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NEW RECORD OF NEOEHRLICHIA MIKURENSIS AND CANDIDATUS NEOEHRLICHIA SP NEW EMERGING THREAT OF ZOONOTIC TICK-BORNE PATHOGEN IN PAKISTAN

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Abstract

Ticks serve as critical vectors of zoonotic pathogens, significantly impacting public health and livestock productivity worldwide. This study explored tick infestation and the prevalence of tick-borne pathogens in livestock from four regions of Pakistan: Bajaur, Khyber, Orakzai, and North Waziristan. Out of 635 examined animals (cattle, goats, and sheep), 384 (60.47%) were infested, yielding 588 ticks. Rhipicephalusmicroplus (32.3%) emerged as the predominant species, accompanied by various Hyalomma and Haemaphysalis species. For the first time in Pakistan, Neoehrlichiamikurensis(13.68%) and CandidatusNeoehrlichia sp. (6.41%) were detected, marking their emergence as significant zoonotic pathogens in the region. These findings underscore their potential threat to public health and livestock, necessitating immediate attention. Seasonal dynamics revealed peak tick activity from June to August, emphasizing the importance of seasonal surveillance and targeted control measures. This research highlights the intricate ecology of ticks as vectors and their pivotal role in disseminating emerging pathogens. Adopting the One Health approach, the study advocates for integrated epidemiological surveillance, advanced molecular diagnostics, and public awareness campaigns to mitigate the risks posed by tick-borne diseases. These findings provide crucial insights into the dual threat ticks pose to human and veterinary health in Pakistan and call for urgent action to curb their impact.

Keywords:
Zoonotic pathogens, Neoehrlichia mikurensis, Candidatus Neoehrlichiasp, Livestock productivity, One Health approach.

Introduction

Ticks (Acari: Ixodoidea) are most prevalent in the tropical and subtropical regions of the world, parasitizing almost all terrestrial and semi-aquatic vertebrates (Hussain et al 2021).Tick are the 2nd most important vector for diseases transmission after mosquitoes (Boulanger et al., 2019). Tick comprises a diverse group of arthropods mainly divided into 3 families namely Ixodidae (hard ticks), Argasidae (soft ticks), and Nuttalliellidae (having a single species) (Nicholson et al., 2019) Ten percent of all Ixodid and Argasid tick species are known to transmit disease to people and domestic animals (Eisen et al., 2022). In addition to spreading disease to animals, ticks can irritate skin during attachment, causing blood loss, bite wounds, and occasionally result in secondary bacterial infections and self-trauma (Kumar et al., 2024). Severe infestations can cause weight loss and anaemia, which lowers production (Hurtado et al., 2018).

Ticks are responsible for the transmission of various bacterial, protozoan and viral pathogens to various animals including humans (Rizzoli et al., 2014). Major bacterial pathogens carried by ticks include species in the genus Anaplasma, Ehrlichia, Rickettsia, and Borrelia (Lindsø et al., 2024,). Among tick-borne bacteria, interest in Neoehrlichiamikurensisand CandidatusNeoehrlichia sp. has recently increased (Hoberg et al., 2022,). These bacteria belong to the family Anaplasmataceae, obligate intracellular parasites whose vertebrate hosts include humans (Buysse et al., 2024).

