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Seasonal Dynamics and Predilection Sites of Ticks (Acari: Ixodidae) Feeding on Cows in the Western Parts of the Djurdjura, Algeria

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The present study aimed to determine the phenology and predilection sites of ticks infesting cattle in the western region of Djurdjura (North Algeria) from November 2018 and October 2020. Nineteen cattle farms located in thirteen localities within four provinces were visited monthly for two years. Among the 289 examined cattle, 64.36 ± 2.81% (n=189) were infested by ticks. Of the 10,243 collected ticks, the most abundant tick species was *Rhipicephalus bursa* (31.57 ± 0.46%), followed by *R. annulatus* (31.26 ± 0.45%), *Hyalomma marginatum* (10.67 ± 0.30%), *H. lusitanicum* (7.02 ± 0.25%), *H. excavatum* (5.52±%0.22), *H. scupense* (4.27 ± 0.19%) and *H. impeltatum* (3.32 ± 0.17%). Ticks of the *Hyalomma* species were present throughout the year but in a limited number during the winter. *H. scupense* and *H. impletatum* showed similar activity from March to October and peaked in April and July, respectively (P<0.001). *H. lusitanicum* and *H. excavatum* were active from April to November and both peaked in September and October respectively. *Rhipicephalus bursa* was collected from April to August and *R. annulatus* from July to October. *R. turanicus* was active between April and June. *Hyalomma* genus had an affinity of attachment for the inguinal region (P<0.001). All parasitic stages of *R. annulatus* had an affinity for the necks (P<0.001). Nymphs of *R. bursa* prefer the neck (P<0.001), the adults attach to the perennial and inguinal regions (P<0.001). *R. turanicus* attach to the ears (P<0.001). As far as we know, this is the first study on the phenology and predilection sites of cattle ticks in the west region of Djurdjura in Algeria.

Keywords: predilection sites, cattle, Djurdjura, phenology, tick, Algeria

INTRODUCTION

Ticks are the most important hematophagous ectoparasites of livestock. They attach to their hosts at preferred sites before taking their blood meals. Several factors influence this attachment, including coat colour, skin thickness, hair length, host odour, environmental factors related to seasonal changes, and the length of the tick's mouthparts (1, 2). *Rhipicephalus turanicus* attaches to the head, immature stages of *R. bursa* attach to the thighs and tail, and adults prefer the udder quarters and the perineum. *Hyalomma marginatum*, *H. excavatum*, *H. lusitanicum* and *H. scupense* attach preferentially to the posterior udder quarters and perineum (3, 4). In tropical areas, *R. sulcatus* and *R. senegalensis* have a tropism for the auricular conch, while *H. truncatum* and *H. rufipes* prefer the ano-genital region (5).

The attachment of ticks to the animal's body causes serious losses to the cattle industry (6). The direct effects of blood-sucking lead to reduced weight gain and anaemia, while the implantation of the rostrum also reduces hides quality. Indirect effects of ticks are the injection of salivary toxins, which cause toxicosis and transmit several pathogens (7).

Eight hundred and ninety-six species of ticks have been described worldwide; they are divided into three families. The Nuttalliellidae are monotropic, with only one species *Nuttalliella namaqua*, the Argasidae totalling 193 species and Ixodidae that consists of 702 species (8, 9). So far, 26 Ixodid species were reported to be present in Algeria (3, 10–14). Among these species, some transmit to cattle several pathogens belonging to *Anaplasma*, *Babesia* and *Theileria* genera (15–17). It is well established that several tick species transmit the Crimean-Congo haemorrhagic fever virus, which was reported in Algeria (18).

The humid Mediterranean climate of the Djurdjura region has four distinct seasons, which favours the development of a perennial flora. More than 42% of the total area of the region consists of forests of Atlas cedar, holm oak, cork oak, holly, mountain maple, Montpellier maple, field maple, *Prunus avium*, Zea oak, black pine, Aleppo pine, yew, and wild and common olive (19). This region is a habitat of a varied fauna of birds, rodents, lagomorphs, carnivores and other mammals (20). Senevet and Rossi (21) listed eight species of ticks that parasitize livestock. Sergent et al. (22) reported the seasonality of these species. These ticks are obligate parasites of animals with a complex developmental cycle, which requires the availability of several hosts (23). Djurdjura region is an agricultural area; livestock farming is the main activity of the population; the semi-intensive cattle system allows for meat and milk production. Natural grasslands and concentrates constitute the bulk of the diet of cattle. Ticks and tick-borne diseases, especially piroplasmosis, are a particular concern for farmers and veterinarians (19, 24). Effective tick control programmes require the determination of the phenology and predilection sites of ticks to use appropriate acaricides (7). Implementation of tick control programmes requires a good knowledge of the ecology of ticks and the epidemiology of tick-associated infections. However, to the best of our knowledge, no comprehensive survey of ticks infesting cattle has been conducted in the western part of the Djurdjura region of northern Algeria. Therefore, we aimed in the present study to determine the identity, seasonal activity, and predilection sites of ticks infesting cattle in the western part of Djurdjura, Algeria.

MATERIAL AND METHODS

Study Area

The study was conducted in the western part of the Djurdjura, North central Algeria. It is a large area (8,905 km^2) with a landscape interspersed with valleys and mountains. Thirteen localities within four provinces were included in the present study, namely Blida, Boumerdes, Bouira and Tizi Ouzou (**Figure 1**).

