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Morphological and Molecular Identification of Acari: Ixodidae in Cattle, and Their Effect on Production.

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Abstract

This study examined the distribution and impact of Ixodid ticks on cattle in Sulaymaniyah Province, Iraq, utilizing both morphological and molecular identification methods. A total of 1,447 ticks were collected from 714 cattle across various districts, including Darbandikhan, Sharazur, Germian, and Qaradax, over three seasons: spring, summer, and autumn from August 2024 to May 2025. The highest infestation rate was observed in spring (58%), followed by summer (45%) and autumn (30%). No hard ticks were discovered on cattle during the winter months during this study. Morphological analysis identified two tick genera: Hyalomma (98%) and Rhipicephalus (2%). Molecular analysis of ITS2 rDNA regions confirmed the presence of one species of genus *Hyalomma*, *Hyalomma anatolicum* and two species of genus Rhipicephalus, *Rhipicephalus bursa*, and *Rhipicephalus sanguineus*. Statistical analysis using t-tests and Z-tests for proportions highlighted significant seasonal discrepancies. Infestation rates varied significantly by season and geography, particularly among imported cattle, with Darbandikhan showing the highest prevalence. A questionnaire survey conducted with 100 cattle owners revealed high awareness of tick presence and associated health issues, including skin irritation and signs of tick-borne diseases. All respondents reported using chemical acaricides, though 30% suspected treatment resistance.

Keywords: Identification, Molecular Technique, Hyalomma. Rhipicephalus, Cattle.

I. Introduction

Hard ticks, from the family Ixodidae, are essential ectoparasites feeding on both humans and animals. They are distinguished by their small, flattened oval bodies and a tough dorsal shield known as the scutum. As members of the class Arachnida, these ticks possess eight legs, segmented bodies, and specialized blood-feeding mouthparts (Klompen *et al*., 2000; Sonenshine & Roe, 2014). The size of adult ticks typically ranges between 3 and 5 mm, depending on their species, sex, and whether they have fed. Their anatomy is divided into two primary sections: the idiosomic, which includes the legs and internal organs, and the gnathostome, which comprises specialized mouthparts for skin penetration and blood extraction without inflicting pain (Walker *et al*, 2003; Jongejan and Uilenberg, 2004). Ticks feed slowly over several days and are capable of transmitting numerous pathogens during this process. The tick life cycle spans several months to years and may involve one, two, or three different hosts, depending on species and environmental conditions (Perumalsamy *et al*., 2024)

Tick populations worldwide are expanding due to climate change and human land-use changes (Dantas-Torres, 2015; Pollet *et al*., 2020). leading to more tick-borne diseases in livestock, wildlife, and humans (Marcelino *et al*., 2012; Kabir *et al*., 2019). The infestation of ticks led to complications in animals, resulting in skin irritation, weight loss, and decreased production (Al-Saeed *et al*., 2010; Vannier and Krause, 2020). Over 250 million cattle face threats from diseases such as Theileria annulata (Leemans *et al*., 1999; Erdemir *et al*., 2012). Crimean-Congo hemorrhagic fever is another dangerous viral disease transmitted by ticks (Bernard *et al*., 2024). Economic losses from tick infestations

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amount to billions of dollars in countries like India, Brazil, and Mexico (Lane *et al*., 2015; Strydom *et al*., 2023). Ticks of the genera Hyalomma, Rhipicephalus, Haemaphysalis, Amblyomma, and Dermacentor are commonly observed in Iraq and the Kurdistan region, with identification often achieved through both morphological and molecular methods(Falih *et al*., 2022; Nubgan *et al*., 2023). The area's geographic diversity, from valleys to highlands, provides a favorable environment for tick proliferation (Forti et al., 2021). However, the local epidemiology and distribution of tick species, along with the diseases they transmit, remain under-investigated (MolecularDetection of Hyalomma spp. Isolated from Sheep and Camel in the AL-Samawah Desert of AL-MuTthanna Province, IRAQ, 2024). Identifying tick species through molecular and morphological methods is crucial for comprehending the local tick population, determining their involvement in spreading diseases, and devising effective management strategies. Simultaneously, evaluating farmers' knowledge and practices about tick infestations can inform public health and veterinary actions (Giunchetti *et al*., 2025; Makwarela *et al*., 2023).