N. mikurensis is opportunistic, primarily causing disease (referred to as neoehrlichiosis) in immunocompromised individuals, and was discovered on Mikura Island in Japan in rats and Ixodesovatus ticks (Kawahara et al., 2004). This pathogen had already been detected in various tick species including Ixodesricinus ticks (Boyer et al., 2021). Ixodesricinus is a primary vector of N. mikurensis in Europe, often infecting humans and animals (Pustijanac et al., 2024). Ixodespersulcatus in China, Japan, and Russia, found to carry N. mikurensis in its natural distribution range in Eurasia (Luan et al., 2023). Ixodes scapularis has been identified as a potential vector of N. mikurensisin north America.Its distribution seems to overlap with that of the two major vectors of Lyme borreliosis in Europe and Asia: I. ricinus and I. persulcatus, respectively. Moreover, several case reports have recently been reported from northern and central Europe and China (Portillo et al., 2018). The word Candidatus meant that the bacterium could not be grown. Ca. N. mikurensis has so far been found to infect seven tick species: Ixodesricinus, Ixodespersulcatus, Ixodesovatus, and Ixodes. Haemaphysalisconcinna, I. hexagonus, D. reticulatus, and frontalis (Wennerås et al., 2015). Ixodes. ricinus and Ixodes. persulcatus had the highest infection rates of Ca. N. mikurensis, suggesting that these ticks were the main vectors of the disease (Jenkins et al., 2019). However, as other ticks hardly ever carry Ca. N. mikurensis, their ability to transmit the disease is still up for debate. Ca. N. micurensis was successfully propagated in I. ricinus and I. scapularis cell lines up till 2019. There have been reports of infections targeting human vascular endothelial cells (Wass et al., 2019).

Despite its clinical significance, Neoehrlichiamikurensisremains under-documented in countries like Pakistan, where tick-borne diseases (TBDs) are often deprioritized in favor of other pressing health concerns (Ghafar et al., 2020). To date, ticks collected from (goat, sheep, cow, Buffaloes), in Pakistan have not been screened for NeoehrlichiamikurensisandCa. N. mikurensis. This study aims to address this gap by investigating ticks infesting goat, sheep, and cow in Pakistan for the presence of this pathogen.

1. Material and Methods

1.1.Study Area

The current research focuses on four districts situated in Khyber Pakhtunkhwa; namelyBajaur, Khyber, Orakzai, and North Waziristan. These

areas are characterized by mountains, rich green woods, deciduous wood lands, frozen grounds and human habitats. Due to the cloud cover the region experiences high precipitation rates and low evaporation, leading to moderate temperatures between -2 and 36°C. Also, variations of humidity range at a yearly level of 61%.

Tick collection preservation and identification

A total of 635 cattle were searched for the task of tick collection in 4 districts from February 2023 to January 2024. 384 host were positive for tick infestation. 171 cow, 112 goat, 101 sheep (Bajaur:97,Khyber 102,Orakzai: 92,North Waziristan: 93). Ticks were collected using a gentle tick removal procedure that included the use of tweezers to minimize any injury to both ticks and cattle. To minimize damage to tick's DNA, the ticks were washed with distilled water then left in Eppendorf tubes containing 70% ethanol and 5% glycerin. These tubes were labeled accordingly and left at the ambienttemperature for further examination. Details concerning the collected specimens such as locality, host, number of ticks collected from the host, and the date of collection were also documented. All collected ticks were morphologically identified and matched to life stages of R. microplusHy. anatolicum, Hy. excavatum, Hae. Sulcata. Hae. Bispinosa. Hy. scupense, Hae. Punctataincluding adult male, adult female, nymphs, and larvae using a stereo-zoom microscope following the morphological characteristics of (Walker, 2003),

1.2. DNA extraction

A total of 588 ticks including R. microplus(190)Hy. Anatolicum(96), Hy. excavatum (56), Hae. Sulcata(79), Hae. bispinosa (65), Hy. Scupense(58), Hae. punctata(44).were chosen for the extraction of genomic DNA. To extract DNA, these ticks were washed with distilled water and dried on sterile filter paper. Using a surgical blade to dissect each tick individually, the ticks were then crushed up into little pieces with a sterilized mortar and pestle. Following that, sterile 1.5 ml Eppendorf tubes were filled with the pellet. The classic phenol-chloroform approach was used to extract genomic DNA, adhering to the procedure described by Sambrook et al. (1989). A NanoDrop spectrophotometer was used to evaluate the extracted DNA's quality and quantity.

