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'Bites of Knowledge': Ticks and Tick-Borne Pathogens Unveiled Through a Citizen Science Programme in Northern Spain

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ABSTRACT

Introduction: We present the findings of a citizen science project conducted in the Autonomous Region of Aragón (northern Spain), which engaged rural schoolchildren, wildlife rangers, hunters, and members of the general public.

Methods: Over the course of one year (April 2022–April 2023; three months for the schoolchildren), participants collected a total of 913 ticks (98.8% adult specimens) representing 13 species from across the region. The contributions included 373 ticks collected by schoolchildren, 319 by hunters, 108 by rangers, 91 by other volunteers, and 22 by the research team. Of these, 54 ticks were gathered from vegetation, 422 from wildlife (spanning nine animal species), 362 from pets, 44 from humans, and 30 from livestock.

Results: Species identified were *Rhipicephalus sanguineus* s.l., *Rhipicephalus sanguineus* s.s., *Rhipicephalus pusillus, Rhipicephalus bursa, Dermacentor marginatus, Ixodes hexagonus, Ixodes ricinus, Ixodes frontalis, Haemaphysalis punctata, Haemaphysalis sulcata, Haemaphysalis inermis, Hyalomma marginatum,* and *Hyalomma lusitanicum*. DNA of a subset of 349 individual ticks was extracted and the presence of *Rickettsia* spp., Anaplasmataceae, and *Borrelia burgdorferi* sensu lato (the latter, only in *Ixodes* spp.) was analysed either individually ($n=62$) or in pools containing 2–5 ticks ($n=79$), of which 101 resulted positive: 88 were positive for *Rickettsia* spp., revealing the presence of *Rickettsia massiliae*, *Rickettsia slovaca, Rickettsia conorii* subsp. *raoultii, Rickettsia aeschlimannii*, *Rickettsia hoogstraalii, Rickettsia helvetica,* and *Candidatus* Rickettsia barbariae; 34 for Anaplasmataceae (both *Ehrlichia* spp. and *Anaplasma* spp., but in many cases was identified as endosymbionts); and three for *B. burgdorferi* s.l., with only one readable sequence (*Borrelia valaisiana*). Pathogens detected in ticks from humans included *R. massiliae* ($n=3$), *R. conorii raoultii, R. aeschlimannii* and *Ca.* R. barbariae.

Conclusions: This study identified previously unreported tick-host-pathogen associations and contributed to raising awareness about the public health significance of ticks and strategies for their prevention.

Ruth Rodríguez-Pastor and Clara Muñoz-Hernández contributed equally to this study.

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Impacts

- This project shows how citizens, including schoolchildren, hunters, and rangers, can contribute to scientific research, helping map where different tick species live and which pathogens they carry in Aragón, northern Spain.
- This study uncovers previously unknown links between ticks, their hosts and pathogens, providing information that improves public health awareness and future tick-borne disease monitoring efforts.
- The discovery of several bacteria capable of causing disease in humans highlights the need for continued vigilance and preventive measures against tick bites in rural and outdoor settings.

1 | Introduction

Ticks (Ixodoidea) are blood-feeding arthropods with a global distribution, parasitizing virtually all terrestrial vertebrates, including humans. In temperate regions, they are considered the most significant vectors of pathogens affecting both humans and livestock, in terms of disease burden (Jongejan and Uilenberg 2004). Due to their relevance to public and animal health, understanding the geographic distribution of tick species is essential for tracking the circulation of tick-borne pathogens and evaluating strategies to mitigate their impact. Tick distribution is strongly influenced by climatic factors, particularly temperature and humidity, which regulate both their developmental cycles and questing behaviour (Estrada-Peña and de la Fuente 2014). In this context, and under the current scenario of global warming, several species have been observed to expand their ranges and alter their seasonal activity patterns (Gray et al. 2009).

Citizen science (also known as community science) refers to the active involvement of the public in scientific research activities (Vohland et al. 2021). In the natural sciences, it offers a practical means of achieving the broad geographic coverage necessary to document ecological patterns and address research questions at scales relevant to regional population dynamics and environmental processes (Frigerio et al. 2021). One area where citizen science has proven particularly valuable is in generating spatial data on tick occurrences (Eisen and Eisen 2021). Using citizen science in tick research offers numerous benefits. It enables large-scale and cost-effective data collection across broad geographic areas, including remote or under-sampled regions that researchers may not easily reach. Citizen participation helps track seasonal and long-term trends in tick populations and can lead to early detection of tick-borne disease outbreaks. By involving the public, citizen science also raises awareness about tick ecology and prevention, fostering better public health communication. Additionally, volunteers provide access to diverse habitats—urban, rural, and suburban—enriching the data and supporting more comprehensive ecological analyses (Koffi et al. 2012; Nieto et al. 2018; Eisen and Eisen 2021; Földvári et al. 2022).

Aragón is an autonomous region in northeastern Spain characterised by strong contrasts in habitat types, land use, and climatic conditions. Despite this environmental diversity, few systematic studies have been conducted on ticks in the region (Estrada-Peña et al. 1992, 1995). The limited existing research includes studies on ticks parasitizing birds in parts of the region (Osácar-Jimenez et al. 1998), contributions to a national survey on ticks affecting pets (Estrada-Peña, Roura, et al. 2017), and a region-wide survey of ticks on sheep based on a UTM grid system (Estrada-Peña et al. 2004). Information on tick-borne pathogens in Aragón is even more limited. Despite the region's ecological variety, the prevalence of livestock, and abundant wildlife—particularly in this predominantly rural area—our current knowledge of tick species and their distribution in Aragón is inadequate. Given the lack of comprehensive baseline data, our objective was to update the regional tick catalogue by incorporating data collected through active public participation. Rather than conducting a systematic survey—which would be logistically unfeasible—we aimed to improve the understanding of tick identity and distribution, as well as the occurrence of tick-borne pathogens in Aragón, through a citizen science approach that enhanced spatial data coverage.