This study seeks to identify and categorize Ixodid ticks that affect cattle in the Sulaymaniyah Province of Iraq using both morphological and molecular methods, and evaluate cattle owners' understanding, experiences with infestations, and tick management practices via a structured survey. The results will aid in developing focused strategies to reduce the effects of tick infestations on cattle health and productivity in the area. Nonetheless, data on the types of hard ticks present in this region and their distribution remains insufficient; it is crucial to accurately classify this ectoparasite and examine its influence on both humans and animals.

II. Materials and Methods

Study area

The research took place in various zones within the Al-Sulaymaniyah province of Iraq, such as the southern and southeastern areas, along with the Sharazur area, Kalar, Darbandikhan, Qaradagh, and Halabja. During the period from August 2024 to May 2025.

Ticks' collection

From both domestic and imported cow breeds, which were housed in communities and small farms, with an average of 1 to 100 heads per farmer, 1447 ticks in their nymph and adult stages were gathered, the animals' ticks were carefully removed from various body areas, primarily from the udder and between the back legs, using tweezers to detach them from animals (Kocon *et al*., 2022). The process was carried out in all four seasons of the year to differentiate the presence of the ticks in different seasons, then kept and preserved in a plastic container inside 70% ethanol (Ammazzalorso *et al*., 2015).

Questionnaire survey

A questionnaire survey targeting farmers and cattle owners in the area. Participants were chosen based on their readiness to engage and their active role in cattle farming. The survey included both local and international cattle breeds. The responses collected were recorded, summarized, and analyzed to detect common trends in knowledge, observed symptoms, and control methods.

Identification by Morphology

Following the procedures described by Walker *et al*. (2003) and (Estrada-pena *et al*., 2018) in their tick identification investigations, the ticks were transferred to the agricultural college laboratory's Animal Science department and

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evaluated using a light microscope for morphological identification. The gathered samples were classified into the genera Hyalomma and Rhipicephalus. Each tick underwent separate examination using a microscope. The differentiation between genera was primarily based on size, coxae, mouthparts, scutum, legs, adanal plates, and pigmentation.

Molecular Identification

- **DNA Extraction**

Utilizing the DNA extraction tissue kit from Addbio and adhering to the manufacturer's instructions, genomic DNA was isolated from individual ticks. A small sample, such as legs or half of the tick's body, was placed into a 1.5 mL microcentrifuge tube. The samples underwent homogenization with proteinase K and lysis buffer and were then incubated at 56°C for a period ranging from 30 minutes to 1 hour. The obtained DNA was purified and eluted in 50–100 µL of elution buffer and subsequently stored at −20°C until further use.

- **PCR Amplification of the ITS2 Region**

The amplification of the Internal Transcribed Spacer 2 (ITS2) section of rDNA was carried out employing tick-specific degenerative primers. The forward primer (ITS2F) has the sequence 5´-YTGCGARACTTGGTGTGAAT-3', and the reverse primer (ITS2R) is 5´-TATGCTTAARTTYAGSGGGT-3 (Abdigoudarzi *et al*., 2011).

Statistical analysis

Seasonal variations in tick infestation rates between domestic and foreign cattle breeds were assessed statistically using t-tests. Statistical significance was determined at a significance level of $p < 0.05$. To determine whether the mean infection levels throughout each season differed significantly from reference values or other times, the t-test was utilized. Additionally, Z-tests were used to compare the infestation rate proportions between seasons, enabling a more thorough analysis of notable variations over time.

III. Result

Throughout the year, 704 cattle of some cattle breeds, from Al-Sulaymanyah province, were examined for the detection of hard tick (Ixodidae) infestation across various areas, namely Darbandikhan, Sharazur, Germian, and Qaradax. not appear "Ixodidae" of cattle in winter, as shown in Table 1. The prevalence rate of tick infestation was 47% during different seasons. Investigate the high distribution of ticks in spring, accounting for 67%, followed by summer and autumn at 40% and 34%, respectively.

