) x Phase (peak) | -0.530 ± 1.662 | -0.319 | 0.750 |
| | | Month (summer)x Phase (crash) | 4.182 ± 1.897 | 2.205 | 0.028 |
| | | Month (autumn) x Phase (crash) | -1.186 ± 1.712 | -0.692 | 0.489 |
| | *Syphacia* sp. | Intercept | 0.495 ± 0.641 | 0.772 | 0.440 |
| | | Phase (peak) | -0.921 ± 0.729 | -1.263 | 0.207 |
| | | Phase (crash) | 0.618 ± 0.835 | 0.741 | 0.459 |
| | *Anocephaloides dentata* | Intercept | -0.995 ± 0.768 | -1.197 | 0.195 |
| | | Month (summer) | -35.560 ± 4.917x$10^6$ | 0.000 | 1.00 |
| | | Month (autumn) | -1.107 ± 0.592 | -1.870 | 0.061 |
| | | Phase (peak) | -0.871 ± 0.806 | -1.081 | 0.280 |
| | | Phase (crash) | 0.896 ± 0.716 | 1.252 | 0.211 |

SE, standard error

### Associations between vole condition, organ weights, litter size and helminth burdens

We tested for associations between body mass and helminth abundance in males and females separately, including size as a covariate (Table 3). Male body mass was positively associated with helminth abundance ($X^2 = 7.68$, df = 1, P = 0.006), whereas no significant association was detected in females. We found no significant correlations between the weight of immune-related organs and the abundance of helminth, the season or the phase of the host population cycle. Regarding female fecundity, larger females have bigger litters and we found a negative association between litter size and helminth abundance, depending on the season (significant season x helminth interaction; $X^2 = 8.40$, df = 2, P = 0.015). This interaction revealed a negative association between litter size and helminth abundance during summer (slope ± standard error: -1.58 ± 0.94) while no significant associations were found in spring (-0.26 ± 0.34) or autumn (0.54 ± 0.46).

Table 3. Results of general linear models explaining variation in litter size of common voles in NW Spain (2010-2015).

| Predictor | Estimate ± SE | Z-value | *P* |
|---|---|---|---|
| Intercept | -1.692 ± 1.158 | -1.462 | 0.144 |
| Body length | 0.026 ± 0.011 | 2.498 | 0.013 |
| Season (summer) | -0.474 ± 0.340 | -1.397 | 0.162 |
| Season (autumn) | -1.164 ± 0.375 | -3.101 | 0.002 |
| Log (total helminths) | -0.259 ± 0.340 | -0.761 | 0.446 |
| Season (summer) x Log (total helminths) | -1.324 ± 0.938 | -1.412 | 0.158 |
| Season (autumn) x Log (total helminths) | 0.796 ± 0.464 | 1.715 | 0.086 |

SE, standard error

## Discussion

### Helminths infecting common voles

Despite identifying eight different helminths, practically all of them belonged to a single species, with a remarkable prevalence (see Figure 1 and Table 1). We identified up to five nematode species, with a direct cycle; and three species of cestodes, with an indirect cycle that need mites and collembolans as intermediate hosts and herbivorous mammals as definitive hosts (equids, ruminants, rodents, lagomorphs and some birds) (2). The species identified are frequently found in common vole populations and nematodes were the dominant helminth group, although results showed a lower GI parasite species richness compared to other studies in the common vole (43,44,46). The taxonomic diversity of voles has been considered a cause driving differences in helminth diversity between regions (57). In the study area, the common vole is virtually the only vole species (37,53) which may

explain the poor helminth richness and diversity patterns found. The overall mean helminth prevalence (24.9%) was similar to those reported in other European populations of common voles (ranging from 5% to 32%) and *Syphacia* sp. was the predominant helminth (44). *Paranocephaloides omphaloides* had a very low prevalence, despite being a typical parasite of *Microtus* spp. in western Europe (58). Local unfavorable climatic conditions for its intermediate host (i.e. collembolans), such as cold winters and summer drought, could reduce the abundance of this arthropod (59,60) and thus, the cestode too. The absence of metacestodes specimens and larvas is easily explained because they are rarely found in the GI tract (61,62). The nonappearance of certain species, such as taeniid cestodes, or the low number of *Heligmosomum* sp. (the other dominant helminth group in European common vole populations along with *Syphacia*), may be explained by the continental-Mediterranean climate of NW Spain. Eggs of cestodes and free-living larvae of *Heligmosomum* are critical stages in the development of these helminths with an indirect cycle. They are highly sensitive to climatic factors that increase the desiccation risk and compromise their success (63). The small sample size during the low phase of the vole cycle could mean that rare species present only under restricted conditions may have been overlooked (64,65). Of the identified species, *Syphacia* sp. could represent a potential minor zoonotic risk, causing anal pruritus (the typical symptom of pinworms in humans (66)). However, identification at the species level would be needed to clarify if the species involved is indeed *Syphacia obvelata*, the zoonotic agent that usually infects common vole (44).

The helminth aggregation pattern found is in accordance with results obtained in rodents and other hosts worldwide (62,67–69). The D Index close to one and VMR values were indicative of a high level of helminth aggregation among voles (in which 91% of helminths were hosted by 5% of the hosts). The comparison between the aggregation index and the mean intensity shows the differences between mean intrapopulation intensity (within each host) and the mean intensity of the component community (within the common vole population) respectively (70). Based on this, the higher value of the VMR compared to the mean intensity in *Syphacia* sp. indicated that a few individual hosts could suffer acute infection levels.

**Variation according to host sex, season and host population cycle**

The higher prevalence found in males may be a consequence of their more mobile lifestyle while seeking females or defending their home range (71), although it may depend on immunological differences (2,72) or the local conditions too (43,73–75). Despite the higher probability of males getting infected than females, here, the social behavior of female hosts could switch the intensity values. As parasites seldom reproduce within rodents, infestation values represent accumulating parasite burdens due to the number of successive contacts with transmission stages (2). In a host species in

which females aggregate in colonies (76), the closer relationships between them could favor an increased transmission of endoparasites with a direct cycle such as *Syphacia* while grooming and increase the parasite intensity in females during certain periods. Many helminth studies take into account the age of the host since older animals are usually more heavily parasitized (73,77–80). However, the sample size of small animals in our sample (since mass can be used as a proxy for age) is too small to analyze them independently.

In general, autumn was the season when helminths reached higher prevalence but lower intensity values. High infestation levels in other helminth species have been also detected during cold months in similar studies (43). Some authors suggest that high infestation rates after summer could be the result of better conditions for the parasite transmission in the previous months (77). Dispersal movements of juveniles after the breeding season (i.e., summer) could also increase the probabilities of infection. In the region, winter is characterized by harsh, cold climatic conditions (47) and vole densities are typically low during this season (38). Both circumstances would reduce reproduction activity (81) and mobility in voles (82), decreasing the opportunities to get in contact with infective helminth stages.

Regarding the host population cycle, helminth abundance in the vole population was higher in the summer of crash years when the density is minimum but reproduction, feeding activity and mobility of voles are very high (81,82). This pattern of high parasitation levels during low host density periods has been detected in other studies (20,31,83). Collapse periods favor high levels of parasite intensity in the surviving hosts (83), increasing the probabilities of contact between infested animals in small populations and consequently, a greater possibility of infestation with directly transmitted helminths. GI helminths compete for the absorption of nutrients with their host, potentially reducing the energy obtained from the diet. Parasitized hosts must then achieve an energy trade-off between immune response against parasites and the investment in other biological processes such as the metabolic rate, feeding or reproduction (2). Parasitation could hence favor the physiological stress in the host, reducing and compromising other biological processes essential for the recovery of the host population density after a crash.

**Helminth burdens, vole condition, organ weights and reproduction parameters**

We found that litter size was negatively associated with the abundance of helminths in summer, the season with the higher reproduction activity in voles (81), regardless of the phase of the cycle. Pregnant rodents can reabsorb or even produce no viable embryos during the first two weeks of pregnancy as a consequence of parasitic infection (84,85). Hence, litter size could not show the total loss of embryos owing to infection here, underestimating the real influence of helminths on vole

reproduction parameters. The negative correlation between helminth load and common vole reproduction could be particularly important because it was detected during the crash year of the vole cycle. In the declining density periods, the stress level in hosts is higher (86), which has been linked to decreases in immunocompetence. Synergies between parasitism and other factors have been suggested to explain the negative effects of parasites on host fitness (19,27–31), including host reproduction (13). We found a greater aggregation of helminths in the small populations of common vole from crash years as well as a more intense parasitation burden in female hosts. Thus, parasitation may act here synergistically with a high stress level, resulting in a negative effect on reproduction. The potential negative delayed density-dependence pattern of helminth infection on common vole fecundity should be confirmed by experiment, either infecting voles or using antihelminthics to reduce parasite infestation levels and test effects on fecundity. Effects of co-infections should be considered in further investigation because they have a relevant role in similar systems (87–89).

We did not find any organ weight anomaly or negative correlation between the body-mass condition and high parasite burden, possibly because of two reasons: 1) infection intensity was low, and 2) *Syphacia* sp. is the most common helminth infesting our vole population. A minimum helminth threshold appears to be critical in other small mammals in order to cause negative effects (78) since hosts show tolerance under low helminth burdens (2). The most common pathology caused by a severe infection of *Syphacia* sp. (the most abundant helminth) would be occasional rectal prolapsed (21), but severe damage owing to migration through host tissues is not expected (90,91). Helminths identified here could provoke increases in the weight of organs but only with very high infection burdens. The helminth group usually linked to organ hypertrophy in rodents is the cestodes (21,24). However, taxa such as *Heligmosomum* sp. and *Heligmosomoides* sp. are rarely found in our vole sample and other relevant cestodes species causing hypertrophy do not parasitize the GI tract (2,61,62). It is therefore understandable that the prevalence of cestodes specimens in our vole sample was low and hence the effects on host conditions were mild. Further research on extra-intestinal helminths would be required to determine the whole range of endoparasites infecting this vole population and their effect on the host condition.

## Conclusions

Theoretical works have highlighted under which conditions parasites could regulate and in some cases destabilize host populations (2,7,12,13): aggregated distribution, delayed host density-dependence, sublethal effects on hosts and reduction in host survival or reproduction. In cyclic voles from NW Spain, we have shown that the main helminth parasites are aggregated among hosts. The abundance of helminths was higher during the crash years of the vole cycle and it showed a negative correlation

with litter size in summer, a pattern that is consistent with delayed density-dependent parasite recruitment. Correlative evidence suggests a negative influence of helminth burden on vole reproduction, which should be confirmed by experiment. Altogether, these observations indicate that helminth parasites may have a role to play in the cyclic population dynamics of common voles as a constrain factor during the low phase that could prevent the vole population from quickly bouncing back after a population crash.

## Acknowledgments

We thank Voitto Haukisalmi, Heikki Hentonnen and Jukka Niemimaa for helping with helminth identification. This work was funded by the projects ECOCYCLES (Partner 5-EUI2008-03658), ECOVOLE (CGL2012-35348), ECOTULA (CGL2015-66962-C2-1-R) and BOOMRAT (PID2019-109327RB-I00) by the Government of Spain. Silvia Herrero-Cófreces was supported by a PhD studentship from Junta de Castilla-y-León (co-funded by European Social Fund, Orden 10/11/2016).

## Ethics approval and permits

The trapping methods applied in this study were approved by our institution ethics committee (CEEBA, Universidad de Valladolid; authorization code: 4801646) and we held the official trapping permits from DGMN (Junta de Castilla-y-León) as well as compulsory national certificates (B and C categories) to manipulate living animals for research.

## References

1. Castro GA. Helminths: structure, classification, growth, and development, in *Medical Microbiology*, ed. S. Baron (1996). Available at: https://www.ncbi.nlm.nih.gov/books/NBK8282/ [Accessed April 20, 2022]
2. Morand S, Krasnov BR, Poulin R. *Micromammals and macroparasites: from evolutionary ecology to management*. Tokyo: Springer Japan (2006). doi:10.1007/978-4-431-36025-4
3. Krebs CJ. Two paradigms of population regulation. *Wildl Res* (1995) **22**:1. doi:10.1071/WR9950001
4. Krebs CJ. *Population fluctuations in rodents*. Chicago: University of Chicago Press (2013). doi:10.7208/chicago/9780226010496.001.0001
5. Boonstra R, Krebs CJ, Stenseth NC. Population cycles in small mammals: the problem of explaining the low phase. *Ecology* (1998) **79**:1479. doi:10.2307/176770
6. Anderson RM, May RM. Regulation and stability of host-parasite population interactions: I. Regulatory processes. *J Anim Ecol* (1978) **47**:219–247. doi:10.2307/3933
7. May RM, Anderson RM. Regulation and stability of host-parasite population interactions: II. Destabilizing processes. *J Anim Ecol* (1978) **47**:249. doi:10.2307/3934

8. Scott ME, Dobson A. The role of parasites in regulating host abundance. *Parasitol Today* (1989) **5**:176–183. doi:10.1016/0169-4758(89)90140-3

9. Hatcher MJ, Dick JTA, Dunn AM. Diverse effects of parasites in ecosystems: linking interdependent processes. *Front Ecol Environ* (2012) **10**:186–194. doi:10.1890/110016

10. Telfer S, Lambin X, Birtles RJ, Beldomenico PM, Burthe SJ, Paterson S, Begon M. Species interactions in a parasite community drive infection risk in a wildlife population. *Science* (2010) **330**:243–246. doi:10.1126/science.1190333

11. Preston D, Johnson PTJ. Ecological consequences of parasitism. *Nat Educ Knowl* (2010) **1**:39.

12. Hudson PJ, Dobson AP, Newborn D. Prevention of population cycles by parasite removal. *Science* (1998) **282**:2256–2258. doi:10.1126/science.282.5397.2256

13. Tompkins DM, Dobson AP, Arneberg P, Begon M, Cattadori IM, Greenman JV, Heerterbeek JAP, Hudson PJ, Newborn D, Pugliese A, et al. "Parasites and host population dynamics," in *The ecology of wildlife diseases*, eds. P. J. Hudson, A. Rizzoli, B. T. Grenfell, H. Heesterbeek, A. P. Dobson. Oxford: Oxford University Press (2002) 45–62.