1.3. 12S rDNA and gltA gene Amplification

Using specific primers specified in Table 1, a segment of 460 bp of the 12S rDNA was amplified for the molecular identification of tick species. Additionally, screening was done for tick borne pathogensthrough amplification of the345bp partial fragment of the 16S rDNA. PCR reactions were carried out in total volumes of 25.6µL containing 13 µL of the Master mix, 0.4 pmol of the forward and reverse primers, 8 µL of nuclease-free PCR water and 2.4µL of the 50ng genomic DNA template. Negative controls were added by substituting PCR water for the DNA template,while as positive controls for the 12S primersutilized the IxodeskashmricusDNA.Previously amplified Rickettsia sp. 16S rDNA were used as positive control for the 16SrDNA primer used in this study. The amplified DNA fragments were subsequently electrophorized in 1.6% agarose gel, stained with 2% ethidium bromide to reveal the presence of different bands in the samples. The gels were further observed in UV light using GelDoc software of BioDoc-It™ Imaging Systems, UVP, LL

Table 1. PCR Primer Sequences And Amplicon Sizes For The Identification Of Lizard-Related Tick Species And Associated Pathogens.

Target organisms (Genetic markers)	Sequences (5′ to 3′)	Amplicon sizes (bp)	References
Tick (12S rDNA)	5'-GAGGAATTTGCTCTGTAATGG -3'	337 – 335	(Norris *et al.*, 1999)
	5'-AAGAGTGACGGGCGATATGT-3'		
	5'-ATTGCAAAAAGTACCGTAAACA-3′		
EHR16S	5'-GGTACC(C/T)ACAGAAGAAGTCC-3′	345	(Parola et al., 2000)
	5'-TAGCACTCATCGTTTACAGC-3′		

1.4.Sequencing and phylogenetic analysis

All PCR products of the expected size were purified using ExoSap-IT from Thermo Fisher Scientific. The ABI Prism 310 Genetic Analyzer capillary sequencer (Applied Biosystems) was used to perform bidirectional Sanger sequencing on the purified PCR products. First, primer-contaminated regions and any misread nucleotides at the start and finish of the sequences were eliminated using FinchTV(version 1.4.0). These sequences were then submitted to NCBI's GenBankand were assigned accession numbers asR. microplus: PQ892134 –PQ892135, Hy. anatolicum: PQ892130, Hy. excavatum:PQ892132, Hy.scupense: PQ892136. Hae. sulcate:PQ892131, Hae. punctata: PQ892131, Neoehrlichiamikurensisand CandidatusNeoehrlichia:OR668787 – OR668789(Table 2). Additional similar sequences were downloaded using the BLAST tool on the NCBI platform. The ClustalW multiple sequence alignment technique in MEGA 11 was then used to align all obtained sequences (Kumar et al., 2018). A model selection test was conducted for all sequences using MEGA's integrated model finder. The best-fit substitution model was chosen based on Akaike information criterion (AIC), and the Bayesian information criterion (BIC) values, with the "best-fit" replacement model being identified as the one with the lowest BIC, or AIC. The Maximum Likelihood technique was usedto create phylogenetic trees, together with the 1000 bootstrap approximation.

Table 2: Ticks and its associated pathogens sequence that submitted to Gene Bank.

	Specie Name	Host	Collection region	Accession number

Tick	(*R.microplus*), *(Hy.anatolicum),(* *Hy.excavatum),* *(Hy.scupense).* (*Hae.Sulcata),* (*Hae. Punctata),*	Cow Cow Cow Sheep Goat Goat		PQ892134, PQ892135 PQ892130, PQ892132, PQ892136 PQ892131 PQ892131
Pathogens	***Neoehrlichiamikurensis***	(*R.microplus*)	Bajaur, Khyber	OR668787, OR668787, – OR668789,
	***CandidatusNeoehrlichia*sp**	(*R.microplus*)	Orakzai. Bajaur,	PQ867838, PQ867837

1.5.Statistical analysis

All data related to tick infestation were inserted into MS Excel spreadsheet (version 2108). The data were then analyzed in Excel to calculated the total prevalence: (infested cattle/total cattle) × 100; the overall mean intensity: total ticks / infested cattle; and the mean abundance: total ticks / total cattle

Results

Ticks were examined on 635 hosts, with 384 hosts testing positive for tick infestation. The breakdown of infested animals includes 171 cows, 112 goats, and 101 sheep, resulting in a total of 588 ticks collected. This data indicates a 60.47% tick prevalence and an average infestation level of 1.53 ticks per infected goat. Overall, the mean tick abundance was 1.07% per host.