2 | Materials and Methods

2.1 | Study Area

Aragón covers an area of 47,720 km^2 and has a population of approximately 1.3 million inhabitants (Figure 1), with half residing in the capital city, Zaragoza. The region is made up of three provinces (Huesca in the North, Teruel in the South, and Zaragoza between them) and has a low population density of 27.6 inhabitants per km^2. Notably, one-fourth of its territory lies at altitudes above 1000 m above sea level, yet this highland zone is home to only 3.3% of the population. Land use is divided primarily between agriculture and natural landscapes: 46% of the territory is devoted to dryland farming (mainly wheat, barley, olives, and grapes), while 52% consists of forests and natural open areas (Instituto Aragonés de Estadística 2025). The topography is dominated by mountain ranges to the north and south of the Ebro River basin, which runs east to west through the region. In the north, the Pyrenees rise to elevations exceeding 3000 m, with the pre-Pyrenees gradually descending towards the central basin. The Iberian System, one of the major systems of mountain ranges in Spain, dominates the southern landscape. Annual precipitation is generally low, averaging around 350 mm, with most rainfall occurring in spring and autumn. The climate is predominantly Mediterranean, becoming more continental in inland areas, with Atlantic influences in the western Pyrenees (Instituto Aragonés de Estadística 2025). Over recent decades, Aragón has undergone significant rural depopulation, which has had major impacts on land use and wildlife abundance (Gortázar et al. 2000; González et al. 2013). This rural abandonment is considered a key factor contributing to localised increases in wildlife densities and, consequently, to rising tick populations supported by these hosts (e.g., Diuk-Wasser et al. 2021).

2.2 | Sample Collection

According to Eisen and Eisen (2021), our type of approach can be classified as opportunistic, semi-quantitative. Ticks were collected between April 2022 and April 2023 as part of the citizen science initiative ‘Bites of Knowledge’ (‘Picaduas de

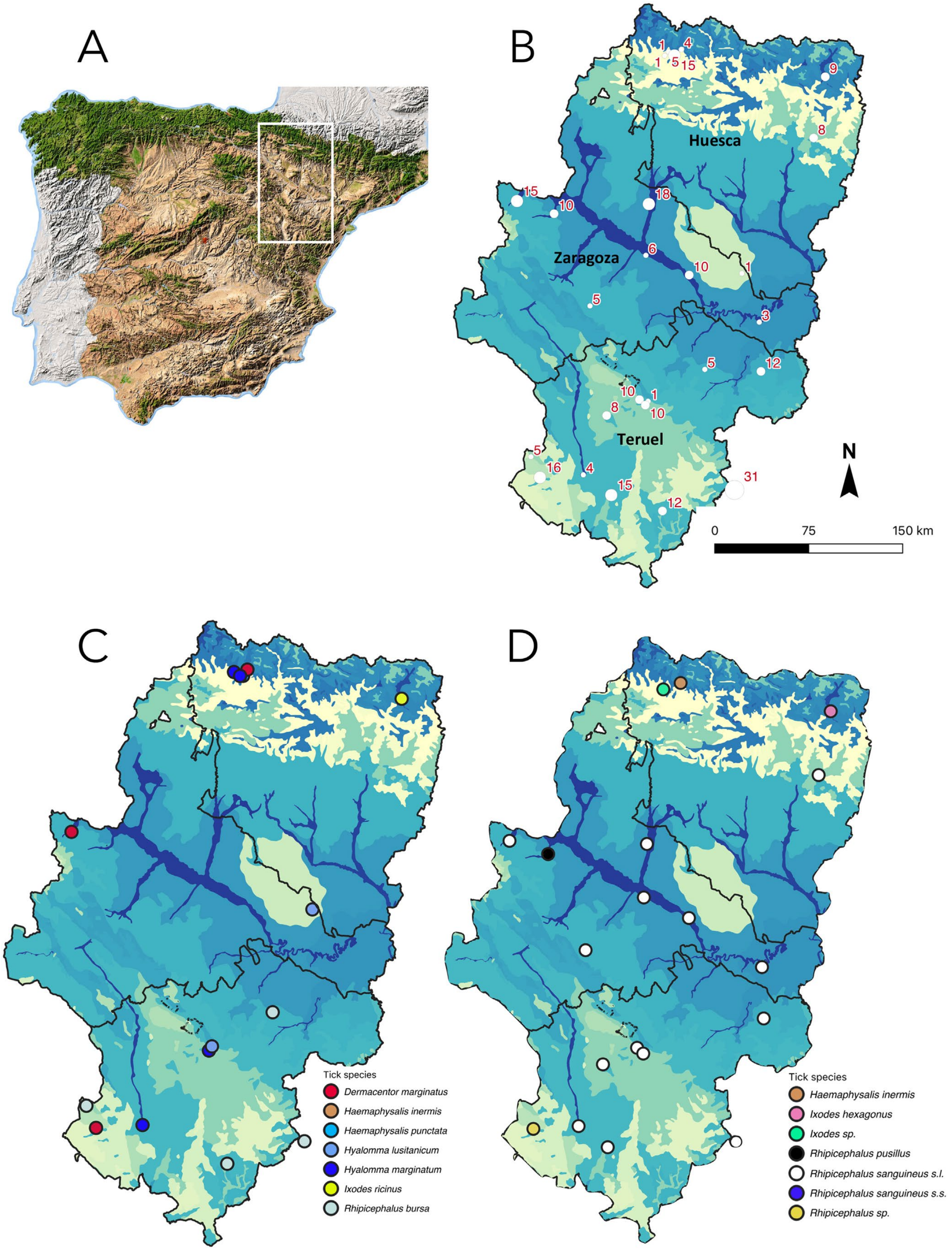

FIGURE 1 | (A) Map of peninsular Spain, showing the location of Aragón. (B) Map of Aragón, showing its biogeographic regions and the collection locations (numbers indicate collection events). (C and D) Location of the retrieved tick species.

Conocimiento' in Spanish; https://alimentandolaciencia.esciencia.es/picaduras-de-conocimiento). The programme was designed to raise awareness about the risks of tick bites, engage and collect tick specimens and associated data from four primary citizen groups: rural schoolchildren, wildlife rangers, hunters, and members of the general public who voluntarily participated. Rural residents have been chosen as participants because they are the ones who have the most frequent contact with ticks. Due to their close relationship with nature, agriculture, and outdoor activities, people living in rural areas are more likely to encounter ticks in their everyday life. This makes their observations and experiences especially valuable for monitoring tick populations and understanding the risks of tick-borne diseases.

For the school-based component, we collaborated with the Regional Department of Education, submitting a letter explaining the project and inviting participation from rural schools across Aragón. Twenty schools responded positively and were subsequently involved in the programme, engaging students aged 12 to 16. In March 2022, one or more researchers visited each participating school. These visits included an educational session with a video presentation (available on the project website), visual materials, and a hands-on introduction to ticks and other arthropod vectors. Students examined ticks at various life stages under stereomicroscopes and received training on how to safely collect and preserve specimens. A teaching guide was also provided to educators.