Climate and Vegetation

The western part of Djurdjura has a Mediterranean climate. Its annual rainfall varies between 600 and 1.200 mm, with a relative humidity of 50%–75% during the summer season. The region is sub-humid to humid with hot and dry summers and cool and rainy winters. The temperature reaches 35°C in summer and drops to 5°C in winter. The region has four seasons, winter (December to February), spring (March to May), summer (June to August) and autumn (September to November). The northwest winds generate heavy rainfall accompanied by cold waves. It is one of the most wooded regions in Algeria and is the shelter of varied flora and fauna (19, 20).

Farms and Animals

Nineteen semi-intensive farms with a herd size of more than 15 animals were randomly selected for tick's collection. A total number of 289 cows; were monthly examined for the ticks collection between November 2018 and October 2020. The animals graze during the day, and in the evening, they return to the farm. The grazing season extends from February to November. The animals examined were Holstein (n=45, 15.6 ± 2.13%), Montbeliard (n=65, 22.5 ± 2.45%) and crossbred (n=179, 61.9 ± 2.85%). Among the 289 examined cows aged between 1 and 14 years, 109 (37.72 ± 2.85%) were less than 18 months old, and 180 (62.28 ± 2.85%) were over 18 months old. Only animals not treated with acaricides, and present at all visits were included in the study.

Tick Collection

The cattle body was divided into seven anatomical areas (**Figure 2**); the head (head and ears), the neck (neck, dewlap and brisket), the back (withers, back and rump), the thorax (thorax and flank), the inguinal region (belly and inguinal region), the perennial region (perineum, anus and tail) and the legs (four legs and two thighs). All ticks visible on each anatomical region were collected manually, and then stored in labelled tubes containing 70% ethanol. Ticks were transported to the Regional Veterinary Laboratory of Tizi Ouzou. They were identified to species level, developmental stages and sex, and counted accordingly to the key of Estrada Pena et al. (23, 25). All ticks with damaged mouthparts and morphological anomalies were discarded.

FIGURE 1 | Geographic presentation of the study area. Map **(A)** show the location of the western parts of the Djurdjura. Map **(B)** shows the study area and the red points highlight the localities where ticks were collected.

Statistical Analysis

The statistical analyses were carried out in two steps using R software version 4.0.4 (The R Foundation, 1020 Vienna, Austria). The results obtained in this survey were estimated and compared using Bayesian approaches. The comparison of the tick's infestation according to the seasons, months and predilections sites was performed by Chi-square and Fisher's exact tests (the difference was considered significant at 5% threshold). The affinity groups of tick species according to the parasitic stage, sex, season and predilection sites was done by the multiple

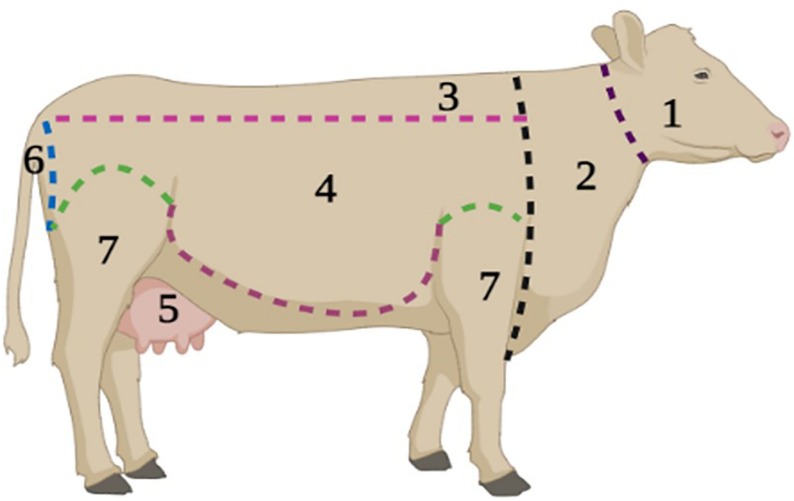

FIGURE 2 | Anatomical subdivision of the cow body for tick's collection in the western part of the Djurdjura, North Central Algeria, between November 2018 and October 2020. 1. Head, 2. Neck, 3. Back, 4. Thorax, 5. Inguinal, 6. Perennial region, 7. Legs.

correspondence analysis (MCA). The MCA graphically represents the relationship between several categorical datasets.

RESULTS

Of the 289 examined cattle, 186 animals were infested by ticks, corresponding to an overall infestation rate of 64.36 ± 2.81%. The rate of infestation is significantly higher (P<0.001) in cattle aged >18 months old (n= 5734, 55.98 ± 0.49) compared to those aged <18 months (n= 4509, 44.02 ± 0.49%). There was no significant difference in infestation rate between exotic (Montbeliard and Holstein) (n=83, 44.62 ± 3.64%) and crossbred (n=103, 55.38 ± 3.64%).

Collected Ticks

A total number of 10,243 ticks were collected during the 24 visits, consisting of 77 (0.75 ± 0.08%) larvae, 2,450 (23.92 ± 0.41%) nymphs and 7,716 (75.33 ± 0.42%) adults. The monthly infestation rate was high, ranging from 2.85% in January to 13.34% in June. The sex ratio of adult ticks (M: F) was 1.66. The number of ticks per animal ranged from zero to 61 and 95 in May and July, respectively. Five tick genera were collected; *Rhipicephalus* (66.74 ± 0.46%) and *Hyalomma* (30.83 ± 0.45%) were the most abundant ticks compared to *Ixodes* (1.64 ± 0.002%), *Dermacentor* (0.70 ± 0.001%) and *Haemphysalis* (0.09 ± 5.80%). Among the listed genera, twelve species were identified and showed a variable infestation rate according to the seasons, of which seven species are perennial and five seasonal (**Tables 1**, **2**).