A total of 588 ticks were identified, comprising the following species: Rhipicephalus microplu s(190), Hyalomma anatolicum (96), Hy.excavatum (56), Haemaphysalis sulcata (79), Haemaphysalis bispinosa (65), Hyalomma scupense (58), and Haemaphysalis punctata (44) as depicted in Figure 2. Tick prevalence was highest in the Bajaur district, followed by Khyber, Orakzai, and North Waziristan, with hosts examined throughout the year (Fig. 2).

Tables 3-7 provides detailed information on tick infestation, including the presence of tick-borne pathogens in cattle, goats, and sheep, as well as the mean tick burden, density, and species infecting each host. In Bajaur agency, 174 animals were studied, with 97 testing positive, leading to the collection of 212 ticks. The tick species identified included Rhipicephalusmicroplus, Hyalomma anatolicum, Hyalomma excavatum, Haemaphysalis sulcata, Haemaphysalis bispinosa, Hyalomma scupense, and Haemaphysalis punctata. Among these, Neoehrlichia mikurensiswas detected in 7 R. microplus ticks (8.97%), while Candidatus Neoehrlichia sp. was found in 5 R. microplus ticks (6.41%).

In Khyber, out of 153 animals examined, 102 were positive for tick infestation, resulting in the collection of 160 ticks. Neoehrlichia mikurensis was identified in 6 samples (8.95%). The prevalence of microorganisms in Khyber was lower than in Bajaur, with an overall infestation rate of 4.58%.

At Orakzai, a cross-sectional study of 151 animals revealed that 93 were infested, with 126 ticks collected. The tick species distribution in Orakzai mirrored those found in other regions. Notably, CandidatusNeoehrlichia sp. was detected in 8 samples (27.58%), indicating a significantly higher prevalence in this region compared to others.

In North Waziristan, 157 animals were examined, and 92 were found to be infested. The tick species reported included R. microplus, Hyalomma anatolicum, Hyalomma excavatum, Haemaphysalis sulcata, and Haemaphysalis bispinosa.

Across all regions, out of the 635 animals examined, 384 were infested, with a total of 588 ticks identified. Rhipicephalusmicroplus was the most common species, accounting for 190 ticks (32.3%). Among the detected microorganisms, Neoehrlichia mikurensis and Candidatus Neoehrlichia sp. were present in 26 samples (13.68%).

These findings underscore the geographical variability in tick species, host infestation rates, and microbial loads. They highlight the complex ecological niches ticks occupy and their role as vectors of zoonotic diseases in Pakistan. Tick infestations were most prevalent from June to August, peaking during this period (Fig. 4). For a detailed overview of the different life stages of ticks collected from various districts throughout the year, refer to Figure 3, which identifies approximately 251 females, 187 males, 105 nymphs, and 45 larvae.

Table 3 Detailed information on tick infestation at Bajaur

Study Locations	Hosts			Morpho-molecularly identified ticks		Screened microorganisms	Number (%)
	Animal type	Examined	Infested (N)	Identified Tick species	Collected Tick		
Bajaur	Cow	65	48	*R.microplus*	78	***Neoehrlichiamikurensis***	7(8.97)
						Candidatus Neoehrlichiasp	5(6.41)
				Hy. anatolicum	27	–	–
				Hy. excavatum	21	–	–
						–	–
	Goats	44	28	*Hae. Sulcata*	26	–	–
				Hae. bispinosa	19	–	–
	Sheep	35	21	*Hy. scupense*	24	–	–
				Hae. punctata	17	–	–

	Total	**174**	**97**		**212**	**12(5.74)**	

Table 4 Detailed information on tick infestation at Khyber

Khyber	Cows	60	46	*R.microplus*	67	***Neoehrlichia mikurensis***	6(8.95)
				Hy. anatolicum	19	_	_
				Hy. excavatum	15	_	_
	Goats	47	29	*Hae. Sulcata*	16	_	_
				Hae. bispinosa	14	_	_
	Sheep	46	27	*Hy. scupense*	16	_	_
				Hae. punctata	13	_	_
	Total	**153**	**102**		**160**	**6(4.58)**	