Each participating teacher received a collection kit containing an instruction flyer, a sample rack, tubes filled with 95% laboratory-grade ethanol, tweezers for each student, and a form to be completed whenever a tick sample was submitted. This form recorded the source of the tick (e.g., human, species of animal, vegetation), as well as the location (municipality) and date of collection. Students were asked to collect ticks opportunistically during their daily life, either attaching to themselves, a member of their family, animals in their household (e.g., when brushing their dogs), or on game animals, if the parent was a hunter. It was explained to them how to safely remove ticks. Teachers were responsible for managing and labelling the samples and submitting them—along with the completed forms—to our laboratory by late June 2023. All but one school submitted appropriately labelled and usable samples meeting the minimum data requirements.

Hunters were recruited through the Aragonese Hunting Association and rangers via the Regional Environmental Office and the staff of Ordesa National Park in the Pyrenees. Both groups received simplified kits, which included a brief information sheet and the necessary materials for collecting and preserving ticks from wildlife or found on themselves in ethanol. The general public was engaged through the project website (which was presented in diverse regional newspapers and TV and radio stations) and received the same simplified kit. Hunters and the general public recorded the host, date, and location of the collection. Ticks collected opportunistically by members of the research team during fieldwork or personal activities were also included. All specimens were preserved in ethanol to facilitate storage and handling by the contributing participants. The samples were brought to our lab by the participants.

Due to the logistical difficulties of obtaining precise geographic coordinates in remote rural areas, we did not request GPS data. Instead, the name of the municipality where the tick was collected was used as the geographic reference. This approach was deemed sufficient, given that Spanish municipalities are relatively small and provide an adequate spatial resolution for our purposes. This was especially important for samples collected by rangers, who typically cover large, ecologically diverse areas on a daily basis, making precise localisation of tick encounters impractical.

2.3 | Tick Identification and Pathogen Detection

Upon arrival at the laboratory, all samples were initially inspected for physical integrity and completeness. Each specimen was assigned a unique identification number and subjected to morphological identification using the taxonomic keys provided by Estrada-Peña, Mihalca, and Petney (2017). In cases where the degree of engorgement or preservation quality hindered accurate identification—particularly within the *Rhipicephalus sanguineus* species group—specimens were conservatively classified as *R. sanguineus* sensu lato or to genus level. Re-examination of the material was not possible following the recent description of *Rhipicephalus hibericus* (Millán et al. 2024), as the specimens had already been crushed and processed for DNA extraction.

Due to a budget limitation, not all the ticks were analysed for pathogens. The ticks to be analysed were chosen trying to have a representation of all the areas studied, emphasising samples collected questing and from humans and wildlife. Therefore, DNA of a subset of 349 individual ticks was extracted following Halos et al. (2004) with some modifications. Briefly, half a tick was placed in a 2-ml sterile microtube containing ten 0.1 mm sterile glass microbeads, and one 3.2 mm sterile stainless steel microbead (BioSpec). The tubes were cooled in liquid nitrogen for 1 min and immediately after crushed by shaking in a Mini Bead Beater-16 (Model 607-EUR, BioSpec) for 2 cycles of 1 min 30 s at 3450 rpm. The tubes were then briefly centrifuged at a maximum speed (10,000 xg) and the pellets were suspended in 180 μL of lysis buffer (ATL buffer, Qiagen). DNA was then extracted using the Qiagen DNeasy Blood & Tissue kit (Qiagen, Valencia, CA, USA) following manufacturer instructions. The final elution volume was 100 μL. The presence of DNA of Anaplasmataceae, *Rickettsia* spp., and *Borrelia burgdorferi* sensu lato (the latter, only in *Ixodes* spp.) was analysed either individually ($n = 62$) or in pools containing between 2 and 5 ticks ($n = 79$) from the same individual host (Table S1).

Primers and PCR conditions are detailed in Table 1. The PCR assays were carried out using PCR Master Mix (Promega Corporation, Madison, WI, USA). Anaplasmataceae screening was performed using two conventional PCR protocols targeting partial fragments of the *16S rRNA* gene. The detection of *B. burgdorferi* s.l. was carried out by nested PCR assays amplifying fragments of the flagellin gene (*flaB*) and the spacer region between the *5S* and *23S rRNA* genes. Tick samples were considered positive for Anaplasmataceae or *B. burgdorferi* s.l. if amplification was observed in at least one of the screening PCR assays. *Rickettsia* spp. was screened by

TABLE 1 | Primers and PCR conditions of assays used in this study.

Bacteria	Gene target	Primer	Primer sequence 5′-3′	Size (bp)	Annealing temperature	References
Rickettsia spp.	*16S rRNA*	F	AGAGTTTGATCCTGGCTCAG	416	54°C	Weisburg et al. (1989)
		R	AACGTCATTATCTTCCTTGC			
	ompA	F	ATGGCGAATATTTCTCCAAAA	650	54°C	Roux et al. (1996)
		R	GTTCCGTTAATGGCAGCATCT			
	ompB	F	GGGTGCTGCTACACAGCAGAA	618	53°C	Fernández de Mera et al. (2009)
		R	CCGTCACCGATATTAATTGCC			
	atpA	F	ACATATCGAGATGAAGGCTCC	731	48°C	Fernández de Mera et al. (2009)
		R	CCGAAATACCGACATTAACG			
	recA	F	TGCTTTTATTGATGCCGAGC	428	52°C	Fernández de Mera et al. (2009)
		R	CTTTAATGGAGCCGATTCTTC			
	gltA	F	GGGGGCCTGCTCACGGCGG	360	45°C	Roux et al. (1996)
		R	ATTGCAAAAAGTACAGTGAACA			
Anaplasmataceae	*16S rRNA*	F	TAGCACTCATCGTTTACAGC	345	54°C	Inokuma et al. (2000)
		R	GGTACCYACAGAAGAAGTCC			
	16S rRNA	F	TTTATCGCTATTAGATGAGCCTATG	452	52.4°C	Battisti et al. (2020)
		R	CTCTACACTAGGAATTCCGCTAT			
Borrelia burgdorferi s.l.	5S-23S intergenic spacer region (nested)	F1	ACCATAGACTCTTATTACTTTGAC	380	52°C	Rijpkema et al. (1995)
		R1	TAAGCTGACTAATACTAATTACCC			
		F2	ACCATAGACTCTTATTACTTTGACCA	225	52°C	
		R2	GAGAGTAGGTTATTGCCAGGG			
	flab (nested)	F1	AARGAATTGGCAGTTCAATC	497	52°C	Clark et al. (2005)
		R1	GCATTTTCWATTTTAGCAAGTGATG			
		F2	ACATATTCAGAGCAGACAGAGGTTCTA	389	55°C	
		R2	GAAGGTGCTGTAGCAGGTGCTGGCTGT			