Table 1: Prevalence of ticks from local cattle in different locations in Sulaymaniyah province, with infestation rate

Season	Examined head	Infested	%
Summer	179	72	40%
Autumn	113	38	34%
Spring	131	88	67%
Total	**423**	**198**	**47%**

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During the Table.2: A seasonal variation in tick infestation was observed among foreign cattle. Out of the 291 cattle examined, 128 (44%) were found to be infested. The infestation rates peaked in summer (67%), then decreased in spring (46%) and further in autumn (28%).

Table 2: Prevalence of ticks from foreign cattle in different locations in Sulaymaniyah province, with infestation rate

Season	Examined head	Infested	%
Summer	85	57	67%
Autumn	94	26	28%
Spring	92	42	46%
Total	**291**	**128**	**44%**

Throughout the study period, 423 local cattle breeds were examined, with 198 showing signs of infestation, corresponding to an infestation rate of 47% in all seasons, as the following, The spring season had the highest infestation rate in cattle was 67% of the 131/ 88, with the highest percentage in Kalar region 82%, followed the summer season 40%, with the highest percentage investigate in Qaradaxr 46%, the lowest infestation detection in autumn was 34%, with the highest rats in Kalar egion were 45%, while in the winter season, we didn't notice hard ticks on the cattle's bodies, Infestation was considerably higher in the summer ($t = 12.590$; $p < 0.05$) and spring ($t = 7.848$; $p < 0.05$) for local breeds, but not in the fall ($t = 3.016$; $p > 0.05$), according to t-test results. These results were corroborated by Z-test analysis, which showed that infection in the spring was much higher than in the summer and fall ($p < 0.001$). These results are described in Table 3.

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Table 3: Prevalence of ticks in local cattle in different locations in Sulaymaniyah province.

Location	Examined cattle	Infested cattle	%
summer			
Darbandikhan	39	17	43%
Kalar	79	28	35%
Halabja	48	21	43%
Qaradax	13	6	46%
Overall	179	72	40%
autumn			
Darbandikhan	30	11	36%
Kalar	42	19	45%
Halabja	22	5	22%
Qaradax	19	3	15%
Overall	113	38	34%
spring			
Darbandikhan	39	22	56%
Kalar	46	38	82%
Halabja	31	20	64 %
Qaradax	15	8	53%
Overall	131	88	67%
Total	**423**	**198**	**47%**

In Table 4. Shows, 291 foreign cattle breeds were examined, with 128 showing with hard ticks, corresponding to an prevalence rate of 44% in all seasons, as the following, The spring season had the highest infestation rate in cattle was 67% of the 131/ 88, with the highest percentage in Kalar region 82%, followed the summer season 40%, with the highest percentage investigate in Qaradaxr r 46%, the lowest infestation detection in autumn was 34%, with the highest percentage in Kalar region were 45%, while in the winter season, we didn't notice hard ticks on the cattle's bodies. The t-test results showed that infestation was statistically significant for foreign cattle in all seasons ($t = 28.567$ in the spring, $t = 6.428$ in the summer, and $t = 4.805$ in the fall), with $p < 0.05$. According to the Z-test results, the infestation was much larger in the summer than in the fall ($p < 0.001$).

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Table 4: Prevalence of ticks in foreign cattle in different locations in Sulaymaniyah province

location	Examined cattle	Infested head, %	
	Summer		
Darbandikhan	21	18	85%
Kalar	15	5	33%
Halabja	57	32	56%
Qaradax	12	5	41%
Overall	85	57	67%
	autummn		
Darbandikhan	20	2	10%
Kalar	17	9	52%
Halabja	44	11	25%
Qaradax	13	4	36%
Overall	94	26	28%
	spring		
Darbandikhan	19	17	89%
Kalar	22	8	36%
Halabja	37	12	32%
Qaradax	14	5	35%
Overall	92	42	46%
Total	**291**	**128**	**44%**

According to Figure 1. Shown the site of tick attachment, some anatomical parts of cattle are more likely to become infested by ticks than others, with 31% of infestations, the udder was the most often found site among the 1,447 ticks that were gathered. The inner thighs (27%), followed by the ears (8%), other places (5%), and the lowest notice in under belly (4%), were less common attachment sites.