14. Gilg O, Bollache L, Afonso E, Yannic G, Schmidt NM, Hansen LH, Hansen J, Sittler B, Lang J, Meyer N, et al. Are gastrointestinal parasites associated with the cyclic population dynamics of their arctic lemming hosts? *Int J Parasitol Parasites Wildl* (2019) **10**:6–12. doi:10.1016/j.ijppaw.2019.06.011

15. Laakkonen J, Kallio-Kokko H, Öktem MA, Blasdell K, Plyusnina A, Niemimaa J, Karataş A, Plyusnin A, Vaheri A, Henttonen H. Serological survey for viral pathogens in Turkish rodents. *J Wildl Dis* (2006) **42**:672–676. doi:10.7589/0090-3558-42.3.672

16. Forbes KM, Stuart P, Mappes T, Henttonen H, Huitu O. Food resources and intestinal parasites as limiting factors for boreal vole populations during winter. *Ecology* (2014) **95**:3139–3148. doi:10.1890/13-2381.1

17. Deter J, Charbonnel N, Cosson J-F, Morand S. Regulation of vole populations by the nematode Trichuris arvicolae: insights from modelling. *Eur J Wildl Res* (2008) **54**:60–70. doi:10.1007/s10344-007-0110-6

18. Lemaître J, Fortin D, Montiglio P-O, Darveau M. Bot fly parasitism of the red-backed vole: host survival, infection risk, and population growth. *Oecologia* (2009) **159**:283–294. doi:10.1007/s00442-008-1219-3

19. Pedersen AB, Greives TJ. The interaction of parasites and resources cause crashes in a wild mouse population. *J Anim Ecol* (2008) **77**:370–377. doi:10.1111/j.1365-2656.2007.01321.x

20. Winternitz JC, Yabsley MJ, Altizer SM. Parasite infection and host dynamics in a naturally fluctuating rodent population. *Can J Zool* (2012) **90**:1149–1160. doi:10.1139/z2012-083

21. Wiger R. Some pathological effects of endoparasites on rodents with special reference to the population ecology of microtines. *Oikos* (1977) **29**:598. doi:10.2307/3543598

22. Ponlet N, Chaisiri K, Claude J, Morand S. Incorporating parasite systematics in comparative analyses of variation in spleen mass and testes sizes of rodents. *Parasitology* (2011) **138**:1804–1814. doi:10.1017/S003118201100028X

23. Morand S, Poulin R. Nematode parasite species richness and the evolution of spleen size in birds. *Can J Zool* (2000) **78**:1356–1360. doi:10.1139/cjz-78-8-1356

24. Tenora F, Wiger R, Barus V. Seasonal and annual variations in the prevalence of helminths in a cyclic population of Clethrionomys glareolus. *Ecography (Cop)* (1979) **2**:176–181.

doi:10.1111/j.1600-0587.1979.tb00698.x

25. Pao-Chang H. The pathology of *Clonorchis sinensis* infestation of the liver. *J Pathol Bacteriol* (1955) **70**:53–64. doi:10.1002/path.1700700106

26. McCuskey RS, McCuskey PA, Urbaschek R, Urbaschek B. Kupffer cell function in host defense. *Clin Infect Dis* (1987) **9**:S619–S619. doi:10.1093/clinids/9.Supplement_5.S616

27. Tompkins DM, Begon M. Parasites can regulate wildlife populations. *Parasitol Today* (1999) **15**:311–313. doi:10.1016/S0169-4758(99)01484-2

28. Keymer AE, Dobson AP. The ecology of helminths in populations of small mammals. *Mamm Rev* (1987) **17**:105–116. doi:10.1111/j.1365-2907.1987.tb00056.x

29. Marcogliese DJ, Pietrock M. Combined effects of parasites and contaminants on animal health: parasites do matter. *Trends Parasitol* (2011) **27**:123–130. doi:10.1016/j.pt.2010.11.002

30. Lambin X. Population cycles and parasitism. *Science* (1999) **286**:2425. doi:10.1126/science.286.5449.2425a

31. Redpath SM, Mougeot F, Leckie FM, Elston DA, Hudson PJ. Testing the role of parasites in driving the cyclic population dynamics of a gamebird. *Ecol Lett* (2006) **9**:410–418. doi:10.1111/j.1461-0248.2006.00895.x

32. Lambin X, Bretagnolle V, Yoccoz NG. Vole population cycles in northern and southern Europe: Is there a need for different explanations for single pattern? *J Anim Ecol* (2006) **75**:340–349. doi:10.1111/j.1365-2656.2006.01051.x

33. Elton CS. Periodic fluctuations in number of animals: their causes and effects. *Br J Exp Biol* (1924) **2**:119–163.

34. Hudson PJ, Dobson AP. "Macroparasites: observed patterns," in *Ecology of Infectious Diseases in Natural Populations*, Cambridge: Cambridge University Press (1995) 144–176. doi:10.1017/CBO9780511629396.006

35. Jacob J, Tkadlec E. "Rodent outbreaks in Europe: dynamics and damage," in *Rodent outbreaks: ecology and impacts*, eds. G. Singleton, S. Belmain, P. Brown, B. Hardy. Los Baños: International Rice Research Institute (2010) 207–223.

36. Oli MK. Population cycles in voles and lemmings: state of the science and future directions. *Mamm Rev* (2019) **49**:226–239. doi:10.1111/mam.12156

37. Rodríguez-Pastor R, Luque-Larena JJ, Lambin X, Mougeot F. "Living on the edge": The role of field margins for common vole (*Microtus arvalis*) populations in recently colonised Mediterranean farmland. *Agric Ecosyst Environ* (2016) **231**:206–217. doi:10.1016/j.agee.2016.06.041

38. Mougeot F, Lambin X, Rodríguez-Pastor R, Romairone J, Luque-Larena JJ. Numerical response of a mammalian specialist predator to multiple prey dynamics in Mediterranean farmlands. *Ecology* (2019) **100**:e02776. doi:10.1002/ecy.2776

39. Herrero-Cófreces S, Mougeot F, Lambin X, Luque-Larena JJ. Linking zoonosis emergence to farmland invasion by fluctuating herbivores: common vole populations and tularemia outbreaks in NW Spain. *Front Vet Sci* (2021) **8**:900. doi:10.3389/fvets.2021.698454

40. Hernández MC, Sánchez-González B, Navarro-Castilla Á, Barja I. Topillo campesino – *Microtus arvalis* (Pallas, 1779). *Encicl Virtual los Vertebr Españoles* (2017) Available at: https://digital.csic.es/bitstream/10261/146730/4/micarv_v1.pdf [Accessed April 12, 2020]

41. González-Esteban J, Villate I. "*Microtus arvalis* (Pallas, 1778)," in *Atlas y Libro Rojo de los*

*Mamíferos Terrestres de España*, eds. L. J. Palomo, J. Gisbert, J. C. Blanco. Madrid: Dirección General para la Biodiversidad-SECEM-SECEMU (2007) 128–130.

42. Boyce CCK, Boyce JL. Population biology of *Microtus arvalis*. III. Regulation of numbers and breeding dispersion of females. *J Anim Ecol* (1988) **57**:737. doi:10.2307/5090

43. Gubányi A, Mészáros F, Murai É, Soltész A. Studies on helminth parasites of the small field mouse (*Apodemus microps*) and the common vole (*Microtus arvalis*) from a pine forest in Hungary. *Parasit Hung* (1992) **25**:37–51.

44. Tenora F, Murai É, Mészáros F. Quantitative and qualitative analysis of helminth fauna of *Microtus arvalis* (Rodentia, Microtinae) in Europe. *Parasit Hung* (1973) **6**:131–148.

45. Kisielewska K, Zubczewska Z. Intestinal helminths as indexes of reproduction dynamics in the host population-common vole. *Acta Theriol (Warsz)* (1973) **18**:237–246.

46. Feliu C, Renaud F, Catzeflis F, Hugot JP, Durand P, Morand S. A comparative analysis of parasite species richness of Iberian rodents. *Parasitology* (1997) **115**:453–466. doi:10.1017/S0031182097001479

47. Rivas-Martínez S, Penas Á, del Río S, Díaz González TE, Rivas-Sáenz S. "Bioclimatology of the Iberian Peninsula and the Balearic Islands," in *The Vegetation of the Iberian Peninsula*, ed. J. Loidi. Cham: Springer International Publishing (2017) 29–80. doi:10.1007/978-3-319-54784-8_2

48. Henttonen H, Fuglei E, Gower CN, Haukisalmi V, Ims RA, Niemimaa J, Yoccoz NG. *Echinococcus multilocularis* on Svalbard: introduction of an intermediate host has enabled the local life-cycle. *Parasitology* (2001) **123**: doi:10.1017/S0031182001008800

49. Haukisalmi V, Henttonen H. Coexistence in helminths of the bank vole *Clethrionomys glareolus*. I. Patterns of co-occurrence. *J Anim Ecol* (1993) **62**:221. doi:10.2307/5353

50. Tkach V V., Hope AG, Greiman SE. Method for the rapid fixation of gastrointestinal helminths in small mammals. *Acta Parasitol* (2019) **64**:406–410. doi:10.2478/s11686-019-00028-w

51. Poulin R. The disparity between observed and uniform distributions: A new look at parasite aggregation. *Int J Parasitol* (1993) **23**:937–944. doi:10.1016/0020-7519(93)90060-C

52. Reiczigel J, Marozzi M, Fábián I, Rózsa L. Biostatistics for parasitologists – A primer to quantitative parasitology. *Trends Parasitol* (2019) **35**:277–281. doi:10.1016/j.pt.2019.01.003

53. Herrero-Cófreces S, Flechoso MF, Rodríguez-Pastor R, Luque-Larena JJ, Mougeot F. Patterns of flea infestation in rodents and insectivores from intensified agro-ecosystems, Northwest Spain. *Parasit Vectors* (2021) **14**:16. doi:10.1186/s13071-020-04492-6

54. Bates D, Mächler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. *J Stat Softw* (2015) **67**: doi:10.18637/jss.v067.i01

55. Bolker B, Skaug H, Maintainer JL. R2admb: "ADMB" to R interface functions. (2017)

56. R Core Team. R: The R project for statistical computing. (2019)

57. Haukisalmi V, Henttonen H, Hardman LM, Hardman M, Laakkonen J, Murueva G, Niemimaa J, Shulunov S, Vapalahti O. Review of tapeworms of rodents in the Republic of Buryatia, with emphasis on anoplocephalid cestodes. *Zookeys* (2009) **8**:1–18. doi:10.3897/zookeys.8.58

58. Rausch RL. Distribution and specificity of helminths in microtine rodents: evolutionary implications. *Evolution* (1957) **11**:361–368.

59. Greenslade P. Survival of Collembola in arid environments: observations in South Australia and the Sudan. *J Arid Environ* (1981) **4**:219–228. doi:10.1016/S0140-1963(18)31563-5

60. Salmon S, Ponge JF, Gachet S, Deharveng L, Lefebvre N, Delabrosse F. Linking species, traits and habitat characteristics of Collembola at European scale. *Soil Biol Biochem* (2014) **75**:73–85. doi:10.1016/j.soilbio.2014.04.002

61. Führer H-P, Schneider R, Walochnik J, Auer H. Extraintestinal helminths of the common vole (*Microtus arvalis*) and the water vole (*Arvicola terrestris*) in Western Austria (Vorarlberg). *Parasitol Res* (2010) **106**:1001–1004. doi:10.1007/s00436-010-1753-x

62. Haukisalmi V, Henttonen H. Variability of helminth assemblages and populations in the bank vole *Clethrionomys glareolus*. *Polish J Ecol* (2000) **48**:219–231.

63. Haukisalmi V, Henttonen H. The impact of climatic factors and host density on the long-term population dynamics of vole helminths. *Oecologia* (1990) **83**:309–315. doi:10.1007/BF00317553

64. Haukisalmi V, Henttonen H. Determinants of helminth aggregation in natural host populations: individual differences or spatial heterogeneity? *Ecography (Cop)* (1999) **22**:629–636. doi:10.1111/j.1600-0587.1999.tb00511.x

65. Haukisalmi V, Henttonen H, Tenora F. Population dynamics of common and rare helminths in cyclic vole populations. *J Anim Ecol* (1988) **57**:807. doi:10.2307/5094

66. Despommier DD, Gwadz RW, Hotez PJ. *Parasitic diseases*. 3rd ed. New York, NY: Springer New York (1995). doi:10.1007/978-1-4612-2476-1

67. Behnke JM, Eira C, Rogan M, Gilbert FS, Torres J, Miquel J, Lewis J. Helminth species richness in wild wood mice, *Apodemus sylvaticus* , is enhanced by the presence of the intestinal nematode *Heligmosomoides polygyrus*. *Parasitology* (2009) **136**:793–804. doi:10.1017/S0031182009006039

68. Haukisalmi V. Frequency distributions of helminths in microtine rodents in Finnish Lapland. *Ann Zool Fenn* (1986) **23**:141–150.