the partial amplification of the *16S rRNA* gene, and the 16S-positive samples were further confirmed and characterised using *Rickettsia*-specific PCR assays: outer membrane protein A (*ompA*), outer membrane protein B (*ompB*), adenosine triphosphate synthase alpha subunit (*atpA*), recombinase A (*recA*) and citrate synthase (*gltA*) genes. Positive samples for *Rickettsia* spp. were confirmed by amplifying at least one specific marker. Amplicons were visualised on 1.5% agarose gels, and bands at the expected size were purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) and sequenced by the Sanger method (Macrogen, Madrid, Spain). All nucleotide sequences were analysed and compared with sequences previously deposited in the National Centre for Biotechnology Information (NCBI) GenBank database through the nucleotide BLAST tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to assess the identity level. Maximum Likelihood phylogenetic trees were conducted using MEGA X to confirm the species identification of *Rickettsia* spp. and *B. burgdorferi* s.l. isolates. Unique partial sequences obtained in this study were deposited in the GenBank database under the following accession numbers: PV102480, and PV126191–PV126203.

3 | Results

3.1 | Ticks Collected

A total of 913 ticks were collected. Of these, 373 were gathered by schoolchildren (40.8%), primarily from pets (61.1%), but also from wildlife (24.4%), humans (6.2%), livestock (3.7%), and vegetation (4.3%); 319 by hunters (34.9%, mostly from wildlife), 108 by rangers (11.8%, with a relatively even distribution across all categories), 91 by general citizens (9.9%, mainly from pets), and 22 by the authors (predominantly from vegetation; Figure 2, Table S2). By province, 649 ticks were collected in Zaragoza, 203 in Teruel, and 60 in Huesca (data for one tick was unavailable; Table 2). Of the total, 54 ticks were collected while questing on vegetation, with the remainder collected from hosts. Specifically, 422 ticks were obtained from wildlife (all but two from mammals), 362 from companion animals, 44 from humans, and 30 from livestock (Table 3). The host information for one tick was unknown.

The sample included 13 different tick species of five genera, including *Rhipicephalus* sp. (*n* = 13), *R. sanguineus* s.l. (*n* = 303), *R. sanguineus* s.s. (*n* = 90), *R. pusillus* (*n* = 337), *R. bursa* (*n* = 86), *Dermacentor marginatus* (*n* = 26), *Ixodes* sp. (*n* = 3), *Ixodes hexagonus* (*n* = 6), *I. ricinus* (*n* = 6), *I. frontalis* (*n* = 1), *Haemaphysalis* sp. (*n* = 1), *Ha. punctata* (*n* = 16), *Ha. sulcata* (*n* = 3), *Ha. inermis*, (*n* = 1), *Hyalomma marginatum* (*n* = 17), and *H. lusitanicum* (*n* = 4). The highest variety of species was found in humans (nine species). Tick diversity varied widely amongst animal hosts, ranging from 7 species on dogs to only one on rabbits. Some tick/host associations were found (e.g., rabbit-*R. pusillus*, sheep-*R. bursa*). The vast majority of collected ticks were adult specimens (*n* = 901, 98.8%), of which 435 were males and 466 were females. Only 12 immature ticks (11 nymphs and one larva) were collected.

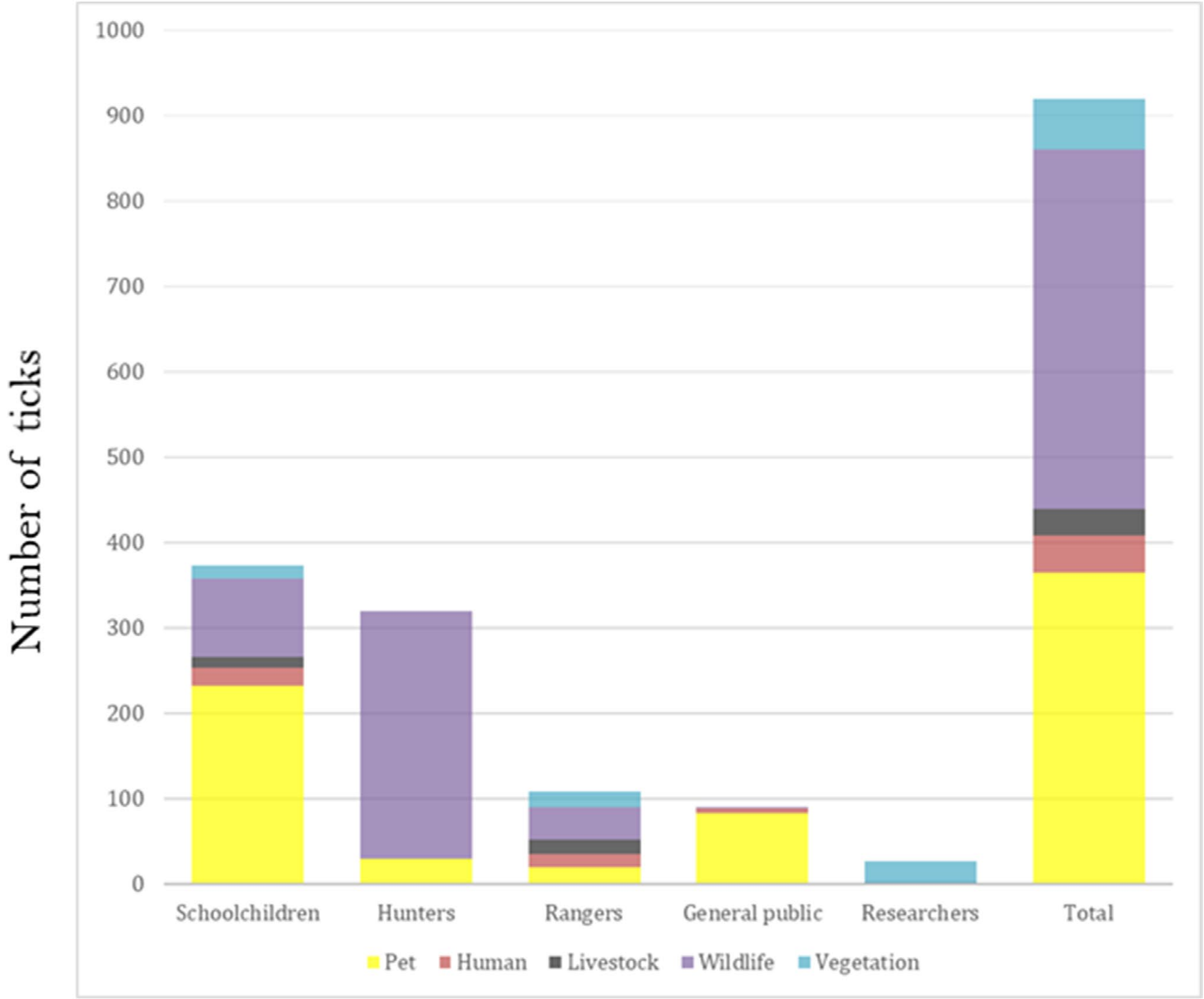

FIGURE 2 | Number of ticks collected depending on the type of volunteer and the host.