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Fig. 1

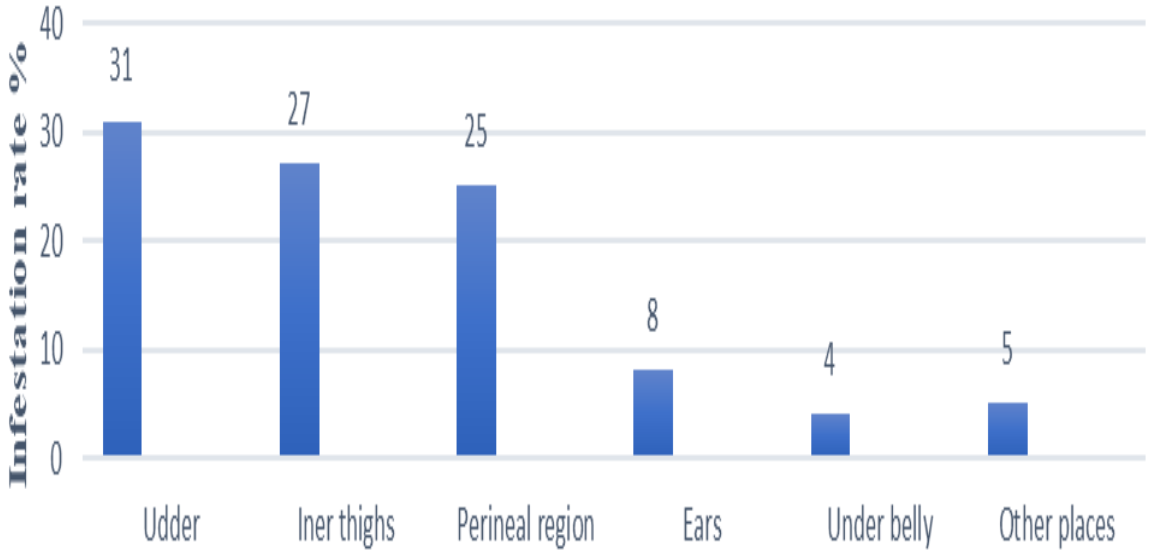


Figure 1 Distribution of tick attachment sites on cattle

During the Morphological study of the hard ticks, it shows that the Male Hyalomma ticks are characterized by their extended mouthparts and the presence of three pairs of adanal plates, with conspicuous dark adanal plates encircling the anal groove. Conversely, Rhipicephalus ticks usually have shorter mouthparts and only two pairs of adanal plates, figures 2 and 3

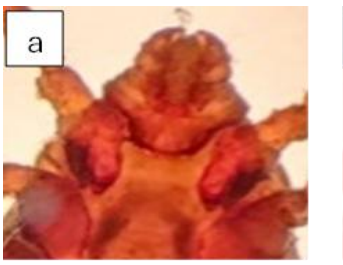
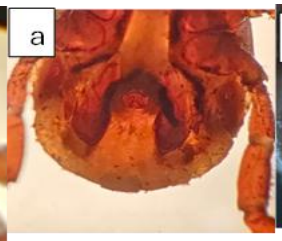


Figure.2 Mouth parts in bouth Hyalomma and Rhipicephalus, a mouthpart of male Rhipicephalus, b mouthpart of male Hyalomma genera



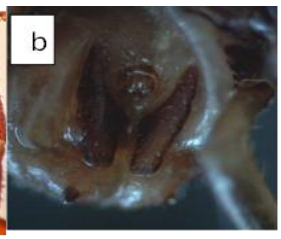


Figure.3 Adanal plates in Hyalomma and Rhipicephalus ticks, F3a ale Rhipicephalus tick with tow paire of adanal plates, b male yalomma ticwith three paire adanal plate

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Table 5 shows the two genera of Ixodidae, Hyalomma and Rhipicephalus, and investigates the genus Hyalomma, 98%; the highest number collected follows the genus Rhipicephalus, 2%

Table 5: Morphological identification and distribution of hard ticks in different seasons.