69. Shaw DJ, Dobson AP. Patterns of macroparasite abundance and aggregation in wildlife populations: a quantitative review. *Parasitology* (1995) **111**:S111–S133. doi:10.1017/S0031182000075855

70. Reiczigel J, Lang Z, Rózsa L, Tóthmérész B. Properties of crowding indices and statistical tools to analyze parasite crowding data. *J Parasitol* (2005) **91**:245–252. doi:10.1645/GE-281R1

71. Krasnov BR, Bordes F, Khokhlova IS, Morand S. Gender-biased parasitism in small mammals: patterns, mechanisms, consequences. *Mammalia* (2012) **76**:1–13. doi:10.1515/mammalia-2011-0108

72. Jackson JA, Hall AJ, Friberg IM, Ralli C, Lowe A, Zawadzka M, Turner AK, Stewart A, Birtles RJ, Paterson S, et al. An immunological marker of tolerance toinfection in wild rodents. *PLoS Biol* (2014) **12**:e1001901. doi:10.1371/journal.pbio.1001901

73. Haukisalmi V, Henttonen H, Batzli GO. Helminth parasitism in the voles *Microtus oeconomus* and *M. miurus* on the North slope of Alaska: host specificity and the effects of host sex, age and breeding status. *Ann Zool Fennici* (1995) **32**:193–201. Available at: https://www.jstor.org/stable/23735525

74. Bordes F, Ponlet N, de Bellocq JG, Ribas A, Krasnov BR, Morand S. Is there sex-biased resistance and tolerance in Mediterranean wood mouse (*Apodemus sylvaticus*) populations facing multiple helminth infections? *Oecologia* (2012) **170**:123–135. doi:10.1007/s00442-012-2300-5

75. Bordes F, Morand S. Helminth species diversity of mammals: parasite species richness is a

host species attribute. *Parasitology* (2008) **135**:1701–1705. doi:10.1017/S0031182008005040

76. Rozenfeld F, Dobly A. Burrowing by common voles (*Microtus arvalis*) in various social environments. *Behaviour* (2000) **137**:1443–1461. doi:10.1163/156853900502664

77. Behnke JM, Lewis JW, Zain SNM, Gilbert FS. Helminth infections in *Apodemus sylvaticus* in southern England: interactive effects of host age, sex and year on the prevalence and abundance of infections. *J Helminthol* (1999) **73**:31–44. doi:10.1017/S0022149X99000049

78. Cowan KM, Shutler D, Herman TB, Stewart DT. Splenic mass of masked shrews, Sorex cinereus, in relation to body mass, sex, age, day of the year, and bladder nematode, *Liniscus* (=*Capillaria*) *maseri*, infection. *J Parasitol* (2009) **95**:228–230. doi:10.1645/ge-1566.1

79. Barnard CJ, Behnke JM, Bajer A, Bray D, Race T, Frake K, Osmond J, Dinmore J, Sinski E. Local variation in endoparasite intensities of bank voles (*Clethrionomys glareolus*) from ecologically similar sites: morphometric and endocrine correlates. *J Helminthol* (2002) **76**:103–112. doi:10.1079/JOH2001109

80. Rossin MA, Malizia AI, Timi JT, Poulin R. Parasitism underground: determinants of helminth infections in two species of subterranean rodents (Octodontidae). *Parasitology* (2010) **137**:1569–1575. doi:10.1017/S0031182010000351

81. Eccard JA, Herde A. Seasonal variation in the behaviour of a short-lived rodent. *BMC Ecol* (2013) **13**:43. doi:10.1186/1472-6785-13-43

82. Brügger A, Nentwig W, Airoldi J-P. The burrow system of the common vole (*M. arvalis*, Rodentia) in Switzerland. *Mammalia* (2010) **74**:311–315. doi:10.1515/mamm.2010.035

83. Cerqueira D, Delattre P, De Sousa B, Gabrion C, Morand S, Quere JP, Quéré J-P. Numerical response of a helminth community in the course of a multi-annual abundance cycle of the water vole (*Arvicola terrestris*). *Parasitology* (2006) **134**:705–711. doi:10.1017/S0031182006001946

84. Shaw GL, Quadagno D. *Trypanosoma lewisi* and *T. cruzi*: Effect of infection on gestation in the rat. *Exp Parasitol* (1975) **37**:211–217. doi:10.1016/0014-4894(75)90072-7

85. Shaw GL, Dusanic DG. *Trypanosoma lewisi*: Termination of pregnancy in the infected rat. *Exp Parasitol* (1973) **33**:46–55. doi:10.1016/0014-4894(73)90008-8

86. Charbonnel N, Chaval Y, Berthier K, Deter J, Morand S, Palme R, Cosson J-F. Stress and demographic decline: a potential effect mediated by impairment of reproduction and immune function in cyclic vole populations. *Physiol Biochem Zool* (2008) **81**:63–73. doi:10.1086/523306

87. Lello J, Boag B, Fenton A, Stevenson IR, Hudson PJ. Competition and mutualism among the gut helminths of a mammalian host. *Nature* (2004) **428**:840–844. doi:10.1038/nature02490

88. Ezenwa VO. Helminth-microparasite co-infection in wildlife: lessons from ruminants, rodents and rabbits. *Parasite Immunol* (2016) **38**:527–534. doi:10.1111/pim.12348

89. Turner AK, Beldomenico PM, Bown KJ, Burthe SJ, Jackson JAA, Lambin X, Begon M. Host–parasite biology in the real world: the field voles of Kielder. *Parasitology* (2014) **141**:997–1017. doi:10.1017/S0031182014000171

90. Stahl W. Studies on the life cycle of *Syphacia muris*, the rat pinworm. *Keio J Med* (1963) **12**:55–60. doi:10.2302/kjm.12.55

91. Whary MT, Baumgarth N, Fox JG, Barthold SW. "Biology and diseases of mice," in *Laboratory Animal Medicine*, eds. J. G. Fox, L. C. Anderson, G. M. Otto, K. R. Pritchett-Corning, M. T. Whary. London: Academic Press (2015) 43–149. doi:10.1016/B978-0-12-409527-4.00003-1

# APPENDIX

Table S1. Number of common voles analyzed from each trapping session in NW Spain, 2010–2015.

| Year | Month | Phase | Host | | |
|---|---|---|---|---|---|
| | | | Female | Male | All |
| 2010 | All months | Increase | 7 | 5 | 12 |
| | July | | 0 | 1 | 1 |
| | November | | 7 | 4 | 11 |
| 2011 | All months | Peak | 25 | 35 | 60 |
| | March | | 5 | 7 | 12 |
| | July | | 5 | 13 | 18 |
| | November | | 15 | 15 | 30 |
| 2012 | All months | Crash | 17 | 28 | 45 |
| | March | | 4 | 13 | 17 |
| | July | | 13 | 14 | 27 |
| | November | | 0 | 1 | 1 |
| 2013 | All months | Increase | 29 | 23 | 52 |
| | March | | 3 | 1 | 4 |
| | July | | 4 | 12 | 16 |
| | November | | 22 | 10 | 32 |
| 2014 | All months | Peak | 113 | 90 | 203 |
| | March | | 35 | 28 | 63 |
| | July | | 61 | 41 | 102 |
| | November | | 17 | 21 | 38 |
| 2015 | All months | Crash | 2 | 6 | 8 |
| | March | | 2 | 6 | 8 |
| All years | All months | | 193 | 187 | 380 |

# GENERAL DISCUSSION

Zoonoses are a major concern for public health and human interests, and both natural and human-induced causes are driving the emergence of zoonoses worldwide. Human-driven modifications in the ecosystems are the most frequent events that alter relationships between species, including hosts, vectors, and pathogens (15,17). These anthropogenic alterations trigger cascading effects that change the dynamics of infectious diseases. Anthropic land-use changes and human activities in nature involve an increase in the transmission and spillover risk to humans, who are competent hosts for many zoonoses whose reservoirs are mammals. Detecting the zoonotic pathogens that circulate in the system, identifying their main reservoirs and vectors, determining their possible circulation routes, and unveiling the spatial-temporal patterns are essential to understand the role of pathogens in the system and develop an effective prevention program against zoonoses.

In this thesis, I have investigated the role of rodents from a Disease Ecology perspective (a view that considers different levels of complexity in the study of zoonoses, from individual to ecosystem (25,26)), with the aim of producing new knowledge on the pathogens that are circulating in the fluctuating *Microtus arvalis* and the small mammal community that co-exist with them in the intensive farmland of NW Spain. Specifically, I have been able to: i) review the current knowledge on the "*Francisella–Microtus*" system and underlie four relevant knowledge gaps in the ecological epidemiology of tularemia; ii) identify and quantify the presence of some micropathogens, macroparasites and vector species in the small mammal community that co-habits with *M. arvalis*, a small rodent whose populations greatly fluctuate in abundance in the study region and drive zoonotic risk of tularemia epidemics among humans; iii) determine patterns of parasitological parameters in small mammal hosts according to habitat, seasonality and abundance fluctuations; and iv) shed some light onto the potential role that pathogens and vectors might play in the regulation of vole populations.

## Zoonoses in the agricultural system and public health implications

### Pathogens and small mammal hosts

The review of the "*Francisella tularensis – Microtus arvalis"* system (**Chapter 1**) compiled all the knowledge on this system, mostly acquired in the last decade (95). Throughout Europe, this bacterium has been linked to fluctuating *Microtus* species in terrestrial cycles (98,109–112) or the presence of water masses in aquatic cycles (113–116). *Microtus arvalis* with fluctuating dynamics occur in agricultural areas (117,118) with fodder and protein-rich crops such as alfalfa (94,119). This review shows that five conditions have aligned to trigger tularemia epidemics in the NW Spain, facilitating the current endemic state of this zoonosis: i) land-use changes due to agriculture intensification; ii) irrigation system development; iii) highly infective and generalist bacterium; iv) colonization and

expansion of a competent mammalian host (i.e. *M. arvalis*); v) fluctuating dynamics of this key host. The result was the emergence of tularemia outbreaks, which have become endemic in the region since then 1997. I also corroborate the trend observed in *M. arvalis* dynamics by Luque-Larena (120), showing that large density peaks (associated with tularemia epidemics) alternate with lesser peaks that do not cause epidemic events. The circulation of *F. tularensis* in the sympatric community is likely to occur, due to the high infectivity of the bacterium and the habitat sharing of *M. arvalis* with other small mammals (i.e. *A. sylvaticus*, *M. spretus* and *C. russula*) (94). Nevertheless, the virtual absence of the bacterium in other hosts from the sympatric community suggests that these species that co-habit with *M. arvalis* play no relevant role in the circulation of the bacterium (**Chapter 3.1**). *Francisella tularensis* is thought to circulate here in a terrestrial-aquatic intertwined cycle since the bacterium has been detected in lagomorphs (121) and *M. arvalis* (98), and tularemia transmission have been also linked to water and crayfish fishing (116). This thesis (**Chapter 1**) highlighted some knowledge gaps that need to be addressed to fully understand the circulation of the bacterium in the system.

Agricultural intensification can modify the composition and functioning of the ecosystem, enhancing the emergence of zoonotic diseases (19,20). The amplification potential of the colonizing *M. arvalis* throughout the region during population outbreaks triggered the emergence of *F. tularensis*. This prompted us to screen for other zoonotic pathogens that could potentially be circulating in the system. This thesis provided new information on the pathogen pool that is circulating in the small mammals of this agro-ecosystem (Table 1), specifically, two types of bacteria (**Chapter 3.1**), three viruses (**Chapter 3.2**), and the GI helminth community (**Chapter 4**), revealing some of these species as zoonotic and concerning pathogens.

**Vectors and pathogen circulation**

Regarding vectors, I have identified six species of fleas and four ticks infesting the small mammal guild (Table 1). The commonest ones are three species of fleas (*C. apertus*, *N. fasciatus* and *L. taschenbergi*) and one of tick (*R. turanicus*), shared by the sympatric rodent community. The low prevalence and diversity of ticks among the small mammal community make fleas a more suitable candidate for being involved in the circulation of pathogens linked with fluctuating voles. Note that the diversity of *Bartonella* species is known to be related to a high diversity of fleas since there is a high adaptation level between both (122). This thesis (**Chapters 2.1 and 3.1**) showed that the higher diversity of *Bartonella* co-infecting small mammals co-occurred within periods of high prevalence and abundance of the most frequently shared flea species. This is consistent with the hypothesis that when there are more fleas in the system, more flea-borne pathogens circulate among hosts. *Nosopsyllys fasciatus* has been suggested as a good candidate for *Bartonella* circulation among the guild (97,108)

because of its generalist requirements. The apparent high tolerance to cohabit with other flea species in any of the four studied small mammal hosts supports a vectoring role of this flea. *Rattus* spp. and *Mus domesticus* are common rodents inhabiting anthropic habitats and can also harbor *N. fasciatus* and *Bartonella* (62,73,123,124). Since the rural settlements in the study area are embedded in the farming landscape, the circulation of pathogens carried by *N. fasciatus* may involve synanthropic and wild rodents, with the consequent implications for human health.