TABLE 2 | Province in which the ticks were collected. Data on the province was missing for one tick.

Province	Pets	Wildlife	Livestock	Human	Vegetation	Total	Species[a]
Huesca	31	1	1	6	21	60	Rssl, Rsss, Rsp, Dm, Ir, Ih, Isp, Hap, Hai, Hym, Hyl.
Zaragoza	242	386	2	11	7	649[b]	Rssl, Rsss, Rp, Rb, Rsp, Dm, If, Has, Hasp
Teruel	89	34	27	27	26	203	Rssl, Rsss, Rp, Rb, Rsp, Dm, Hap, Hym

[a] Dm, *Dermacentor marginatus*; Hai, *H. inermis*; Hap, *Haemaphysalis punctata*; Has, *H. sulcata*; Hasp, *Haemaphysalis* sp.; Hyl, *H. lusitanicum*; Hym, *Hyalomma marginatum*; Hysp, *Hyalomma* sp.; If, *I. frontalis*; Ih, *Ixodes hexagonus*; Ir, *I. ricinus*; Isp, *Ixodes* sp.; Rb, *R. bursa*; Rp, *R. pusillus*; Rsp, *Rhipicephalus* sp.; Rssl, *Rhipicephalus sanguineus* s.l.; Rsss, *R. sanguineus* s.s.

[b] There was one tick collected in Zaragoza province with no host data (not included in the table).

TABLE 3 | Number of individuals inspected, and ticks collected according to the origin (vegetation/host) and the type of host. One tick (*R. bursa* collected in Zaragoza province) is not included in the table due to the lack of data on host species.

Origin	Type of host	Host species	Number of individuals	Number of ticks	Species found
Vegetation			—	54	*R. sanguineus* s.l. (9), *R. bursa* (15), *D. marginatus* (7), *Haemaphysalis* sp. (1), *Ha. punctata* (9), *Ha. inermis* (1), *Hy. marginatum* (10), *Hy. lusitanicum* (2).
On host			273	823	
	Human		37	44	*R. sanguineus* s.l. (10), *R. sanguineus* s.s. (2), *R. bursa* (13), *D. marginatus* (3), *Ixodes* sp. (2), *I. hexagonus* (2), *I. ricinus* (2), *Ha. sulcata* (3), *Hy. marginatum* (6), *Hy. lusitanicum* (1).
	Pet		158	364	
		Dog	116	272	*Rhipicephalus* sp. (4), *R. sanguineus* s.l. (177), *R. sanguineus* s.s. (63), *R. pusillus* (20), *R. bursa* (2), *Ixodes* sp. (1), *I. hexagonus* (2), *I. ricinus* (2), *Hy. marginatum* (1).
		Cat	42	90	*Rhipicephalus* sp. (6), *R. sanguineus* s.l. (41), *R. sanguineus* s.s. (5), *R. pusillus* (34), *I. hexagonus* (2), *I. ricinus* (2)
	Livestock		10	30	
		Sheep	4	19	*R. sanguineus* s.s. (2), *R. pusillus* (2), *R. bursa* (15)
		Cattle	5	9	*Rhipicephalus* sp. (1), *R. sanguineus* s.l. (2), *R. bursa* (2), *Ha. punctata* (4)
		Horse	1	2	*R. bursa* (2)
	Wildlife		74	422	
		Red deer	6	17	*Rhipicephalus* sp. (1), *D. marginatus* (13), *Ha. punctata* (3)
		Roe deer	16	77	*Rhipicephalus* sp. (1), *R. sanguineus* s.l. (35), *R. sanguineus* s.s. (4), *R. bursa* (37),
		Wild boar	3	4	*D. marginatus* (3), *Hy. lusitanicum* (1)
		Hedgehog	1	13	*R. sanguineus* s.s. (13)
		Wild rabbit	36	248	*R. pusillus* (248)
		Red fox	9	57	*R. sanguineus* s.l. (25), *R. pusillus* (32)
		Stone marten	1	4	*R. sanguineus* s.l. (4)
		Birds[a]	2	2	*R. sanguineus* s.s. (1), *I. frontalis* (1)

[a] *Luscinia svecica, Milvus migrans.*

The gradient of climate and vegetation in the target region revealed a clear pattern of tick distribution (Figure 1). Both *Rhipicephalus* spp. and *Hyalomma* spp. were most abundant in the dry, warm areas of the mid Ebro valley, while *D. marginatus* and *R. bursa* were more commonly found in Mediterranean-type forests and low-altitude hills in the southern part of the region. *Ixodes* spp. was restricted to the medium-altitude areas of the Pyrenees, the region with the highest humidity within the study region. *Hyalomma* spp. was also found in these areas due to local livestock farming in the low-altitude valleys. *Rhipicephalus pusillus* is found wherever wild rabbit (*Oryctolagus cuniculus*) populations are present.

3.2 | Pathogens Detected

Molecular screening for pathogens revealed DNA of any of the studied pathogens in 101 samples. *Rickettsia* spp. DNA was found in 31 individual ticks and 57 pools; Anaplasmataceae DNA, in 14 individual ticks and in 20 pools; and *B. burgdorferi* s.l. DNA, in 3 of the individually analysed *Ixodes* spp. (Table 4, Table S1).

The most frequently detected *Rickettsia* species was *R. massiliae*, identified in 43 ticks or pools, and found mostly in

TABLE 4 | Samples included in the study and results of the molecular analysis of ticks. In parenthesis, characterised isolates (see Supporting Information for details).