Seasonal Ticks

The five seasonal ticks showed varying levels of infestation rate by month and season (**Tables 1**, **2**). *R. turanicus* was most active between March and July (**Table 1**), with its peak occurring in May (P<0.001, **Table 2**). Four *H. rufipes* were collected during summer (**Tables 1**, **2**). Infestation with *I. ricinus* peaked in October, following a period of significant activity between October and January (P<0.001 **Tables 1**, **2**). *Dermacentor marginatus* was found on cattle between September and February and peaked in October (P<0.001, **Table 1**, **2**). Only nine specimens of *Hae. punctata* were collected from October to January and peaked in October (**Tables 1**, **2**).

Perennial Ticks

Monthly and seasonal infestation rates of the perennial ticks were presented in **Tables 1** and **2**. *Rhipicephalus bursa* (31.57 ± 0.46%) and *R. annulatus* (31.26 ± 0.45%) have the highest infestation rate (**Table 1**). The adult activity of *R. bursa* is from March to November, with a peak in June. The abundance of this stage is significantly high in April, June, July and August (P<0.001, **Tables 1** and **2**). The lower larval activity occurred between November to January and from March to April. However, the nymph activity was observed from October to April and peaked in December (P<0.001, **Tables 1**, **2**). Adults *R. annulatus* occurs throughout the year with significant activity between July and October (P<0.001, **Tables 1**, **2**); this stage peaked in July and September. Larvae were active from July to November and peaked in September. Nymphs peaked in August with high activity from July to October (P<0.001, **Tables 1**, **2**).

Hyalomma species occurs all the year; each species has a monthly and seasonal infestation rate. They were active in spring, summer and autumn compared to the winter (P ≤ 0.05, **Table 2**). *H. marginatum* was the most collected *Hyalomma* species with significant activity from March to August and reached a peak in May (P<0.001, **Tables 1**, **2**). *H. lusitanicum* and *H. excavatum* show a similar pattern of activity from February to October (P<0.001) and peaked respectively in September and October (**Tables 1**, **2**). *H. scupense* and *H. impletatum* showed the same pattern of activity from March to October; both ticks peaked respectively in July and April (P<0.001, **Tables 1**, **2**).

Distribution of Ticks on the Cattle Body

The 12 tick species collected share the same predilection sites with mixed infestations ranging from five to eleven tick species on the same anatomical site. According to the MCA, the tick population is homogeneous, and their spatial distribution on the cattle body depends on the season, the species and the parasitic stages (**Figure 3**).

The MCA identified three groups of ticks according to the qualitative variables studied (**Figure 4**). The larval and nymphal stages of *R. annulatus* attach to the neck in autumn (**Table 3** and **Figure 4**). In contrast, adults of this species have a high frequency of attachment to the neck in autumn and the inguinal region in summer (**Table 3**).

The nymph of *R. bursa* has an affinity for attachment to the neck and back in winter (**Table 3** and **Figure 4**). The adult attaches to the perineum in summer and the inguinal in spring (**Table 3**). All *R. turanicus* ticks were collected from the head region, especially the ears in spring (**Table 3** and **Figure 4**).

Hyalomma species had a similar spatial distribution; they have a strong affinity for the inguinal region and a lesser degree for the perineum and legs. According to MCA, *H. impeltatum* and *H. marginatum* are spring ticks with the inguinal region as their predilection site (**Figure 4** and **Table 3**). *H. excavatum* and *H. lusitanicum* are autumnal ticks with an affinity of attachment for legs (**Table 3** and **Figure 4**). The adults of *I. ricinus* prefer the inguinal region, and *D. marginatus* prefer to attach to the head, neck and back (**Table 3**).

DISCUSSION

Effective control measures against ticks and tick-borne diseases; requires a detailed account of the distribution, seasonal dynamics, and predilection sites of ticks on livestock. The latter is crucial because ticks choose areas that are non-accessible to grooming and licking, rich in blood vessels, dark and hidden to avoid predators and deceive the vigilance of owners (4, 7). The nature of the host coat can influence the attachment site preference of different tick species, as can the seasons (26); knowing the attachment site of ticks allows us to recommend the appropriate acaricide and application method with its optimal concentration (4, 27).

Rhipicepahlus turanicus was collected from the head with a relative abundance of 79.25 ± 2.02% of specimens in-ears in spring and 20.75 ± 2.02% in early summer (P<0.001; **Table 1**). Similar results were reported in western (3, 10) and eastern

TABLE 1 | Number and monthly distribution of tick species (number. % ± SE) identified in the western part of the Djurdjura, North Central Algeria, between November 2018 and October 2020.