Table 1. Detection of all pathogens and vectors identified in the small mammal guild studied from NW Spain, 2013–2015.

| Pathogen/vector group | Species | Host | | | |
|---|---|---|---|---|---|
| | | AS | CR | MA | MS |
| **Bacteria** | *Bartonella birtlesii* | ✗ | ✗ | ✗* | ✓ |
| | *B. chomelii* | ✓ | ✓ | ✗* | ✗ |
| | *B. clarridgeae* | ✗ | ✗ | ✓* | ✗ |
| | *B. cooperplainsense* | ✗ | ✗ | ✗* | ✓ |
| | *B. doshiae* | ✗ | ✗ | ✓* | ✗ |
| | *B. elisabethae* | ✓ | ✓ | ✗* | ✓ |
| | *B. grahamii* | ✓ | ✓ | ✓* | ✓ |
| | *B. rochalimae* | ✓ | ✗ | ✓* | ✗ |
| | *B. taylorii* | ✓ | ✗ | ✓* | ✓ |
| | *B. tribocorum* | ✗ | ✓ | ✗* | ✗ |
| | *B. vinsonii* | ✗ | ✗ | ✗* | ✓ |
| | *Francisella tularensis* | ✓ | ✗ | ✓* | ✗ |
| **Viruses** | Hantavirus | ✗ | ✗ | ✓ | ✗ |
| | Lymphocytic choriomeningitis virus | ✓ | ✓ | ✓ | ✗ |
| | Orthopoxvirus | ✗ | ✗ | ✓ | ✓ |
| **Helminths** | *Anocephaloides dentata* | NA | NA | ✓ | NA |
| | *Heligmosomoides laevis* | NA | NA | ✓ | NA |
| | *Heligmosomoides* sp. | NA | NA | ✓ | NA |
| | *Heligmosomum* sp. | NA | NA | ✓ | NA |
| | *Paranocephaloides gracilis* | NA | NA | ✓ | NA |
| | *Paranocephaloides omphaloides* | NA | NA | ✓ | NA |
| | *Syphacia* sp. | NA | NA | ✓ | NA |
| | *Trichuris* sp. | NA | NA | ✓ | NA |
| **Ticks** | *Dermacentor* sp. | ✓ | ✗ | ✗ | ✗ |
| | *Hyalomma* sp. | ✓ | ✗ | ✓ | ✗ |
| | *Rhipicephalus* sp. | ✓ | ✓ | ✓ | ✓ |
| | *Rhipicephalus pusillus* | ✗ | ✓ | ✗ | ✗ |
| | *Rhipicephalus turanicus* | ✓ | ✓ | ✓ | ✓ |
| **Fleas** | *Ctenophthalmus* sp. | ✓ | ✗ | ✓ | ✓ |
| | *Ctenophthalmus apertus apertus* | ✗ | ✗ | ✓ | ✗ |
| | *Ctenophthalmus apertus gilcolladoi* | ✓ | ✓ | ✓ | ✓ |
| | *Ctenophthalmus baeticus* | ✗ | ✗ | ✓ | ✗ |
| | *Leptopsylla taschenbergi amitina* | ✓ | ✗ | ✓ | ✓ |
| | *Nosopsyllus fasciatus* | ✓ | ✓ | ✓ | ✓ |
| | *Rhadinopsylla beillardae* | ✓ | ✗ | ✓ | ✓ |

AS, *Apodemus sylvaticus*; CR, *Crocidura russula*; MA, *Microtus arvalis*; MS, *Mus spretus*; NA, not analyzed; green check, pathogen/vector species detected; red cross, pathogen/vector species not detected; *From Rodríguez-Pastor et al. (2019). Zoonotic pathogens in fluctuating common vole (*Microtus arvalis*) populations: occurrence and dynamics. *Parasitology*, *146*(3), 389–398. doi:10.1017/S0031182018001543

Preliminary screenings performed during this thesis showed that *F. tularensis* was not present in ticks collected from the small mammal hosts (*M. arvalis*: n = 89; *A. sylvaticus*: n = 34, *M. spretus*: n = 8; *C. russula*: n = 12; unpublished data). Tularemia prevalence in fleas collected from the *M. arvalis* was also found to be low (estimated at 6%) (108). This supports the hypothesis that these potential vectors have a secondary role in the terrestrial tularemia cycle, and may be involved in the circulation rather than acting as a key reservoir for the bacterium.

**Public health implications**

My first contribution has been to raise awareness regarding which pathogens circulate within the study farming system (**Chapters 2-4**). Results unveiled that, apart from *F. tularensis*, five species of *Bartonella*, three viruses (hantavirus, orthopoxvirus and lymphocytic choriomeningitis virus [LCMV]), and one species of helminths (*Syphacia* sp.) should be considered as novel zoonotic risks. These pathogens are the etiological agents of concerning emerging zoonoses throughout Europe, especially *F. tularensis*, hantavirus and orthopoxvirus, because of the epidemic events that they cause, or their high potential impact if they emerge (102,120,125–127). The unspecific symptomatology of many zoonoses can mask the real incidence in human populations (128). The high number of cases in the two first tularemia epidemics recorded in Spain (589 in 1997 and 497 in 2007) compared to the following events (105 in 2014 and 187 in 2019) could be explained by two facts: i) a lack of evidence of tularemia cases until the colonization of *M. arvalis* and ii) a lack of knowledge of the zoonotic pathogen pool in the colonizing *M. arvalis* and native species. The unawareness of pathogens circulating in the wild fauna, combined with the unfamiliarity with the symptomatology of many emerging diseases, explain the difficulty in the diagnoses of the human cases and the lack of prevention measures, enhancing the negative impacts of epidemic events on people. The tularemia case study (**Chapter 1**) highlighted the importance of surveillance as a preventive tool against zoonotic disease emergences. Identifying new pathogens circulating among local fauna is the first step towards the development and implementation of new protocols of zoonoses, which should include specific diagnostic proofs of these diseases in patients with compatible symptomatology.

Human cases caused by the pathogens that I have detected are often linked to rodent dynamics (102,120,125–127), so understanding and predicting the population dynamics of these key hosts and reservoirs are essential to prevent future zoonotic epidemics. Establishing patterns in the prevalence and intensity of pathogens, vectors and hosts can help to detect high and low-risk periods, essential for the design and implementation of preventive measures. The cyclic dynamics of *M. arvalis* are the origin of the amplification role of the bacterium driving the recurrent tularemia epidemics (98,120). Cyclic dynamics may be predictable, so there is also an opportunity to take advantage of this

predictability for prevention. This knowledge should be used to anticipate future outbreaks and implement preventive actions at the right time to avoid negative impacts on public health.

Climatic conditions are crucial for arthropod vectors because they are cool-blooded animals whose activity is necessarily linked to suitable external factors. The vegetation cover determines the microclimatic conditions and the type of animals visiting the places where arthropod vectors reside (129). Despite that, I found a poor effect of habitat (crop types) on vector parasitism of small mammal hosts, other than a decrease in the prevalence and intensity of fleas among voles trapped in alfalfas (**Chapter 2.1**). This could be due to a dilution effect among voles inhabiting this crop since it is the most favorable habitat for voles, where they reach higher abundances (94). There does not appear to be a spatial pattern stemming from the crop distribution that implies a higher vector-borne zoonotic risk to humans.

Seasonality is relevant to the pathogen hazard and vector activity in this system, though. I found that summer is the season with a higher presence of LCMV (**Chapter 3.2**) and fleas (especially the synanthropic and generalist *N. fasciatus*; **Chapter 2.1**). They follow a typical seasonal pattern, with an increasing trend in spring, a peak during the summer, and a decline in autumn. These seasonal patterns occurred in LCMV (130) and in most flea species (131,132). By contrast, autumn was the season with heavier helminth infestation (**Chapter 4)** and *Bartonella* prevalence (**Chapter 3.1** and (97)). These patterns are consistent with accumulating parasite burdens after the reproductive period and population growth of *M. arvalis* (spring and summer), a pattern usually found in helminthological studies (133). Summer and autumn are the seasons with higher pathogen risk, coinciding with the highest abundance of *M. arvalis* and mouse hosts respectively (106). Summer is also the season with a higher exposure rate of humans in the region (through outdoor leisure activities or farming activities, in particular, crop harvesting). In terms of seasonality, flea-borne diseases and LCMV might thus be the most relevant emerging zoonoses and would require more surveillance. Effective prevention policies should consider the temporal dynamics of pathogens, vectors and hosts, as well as the different levels of exposure of the human population in each season.

## The role of host population dynamics in the circulation of pathogens

The distribution of pathogens within the host population is a key feature in the circulation and spillover of zoonoses, but the host population density is also crucial (24). Pathogens are highly influenced by host population dynamics. Host density can be either a negative or a positive factor influencing the transmission of a pathogen because it determines the availability of potential competent hosts (26) and the potential for disease amplification, spill over to other hosts and environmental contamination. Nevertheless, it depends on whether the host species is a suitable host for the vector and a competent

host transmitting the pathogen (134). I provide evidence that the circulation of microparasites throughout the ecosystem was affected by the population dynamics of *M. arvalis*. Even when disease prevalence is low (i.e. hantavirus and LCMV; **Chapter 3.2**), a very high host abundance means that there are many infected hosts that can amplify or transmit the disease, either directly or indirectly, via vectors or environmental contamination. *Bartonella* prevalence among *M. arvalis* (97) and the sympatric hosts (**Chapter 3.1**) are also higher during outbreaks of *M. arvalis*, as referred for *F. tularensis* infections in *M. arvalis* (98). And the circulation and possible spillover of flea-borne pathogens could be higher during those periods, based on a higher prevalence and abundance of fleas among the small mammals, specifically those flea species shared among de host community (**Chapter 3.1**). The transmission of pathogens with a direct infection route (such as viruses) or mediated by the host-shift of mobile vectors (such as fleas) requires either close contact between hosts or the use of the same habitats as the vector (135). Thus, the prevalence of pathogens will increase with the abundance of hosts because more cohabitating individuals facilitate the horizontal transmission (67) and the exchange of fleas (135). Density-dependent transmission patterns have been suggested for hantavirus (126,136), LCMV (130) and *Bartonella* (137) in similar systems with fluctuating voles. Multiple pathogens might circulate during *M. arvalis* population outbreaks and spillover from voles to co-habiting small mammals, and eventually to humans, may likely occur.

Rodents are optimal species for immature stages of ticks (all ticks collected and identified here were indeed immature stages; Ch**apter 2.2**) and are competent hosts for fleas (73,129). Consequently, the variation in the abundance of rodents will affect these vectors. I found that fleas were also more abundant and prevalent in the small mammal community during high-density *M. arvalis* periods – the increasing or peak phases of the vole cycle – (**Chapter 2.1**), so a greater circulation and spreading of flea-borne pathogens may occur during *M. arvalis* outbreaks. Since *Bartonella* is a pathogen vectored by fleas, this could contribute to the spillover transmission from voles to mice during the increase and peak phases of the vole cycle. Helminths and ticks were also more prevalent in voles during the crash phase of the vole cycle, suggesting that these could also play a role in the regulation of host populations (see below).

I suggest that the fluctuating population dynamics of *M. arvalis* affect both the dynamic of vectors and the circulation of pathogens. The presence of other competent host species may favor a dilution effect for ticks (**Chapter 2.2**) and *Bartonella* (**Chapter 3.1** and (97)), supporting the hypothesis that the risk of zoonosis emergence decreases when non-competent or dead-end hosts are present (138). Unless fluctuating dynamics of hosts are included in the disease system, crucial information in the understanding of pathogen dynamics would be missing to develop effective preventive recommendations.

## The role of pathogens on *Microtus arvalis* population dynamics

After a high-density (peak) phase, voles typically have a high prevalence of pathogens (**Chapter 3**) and carry greater flea burdens (**Chapter 2.1**) that negatively affect them. For example, some viruses are known to delay female maturation (139) and reduce survival rate (140). *Francisella tularensis*, whose virulence can vary among genotypes (141), also has negative effects on vole hosts, causing chronic effects (142) or death (143). Infestation by fleas increases the energy cost for maintenance concerning a non-parasitized host (144) and has been shown experimentally to reduce future reproductive success (145) and the life span of hosts (146). During the crash phase of the vole cycle, voles must face additional negative effects due to additional pathogen infestations. At this point, host sex should be also considered and I report different patterns of pathogen infections in males and females of *M. arvalis*. Females harbored more ticks (**Chapter 2.2**) and a higher intensity of helminths (**Chapter 4**), which could contribute to the reduction of fecundity during the crash phase of the vole cycle. Consistent with this idea, greater helminth abundance was associated with a reduction in vole litter size. Males seemed more relevant in terms of LCMV infection (**Chapter 3.2**), the prevalence of helminths (**Chapter 4**), and the circulation of pathogens transmitted by the flea *L. taschenbergi* (**Chapters 2.1**). Effects on females were apparently more severe than on males since LCMV causes a mild infection in voles with subclinical effects (49), and there is no apparent worsening of body condition due to helminths (**Chapter 4**). Nothing is known about the pathogens transmitted by the flea *L. taschenbergi*.

Parasitism is costly for hosts and represents a challenge for the individual energy trade-off between immune response against parasites and the investment in other biological processes such as reproduction or survival. The greater levels of infection found in voles after peak density are consistent with a regulatory role of diseases that could contribute to the maintenance of *M. arvalis* at low numbers, preventing the population from a rapid recovery after a population crash. However, this should be confirmed by experimental studies on the effects of specific pathogens on vole fitness (survival or reproduction).

## Preliminary exploration of co-infection patterns and future investigation lines

I would like to highlight the difficulty of this multi-parasitism work in a system where little previous surveillance effort has been made for the detection of zoonotic pathogens in the wildlife and human inhabitants. Thus, most emphasis has been put into identifying which zoonotic pathogens select

according to species previously detected in the system (97,108) or in similar *Microtus* vole systems (147–152).