Table 5 Detailed information on tick infestation at Orakzai

Orakzai	Cow	62	38	*R.microplus*	29	***Candidatus Neoehrlichia sp***	8(27.58)
				Hy. anatolicum	27	_	_
				Hy. excavatum	13	_	_
	Goats	41	23	*Hae. Sulcata*	14	_	_
				Hae. bispinosa	11	_	_
	Sheep	48	32	*Hy. scupense*	18	_	_
				Hae. punctata	14	_	_
	Total	**151**	**93**		**126**	**8 (6.34)**	

Table 6 Detailed information on tick infestation at North Waziristan

North Waziristan	Cow	64	39	*R.microplus*	16	–	–
				Hy. anatolicum	23	–	–
				Hy. excavatum	7	–	–
	Goats	54	32	*Hae. Sulcata*	23	–	_
	Sheep	40	21	*Hae. bispinosa*	21	_	_
	Total	**157**	**92**		**90**	=	

Table 7 Overall Detailed information on tick infestation at all sites studied

Overall	**635**	**384**	*R.*microplus	190	26 (13.68)
			Hy. anatolicum	96	–
			Hy. excavatum	56	–
			Hae. Sulcata	79	–
			Hae. bispinosa	**65**	–
			Hy.scupense	**58**	–
			Hae. punctata	**44**	–
				588	26(13.68)

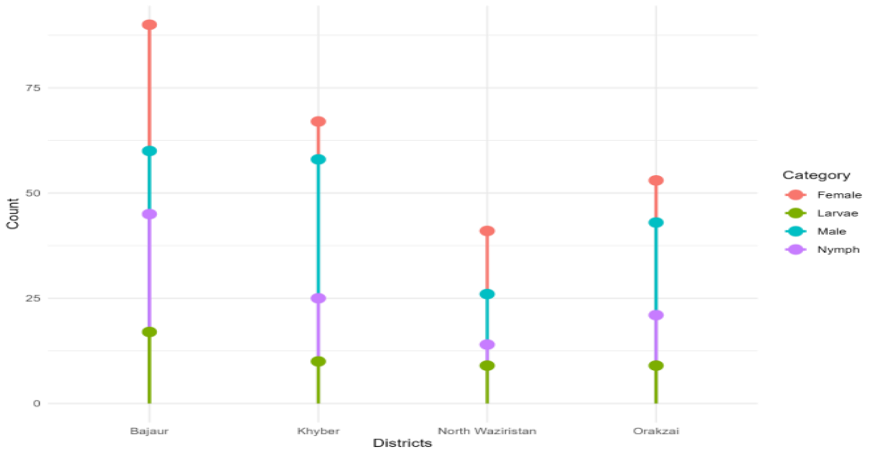

Figure. 2. District wise collection of 7 tick species, R. microplus, Hy. anatolicum, Hy. excavatum, Hae. Sulcata, Hae. Bispinosa, Hy. scupense, Hae. Punctata.

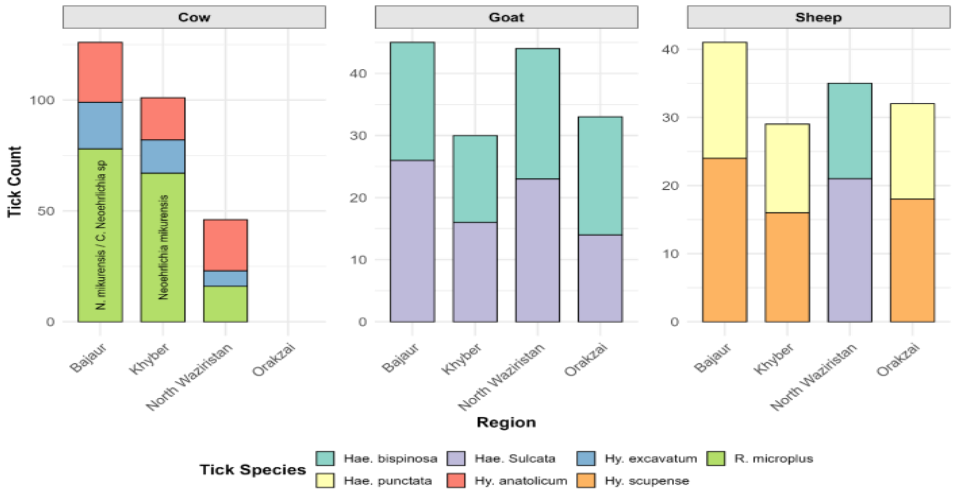

Figure. 3. Different life stages of ticks species Male, Female, Larva, and Nymph.