Ticks species	Dec.	Jan.	Feb.	March	April	May	June	July	Aug.	Sep.	Oct.	Nov.	Total
D. marginatus	7	12	8	0	2	2	1	0	0	2	22	16	72
	9.72 ± 3.49	16.66 ± 4.38	11.11 ± 3.70		2.77 ± 1.93	2.77 ± 1.93	1.38 ± 1.37			2.77 ± 1.93	30.5 ± 5.42	22.22 ± 4.89	0.70 ± 0.08
I. ricinus	6	9	3	1	1	1	0	0	0	0	95***	52***	168
	3.57 ± 1.43	5.35 ± 1.73	1.78 ± 1.02	0.59 ± 0.59	0.59 ± 0.59	0.59 ± 0.59					56.54 ± 3.82	30.9 ± 3.56	1.64 ± 0.12
H. punctata	1	2	0	0	0	0	0	0	0	0	4	2	9
	11.11 ± 10.04	22.22 ± 13.79									44.44 ± 16.56	22.22 ± 13.79	0.09 ± 0.02
H. excavatum	1	2	4	5	38	38	18	53	65***	154***	172***	15	565
	0.17 ± 0.17	0.35 ± 0.24	0.70 ± 0.35	0.88 ± 0.39	6.72 ± 1.05	6.72 ± 1.05	3.18 ± 0.73	9.38 ± 1.22	11.5 ± 1.34	27.25 ± 1.87	30.44 ± 1.93	2.65 ± 0.67	5.52 ± 0.22
H. scupense	0	1	0	33	70***	40	97***	117***	49	10	18	2	437
		0.22 ± 0.22		7.55 ± 1.26	16.01 ± 1.75	9.15 ± 1.37	22.2 ± 1.98	26.77 ± 2.11	11.21 ± 1.5	2.28 ± 0.71	4.11 ± 0.94	0.45 ± 0.32	4.27 ± 0.19
H. impletatum	2	1	2	40	63***	55***	45	47	29	34	18	4	340
	0.5 ± 0.38	0.29 ± 0.29	0.5 ± 0.38	11.67 ± 1.74	18.52 ± 2.1	16.2 ± 1.99	13.23 ± 1.83	13.82 ± 1.87	8.52 ± 1.51	10 ± 1.62	5.29 ± 1.21	1.17 ± 0.58	3.32 ± 0.17
H. lusitanicum	4	2	14	18	39	50	36	55	102***	178***	176***	45	719
	0.56 ± 0.27	0.28 ± 0.19	1.95 ± 0.51	2.50 ± 0.58	5.42 ± 0.84	6.95 ± 0.94	5.01 ± 0.81	7.65 ± 0.99	14.19 ± 1.3	24.76 ± 1.6	24.48 ± 1.6	6.26 ± 0.90	7.02 ± 0.25
H. marginatum	0	1	2	38	232***	440***	188***	116***	50	0	23	3	1093
	0,00	0.09 ± 0.09	0.18 ± 0.12	3.48 ± 0.55	21.23 ± 1.23	40.26 ± 1.48	17.20 ± 1.14	10.61 ± 0.93	4.57 ± 0.63	0,00	2.10 ± 0.43	0.27 ± 0.15	10.67 ± 0.30
H. rufipes	0	0	0	0	0	0	0	0	4	0	0	0	4
	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	100,00	0,00	0,00	0,00	0.04 ± 0.019
Adults_*R. annulatus*	28	41	38	106	63	13	11	397***	298***	670***	533***	82	2280
	1.23 ± 0.23	1.80 ± 0.27	1.67 ± 0.26	4.65 ± 0.44	2.76 ± 0.34	0.57 ± 0.15	0.48 ± 0.14	17.41 ± 0.79	13.07 ± 0.70	29.39 ± 0.95	23.38 ± 0.88	3.60 ± 0.39	22.26 ± 0.41
Larvae_R. annulatus	0	0	0	0	0	0	0	2	21***	32***	1	1	57
	0,00	0,00	0,00	0,00	0,00	0,00	0,00	3.51 ± 2.43	36.84 ± 6.38	56.14 ± 6.57	1.75 ± 1.73	1.75 ± 1.73	0.56 ± 0.07
Nymph_*R. annulatus*	3	1	4	26	16	0	0	34	390***	273***	114***	4	865
	0.35 ± 0.20	0.12 ± 0.11	0.46 ± 0.23	3.01 ± 0.58	1.85 ± 0.45	0,00	0,00	3.93 ± 0.66	45.09 ± 1.69	31.56 ± 1.58	13.18 ± 1.15	0.46 ± 0.23	8.44 ± 0.27
Adults_*R. bursa*	0	0	0	1	186***	42	891***	290***	195***	11	11	2	1629
	0,00	0,00	0,00	0.06 ± 0.06	11.42 ± 0.78	2.58 ± 0.39	54.70 ± 1.23	17.80 ± 0.94	11.97 ± 0.80	0.68 ± 0.20	0.68 ± 0.20	0.12 ± 0.08	15.9 ± 0.36
Larvae_ *R. bursa*	5	1	0	2	4	0	0	0	0	0	0	8	20
	25.00 ± 9.68	5.00 ± 4.87	0,00	10.00 ± 6.70	20.00 ± 8.94	0,00	0,00	0,00	0,00	0,00	0,00	40.00 ± 10.95	0.20 ± 0.04
Nymph_*R. bursa*	398***	219***	245***	177***	147***	0	0	0	2	0	33	364***	1585
	25.1 ± 1	13.80 ± 0.86	15.50 ± 0.90	11.20 ± 0.79	9.30 ± 0.72	0,0	0,0	0,0	0.10	0,0	2.10 ± 0.36	23.00 ± 1	15.47 ± 0.35
R. turanicus	0	0	0	6	105^{NS}	206^{NS}	79^{NS}	4	0	0	0	0	400
	0	0	0	1.5 ± 0.6	26.25 ± 2.19	51.50 ± 2.49	19.75 ± 1.99	1.00 ± 0.49	0	0	0	0	3.91 ± 0.19
Total	455	292	320	453	966	887	1366	1115	1205	1364	1220	600	10243
	4.44 ± 0.20	2.85 ± 0.16	3.12 ± 0.17	4.42 ± 0.20	9.43 ± 0.28	8.66 ± 0.27	13.33 ± 0.33	10.89 ± 0.30	11.76 ± 0.31	13.32 ± 0.33	11.91 ± 0.31	5.86 ± 0.23	

*N, number of collected ticks; %, Rate of infestation; SE, standard error; ***P, <0.001; NS, Not significant.*

Algeria (12), Morocco (28), Tunisia (29), Egypt (30), Turkey (31), Republic of Guinea (32), Zimbabwe (33) and southern Europe (23). *R. turanicus* was reported with a relative abundance of 82.30%, and they likely prefer to feed on wild boar (34). This species is not known as the main vector of any pathogens for cattle (23) but transmits *Hepatozoon canis* (35). It causes a warm and painful oedematous reaction of the auricular concha in infested sheep (our unpublished data).