For the study of pathogens at the community scale, we should ideally focus on the four small mammals studied, that co-occurred in time and space, and try to screen the same vectors and pathogens in each of these species. A total of 160 *M. arvalis*, 20 *A. sylvaticus*, 9 *C. russula,* and 19 *M. spretus* have been screened in this thesis for fleas, ticks, *F. tularensis*, *Bartonella* sp., hantavirus, LCMV and orthopoxvirus. Preliminary results show that half of the *M. arvalis* and *A. sylvaticus* were infected with at least one micropathogen, and the percentage reached 60% if helminths are considered in *M. arvalis* (unpublished data). The highest percentage of infected animals was found in *M. spretus*, reaching nearly 90% of the animals screened. Thus, this rodent species studied had an important role as a host for micropathogens. More than half of the *M. arvalis* (58.8%) harbored at least one vector when processed in the lab, although the percentage was lower in the sympatric small mammals (35.0% in *A. sylvaticus*, 33.3% in *C. russula*, and 5.3% in *M. spretus*). This high infestation rate by vectors in *M. arvalis*, though, represents a high potential risk of vector-borne zoonoses to humans. Community results improve the understanding of pathogen and vector preferences that likely contribute to the circulation and maintenance of zoonoses in the system. The results obtained in this thesis show new zoonotic risks in the region and support the hypothesis that rodents play a role as reservoirs and amplifiers of zoonoses in this farming ecosystem. Nevertheless, the small sample size in some screenings (especially for *C. russula*, *M. spretus* and in the preliminary screening of viruses) was a limitation for some analyses, so replication with larger samples should be carried out for more accurate and reliable results. The helminth community screening should be performed in the accompanying community of *M. arvalis* and could be complemented with an extraintestinal helminth survey. It would be interesting to screen for other groups of helminths that involve some zoonotic and veterinary species of interest, such as taeniid cestodes and *Echinococcus multilocularis* (104). Another aspect that needs to be addressed is the complete pathogen pool harbored by each type of vector and their role in their transmission. In this thesis, some associations between vectors and pathogens were reported but to confirm a vectoring role, it is necessary to demonstrate, by experiment, that certain flea and tick species can efficiently transmit specific pathogens to a competent host.

Once the pathogens and vectors are identified at the individual host level, co-infections and relationships between co-occurring pathogens can be further investigated. Preliminary results show that co-infections of pathogens are especially frequent in *M. spretus* (42.1%), but also relevant in *M. arvalis* (17.5%). In *M. arvalis*, I found that up to three pathogen species from the three different groups can coexist (i.e., bacterium, virus and helminth); *C. russula* can harbor up to four species from the two different groups screened (i.e., bacterium and virus); *M. spretus*, up to four species, but within the

same group of pathogens (i.e. *Bartonella* sp.); and two species from the same group of pathogens (i.e. *Bartonella* sp.) can be found in *A. sylvaticus* at a time (unpublished data). Note that the survey of GI helminths was only performed in *M. arvalis*, so the number of co-infecting pathogens could be different in the sympatric species. The next step would be to investigate the patterns of co-infection and the relationship between the different pathogens. Relationships between pathogens can be negative through competition or positive via facilitation, modulating the prevalence and abundance of the rest of the pathogens infecting the host (38,153–155). Hence, co-infections control the circulation of pathogens and the progression of diseases at a population and community levels too. Co-infection patterns and mechanisms (competition or facilitation) deserve further studies.

# CONCLUSIONS

1. The "*Francisella–Microtus*" case study showed how agricultural intensification and irrigation changed the distribution of a key host with cyclic dynamics and led to the emergence of the zoonotic disease, tularemia. It also illustrated how abundance fluctuations are key to understand disease spillover and transmission, and highlighted that a more integral, community-based disease knowledge will help to better understand the dynamics of disease circulation. A better understanding of the pathogen pool circulating in the wild fauna and environment will contribute to the reduction of the negative impacts of tularemia epidemics in the region.

2. The small mammal guild of NW Spain (*A. sylvaticus*, *C. russula*, *M. arvalis* and *M. spretus*) shared one species of tick (*R. turanicus*) and three species of flea (*C. apertus*, *N. fasciatus* and *L. taschenbergi*) that are potential pathogen vectors. Vectoring roles should be further investigated, combined with climate effects and the recurrent vole outbreaks, because these ectoparasites could circulate concerning pathogens.

3. The fluctuating vole population dynamics affect both the dynamic of vectors and the circulation of micropathogens. They act as amplifiers of pathogens throughout the ecosystem, increasing the zoonotic risk for humans that share the habitat with them, especially in summer and during vole outbreaks.

4. This thesis unraveled the occurrence of many pathogens in the small mammal guild: F. tularensis, eleven *Bartonella* species, three types of viruses and eight different helminth taxa. Among these, *F. tularensis*, five species of *Bartonella*, the three viruses, and, possibly, one species of helminths represent zoonotic risks.

5. *Francisella tularensis* was almost absent from the surveyed small mammal community that lives in sympatry with *M. arvalis* suggesting that, unlike voles, they are unlikely to play an important role in the circulation and maintenance of this disease.

6. During periods of high abundance (population peaks), *M. arvalis* represent a spillover risk to sympatric mice. Furthermore, fleas were also more abundant on the guild during vole peak periods, enhancing a possible vectoring role of these ectoparasites and the circulation of flea-borne pathogens.

7. Common voles were frequently infected by helminths, in particular *Syphacia* sp. The level of parasite aggregation delayed response of worm burdens to changes in vole abundance, and a potential negative impact of helminths on vole reproduction (reduced litter size) are consistent with a regulatory role for helminths in vole population dynamics.

# CONCLUSIONES

1. El caso de estudio "*Francisella-Microtus*" mostró cómo la intensificación de la agricultura y la irrigación cambiaron la distribución de un huésped clave con una dinámica cíclica y condujo a la aparición de una enfermedad zoonótica, la tularemia. También ilustró cómo las fluctuaciones en la abundancia son clave para entender la expansión y la transmisión de la enfermedad, y puso de relieve que un conocimiento más integral de la enfermedad, basado en la comunidad, ayudará a comprender mejor la dinámica de la circulación de esta enfermedad. Una mejor comprensión del conjunto de patógenos que circulan en la fauna silvestre y el medio ambiente contribuirá a reducir los impactos negativos de las epidemias de tularemia en la región.

2. El gremio de pequeños mamíferos del noroeste de España (*A. sylvaticus*, *C. russula*, *M. arvalis* y *M. spretus*) comparte una especie de garrapata (*R. turanicus*) y tres especies de pulga (*C. apertus*, *N. fasciatus* y *L. taschenbergi*) que son potenciales vectores de patógenos. El papel de los vectores debe investigarse más a fondo, en combinación con los efectos del clima y los brotes recurrentes de topillos, porque estos ectoparásitos podrían estar implicados en la circulación de patógenos de interés en salud pública.

3. La dinámica fluctuante de las poblaciones de topillos afecta tanto a la dinámica de los vectores como a la circulación de los micropatógenos. Actúan como amplificadores de patógenos en todo el ecosistema, aumentando el riesgo zoonótico para los humanos que comparten el hábitat con ellos, especialmente en verano y durante los brotes de topillos.

4. Esta tesis ha desvelado la presencia de muchos patógenos en el gremio de los pequeños mamíferos: *F. tularensis*, once especies de *Bartonella*, tres tipos de virus y ocho taxones de helmintos diferentes. Entre ellos, *F. tularensis*, cinco especies de *Bartonella*, los tres virus estudiados y, posiblemente, una especie de helmintos representan riesgo zoonótico.

5. La casi total ausencia de *Francisella tularensis* en la comunidad de pequeños mamíferos analizados que viven en simpatría con *M. arvalis* sugiere que, a diferencia de los topillos, es poco probable que aqullos desempeñen un papel importante en la circulación y el mantenimiento de esta enfermedad.

6. Durante los periodos de alta abundancia (picos de población), *M. arvalis* representa un riesgo de propagación del patógenos para los ratones simpátricos. Las pulgas también fueron más

abundantes en el gremio durante los periodos de pico de población de topillos, potenciando un posible papel vectorial de estos ectoparásitos y la circulación de patógenos transmitidos por pulgas.

7. Los topillos estaban frecuentemente infectados por helmintos, en particular *Syphacia* sp. El nivel de agregación, la respuesta diferida de la carga parasitaria respecto a los cambios en la abundancia de topillos, y el potencial impacto negativo de los helmintos en la reproducción de los topillos (reducción del tamaño de las camadas) son congruentes con un posible papel regulador de los helmintos en la dinámica de las poblaciones de topillos.

# References


1. World Health Organization. *A brief guide to emerging infectious diseases and zoonoses*. New Delhi: WHO Regional Office for South-East Asia (2014). Available at: https://apps.who.int/iris/bitstream/handle/10665/204722/B5123.pdf?sequence=1&isAllowed=y [Accessed February 14, 2022]
2. Morens DM, Folkers GK, Fauci AS. The challenge of emerging and re-emerging infectious diseases. *Nature* (2004) **430**:242–249. doi:10.1038/nature02759
3. Binder S, Levitt AM, Sacks JJ, Hughes JM. Emerging infectious diseases: public health issues for the 21st century. *Science* (1999) **284**:1311–1313. doi:10.1126/science.284.5418.1311
4. McCloskey B, Dar O, Zumla A, Heymann DL. Emerging infectious diseases and pandemic potential: status quo and reducing risk of global spread. *Lancet Infect Dis* (2014) **14**:1001–1010. doi:10.1016/S1473-3099(14)70846-1
5. Pike J, Bogich T, Elwood S, Finnoff DC, Daszak P. Economic optimization of a global strategy to address the pandemic threat. *Proc Natl Acad Sci U S A* (2014) **111**:18519–23. doi:10.1073/pnas.1412661112
6. World Health Organization. The true death toll of COVID-19: estimating global excess mortality. (2020) Available at: https://www.who.int/data/stories/the-true-death-toll-of-covid-19-estimating-global-excess-mortality [Accessed January 25, 2022]
7. The Institut National d'Etudes Démographiques. Population, births and deaths in Europe and other developed OECD countries. (2020) Available at: https://www.ined.fr/en/everything_about_population/data/europe-developed-countries/population-births-deaths/ [Accessed January 25, 2022]
8. Cutler DM, Summers LH. The COVID-19 pandemic and the $16 trillion virus. *JAMA* (2020) **324**:1495. doi:10.1001/jama.2020.19759
9. McKibbin WJ, Fernando R. Global macroeconomic scenarios of the COVID-19 pandemic. *SSRN Electron J* (2020) **62**: doi:10.2139/ssrn.3635103
10. Kruse H, Kirkemo A-M, Handeland K. Wildlife as source of zoonotic infections. *Emerg Infect Dis* (2004) **10**:2067–2072. doi:10.3201/eid1012.040707
11. Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, Daszak P. Global trends in emerging infectious diseases. *Nature* (2008) **451**:990–993. doi:10.1038/nature06536
12. Taylor LH, Latham SM, Woolhouse MEJ. Risk factors for human disease emergence. *Philos Trans R Soc London Ser B Biol Sci* (2001) **356**:983–989. doi:10.1098/rstb.2001.0888
13. Bonita R, Beaglehole R, Kjellström T. Basic Epidemiology. *World Heal Organ* (2006)212. Available at: https://apps.who.int/iris/bitstream/handle/10665/43541/9241547073_eng.pdf;sequence=1 [Accessed February 14, 2022]
14. Daszak P, Cunningham AA, Hyatt AD. Emerging infectious diseases of wildlife- threats to biodiversity and human health. *Science* (2000) **287**:443–449. doi:10.1126/science.287.5452.443
15. Karesh WB, Dobson A, Lloyd-Smith JO, Lubroth J, Dixon MA, Bennett M, Aldrich S, Harrington T, Formenty P, Loh EH, et al. Ecology of zoonoses: natural and unnatural histories. *Lancet*

(2012) **380**:1936–1945. doi:10.1016/S0140-6736(12)61678-X

16. Woolhouse MEJ, Haydon DT, Antia R. Emerging pathogens: the epidemiology and evolution of species jumps. *Trends Ecol Evol* (2005) **20**:238–244. doi:10.1016/j.tree.2005.02.009

17. McMahon BJ, Morand S, Gray JS. Ecosystem change and zoonoses in the Anthropocene. *Zoonoses Public Health* (2018) **65**:755–765. doi:10.1111/zph.12489

18. Jánová E. Emerging and threatening vector-borne zoonoses in the world and in Europe: a brief update. *Pathog Glob Health* (2019) **113**:49–57. doi:10.1080/20477724.2019.1598127

19. Jones BA, Grace D, Kock R, Alonso S, Rushton J, Said MY, McKeever D, Mutua F, Young J, McDermott J, et al. Zoonosis emergence linked to agricultural intensification and environmental change. *Proc Natl Acad Sci* (2013) **110**:8399–8404. doi:10.1073/pnas.1208059110

20. Rohr JR, Barrett CB, Civitello DJ, Craft ME, Delius B, DeLeo GA, Hudson PJ, Jouanard N, Nguyen KH, Ostfeld RS, et al. Emerging human infectious diseases and the links to global food production. *Nat Sustain* (2019) **2**:445–456. doi:10.1038/s41893-019-0293-3

21. Wolfe ND, Dunavan CP, Diamond J. Origins of major human infectious diseases. *Nature* (2007) **447**:279–283. doi:10.1038/nature05775