Type of ticks	Summer	Autumn	Spring	**Total**	**%**
Hyalomma spp	557	329	537	1423	%98
Rhipicephalus spp	-	24	-	24	%2
Over all	**557**	**353**	**537**	**1447**	**%100**

PCR Amplification of the ITS2 Region

The amplification of the Internal Transcribed Spacer 2 (ITS2) section of rDNA was carried out employing tick-specific degenerative primers. The forward primer (ITS2F) has the sequence 5´-YTGCGARACTTGGTGTGAAT-3', and the reverse primer (ITS2R) is 5´-TATGCTTAARTTYAGSGGGT-3 (**Abdigoudarzi et al., 2011).**

PCR (Polymerase Chain Reaction) examination.

During the Polymerase Chain Reaction examination. The ITS2 region of rDNA was successfully amplified from all tick DNA samples using degenerate primers ITS2F and ITS2R. Clear, single bands were observed in 1.5% agarose gel electrophoresis, with product sizes ranging between 450 to 1300 base pairs, depending on the tick species. The ITS2 primer set is reported to give bands broadly in the (450–1300) bp window for tick detection (this is a common working range. Estimated species-specific ITS2 amplicon ranges you supplied: *Hyalomma anatolicum anatolicum*: (273–960 bp), Rhipicephalus spp.202–916 bp (Abdigoudarzi *et al*., 2011).

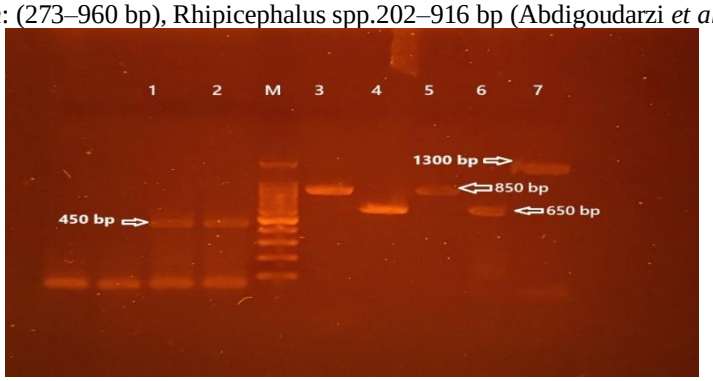

Figure 4: Gel electrophoresis of ITS2 PCR products for tick spp. Lane M: molecular weight marker (bands shown from 45-1300 bp). Lanes 1–7: PCR products amplified with ITS2 primers. Estimated species-specific ITS2 amplicon size ranges used for interpretation: *Hyalomma anatolicum anatolicum* 273–960 bp; Rhipicephalus spp. 202–916 bp.

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In Table 5, A questionnaire was conducted among 100 cattle owners in the region to assess their observations and experiences related to tick infestation. The majority (70%) reported observing ticks on cattle daily, with ticks most frequently located on the udder (100%), ears (70%), and other body parts (100%). All respondents indicated that ticks were visibly detectable. Common complications included skin irritation (70%), other health problems (70%), and signs of tick-borne diseases (70%), while only 20% reported weight loss in infested animals. In terms of control measures, all participants used chemical acaricides, while 50% used powder treatments and 40% employed other control methods. Notably, 30% believed tick resistance to treatment had developed, while 70% were uncertain. These findings highlight the widespread awareness of tick presence and associated health issues among livestock owners, as well as a reliance on chemical control methods (Table 6).