TABLE 2 | Seasonal distribution of tick's species (N, % ± SE) identified in the western part of the Djurdjura, North Central Algeria, between November 2018 and October 2020.

Tick species	Season			
	Winter	**Spring**	**Summer**	**Autumn**
D. marginatus	27	4	1	40**
	37.50 ± 5.70	5.56 ± 2.70	1.39 ± 1.37	55.56 ± 2.69
I. ricinus	18	3	0	147**
	10.71 ± 2.38	1.79 ± 1.00	0	87.50 ± 2.5
H. punctata	3	0	0	6
	33.33 ± 15.70	0	0	66.67 ± 15.70
H. excavatum	7	81 ^{NS}	136**	341**
	1.24 ± 0.46	14.34 ± 1.44	24.07 ± 1.79	60.35 ± 2.05
H. scupense	1	143**	263**	30
	0.23 ± 0.22	32.72 ± 2.23	60.18 ± 3.32	6.86 ± 1.20
H. impletatum	5	158**	121**	56
	1.47 ± 0.65	46.47 ± 2.70	35.59 ± 2.59	16.47 ± 2.01
H. lusitanicum	20	107**	193**	399***
	2.78 ± 0.13	14.88 ± 1.32	26.84 ± 1.64	55.49 ± 1.85
H. marginatum	3	710***	354**	26
	0.27 ± 0.15	64.96 ± 1.43	32.39 ± 1.41	2.38 ± 0.45
H. rufipes	0	0	4	0
	0	0	100.00	0
Adults_*R. annulatus*	107^{NS}	182 ^{NS}	706**	1285***
	4.70 ± 0.43	7.98 ± 0.56	30.96 ± 0.96	56.36 ± 1.03
Larvae_ *R. annulatus*	0	0	23*	34*
	0	0	40.35 ± 6.49	59.65 ± 6.49
Nymph_ *R. annulatus*	8	42 ^{NS}	424**	391**
	0.92 ± 0.32	4.86 ± 0.72	49.02 ± 1.69	45.2 ± 1.68
Adults _*R. bursa*	0	229**	1376***	24
	0	14.06 ± 0.85	84.47 ± 0.89	1.47 ± 0.29
Larvae_ *R. bursa*	6 ^{NS}	6 ^{NS}	0	8**
	30.00 ± 10.20	30.00 ± 10.20		40.00 ± 10.95
Nymph_ *R. bursa*	862	324**	2	397**
	54.38 ± 1.25**	20.44 ± 1.01	0.13 ± 0.08	25.05 ± 1.02
R. turanicus	0	317**	83	0
	0	79.25 ± 2.02	20.75 ± 2.02	0
Total	1067	2306**	3686***	3184***
	10.41 ± 0.30	22.51 ± 0.41	35.99 ± 0.47	31.08 ± 0.45

*N, number of collected ticks; %, Rate of infestation; SE, standard error; *P, ≤0.05; **P, <0.01; ***P, <0.001; NS, Not significant.*

The activity of *R. bursa* in the western part of the Djurdjura is similar to that reported in Mila and El Tarf (12), north-central of Algeria (24) and Tiaret (10). However, these results are different from those reported in the western part of the country (3) and Morocco (28). The predilection site of *R. bursa* on cattle varies according to the season and the parasitic stage. Immatures are sensitive to desiccation, their activity starts from late autumn to beginning spring (**Table 2**), as was reported by Boulkaboul (10), they settle on sun-exposed areas such as the neck and back, as it seems that the low winter temperatures are unfavorable to their engorging. The adults are active from late spring to late summer and settle on the perineum and inguinal area, which corresponds to observations reported in the western part of the country (3).

The cattle tick *R. annulatus* is active all the year, shared between the different parasitic stages. This seasonal activity is favoured by the humid climate of the study area. Our results are similar to those reported in the eastern part of Algeria (12), Tunisia (36), Libya (37) and Southern Europe (23). However, the *R. annulatus* activity in the Djurdjura region is different from that reported in the western part of the country (3) and Morocco (28).

The number of larvae of *R. annulatus* and *R. bursa* collected from cattle is low because these parasitic stages prefer to feed on small mammals, which are numerous in the Djurdjura region (20), and their small size deceives our vigilance. The larvae and nymph of *R. annulatus* feed on the neck of cattle of the Djurdjura. Whereas they attached on the perineal region in the cattle of the western Algeria (3). This difference in predilection site is probably due to the hot-humid climate in the west and the cold-humid climate in the study area. In contrast, *R. bursa* immatures stages feed on the neck and back, similar to the results reported by Yousfi Monod et al. (3).