22. Hassell JM, Begon M, Ward MJ, Fèvre EM. Urbanization and disease emergence: dynamics at the wildlife–livestock–human interface. *Trends Ecol Evol* (2017) **32**:55–67. doi:10.1016/j.tree.2016.09.012

23. Kołodziej-Sobocińska M. Factors affecting the spread of parasites in populations of wild European terrestrial mammals. *Mammal Res* (2019) **64**:301–318. doi:10.1007/s13364-019-00423-8

24. Plowright RK, Parrish CR, McCallum HI, Hudson PJ, Ko AI, Graham AL, Lloyd-Smith JO. Pathways to zoonotic spillover. *Nat Rev Microbiol* (2017) **15**:502–510. doi:10.1038/nrmicro.2017.45

25. Hassell JM, Newbold T, Dobson AP, Linton Y-M, Franklinos LH V., Zimmerman D, Pagenkopp Lohan KM. Towards an ecosystem model of infectious disease. *Nat Ecol Evol* (2021) **5**:907–918. doi:10.1038/s41559-021-01454-8

26. Tompkins DM, Dunn AM, Smith MJ, Telfer S. Wildlife diseases: from individuals to ecosystems. *J Anim Ecol* (2011) **80**:19–38. doi:10.1111/j.1365-2656.2010.01742.x

27. Johnson PTJ, de Roode JC, Fenton A. Why infectious disease research needs community ecology. *Science (80- )* (2015) **349**: doi:10.1126/science.1259504

28. Lloyd-Smith JO, George D, Pepin KM, Pitzer VE, Pulliam JRC, Dobson AP, Hudson PJ, Grenfell BT. Epidemic dynamics at the human-animal interface. *Science* (2009) **326**:1362–1367. doi:10.1126/science.1177345

29. Allen T, Murray KA, Zambrana-Torrelio C, Morse SS, Rondinini C, Di Marco M, Breit N, Olival KJ, Daszak P. Global hotspots and correlates of emerging zoonotic diseases. *Nat Commun* (2017) **8**:1124. doi:10.1038/s41467-017-00923-8

30. Morens DM, Fauci AS. Emerging infectious diseases: threats to human health and global stability. *PLoS Pathog* (2013) **9**:e1003467. doi:10.1371/journal.ppat.1003467

31. de Wit E, van Doremalen N, Falzarano D, Munster VJ. SARS and MERS: recent insights into emerging coronaviruses. *Nat Rev Microbiol* (2016) **14**:523–534. doi:10.1038/nrmicro.2016.81

32. Coltart CEM, Lindsey B, Ghinai I, Johnson AM, Heymann DL. The Ebola outbreak, 2013–2016: old lessons for new epidemics. *Philos Trans R Soc B Biol Sci* (2017) **372**:20160297.

doi:10.1098/rstb.2016.0297

33. Maxmen A. Has COVID taught us anything about pandemic preparedness? *Nature* (2021) **596**:332–335. doi:10.1038/d41586-021-02217-y

34. Anderson RM, May RM. Population biology of infectious diseases: Part I. *Nature* (1979) **280**:361–367.

35. May RM, Anderson RM. Population biology of infectious diseases: Part II. *Nature* (1979) **280**:455–461.

36. Cox FEG. Concomitant infections, parasites and immune responses. *Parasitology* (2001) **122**:S23–S38. doi:10.1017/S003118200001698X

37. Woolhouse MEJ. Population biology of multihost pathogens. *Science* (2001) **292**:1109–1112. doi:10.1126/science.1059026

38. Telfer S, Lambin X, Birtles RJ, Beldomenico PM, Burthe SJ, Paterson S, Begon M. Species interactions in a parasite community drive infection risk in a wildlife population. *Science* (2010) **330**:243–246. doi:10.1126/science.1190333

39. Vaumourin E, Vourc'h G, Gasqui P, Vayssier-Taussat M. The importance of multiparasitism: examining the consequences of co-infections for human and animal health. *Parasit Vectors* (2015) **8**:545. doi:10.1186/s13071-015-1167-9

40. Power AG, Mitchell CE. Pathogen spillover in disease epidemics. *Am Nat* (2004) **164**:S79–S89. doi:10.1086/424610

41. One Health Commission. What is One Health? Available at: https://www.onehealthcommission.org/en/why_one_health/what_is_one_health/ [Accessed March 11, 2022]

42. Seabloom EW, Borer ET, Gross K, Kendig AE, Lacroix C, Mitchell CE, Mordecai EA, Power AG. The community ecology of pathogens: coinfection, coexistence and community composition. *Ecol Lett* (2015) **18**:401–415. doi:10.1111/ele.12418

43. Thompson RCA. Parasite zoonoses and wildlife: One health, spillover and human activity. *Int J Parasitol* (2013) **43**:1079–1088. doi:10.1016/j.ijpara.2013.06.007

44. Pilosof S, Morand S, Krasnov BR, Nunn CL. Potential parasite transmission in multi-host networks based on parasite sharing. *PLoS One* (2015) **10**:e0117909. doi:10.1371/journal.pone.0117909

45. Holmes EC, Rambaut A, Andersen KG. Pandemics: spend on surveillance, not prediction. *Nature* (2018) **558**:180–182. doi:10.1038/d41586-018-05373-w

46. Carroll D, Daszak P, Wolfe ND, Gao GF, Morel CM, Morzaria S, Pablos-Méndez A, Tomori O, Mazet JAK. The Global Virome Project. *Science* (2018) **359**:872–874. doi:10.1126/science.aap7463

47. Morse SS, Mazet JAK, Woolhouse M, Parrish CR, Carroll D, Karesh WB, Zambrana-Torrelio C, Lipkin I, Daszak P. Prediction and prevention of the next pandemic zoonosis. *Lancet* (2012) **380**:1956–1965.

48. Kaplan B, Kahn LH, Monath TP, Woodall J. "ONE HEALTH" and parasitology. *Parasit Vectors* (2009) **2**:36. doi:10.1186/1756-3305-2-36

49. Meerburg BG, Singleton GR, Kijlstra A. Rodent-borne diseases and their risks for public health. *Crit Rev Microbiol* (2009) **35**:221–270. doi:10.1080/10408410902989837

50. Dickman C. "Rodent-ecosystem relationships: a review," in *Ecologically-based Rodent*

*Management*, eds. Grant R Singleton, L. A. Hinds, H. Leirs, Z. Zhang. Canberra: Australian Centre for International Agricultural Research (1999) 113–133.

51. Delibes-Mateos M, Smith AT, Slobodchikoff CN, Swenson JE. The paradox of keystone species persecuted as pests: A call for the conservation of abundant small mammals in their native range. *Biol Conserv* (2011) **144**:1335–1346. doi:10.1016/j.biocon.2011.02.012

52. Hanski I, Hansson L, Henttonen H. Specialist predators, generalist predators, and the Microtine rodent cycle. *J Anim Ecol* (1991) doi:10.2307/5465

53. Yoshihara Y, Okuro T, Undarmaa J, Sasaki T, Takeuchi K. Are small rodents key promoters of ecosystem restoration in harsh environments? A case study of abandoned croplands on Mongolian grasslands. *J Arid Environ* (2009) **73**:364–368. doi:10.1016/j.jaridenv.2008.09.017

54. Haim A, Izhaki I. Changes in rodent community during recovery from fire: relevance to conservation. *Biodivers Conserv* (1994) doi:10.1007/BF00114202

55. Borchert M, Johnson M, Schreiner DS, Vander Wall SB. Early postfire seed dispersal, seedling establishment and seedling mortality of *Pinus coulteri* (D. Don) in central coastal California, USA. *Plant Ecol* (2003) **168**:207-220. doi:10.1023/A:1024447811238

56. Jahan NA, Lindsey LL, Larsen PA. The role of peridomestic rodents as reservoirs for zoonotic foodborne pathogens. *Vector-Borne Zoonotic Dis* (2021) **21**:133–148. doi:10.1089/vbz.2020.2640

57. Stenseth NC, Leirs H, Skonhoft A, Davis SA, Pech RP, Andreassen HP, Singleton GR, Lima M, Machang'u RS, Makundi RH, et al. Mice, rats, and people: the bio-economics of agricultural rodent pests. *Front Ecol Environ* (2003) **1**:367. doi:10.2307/3868189

58. Han BA, Kramer AM, Drake JM. Global patterns of zoonotic disease in mammals. *Trends Parasitol* (2016) **32**:565–577. doi:10.1016/j.pt.2016.04.007

59. Han BA, Schmidt JP, Bowden SE, Drake JM. Rodent reservoirs of future zoonotic diseases. *Proc Natl Acad Sci* (2015) **112**:7039–7044. doi:10.1073/pnas.1501598112

60. Mills JN, Childs JE. Ecologic studies of rodent reservoirs: their relevance for human health. *Emerg Infect Dis* (1998) **4**:529–537. doi:10.3201/eid0404.980403

61. Himsworth CG, Parsons KL, Jardine C, Patrick DM. Rats, cities, people, and pathogens: A systematic review and narrative synthesis of literature regarding the ecology of rat-associated zoonoses in urban centers. *Vector-Borne Zoonotic Dis* (2013) **13**:349–359. doi:10.1089/vbz.2012.1195

62. Hornok S, Földvári G, Rigó K, Meli ML, Gönczi E, Répási A, Farkas R, Papp I, Kontschán J, Hofmann-Lehmann R. Synanthropic rodents and their ectoparasites as carriers of a novel haemoplasma and vector-borne, zoonotic pathogens indoors. *Parasites and Vectors* (2015) **8**:1–6. doi:10.1186/s13071-014-0630-3

63. Mendoza H, Rubio A V., García-Peña GE, Suzán G, Simonetti JA. Does land-use change increase the abundance of zoonotic reservoirs? Rodents say yes. *Eur J Wildl Res* (2020) **66**:1–5. doi:10.1007/S10344-019-1344-9/FIGURES/1

64. Bordes F, Blasdell K, Morand S. Transmission ecology of rodent-borne diseases: New frontiers. *Integr Zool* (2015) **10**:424–435. doi:10.1111/1749-4877.12149

65. Lõhmus M, Janse I, van de Goot F, van Rotterdam BJ. Rodents as potential couriers for bioterrorism agents. *Biosecurity Bioterrorism Biodefense Strateg Pract Sci* (2013) **11**:S247–S257. doi:10.1089/bsp.2012.0085

66. Ostfeld RS, Mills JN. “Social behavior, demography, and rodent-borne pathogens,” in *Rodent Societies: An ecological and evolutionary perspective*, eds. J. O. Wolff, P. W. Sherman. Chicago: Chicago University Press (2007) 478–486. doi:10.7208/9780226905389-043

67. Davis SA, Calvet E, Leirs H. Fluctuating rodent populations and risk to humans from rodent-borne zoonoses. *Vector-Borne Zoonotic Dis* (2005) **5**:305–314. doi:10.1089/vbz.2005.5.305

68. Gibb R, Redding DW, Chin KQ, Donnelly CA, Blackburn TM, Newbold T, Jones KE. Zoonotic host diversity increases in human-dominated ecosystems. *Nature* (2020) **584**:398–402. doi:10.1038/s41586-020-2562-8

69. Singleton GR, Belmain SR, Brown PR, Aplin K, Htwe NM. Impacts of rodent outbreaks on food security in Asia. *Wildl Res* (2010) **37**:355. doi:10.1071/WR10084

70. Diagne C, Galan M, Tamisier L, D’Ambrosio J, Dalecky A, Bâ K, Kane M, Niang Y, Diallo M, Sow A, et al. Ecological and sanitary impacts of bacterial communities associated to biological invasions in African commensal rodent communities. *Sci Rep* (2017) **7**:14995. doi:10.1038/s41598-017-14880-1

71. Morand S, Bordes F, Chen H-W, Claude J, Cosson J-F, Galan M, Czirják GÁ, Greenwood AD, Latinne A, Michaux J, et al. Global parasite and *Rattus* rodent invasions: The consequences for rodent-borne diseases. *Integr Zool* (2015) **10**:409–423. doi:10.1111/1749-4877.12143

72. Meerburg BG. Rodents are a risk factor for the spreading of pathogens on farms. *Vet Microbiol* (2010) **142**:464–465. doi:10.1016/j.vetmic.2009.06.038

73. Bitam I, Dittmar K, Parola P, Whiting MF, Raoult D. Fleas and flea-borne diseases. *Int J Infect Dis* (2010) **14**:e667–e676. doi:10.1016/j.ijid.2009.11.011

74. Swei A, Couper LI, Coffey LL, Kapan D, Bennett S. Patterns, drivers, and challenges of vector-borne disease emergence. *Vector-Borne Zoonotic Dis* (2020) **20**:159–170. doi:10.1089/vbz.2018.2432

75. Vorou RM, Papavassiliou VG, Tsiodras S. Emerging zoonoses and vector-borne infections affecting humans in Europe. *Epidemiol Infect* (2007) **135**:1231–1247. doi:10.1017/S0950268807008527

76. Colwell DD, Dantas-Torres F, Otranto D. Vector-borne parasitic zoonoses: Emerging scenarios and new perspectives. *Vet Parasitol* (2011) **182**:14–21. doi:10.1016/j.vetpar.2011.07.012

77. Lambin EF, Tran A, Vanwambeke SO, Linard C, Soti V. Pathogenic landscapes: Interactions between land, people, disease vectors, and their animal hosts. *Int J Health Geogr* (2010) **9**:54. doi:10.1186/1476-072X-9-54

78. Cator LJ, Johnson LR, Mordecai EA, El Moustaid F, Smallwood TRC, LaDeau SL, Johansson MA, Hudson PJ, Boots M, Thomas MB, et al. The role of vector trait variation in vector-borne disease dynamics. *Front Ecol Evol* (2020) **8**:189. doi:10.3389/fevo.2020.00189

79. Semenza JC, Suk JE. Vector-borne diseases and climate change: a European perspective. *FEMS Microbiol Lett* (2018) **365**:fnx244. doi:10.1093/femsle/fnx244

80. Rocklöv J, Dubrow R. Climate change: an enduring challenge for vector-borne disease prevention and control. *Nat Immunol* (2020) **21**:479–483. doi:10.1038/s41590-020-0648-y

81. Gratz N. *Vector-and rodent-borne diseases in Europe and North America: distribution, public health burden, and control*. Geneva: World Health Organization (2006).