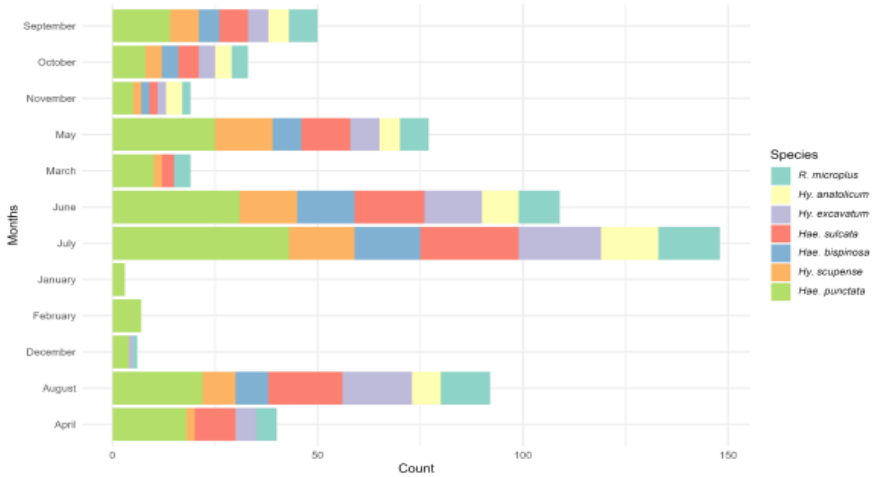

Figure 4. Seasonal wise and monthly collection of tick species

Sequence similarity and BLAST analysis

In total 588 ticks were used to extract DNA, and at least 370 ticks produced effective ampliconsFor the 12S RNA gene (337 – 335 bp), 7 sequences were acquired. The 2R.microplus 12S rDNA amplicons showed a 99–100% similarity range for 12S rDNA after repeated sequence alignment. Compared to the BLAST analysis's sequences, the 12S RNA partial sequence displayed a 99.15% or more identity with the R.microplus partial 12S sequence from Myanmar (MG459960, MG459969), Pakistan (PQ226727), The Hy. anatolicum (PQ892130), showed 99–100% similarity with Hy. anatolicumthat previously reported from China (MG645367, MG645340). The Hy. excavatum (PQ892132), 98–99% identity with Hy. excavatumthat previously reported from Turkey (MG418639, MG418641). Hy. scupense (PQ892136) showed 99–100% identity with Hy. scupensethat collected from goat in France(OM744171) and from horse in Tunisia (OR387869). The Hae. Sulcata (PQ892131) showed 99–100% identity with Hae. Sulcatat hat previously reported from Pakistan (OR665364, OR665365). The Hae. Punctata (PQ892133) showed 98–100% similarity with Hae. Punctata that collected from sheep in China (MN267437, MN267438)

Pathogens DNA analysis

The extracted DNA amplified for the presence of pathogens yielded 26(13.68) Neoehrlichiamikurensis13 (6.84%), and Candidatus Neoehrlichia sp13 (6.84%) positive amplicons for 16s primers respectively. Ticks collected from cow in Bajaur district tested positive for Neoehrlichia mikurensis and Candidatus Neoehrlichia sp. In contrast, ticks from Khyber district were found to carry, Neoehrlichia mikurensis Ticks infesting cow in Orakzai district tested positive for only Candidatus Neoehrlichia sp. The positive amplicons for 16s primer yielded 26 bacterial pathogens Neoehrlichia mikurensis and Candidatus Neoehrlichiasp.The BLAST analysis revealed that Neoehrlichia mikurensis isolated from R.microplus displayed a higher percent identity to other Neoehrlichia mikurensis reported in GenBank. Specifically, the species was found to have 100% identity and query cover with sequence of Neoehrlichia mikurensis (MW922773, MW922774) isolated from tick infesting cattle of Estonia. Similarly, the sequence had a 99.50% identity to Neoehrlichia mikurensis. isolated directly from cattle of China (MH722234, MH722225). The Candidatus Neoehrlichiasp (PQ867838, PQ867837) displayed 100% identity and query cover with Candidatus Neoehrlichia sp reported from Algeria (OM692211, OM692212,).