			Analysed		**Positive for Anaplasmataceae**		**Positive for *Rickettsia* spp.**		**Positive for *B. burgdorferi* s.l.**	
Tick sp.	**Host**	**Province**	**Individually**	**Pooled (ticks in the pool)**	**Individual**	**Pools**	**Individual**	**Pools**	**Individual**	**Pools**
R. sanguineus s.l.	Vegetation	Zaragoza	2	1 (2)				1		
	Vegetation	Teruel	1	2 (2)		1		1		
	Human	Zaragoza	5	1 (2)	1 (CaMm)		2 (Rmas)	1 (Rmas)		
	Human	Teruel	3							
	Dog	Zaragoza		7 (2–5)				6 (Rmas)		
	Dog	Huesca		1 (4)				1 (Rmas)		
	Dog	Teruel		3 (3–5)		1		2		
	Cat	Zaragoza		2 (5)				2 (Rmas)		
	Cat	Huesca		2 (5)				2 (Rmas)		
	Cattle	Zaragoza		1 (2)				1 (Rmas)		
	Red fox	Zaragoza	1	7 (3–5)			1 (Rmas)	7 (Rmas)		
	Stone marten	Zaragoza		1 (3)				1 (Rmas)		
	Stone marten	Teruel		3 (5)		1		3 (Rmas)		
	Roe deer	Zaragoza		3 (4–5)		1 (Abov)		1		
	Roe deer	Teruel		2 (4–5)		2		1 (Rmas)		
R. sanguineus s.s.	Human	Teruel	1							
	Roe deer	Teruel		1 (4)				1 (Rmas)		
	Hedgehog	Zaragoza		2 (5)				2 (Rmas)		
R. pusillus	Cat	Zaragoza		1 (4)						
	Red fox	Zaragoza		2 (5)		1 (Ehsp)		1		

(Continues)

TABLE 4 | (Continued)

			Analysed		Positive for Anaplasmataceae		Positive for *Rickettsia* spp.		Positive for *B. burgdorferi* s.l.	
Tick sp.	**Host**	**Province**	**Individually**	**Pooled (ticks in the pool)**	**Individual**	**Pools**	**Individual**	**Pools**	**Individual**	**Pools**
R. bursa	Vegetation	Zaragoza	1							
	Vegetation	Teruel	1	1 (5)						
	Human	Teruel	6	3 (2–3)	1		1 (CaRb)	1		
	Dog	Teruel		1 (2)						
	Cattle	Teruel		1 (3)						
	Sheep	Teruel		4 (4–5)		1		2		
	Roe deer	Zaragoza		6 (2–5)		3 (3 Asp)		6 (Rmas)		
		Teruel		1 (3)				1 (Rmas)		
Rhipicephalus sp.	Cattle	Huesca	1				1			
	Red deer	Teruel	1		1		1			
R. sanguineus s.l. + *R. bursa*	Roe deer	Zaragoza		2 (5)				2 (Rmas)		
R. sanguineus s.l. + *R. pusillus*	Dog	Teruel		1 (5)				1 (Rmas)		
D. marginatus	Vegetation	Zaragoza	1		1 (Asp)		1 (Rmas)			
	Vegetation	Huesca		3 (2)				3 (2 Rcor, 1 Rslo)		
	Human	Teruel	1	1 (2)			1 (Rcor)	1 (Rslo)		
	Red deer	Teruel	2	3 (2–5)			2 (Rcor, Rslo)	3 (2 Rcor)		
	Wild boar	Teruel	1	1 (2)			1 (Rslo)	1		
Ha. inermis	Vegetation	Huesca	1							

(Continues)

TABLE 4 | (Continued)

Tick sp.	Host	Province	Analysed		Positive for Anaplasmataceae		Positive for *Rickettsia* spp.		Positive for *B. burgdorferi* s.l.	
			Individually	Pooled (ticks in the pool)	Individual	Pools	Individual	Pools	Individual	Pools
Ha. punctata	Vegetation	Teruel	1	2 (2)		2	1 (Rsp)	2 (Rmas, Rhoo)		
	Vegetation	Huesca	2	1 (2)		1 (Ehsp)				
	Cattle	Teruel		1 (4)				1		
	Red deer	Teruel		1 (2)		1 (CaLe)				
Haempahysalis sp.	Vegetation	Zaragoza	1							
Hy. lusitanicum	Vegetation	Teruel	1							
	Vegetation	Huesca		1 (2)				1		
	Human	Teruel	1							
	Wild boar	Huesca	1							
Hy. marginatum	Vegetation	Teruel	1							
	Vegetation	Huesca	1	2 (4)			1 (Rcor)	2 (1 Raes)		
	Human	Teruel	6		1		1 (Raes)			
	Dog	Teruel	1				1 (Raes)			
Hyalomma sp.	Dog	Teruel	1				1			
I. ricinus	Vegetation	Huesca	1	1 (4)	1			1		
	Human	Huesca	2		1 (CaMm)		1 (Rhel)			
	Dog	Huesca	2	1 (2)	2 (CaMm. CaLe)	1			1	
	Cat	Huesca	1				1 (Rslo)			
I. frontalis	Bird	Zaragoza	1		1 (CaMm)		1			
I. hexagonus	Human	Huesca	2		1 (CaMm)		1		1	
	Dog	Huesca	2		2 (CaMm)		1		1 (Bval)	
	Cat	Huesca	2		2 (Ehsp, CaMm)		1			
Ixodes sp.	Human	Huesca	2				1			
	Dog	Huesca	1		1		1			

Abbreviations: Abov: *Anaplasma bovis*; Asp: *Anaplasma* sp.; Bval: *Borrelia valaisiana*; CaLe: *Ca.* Lariskella arthropodarum; CaMm: *Ca.* Midichloria mitochondrii; CaRb: *Ca.* Rickettsia barbariae; Ehsp: *Ehrlichia* sp.; Raes: *Rickettsia aeschlimannii*; Rcor: *Rickettsia conorii* subsp. *raoultii*; Rhel: *Rickettsia helvetica*; Rhoo: *Rickettsia hoogstraalii*; Rmas: *Rickettsia massiliae*; Rslo: *Rickettsia slovaca*.

R. sanguineus s.l. (including two removed from humans) but also in *R. sanguineus* s.s. and *R. bursa* from wildlife, and questing *Ha. punctata* and *D. marginatus*. Other species identified were *R. slovaca* found in four *D. marginatus* pools but also in an *I. ricinus* from a cat; *R. conorii* subsp. *raoultii* in *R. bursa* removed from a human and one *D. marginatus* from a red deer, and also in questing *D. marginatus* and *Hy. marginatum*; *R. aeschlimannii* was detected in two feeding *Ha. marginatum* from a dog and a human; *R. hoogstraalii* in a pool containing two questing *Ha. punctata*; *Candidatus* Rickettsia barbariae in a *R. bursa* removed from a human; and *R. helvetica* in an *I. ricinus* also from a human (Figure 3).