82. Kilpatrick AM, Randolph SE. Drivers, dynamics, and control of emerging vector-borne zoonotic diseases. *Lancet* (2012) **380**:1946–1955. doi:10.1016/S0140-6736(12)61151-9

83. Ostfeld RS, Keesing F. The function of biodiversity in the ecology of vector-borne zoonotic diseases. *Can J Zool* (2000) doi:10.1139/cjz-78-12-2061

84. Radolf JD, Caimano MJ, Stevenson B, Hu LT. Of ticks, mice and men: understanding the dual-host lifestyle of Lyme disease spirochaetes. *Nat Rev Microbiol* (2012) **10**:87–99. doi:10.1038/nrmicro2714

85. Stenseth NC, Atshabar BB, Begon M, Belmain SR, Bertherat E, Carniel E, Gage KL, Leirs H, Rahalison L. Plague: past, present, and future. *PLoS Med* (2008) **5**:e3. doi:10.1371/journal.pmed.0050003

86. Stenseth NC, Samia NI, Viljugrein H, Kausrud KL, Begon M, Davis SA, Leirs H, Dubyanskiy VM, Esper J, Ageyev VS, et al. Plague dynamics are driven by climate variation. *Proc Natl Acad Sci* (2006) **103**:13110–13115. doi:10.1073/pnas.0602447103

87. Kilpatrick AM. Globalization, land use, and the invasion of West Nile virus. *Science* (2011) **334**:323–327. doi:10.1126/science.1201010

88. Centers for Disease Control and Prevention. Final cumulative maps and data, West Nile virus. (2021). Available at: https://www.cdc.gov/westnile/statsmaps/cumMapsData.html#two [Accessed March 11, 2022]

89. Sejvar JJ. West Nile virus infection. *Microbiol Spectr* (2016) **4**: doi:10.1128/microbiolspec.EI10-0021-2016

90. Moutailler S, Valiente Moro C, Vaumourin E, Michelet L, Tran FH, Devillers E, Cosson J-F, Gasqui P, Van VT, Mavingui P, et al. Co-infection of ticks: the rule rather than the exception. *PLoS Negl Trop Dis* (2016) **10**:e0004539. doi:10.1371/journal.pntd.0004539

91. Randolph SE, Gern L, Nuttall PA. Co-feeding ticks: Epidemiological significance for tick-borne pathogen transmission. *Parasitol Today* (1996) **12**:472–479. doi:10.1016/S0169-4758(96)10072-7

92. Instituto Nacional de Estadística. Estadísticas territoriales: agricultura y medio ambiente. (2016) Available at: https://ine.es/dyngs/INEbase/es/categoria.htm?c=Estadistica_P&cid=1254735570567 [Accessed March 12, 2022]

93. Nafría García DA, Garrido del Pozo N, Álvarez Arias MV, Cubero Jiménez D, Fernández Sánchez M, Villarino Barrera I, Gutiérrez García A, Abia Llera I. *Atlas agroclimático - Castilla y León*. Agencia Estatal de Meteorología ; Instituto Tecnológico Agrario de Castilla y León (2013). doi:10.31978/281-13-008-5

94. Rodríguez-Pastor R, Luque-Larena JJ, Lambin X, Mougeot F. "Living on the edge": The role of field margins for common vole (*Microtus arvalis*) populations in recently colonised Mediterranean farmland. *Agric Ecosyst Environ* (2016) **231**:206–217. doi:10.1016/j.agee.2016.06.041

95. Luque-Larena JJ, Mougeot F, Viñuela J, Jareño D, Arroyo L, Lambin X, Arroyo B. Recent large-scale range expansion and outbreaks of the common vole (*Microtus arvalis*) in NW Spain. *Basic Appl Ecol* (2013) **14**:432–441. doi:10.1016/j.baae.2013.04.006

96. Luque-Larena JJ, Mougeot F, Arroyo B, Vidal MD, Rodríguez-Pastor R, Escudero R, Anda P, Lambin X. Irruptive mammal host populations shape tularemia epidemiology. *PLOS Pathog* (2017) **13**:e1006622. doi:10.1371/journal.ppat.1006622

97. Rodríguez-Pastor R, Escudero R, Lambin X, Vidal MD, Gil H, Jado I, Rodríguez-Vargas M, Luque-Larena JJ, Mougeot F. Zoonotic pathogens in fluctuating common vole (*Microtus*

*arvalis*) populations: occurrence and dynamics. *Parasitology* (2019) **146**:389–398. doi:10.1017/S0031182018001543

98. Rodríguez-Pastor R, Escudero R, Vidal MD, Mougeot F, Arroyo B, Lambin X, Vila-Coro AM, Rodríguez-Moreno I, Anda P, Luque-Larena JJ. Density-dependent prevalence of *Francisella tularensis* in fluctuating vole populations, northwestern Spain. *Emerg Infect Dis* (2017) **23**:1377–1379. doi:10.3201/eid2308.161194

99. González-Barrio D, Jado I, Viñuela J, García JT, Olea PP, Arce F, Ruiz-Fons F. Investigating the role of micromammals in the ecology of *Coxiella burnetii* in Spain. *Animals* (2021) **11**:654. doi:10.3390/ani11030654

100. Jeske K, Emirhar D, García JT, González-Barrio D, Olea PP, Fons FR, Schulz J, Mayer-Scholl A, Heckel G, Ulrich RG. Frequent *Leptospira* spp. detection but absence of tula orthohantavirus in *Microtus* spp. voles, northwestern Spain. *J Wildl Dis* (2021) **57**: doi:10.7589/JWD-D-20-00109

101. Schmidt S, Essbauer SS, Mayer-Scholl A, Poppert S, Schmidt-Chanasit J, Klempa B, Henning K, Schares G, Groschup MH, Spitzenberger F, et al. Multiple infections of rodents with zoonotic pathogens in Austria. *Vector-Borne Zoonotic Dis* (2014) **14**:467–475. doi:10.1089/vbz.2013.1504

102. Kallio-Kokko H, Uzcategui N, Vapalahti O, Vaheri A. Viral zoonoses in Europe. *FEMS Microbiol Rev* (2005) **29**:1051–1077. doi:10.1016/j.femsre.2005.04.012

103. Pawelczyk A, Bajer A, Behnke JM, Gilbert FS, Sinski E. Factors affecting the component community structure of haemoparasites in common voles (*Microtus arvalis*) from the Mazury Lake District region of Poland. *Parasitol Res* (2004) **92**:270–284. doi:10.1007/s00436-003-1040-1

104. Führer H-P, Schneider R, Walochnik J, Auer H. Extraintestinal helminths of the common vole (*Microtus arvalis*) and the water vole (*Arvicola terrestris*) in western Austria (Vorarlberg). *Parasitol Res* (2010) **106**:1001–1004. doi:10.1007/s00436-010-1753-x

105. Guerra D, Hegglin D, Bacciarini L, Schnyder M, Deplazes P. Stability of the southern European border of *Echinococcus multilocularis* in the Alps: evidence that *Microtus arvalis* is a limiting factor. *Parasitology* (2014) **141**:1593–1602. doi:10.1017/S0031182014000730

106. Mougeot F, Lambin X, Rodríguez-Pastor R, Romairone J, Luque-Larena JJ. Numerical response of a mammalian specialist predator to multiple prey dynamics in Mediterranean farmlands. *Ecology* (2019) **100**:e02776. doi:10.1002/ecy.2776

107. Roos D, Caminero-Saldaña C, Elston D, Mougeot F, García-Ariza M, Arroyo B, Luque-Larena JJ, Revilla FR, Lambin X. From pattern to process? Dual travelling waves, with contrasting propagation speeds, best describe a self-organised spatio-temporal pattern in population growth of a cyclic rodent. *Ecol Lett* (under review). doi:10.22541/AU.164302552.25540752/V1

108. Rodríguez-Pastor R, Mougeot F, Vidal MD, Jado I, González-Martín-Niño RM, Escudero R, Luque-Larena JJ. Zoonotic bacteria in fleas parasitizing common voles, northwestern Spain. *Emerg Infect Dis* (2019) **25**:1423–1425. doi:10.3201/eid2507.181646

109. Rossow H, Sissonen S, Koskela KA, Kinnunen PM, Hemmilä H, Niemimaa J, Huitu O, Kuusi M, Vapalahti O, Henttonen H, et al. Detection of *Francisella tularensis* in voles in Finland. *Vector-Borne Zoonotic Dis* (2014) **14**:193–198. doi:10.1089/vbz.2012.1255

110. Tokarz R, Markotić A, Margaletić J, Lipkin WI, Habuš J, Jain K, Turk N, Svoboda P, Vucelja M, Desai A, et al. Molecular survey of zoonotic agents in rodents and other small mammals in Croatia. *Am J Trop Med Hyg* (2016) **94**:466–473. doi:10.4269/ajtmh.15-0517

111. Gyuranecz M, Reiczigel J, Krisztalovics K, Monse L, Szabóné GK, Szilágyi A, Szépe B, Makrai L, Magyar T, Bhide M, et al. Factors influencing emergence of tularemia, Hungary, 1984–2010. *Emerg Infect Dis* (2012) **18**:1379–1381. doi:10.3201/eid1808.111826

112. Kaysser P, Seibold E, Mätz-Rensing K, Pfeffer M, Essbauer S, Splettstoesser WD. Re-emergence of tularemia in Germany: presence of *Francisella tularensis* in different rodent species in endemic areas. *BMC Infect Dis* (2008) **8**:157. doi:10.1186/1471-2334-8-157

113. Broman T, Thelaus J, Andersson AC, Bäckman S, Wikström P, Larsson E, Granberg M, Karlsson L, Bäck E, Eliasson H, et al. Molecular detection of persistent *Francisella tularensis* subspecies *holarctica* in natural waters. *Int J Microbiol* (2011) **2011**:1–10. doi:10.1155/2011/851946

114. Kilic S, Birdsell DN, Karagöz A, Çelebi B, Bakkaloglu Z, Arikan M, Sahl JW, Mitchell C, Rivera A, Maltinsky S, et al. Water as source of *Francisella tularensis* infection in humans, Turkey. *Emerg Infect Dis* (2015) **21**:2213–2216. doi:10.3201/eid2112.150634

115. Rydén P, Björk R, Schäfer ML, Lundström JO, Petersén B, Lindblom A, Forsman M, Sjöstedt A, Johansson A. Outbreaks of tularemia in a boreal forest region depends on mosquito prevalence. *J Infect Dis* (2012) **205**:297–304. doi:10.1093/infdis/jir732

116. Anda P, Segura del Pozo J, García JMD, Escudero R, Peña FJG, Velasco MCL, Sellek RE, Chillarón MRJ, Serrano LPS, Navarro JFM. Waterborne outbreak of tularemia associated with crayfish fishing. *Emerg Infect Dis* (2001) **7**:575–582. doi:10.3201/eid0707.017740

117. Heroldová M, Bryja J, Zejda J, Tkadlec E. Structure and diversity of small mammal communities in agriculture landscape. *Agric Ecosyst Environ* (2007) **120**:206–210. doi:10.1016/j.agee.2006.09.007

118. Jacob J, Manson P, Barfknecht R, Fredricks T. Common vole (*Microtus arvalis*) ecology and management: implications for risk assessment of plant protection products. *Pest Manag Sci* (2014) **70**:869–878. doi:10.1002/ps.3695

119. Lantová P, Lanta V. Food selection in *Microtus arvalis*: the role of plant functional traits. *Ecol Res* (2009) **24**:831–838. doi:10.1007/s11284-008-0556-3

120. Luque-Larena JJ, Mougeot F, Vidal Roig D, Lambin X, Rodríguez-Pastor R, Rodríguez-Valín E, Anda P, Escudero R. Tularemia outbreaks and common vole (*Microtus arvalis*) irruptive population dynamics in Northwestern Spain, 1997–2014. *Vector-Borne Zoonotic Dis* (2015) **15**:568–570. doi:10.1089/vbz.2015.1770

121. Rodríguez Ferri E-F. *Tularemia: una aproximación a su estudio integral en Castilla y León*. León: Academia Ciencias Veterinarias de Castilla y León (2017).