Table 6. Questionnaire survey among cattle owners about tick infestation and complication on Cattle

Survey Question	Answered with Yes	Percentage (%)
Observed Ticks Daily	70	70
Observed Ticks Monthly	30	30
Ears	70	70
Udder	100	100
Other Places	100	100
Visible Ticks	100	100
Skin Irritation	70	70
Weight Loss	20	20
Other Signs	50	50
Chemical Acaricides	100	100
Powder Treatment	50	50
Other Control	40	40
Tick Resistance: Yes	30	30
Tick Resistance: Not Sure	70	70
Tick-borne Diseases	70	70
Other Health Problems	70	70

IV. Discussion

This research details a morphological categorization alongside a seasonal examination of Ixodidae ticks gathered from cattle across different parts of Sulaymaniyah province, Iraq. We collected 1,447 ticks from 714 cattle, revealing infestation in 45% (326 of 714) of the assessed animals. Including both indigenous and imported breeds, the study identified a distinct seasonal pattern in tick infestation rates. Unlike in warmer periods, ticks were absent during winter, highlighting a lack of tick activity in colder months within this area, same result mentioned by (Bedouhene *et al*., 2022), that Hyalomma ticks were absent in winter or very rare in this season. The greatest infestation rate appeared in spring, consistent with findings by (Mohammed *et al*., 2019), impacting 58% of the cattle observed (130 out of 223), with 537 ticks collected. Summer followed, with 45% of the cattle (132 out of 284) infested and a collection of 557 ticks, the largest quantity documented. In autumn, infestation rates decreased to 30% (64 out of 207), with 353 ticks recorded. These results verify that tick engagement and infestation levels reach their peak in the warmer seasons, notably spring

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and summer. Similar seasonal trends have also been reported by (Banafshi *et al*., 2018), and reduced with cooler weather, in accord with Sulaymaniyah's semi-arid climate characteristics.

Morphological analysis of the collected samples confirmed that the Hyalomma genus was predominant, constituting 98% (1,223 out of 1,447) of the ticks, whereas Rhipicephalus accounted for a mere 2% (24 out of 1,447), consistent with findings by (Kakabwa *et al*., 2021) in Iraq. Hyalomma ticks were identified by their larger size, longer mouthparts, and three sets of adanal plates, compared to the Rhipicephalus ticks, which featured smaller bodies, shorter mouthparts, and only two sets of adanal plates. These distinct morphological features allowed for precise identification using light microscopy and established taxonomic keys. Regional tick distribution also varied; during the summer, Darbandikhan and Halabja experienced the highest infestation rates (58% and 50%, respectively), while in the spring, Kalar and Darbandikhan had high rates (68% and 67%). These differences may result from variations in microclimates, livestock density, and farm hygiene practices within the surveyed regions.

Among the 423 local cattle assessed, 198 were found to have infestations, resulting in an overall prevalence rate of 47%. This is exactly what (Shubber *et al*., 2014) found in the middle and south of Iraq, in cattle infestation by ticks, mostly by Hyalomma species. The peak infestation rate was in the spring, impacting 67% of the animals, while summer saw a 40% infestation rate. The lowest rate, at 34%, was observed in autumn. These results are somewhat similar to those reported by (Shanan *et al*., 2017) in 12 provinces of Iraq, concerning the diversity and seasonal tick infestation in cattle. Favorable climatic circumstances, such as warmer temperatures and more vegetation, are linked to the noticeable rise in ticks in the spring, when the majority of cattle owners in this area breed their animals outdoors with a pasture grazing system. This increases tick activity and facilitates interaction with hosts and pasture, which are preferred by ticks in this season (Davari *et al*., 2017). On the other hand, the decrease in autumn might be due to cooler temperatures and a drop in tick numbers.

Among the 291 foreign cattle tested, 128 were found to be infested, equating to a total prevalence rate of 44%. The infestation reached its peak in the summer, impacting 67% of the cattle, a finding also noted by (Omer *et al*., 2007), with spring showing a 46% rate. The lowest prevalence was observed in autumn, at 28%. The summer peak might be attributed to favorable environmental conditions that encourage tick reproduction and survival. This could also be related to the breeding practices in these regions, where foreign cattle are housed rather than grazed or reared outdoors, making the housing environment more conducive for tick proliferation. foreign breeds that. The reduced infestation in autumn could be associated with a seasonal decline in tick activity as temperatures drop.