Gueye et al. (5) reported that the parasitic stages of *R. decoloratus* attach to the dewlap, while those of *B. microplus* prefer the neck of tropical cattle (7). Our results confirm the above findings and highlight that *R. annulatus* have an affinity for the neck and dewlap in the Mediterranean region (**Table 3**). The low temperatures slow down metamorphosis and development and increase the mortality of ticks, especially immature stages (38). Below a certain temperature threshold, these processes do not take place, and the ticks are in a lethargic state or die (38). The parasitic stages of *R. annulatus* have an affinity for the neck and dewlap because these anatomical areas

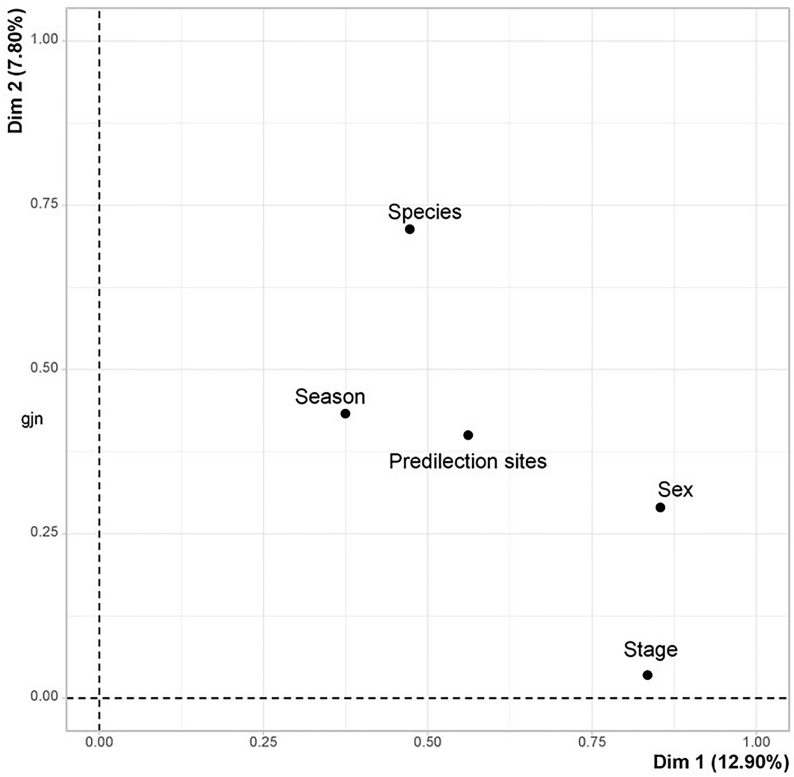

FIGURE 3 | Factorial design of the qualitative variables studied in the western part of the Djurdjura, North Central Algeria, between November 2018 and October 2020.

are exposed to the sun. We have collected *R. annulatus* from the thighs of cattle as reported in the western part of the country (3).

Our results demonstrated that species belonging to the genus *Hyalomma* represent the most abundant ticks on cattle in the Djurdjura region. They were observed throughout the year and are abundant in the summertime than in winter; similar to reported results in the Maghreb (3, 4, 10, 12, 28, 39). Because of the climatic difference between regions in Algeria, *H. marginatum* reached its peak in May in the Djurdjura region in February in the west (10) and in April in the eastern part of Algeria (12). This species is the vector of the virus causing Crimean-Congo haemorrhagic fever in humans (23). *H. marginatum* is closely associated with wild animals, while larvae and nymphs feed on a wide range of ground-feeding birds and medium-sized mammals, and adults prefer large animals (40).

Hyalomma scupense appear on cattle in late spring with a peak in July and disappear by the end of August in Algeria (3, 10, 12, 24). We have observed synchronisation between the *H. scupense* activity and clinical cases of tropical theileriosis (15, 24, 41). A few adult males of *H. scupense* were collected from September to early November as reported in Turkey (31). The larvae and nymphs feed on cattle, in autumn, from September to November. This tick species is often associated with livestock barns, stables, sheds and pens (4). We could not find larvae and nymphs of *H. scupense*. Probably, these parasitic stages are engorged and detached for winter diapause. In addition, the farmers clean their stables twice a day and at the same time, they bring out the Immatures in the dung.

H. lusitanicum is significantly more abundant than *H. excavatum* ($P<0.001$). Both species have been reported in the Maghreb (3, 10, 12, 28). There is a period from the beginning of October to mid-November, during which we have confirmed many clinical cases of tropical theileriosis in Algeria (our unpublished data). This period coincides with the activity of both species (**Tables 1** and **2**). These two tick species are likely natural vectors of *Theileria annulata*, especially as the adults of *H. scupense* are not active during this period. *H. lusitanicum* was suspected to be a vector of *T. annulata* in Algeria (12) and Spain (42).

Hyalomma rufipes was reported for the first time in the study area. The immature feed on rodents, birds and lagomorphs, while the adults feed on herbivores (43). Therefore, it seems that the probability of detecting this species on domestic ruminants is very low for this broad host spectrum by taking one or two samples per month. It could also explain, at least in part, the relatively low number of *H. rufipes* reported in the present study, which is similar to results reported in the western part of Algeria (10) and Turkey (31).