122. Gutiérrez R, Krasnov BR, Morick D, Gottlieb Y, Khokhlova IS, Harrus S. *Bartonella* infection in rodents and their flea ectoparasites: an overview. *Vector-Borne Zoonotic Dis* (2015) **15**:27–39. doi:10.1089/vbz.2014.1606

123. Sans-Fuentes MA. Ratón casero – Mus musculus Linnaeus, 1758. *Encicl Virtual los Vertebr Españoles* (2017) Available at: http://digital.csic.es/bitstream/10261/112032/10/musmus_v3.pdf [Accessed April 29, 2020]

124. Harrus S, Bar-Gal GK, Golan A, Elazari-Volcani R, Kosoy MY, Morick D, Avidor B, Baneth G. Isolation and genetic characterization of a *Bartonella* strain closely related to *Bartonella tribocorum* and *Bartonella elizabethae* in Israeli commensal rats. *Am J Trop Med Hyg* (2009) **81**:55–58. doi:10.4269/ajtmh.2009.81.55

125. Kallio ER, Begon M, Henttonen H, Koskela E, Mappes T, Vaheri A, Vapalahti O. Cyclic

hantavirus epidemics in humans — Predicted by rodent host dynamics. *Epidemics* (2009) **1**:101–107. doi:10.1016/j.epidem.2009.03.002

126. Tersago K, Verhagen R, Vapalahti O, Heyman P, Ducoffre G, Leirs H. Hantavirus outbreak in western Europe: reservoir host infection dynamics related to human disease patterns. *Epidemiol Infect* (2011) **139**:381–390. doi:10.1017/S0950268810000956

127. Rossow H, Ollgren J, Hytönen J, Rissanen H, Huitu O, Henttonen H, Kuusi M, Vapalahti O. Incidence and seroprevalence of tularaemia in Finland, 1995 to 2013: regional epidemics with cyclic pattern. *Eurosurveillance* (2015) **20**:21209. doi:10.2807/1560-7917.ES2015.20.33.21209

128. Cleri DJ, Ricketti AJ, Vernaleo JR. Fever of unknown origin due to zoonoses. *Infect Dis Clin North Am* (2007) **21**:963–996. doi:10.1016/j.idc.2007.08.009

129. Estrada-Peña A. Ticks as vectors: taxonomy, biology and ecology. *Rev Sci Tech l'OIE* (2015) **34**:53–65. doi:10.20506/rst.34.1.2345

130. Tagliapietra V, Rosà R, Hauffe HC, Laakkonen J, Voutilainen L, Vapalahti O, Vaheri A, Henttonen H, Rizzoli A. Spatial and temporal dynamics of lymphocytic choriomeningitis virus in wild rodents, Northern Italy. *Emerg Infect Dis* (2009) **15**:1019–1025. doi:10.3201/eid1507.081524

131. Krasnov BR. *Functional and Evolutionary Ecology of Fleas. A Model for Ecological Parasitology*. New York: Cambridge University Press (2008). doi:10.1017/CBO9780511542688

132. Gómez MS, Blasco-Zumeta J. Pulgas (Insecta: Siphonaptera) colectadas en Los Monegros (Zaragoza). *Zapateri Rev Aragon Entomol* (2001) **9**:85–89.

133. Morand S, Krasnov BR, Poulin R. *Micromammals and macroparasites: from evolutionary ecology to management*. Tokyo: Springer Japan (2006). doi:10.1007/978-4-431-36025-4

134. Keesing F, Belden LK, Daszak P, Dobson A, Harvell CD, Holt RD, Hudson P, Jolles A, Jones KE, Mitchell CE, et al. Impacts of biodiversity on the emergence and transmission of infectious diseases. *Nature* (2010) **468**:647–652. doi:10.1038/nature09575

135. Krasnov BR, Khokhlova IS. The effect of behavioural interactions on the transfer of fleas (Siphonaptera) between two rodent species. *J Vector Ecol* (2001) **26**:181–90.

136. Voutilainen L, Kallio ER, Niemimaa J, Vapalahti O, Henttonen H. Temporal dynamics of Puumala hantavirus infection in cyclic populations of bank voles. *Sci Rep* (2016) **6**:21323. doi:10.1038/srep21323

137. Telfer S, Clough HE, Birtles RJ, Bennett M, Carslake D, Helyar S, Begon M. Ecological differences and coexistence in a guild of microparasites: *Bartonella* in wild rodents. *Ecology* (2007) **88**:1841–1849. doi:10.1890/06-1004.1

138. Keesing F, Holt RD, Ostfeld RS. Effects of species diversity on disease risk. *Ecol Lett* (2006) **9**:485–498. doi:10.1111/j.1461-0248.2006.00885.x

139. Telfer S, Bennett M, Bown KJ, Carslake D, Cavanagh RD, Hazel SM, Jones TR, Begon M. Infection with cowpox virus decreases female maturation rates in wild populations of woodland rodents. *Oikos* (2005) **109**:317–322. doi:10.1111/j.0030-1299.2005.13734.x

140. Burthe SJ, Telfer S, Begon M, Bennett M, Smith AT, Lambin X. Cowpox virus infection in natural field vole *Microtus agrestis* populations: significant negative impacts on survival. *J Anim Ecol* (2008) **77**:110–119. doi:10.1111/j.1365-2656.2007.01302.x

141. Kreizinger Z, Erdélyi K, Felde O, Fabbi M, Sulyok KM, Magyar T, Gyuranecz M. Comparison of virulence of *Francisella tularensis* ssp. *holarctica* genotypes B.12 and B.FTNF002-00. *BMC Vet Res* (2016) **13**:46. doi:10.1186/s12917-017-0968-9

142. Bell JF, Stewart SJ. Chronic shedding tularemia nephritis in rodents: possible relation to occurrence of *Francisella tularensis* in lotic waters. *J Wildl Dis* (1975) **11**:421–430. doi:10.7589/0090-3558-11.3.421

143. Rossow H, Forbes KM, Tarkka E, Kinnunen PM, Hemmilä H, Huitu O, Nikkari S, Henttonen H, Kipar A, Vapalahti O. Experimental infection of voles with *Francisella tularensis* indicates their amplification role in tularemia outbreaks. *PLoS One* (2014) **9**:e108864. doi:10.1371/journal.pone.0108864

144. Khokhlova IS, Krasnov BR, Kam M, Burdelova NI, Degen AA. Energy cost of ectoparasitism: The flea *Xenopsylla ramesis* on the desert gerbil *Gerbillus dasyurus*. *J Zool* (2002) **258**:349–354. doi:10.1017/S0952836902001498

145. Richner H, Tripet F. Ectoparasitism and the trade-off between current and future reproduction. *Oikos* (1999) **86**:535. doi:10.2307/3546657

146. Devevey G, Christe P. Flea infestation reduces the life span of the common vole. *Parasitology* (2009) **136**:1351–1355. doi:10.1017/S0031182009990746

147. Gubányi A, Mészáros F, Murai É, Soltész A. Studies on helminth parasites of the small field mouse (*Apodemus microps*) and the common vole (*Microtus arvalis*) from a pine forest in Hungary. *Parasit Hung* (1992) **25**:37–51.

148. Tenora F, Murai É, Mészáros F. Quantitative and qualitative analysis of helminth fauna of *Microtus arvalis* (Rodentia, Microtinae) in Europe. *Parasit Hung* (1973) **6**:131–148.

149. Kallio-Kokko H, Laakkonen J, Rizzoli A, Tagliapietra V, Cattadori IM, Perkins SE, Hudson PJ, Cristofolini A, Versini W, Vapalahti O, et al. Hantavirus and arenavirus antibody prevalence in rodents and humans in Trentino, Northern Italy. *Epidemiol Infect* (2006) **134**:830–836. doi:10.1017/S0950268805005431

150. Kinnunen PM, Henttonen H, Hoffmann B, Kallio ER, Korthase C, Laakkonen J, Niemimaa J, Palva A, Schlegel M, Ali HS, et al. Orthopox virus infections in Eurasian wild rodents. *Vector-Borne Zoonotic Dis* (2011) **11**:1133–1140. doi:10.1089/vbz.2010.0170

151. Scharninghausen JJ, Pfeffer M, Meyer H, Davis DS, Honeycutt RL, Faulde M. Genetic evidence for Tula virus in *Microtus arvalis* and *Microtus agrestis* populations in Croatia. *Vector-Borne Zoonotic Dis* (2002) **2**:19–27. doi:10.1089/153036602760260742

152. Turner AK, Beldomenico PM, Bown K, Burthe SJ, Jackson JA, Lambin X, Begon M. Host–parasite biology in the real world: the field voles of Kielder. *Parasitology* (2014) **141**:997–1017. doi:10.1017/S0031182014000171

153. Lello J, Boag B, Fenton A, Stevenson IR, Hudson PJ. Competition and mutualism among the gut helminths of a mammalian host. *Nature* (2004) **428**:840–844. doi:10.1038/nature02490

154. Jolles AE, Ezenwa VO, Etienne RS, Turner WC, Olff H. Interactions between macroparasites and microparasites drive infection patterns in free-ranging African buffalo. *Ecology* (2008) **89**:2239–2250. doi:10.1890/07-0995.1

155. Ezenwa VO. Helminth-microparasite co-infection in wildlife: lessons from ruminants, rodents and rabbits. *Parasite Immunol* (2016) **38**:527–534. doi:10.1111/pim.12348

# Acknowledgements

This thesis has become a reality thanks to the enthusiasm, help and effort of many people who, direct or indirectly, have given me the strength and support to carry it out.

To my thesis directors, Juancho and François, for the opportunity offered, the trust they have placed in me and their invaluable help during all this time. To Dolo Vidal, for being an exceptional scientist and an even person. To Raquel Escudero, for her kindness and for welcoming me into her laboratory as one more in the team. To Félix Valcárcel and Sonia Olmeda, for how easy and pleasant it is to work with them and for passing on the tick bug of me. And to all the ECOTULA/BOOMRAT team, because not only have they known how to get the best out of me, but also made me feel at home wherever I have gone.

To Ruth, Juan, Eva and Cris a huge thank you for the priceless help and good moments during fieldwork and lab work. Eva, my "personal reviewer", thanks a million!

To Herman Meyer, for putting viruses in my path. To Tarja Sironen and Heikki Henttonen, for opening the door to the complex world of viruses and helminths, and for giving me the opportunity to live the authentic Finnish experience. To Hussein and Sanna, for their infinite patience with my doubts about the IFA positives. And to Fathiah, for her big heart, her kindness, her proximity, and her conversations in a country where words are silver, but silence is gold.

To all the many colleagues and fellows that I have had the pleasure of meeting over these years because they have helped me to learn, improve, reflect, relativize, enjoy, and laugh and they have filled this time with moments that I will always remember.

And of course, to my family, because I owe them everything and they are always here. And to Pepe, for his contagious positivism still in the more miserable moments that you can imagine, because he has always believed in me, even when I do not believe in myself. THANK YOU.

Finally, I would like to thank the University of Valladolid, the iuFOR, the ETSIIAA-Palencia, the Junta de Castilla y León, the European Social Fund, the Erasmus+ Mobility program, the ECOTULA (CGL2015-66962-C2-1-R) and BOOMRAT (PID2019-109327RB-I00) projects, and the GESINTTOP agreement (co-funded by Junta de Castilla y León, Diputación de Palencia, Diputación de Valladolid) for giving me financial support to develop this doctoral thesis.

Life is full of twists and turns and always pushes you forward, wherever that may be; but what you have already lived, can never be taken away from you.

Thanks to all of you.

# Agradecimientos

Esta tesis se ha hecho realidad gracias a la ilusión, la ayuda y el esfuerzo de muchas personas que, directa o indirectamente, me han dado la fuerza y el apoyo para conseguir llevarla a cabo.

A mis directores de tesis, por la oportunidad que me han dado, la confianza que han depositado en mí y su inestimable ayuda durante todo este tiempo. A Dolo Vidal, por ser una científica excepcional y una persona aún mejor. A Raquel Escudero, por su amabilidad y por hacerme un hueco en su laboratorio como a una más. A Félix Valcárcel and Sonia Olmeda, por lo fácil y agradable que es trabajar con ellos y por conseguir contagiarme el gusanillo de las garrapatas. Y a todo el equipo ECOTULA/BOOMRAT, porque no solo han sabido sacar lo mejor de mí, sino que me han hecho sentir como en casa allá donde he ido.

A Ruth, Juan, Eva y Cris unas gracias infinitas por la impagable ayuda y los buenos momentos durante el trabajo de campo y laboratorio. Eva, mi "revisora personal", ¡un millón de gracias!

A Herman Meyer, por colocar los virus delante de mi camino. A Tarja Sironen and Heikki Henttonen, por abrirme la puerta al complejo mundo de los virus y los helmintos, y por brindarme la oportunidad de vivir la auténtica experiencia finlandesa. A Hussein y Sanna, por su infinita paciencia ante mis dudas con los positivos IFA. Y a Fatiha, por su gran corazón, su amabilidad, su cercanía y sus conversaciones en un país donde las palabras son plata, pero el silencio es oro.

A todos los muchos colegas y compañeros de fatigas que he tenido la suerte de conocer a lo largo de estos años, porque me han ayudado a aprender, a mejorar, a reflexionar, a relativizar, a disfrutar, a reír y han llenado esta etapa con momentos que recordaré siempre.

Y por supuesto, a mi familia, que les debo todo y siempre están ahí. Y a Pepe, por su positivismo contagioso hasta en los momentos más oscuros que uno pueda imaginar, porque siempre ha creído y cree en mí, incluso cuando ni yo misma lo hago. GRACIAS.

Por último, agradecer a la Universidad de Valladolid, al iuFOR, a la ETSIIA-Palencia, a la Junta de Castilla, al Fondo Social Europeo, al programa Erasmus+ Mobility, a los proyectos ECOTULA (CGL2015-66962-C2-1-R) y BOOMRAT (PID2019-109327RB-I00), y al convenio GESINTTOP (cofinanciado por la Junta de Castilla y León, Diputación de Palencia, Diputación de Valladolid) por haberme brindado apoyo económico para poder desarrollar esta tesis doctoral.

La vida de muchas vueltas y siempre te empuja hacia delante, sea donde sea eso; pero lo vivido nunca te lo pueden quitar.

Gracias a todos.