The readable sequences obtained from either of the protocols for Anaplasmataceae revealed the presence of *Ehrlichia* sp. and *Anaplasma* sp. in three cases each. These included, amongst others, a sequence with 100% identity with *E. minasensis* and *E. canis* in a questing *Ha. punctata*, a sequence with 99.7% identity with *A. marginale* in a questing *D. marginatus*, sequences with 100% identity with *A. ovis* and other *Anaplasma* spp. in two *R. bursa* pools from two roe deer, and a sequence with 100% identity with *A. bovis* in a *R. sanguineus* s.l. pool from another roe deer. Many other sequences corresponded with tick endosymbionts. Out of the three positive cases of *B. burgdorferi* s.l., only one readable sequence was obtained from an *I. hexagonus* removed from a dog, which corresponded with *B. valaisiana* (Figure 4).

4 | Discussion

4.1 | Outcomes of the Citizen Science Project

In addition to the awareness-raising activities conducted during school visits regarding the risks of tick bites, our citizen science project was highly successful in collecting nearly 1000 ticks in under a year. The economic and human resources required to actively collect such a large number of samples from diverse locations and species would have been considerably higher if carried out solely by the research team. Previous citizen science studies on tick collection have primarily focused on specimens found on humans (e.g., Lernout et al. 2019; Porter et al. 2019), pets (Sgroi et al. 2022) or livestock (Földvári et al. 2022; Uiterwijk et al. 2021), with few examples also in vegetation (Robert et al. 2025). Our study successfully gathered specimens from all of these sources, as well as from wildlife. However, we acknowledge that this study is subject to a common bias inherent to citizen science research (Bird et al. 2014). The data presented here on tick presence and tick-borne pathogens should be interpreted as evidence of presence only, rather than as definitive proof of absence or relative abundance. Similarly, the detection of pathogens in ticks collected from hosts should not be construed as evidence of vectorial capacity (Estrada-Peña et al. 2021).

Schoolchildren were responsible for collecting the majority of the samples, probably because they were the main target group

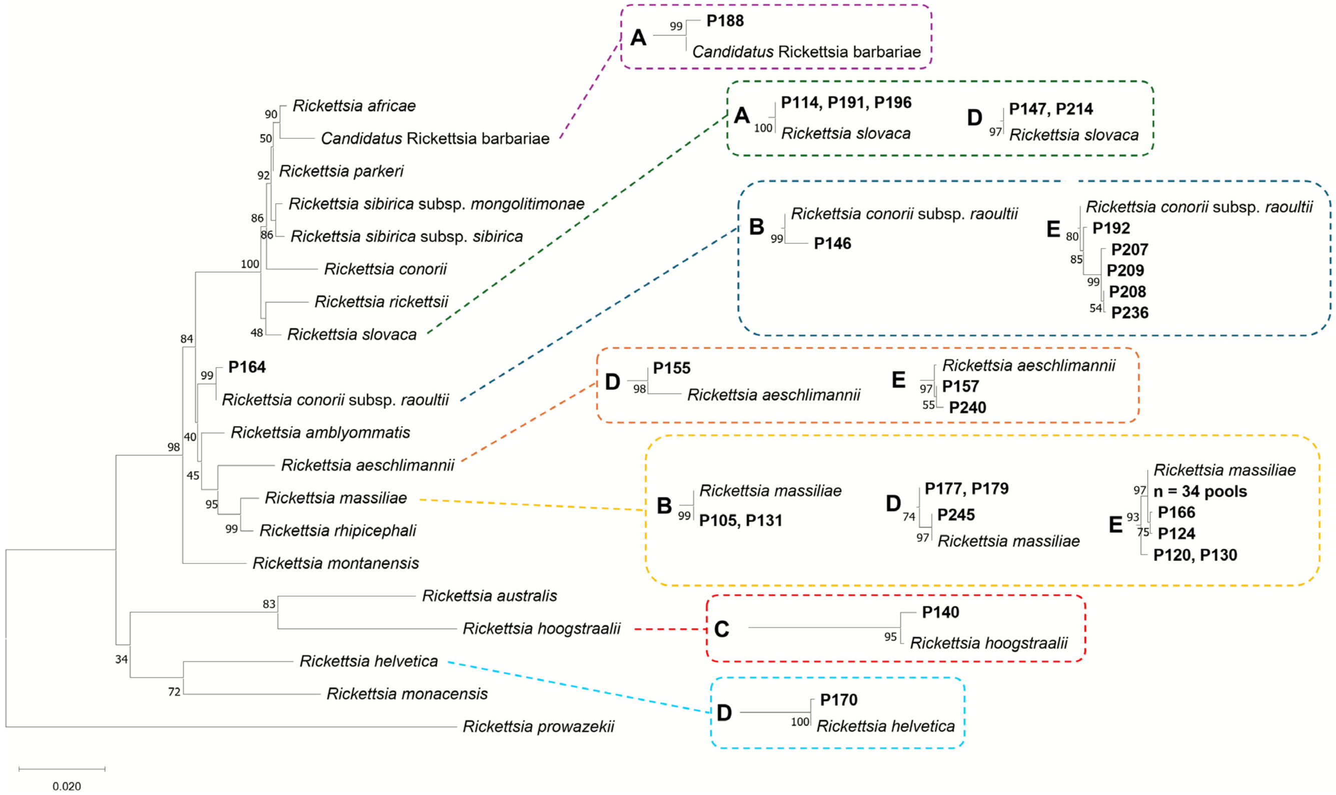

FIGURE 3 | Phylogenetic analysis of the Spotted Fever Group *Rickettsia* sequences identified in our study (highlighted in bold) along with reference sequences from GenBank. A reference tree (shown on the left) was built using nucleotide fragments of Rickettsia-specific genes (atpA, ompA, recA and gltA; 1858 positions). Since not all genes were amplified from every sample, additional alignments and phylogenetic trees were constructed using subsets of genes as follows: (A) atpA, ompA and recA (1517 positions); (B) ompA and recA (894 positions); (C) atpA and gltA (964 positions); (D) atpA and recA (968 positions); and (E) ompB and recA (889 positions). The phylogenetic position of each *Rickettsia* isolate inferred from the additional trees (A–E) is mapped on the reference tree, with *Rickettsia prowazekii* as an external outgroup. Numbers refer to bootstrap values.

FIGURE 4 | Phylogenetic analysis of *Borrelia burgdorferi* s.l. using a partial nucleotide fragment of flagellin (flaB, 344 positions) gene. Reference sequences of complete genomes and isolates from samples obtained in Spain were used, with *Borrelia miyamotoi* as an external outgroup. The sequence obtained in this study is highlighted in bold. Numbers refer to bootstrap values.

of the project. This included personal visits to each participating school and additional efforts by the researchers to emphasise the importance of ticks. It is noteworthy that schoolchildren from 19 rural schools in various locations across Aragón managed to collect nearly 400 ticks within just three months. Furthermore, aside from rangers, schoolchildren were the only group to collect ticks from all types of hosts. Many children in rural areas likely had prior knowledge of ticks, as evidenced during our visits, which may have contributed to both the large number and diversity of ticks they collected. Additionally, we believe that the children's natural curiosity and eagerness to learn, as observed during the school visits, played a key role, as previously highlighted by Makuch and Aczel (2018). While data collected by children are often considered lower quality (Makuch and Aczel 2018), our study demonstrated that children can serve as a reliable source of tick samples.