H. impeltatum was reported in North Africa, the Arabian Peninsula, and the Middle East as far as Pakistan. It is also found in sub-Saharan Africa from Senegal and Mauritania in the west to Eritrea, Djibouti and Somalia in the east. It extends to Cameroon in central Africa and Tanzania in the east (44). We collected this

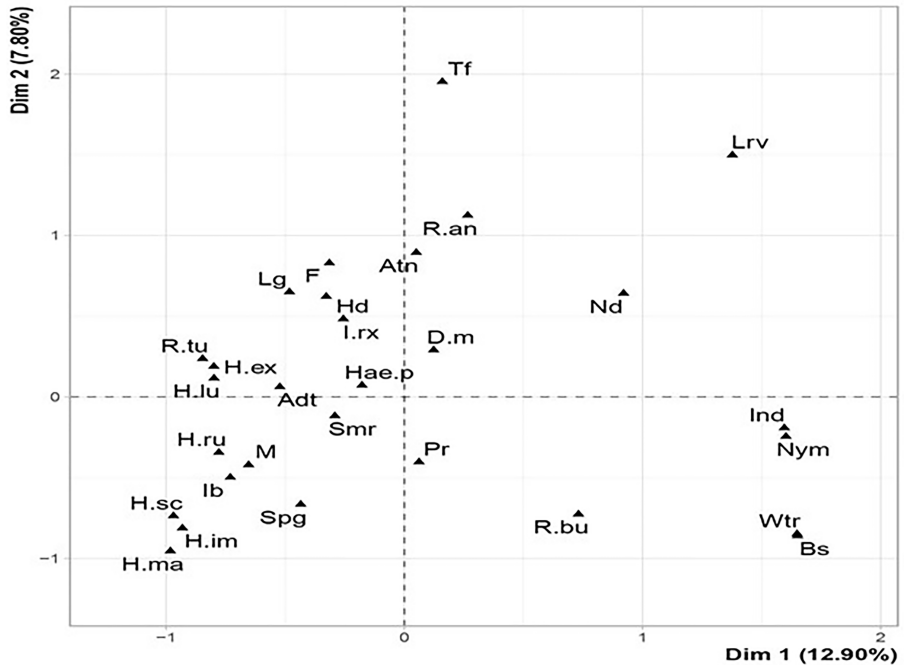

FIGURE 4 | Factorial design of body distribution, seasonality, parasitic stage and tick species in the western part of the Djurdjura, North Central Algeria, between November 2018 and October 2020. Hd, Head; Pr, Perennial region; D.m., Dermacentor marginatus; H.ex., Hyalomma excavatum; H.ma., Hyalomma marginatum; R.an., Rhipicephalus annulatus; Adt, Adulte; Bs, Back; Atn, Autumn; Ib, Inguinal; Wtr, Winter; I.rx., Ixodes ricinus; H.sc, Hyalomma scupense; H.ru., Hyalomma rufipes; R.tu., Rhipicephalus turanicus; F, Female; Nd, Neck; Spg, Spring; Ind, Unidentified.

species from March to October, and it peaked between April and May. In the western part of the country, *H. impeltatum* peaked in June (3). On the other hand, *H. impeltatum* was collected in dromedary with a relative abundance of 19.04% (11).

Dermacentor marginatus is found in the cold and humid parts of the Mediterranean region associated with the Atlas Mountains (45). We collected *D. marginatus* from September to February with a peak in January similar to results reported from Tlemcen (3) and Morocco (28). *D. marginatus* was collected in wild boars from Mostaganem to Tlemcen. This tick ecologically prefers areas with dense bushes and oak and pine trees (3). *Ixodes ricinus* appears on cattle for a short period in autumn and winter, with a peak in October similar to the results reported in Tlemcen (3) and in El Tarf (12).

Yousfi-Monod et al. (3) state that adult *Hyalomma* and *Ixodes* attach in the udder quarters of cows (4). The predilection sites of the six adults *Hyalomma* identified in this work is similar for all the species (**Table 2**). They have a marked preference for the inguinal region. In heavy infestations, *H. marginatum*, *H. lusitanicum* and *H. impletatum* prefer the perianal area. In contrast, *H. excavatum* and *H. scupense* prefer the legs. Our results corroborate the finding reported in the western part of Algeria (3). However, during heavy infestations of the inguinal region by adults of *I. ricinus*, the remaining individuals prefer to attach themselves to the head and neck (**Table 2**).

Some of the identified tick species in the western part of the Djurdjura have already been associated with the transmission of pathogens, such as *Theileria annulata*, *T. buffeli*, *Babesia bovis*, *B. bigemina*, *Anaplasma marginale* (46, 47) as well as in other regions of the country (12, 15, 17, 48) and North Africa in general (36, 49, 50).

CONCLUSION

Since the work conducted in the Pasteur Institute of Algeria during the last century (21, 22, 51), this is the first report describing the intensity, predilection sites, monthly and seasonal activity of twelve species of cattle ticks in the western part of the Djurdjura in Algeria.

Among the identified ticks, eight species are particularly frequent and widespread throughout the study area; *H. marginatum*, *H. lusitanicum*, *H. excavatum*, *H. impeltatum* are annual with medium infestations. However, *R. bursa* and *R. annulatus* achieve massive infestations.

The larvae and nymph of *R. bursa* parasitize cattle in autumn and winter, and those of *R. annulatus* are active in summer and autumn respectively. *R. annulatus* adults ticks are autumnal, and *R. turanicus* are spring ticks. In contrast, adults of *H. scupense* and *R. bursa* are summer ticks.

Three tick species, *D. marginatus*, *I. ricinus* and *Hae. punctate* are parasites of cattle in winter. These cold-climate ticks are adapted to the warm Mediterranean climate of North Africa and have reversed their seasonal activity pattern compared to Europe and have localized to the humid regions of northern Algeria (23).

TABLE 3 | Body distribution of tick species (number, % ± SE) identified in the western part of the Djurdjura, North Central Algeria, between November 2018 and October 2020.