Regarding the prevalence of location differences, in spring, all four locations showed a clear increase in infestation among local and foreign cattle, which were preferred by all types of Ixodidae ticks. across the four surveyed locations. Infestation rates peaked in spring, particularly in Kalar (82%) and Halabja (64%), while autumn showed the lowest overall rates, with Qaradax notably low at 15%. Summer infestation was moderate across all sites, with Darbandikhan, Halabja, and Qaradax showing similar levels (43- 46%). Overall, Kalar consistently exhibited higher infestation levels, whereas Qaradax recorded the lowest in most seasons. These patterns suggest that local climatic conditions and environmental factors play a significant role in tick activity and distribution.

Attachment site by ticks depends on the tick's preferred site. The highest infestation rate was recorded on the udder (31%), followed by the inner thighs (27%) and the perineal region (25%), as mentioned also by (L'Hostis *et al*., 1994) that these sites of cattle are more preferred by ticks. These areas are known by thin skin, higher temperature, and increased blood flow, which provide an optimal microenvironment for tick attachment and feeding. In contrast, less commonly infested sites included the ears (8%), underbelly (4%), and other body parts (5%). The predominance of

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infestations in the udder and surrounding regions is consistent with previous studies, which attribute this pattern to easier access for ticks in these areas and reduced grooming by the host.

Through the use of DNA sequencing and PCR amplification, the molecular identification component of this work effectively enhanced morphological inspection to offer precise species-level confirmation. The ITS2 region of rDNA, as described by Abdigoudarzi *et al.* (2011). The resulting PCR products showed that *Hyalomma anatolicum* was the sole species in this region 98% accompanied by *Rhipicephalus bursa* and *R. sanguineus*, 2%. This result contrasts with the Erbil study by (Aziz, 2022) which discovered that 79% of the cattle had Rhipicephalus and 21% had Hyalomma infestations. While the current study concurs with a study conducted in Sulaimani province (Mustafa, 2020), which found that the Hyalomma genus was the most prevalent tick species in cattle, along with the Rhipicephalus and Bophilus genera, it also concurs with a study conducted along the Iraq-Iran border by (Loui Monfared *et al*., 2015), which found that the infestation rate of cattle was 89% for *Hyalomma anatolicum* and 11% for Rhipicephalus bursa. The current study, which indicated that 70% of cattle were infested with Hyalomma and 20% with Rhipicephalus, is consistent with the Baghdad investigation by (Hasson, 2012).

The survey responses reinforced the observations made in the field. Seventy percent of cattle owners confirmed seeing ticks daily, with the udder (100%), ears (70%), and other body parts (100%) being the most common sites for tick attachment. Seventy percent of respondents noted skin irritation and symptoms of tick-borne diseases. Although weight loss was reported by only 20%, many (70%) observed additional health issues. For control measures, all respondents used chemical acaricides, 50% utilized powder treatments, and 40% applied various other methods. While 30% of the participants suspected treatment resistance, 70% remained uncertain. These findings imply that most cattle owners in the area are aware of the existence of ticks and their immediate consequences, which include skin irritation and common attachment sites. But the lack of awareness of long-term effects, such as weight loss, and the uncertainty around acaricide resistance suggest that tick-related difficulties are only partially understood, and that more education on sustainable control is necessary.

V. Conclusion

This study provides essential insights into the seasonal distribution and species identification of Ixodid ticks affecting cattle in Sulaymaniyah Province, Iraq. A total of 1,447 ticks were collected, with infestation rates highest in spring and summer. Morphological analysis showed a dominance of Hyalomma spp. (98%), and molecular identification using ITS2 sequencing confirmed the presence of *Hyalomma anatolicum*, Rhipicephalus bursa, and *R. sanguineus*. Farmer surveys revealed high awareness of tick presence but limited understanding of acaricide resistance.

VI. References

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