As expected, hunters provided the largest number of wildlife samples, although these results were heavily influenced by the considerable number of *Rhipicephalus pusillus* ticks collected from rabbits. Schoolchildren also contributed wildlife tick samples, although these were typically collected by a parent during hunting activities and preserved at the child's request.

Nearly all of the ticks were collected from mammals, which is not unexpected, as most companion animals, livestock, and game species belong to this taxonomic group. Furthermore, the handling of birds is typically limited to researchers, bird ringers and other specialists, with the exception of game birds, which were not represented in our samples. Amongst mammals, dogs were the most common host, which was anticipated due to their close association with humans and the fact that dogs in rural areas often do not receive regular prophylactic care (Millán et al. 2009). The European wild rabbit was the most represented wild species, as it is the primary small game species in Spain (MITECO 2024). Interestingly, however, the second most common mammal host was the roe deer (*Capreolus capreolus*). This species, which was absent from Aragón 30 years ago, is now experiencing rapid expansion in the region (Acevedo et al. 2005; González et al. 2013). Our study confirms that the roe deer is now an important host for ticks in the region.

4.2 | Ticks Collected and Pathogens Detected

The ticks collected in this study corroborate previous reports, particularly regarding the triad *Rhipicephalus*–*Hyalomma*–*Ixodes*, which are well-established as key markers of different biogeographical regions (Estrada-Peña et al. 2025). These findings also reinforce the Mediterranean association of both *D. marginatus* and *R. bursa* (Estrada-Peña et al. 1992, 1995). While the connection between *R. bursa* and Mediterranean-type environments is widely supported by several studies (Bursali et al. 2012; Coipan et al. 2011; Yeruham et al. 1985), *D. marginatus* has been reported in central Europe (Rubel et al. 2016; Walter et al. 2016), suggesting that its ecological niche may be broader than that of other species considered strictly adapted to semi-arid environments, such as *R. bursa* or *H. marginatum*. Further, *H. punctata* is a widely distributed tick, with a patchy distribution in Europe. Specimens of this tick in Spain have been collected in areas of degraded forest, surrounded by large grass areas, commonly restricted to northern parts of the territory in wet sites (Estrada-Peña et al. 2025), which are similar to the findings reported in this study.

Although our survey was limited to only three bacterial groups amongst the many tick-borne pathogens of potential interest, we found a relatively high number of positive ticks and tick pools. Most of the detected pathogens had not been previously reported in the region, likely due to a lack of surveillance. *Rickettsia massiliae*, a member of the spotted fever group, was the most commonly detected bacterium. It was first isolated from *R. turanicus* ticks in southern France (Beati and Raoult 1993). We previously suggested that the original identification of this bacterium may not have been in a tick belonging to *R. turanicus*, as this species is absent from Europe west of Greece, and that the newly described *R. hibericus* might play a role in the transmission of *R. massiliae* (Millán et al. 2024), although this has yet to be demonstrated in the laboratory. The pathogenic potential of *R. massiliae* for humans was confirmed (Vitale et al. 2006). Other rickettsiae detected during our survey are also pathogenic to humans, such as *R. slovaca*, which causes the DEBONEL/TIBOLA syndrome and has been previously reported in Spain (e.g., Ibarra et al. 2006). Four of the five positive ticks were *D. marginatus*, a known vector (Špitalská et al. 2012), including one from a human. Interestingly, the fifth case was in an adult *I. ricinus* collected from a cat in the Pyrenees. While this does not prove vector competence, it indicates that the agent was present either in the tick or in the host's blood. Other detected species are also zoonotic pathogens, such as *R. aeschlimannii* (Raoult et al. 2002) and *R. helvetica* (Fournier et al. 2000), or have been previously detected in ticks removed from humans in other parts of Spain, such as *R. conorii* subsp. *raoultii* and *Ca. R. barbariae* (Vieira Lista et al. 2024). Special mention should be made of the detection of *R. hoogstraalii* in a questing *H. punctata*. This agent was first described in *H. sulcata* ticks collected from sheep and goats in Croatia in 2006 (Duh et al. 2010). Although this bacterium had already been detected in the Canary Islands, near the west African coast (Abreu-Yanes et al. 2023), our survey marks its first detection in the Iberian Peninsula (Mediterranean Europe), further supporting the utility of this citizen science approach for surveillance.

Regarding the only *Borrelia* identified to species level, we detected *B. valaisiana* in an adult *I. hexagonus* from a dog in the Pyrenees (northern part of the region). This bird-associated agent had previously been detected in northern Spain (Gil et al. 2005; Palomar et al. 2017). In this case, as with *R. slovaca* DNA found in *I. ricinus* feeding on a cat, it is possible that these ticks acquired the bacteria in an earlier life stage.

5 | Conclusion

In an era of climate change and emerging diseases, up-to-date information on tick distribution, host interactions, and associated pathogens is essential. However, the economic and human resources required for comprehensive data collection are often prohibitive. Despite limitations in time and scope, citizen science has proven to be highly effective in identifying new tick species and pathogens in the region. Furthermore, this project played a key role in raising awareness about the health risks posed by ticks and the importance of preventive measures.

Author Contributions

Javier Millán: writing – original draft, writing – review and editing, conceptualisation, funding acquisition, methodology, visualisation. **Ruth Rodríguez-Pastor:** writing – review and editing, data curation, methodology. **Clara Muñoz-Hernández:** writing – review and editing, data curation, methodology. **Marta Sánchez-Sánchez:** writing – review and editing, data curation, methodology. **Alberto Moraga-Fernández:** writing – review and editing, data curation, methodology. **Natalia Fernández-Ruiz:** writing – review and editing, methodology. **Isabel G. Fernández de Mera:** resources, writing – review and editing. **Agustín Estrada-Peña:** writing – review and editing, conceptualisation, methodology, visualisation.

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Ethics Statement

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that supports the findings of this study is available in the Supporting Information of this article.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Database of pathogens identified in ticks collected during a citizen science project in Aragón, N Spain. **Table S2:** Database of ticks collected during a citizen science project in Aragón, N Spain.