Tick species	Stage	Head	Neck	Back	Thorax	Inguinal region	Perennial region	Legs	Total
D. marginatus	Adult	26**	26**	15**	0	2	3	0	72
		36.11 ± 9.41	36.11 ± 9.41	20.83 ± 4.78	0	1.78 ± 1.55	4.17 ± 2.35		0.70 ± 0.08
I. ricinus	Adult	15	22	1	2	121***	7	0	168
		8.93 ± 2.20	13.10 ± 2.60	0.60 ± 0.59	1.19 ± 0.83	72.02 ± 3.46	4.17 ± 1.54		1.64 ± 0.12
H. punctate	Adult	0	0	0	0	5	4	0	9
		0	0	0	0	55.56 ± 16.56	44.44 ± 16.56		0.09 ± 0.02
H. excavatum	Adult	0	0	3	3	365***	76	118***	565
		0	0	0.53 ± 0.29	0.53 ± 0.29	46.60 ± 2.04	13.45 ± 1.39	20.88 ± 1.66	5.52 ± 0.22
H. scupense	Adult	0	0	2	2	356***	30	47	437
		0	0	0.46 ± 0.32	0.46 ± 0.32	81.46 ± 1.85	6.86 ± 1.20	10.67 ± 1.47	4.27 ± 0.19
H. impletatum	Adult	0	1	0	0	261	57	21	340
			0.29 ± 0.29	0	0	76.76 ± 2.29	16.76 ± 2.02	6.18 ± 1.30	3.32 ± 0.17
H. lusitanicum	Adult	0	0	1	1	467***	136***	114***	719
		0	0	0.14 ± 0.13	0.14 ± 0.13	64.95 ± 1.77	18.92 ± 1.46	15.86 ± 1.36	7.02 ± 0.25
H. marginatum	Adult	0	0	0	6	802***	139***	146***	1093
		0	0	0	0.55 ± 0.22	73.38 ± 1.33	12.72 ± 1.00	13.36 ± 1.02	10.67 ± 0.30
H. rufipes	Adult	0	0	0	0	2	2	0	4
		0	0	0	0	50.00 ± 25.00	50.00 ± 25.01	0.00	0.04 ± 0.019
R. annulatus	Adult	176	955	124	143	385	249	248	2280
		7.72 ± 0.55	41.89 ± 1.03	5.44 ± 0.45	6.27 ± 0.50	16.89 ± 0.78	10.92 ± 0.65	10.88 ± 0.62	22.26 ± 0.41
	Larvae	1	41	6	5	2	0	2	57
		1.75 ± 1.73	71.93 ± 5.95	10.53 ± 4.06	8.77 ± 3.74	3.51 ± 2.43	0.00	3.51 ± 2.43	0.56 ± 0.07
	Nymph	47	534**	42	48	46	80	68	865
		5.43 ± 0.77	61.73 ± 1.65	4.86 ± 0.73	5.55 ± 0.77	5.32 ± 0.76	9.25 ± 0.98	7.86 ± 0.91	8.44 ± 0.27
R. bursa	Adult	9	24	1	7	699***	759***	130***	1629
		0.55 ± 0.18	1.47 ± 0.29	0.06 ± 0.06	0.43 ± 0.16	42.91 ± 1.22	46.59 ± 1.23	7.98 ± 0.67	15.9 ± 0.36
	Larvae	0	16	3	0	0	0	1	20
		0.00	80.00 ± 8.94	15 ± 7.98	0	0	0	5.00 ± 4.87	0.20 ± 0.04
	Nymph	83	722***	493***	4	95	163***	25	1585
		5.24 ± 0.55	45.55 ± 1.25	31.1 ± 1.16	0.25 ± 0.12	5.99 ± 0.59	10.28 ± 0.76	1.58 ± 0.31	15.47 ± 0.35
R. turanicus	Adult	392***	8	0	0	0	0	0	400
		98.00 ± 0.70	2.00 ± 0.7.00	0	0	0	0	0	3.91 ± 0.19
Total		749	2349***	691	221	3608***	1705***	920	10243
		7.31 ± 0.25	22.93 ± 0.41	6.74 ± 0.24	2.15 ± 0.14	35.22 ± 0.47	16.65 ± 0.36	8.98 ± 0.28	

*N, number of collected ticks; %, Rate of infestation; SE, standard error; **P, <0.01; ***P, <0.001.*

All parasitic stages of *R. annulatus* had an affinity for the necks. Nymphs of *R. bursa* prefer the neck the adults attach to the perineum and inguinal region. *R. turanicus* attach to the ears. The *Hyalomma* species had an affinity for the inguinal and perennial region.

Despite the Covid 19 pandemic, we reported a high diversity of cattle ticks in the western parts of Djurdjura, and the molecular identification will support this work. Due to the bioclimatic gradient of Algeria, the study needs to be replicated in other biotopes of the country.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by The Ethics Committee of the Directorate of Veterinary Services. Written informed consent for participation was not obtained from the owners because Veterinary clients made their animals available to us for tick collection. No handling was done.

AUTHOR CONTRIBUTIONS

AB: conceptualization, data curation, methodology, and writing-original draft. RK: conceptualization and investigation. BM: software, methodology, and visualization. TK: conceptualization, investigation, methodology, and visualization. FS: validation and visualization. GT: validation and visualization. HZ: conceptualization, data curation, formal analysis, methodology, funding acquisition, project administration, software, supervision, and writing-review & editing. All authors analysed, reviewed, and edited the manuscript's final version and approved it for publication.

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