Discussion

Ticks play a critical role as vectors of numerous pathogens, contributing significantly to the burden of zoonotic diseases worldwide (De la Fuenteet al., 2017). Their adaptability to diverse climatic conditions and ecological systems enables them to thrive and perpetuate diseases in both humans and animals (de Souza et al., 2024). The emergence of Neoehrlichia mikurensis and Candidatus Neoehrlichia spp. as tick-borne pathogens in Pakistan signals a paradigm shift in the epidemiology of tick-borne diseases (TBDs) in the region. These pathogens, previously under-recognized, have drawn attention due to their zoonotic potential and their capacity to complicate public and veterinary health (Pustijanac et al., 2024).

The zoonotic risks associated with Neoehrlich iamikurensis and Candidatus Neoehrlichia spp. underscore the interconnectedness of human, animal, and environmental health as emphasized by the World Health Organization's One Health approach (Wennerås et al., 2024, Binkienė et al., 2023). In Pakistan, where livestock serves as an essential livelihood for rural communities, the presence of these pathogens highlights an urgent need for comprehensive epidemiological surveillance and management strategies. With tick species such as Rhipicephalus microplus and Hyalomma spp. acting as efficient vectors in the region, the likelihood of pathogen dissemination is significantly amplified by the country’s unique

ecosystems and climatic variability (Braam et al., 2023).

The detection of Neoehrlichia mikurensis and Candidatus Neoehrlichia spp. in livestock ticks marks a crucial development in understanding the enzootic cycles of TBDs in Pakistan. Notably, these pathogens have been linked to flu-like symptoms, vascular damage, and life-threatening complications in immune compromised individuals (Andonova et al., 2024, Schötta et al., 2023). The under-diagnosis and misdiagnosis of these infections in animals and humans, compounded by limited diagnostic facilities, further exacerbate the risks of sustained transmission. The zoonotic implications are profound, as these pathogens, localized within the endothelial cells of their hosts, exhibit immune evasion strategies that allow them to establish long-term reservoirs (Zakhamet al., 2023, Egan et al., 2023).

From a veterinary perspective, subclinical infections in livestock contribute to reduced productivity, directly impacting the socioeconomic stability of rural populations dependent on animal husbandry (Bonsiet al., 2023, Saba et al., 2024). Concurrently, human cases of Neoehrlichia mikurensis highlight a significant challenge for public health systems, where non-specific symptoms and co-infections with other pathogens, such as Anaplasma and Rickettsia spp., complicate timely diagnosis and treatment (Pustijanacet al., 2024, Labbéet al., 2022, Sjöwallet al 2021).The discovery of CandidatusNeoehrlichia spp. further expands the genetic and ecological diversity of these pathogens, emphasizing the need for cutting-edge molecular diagnostics and genomic studies (Thakur et al., 2024,).

To mitigate the dual threat posed by Neoehrlichia spp. to public and veterinary health, a multi-faceted approach is imperative (Fleming et al., 2018, Pustijanacet al., 2024). Longitudinal studies examining seasonal variations in tick populations and pathogen prevalence should form the basis of an integrated surveillance system (Little et al., 2019, Deshpande et al., 2023). Public awareness campaigns focused on preventive measures, such as tick control in livestock and personal protective strategies for humans, are vital to reducing transmission risks (Eisenet al., 2022, Eisenet al., 2021).

Moreover, establishing collaborative networks among public health authorities, veterinarians, ecologists, and researchers will foster a holistic response to TBDs (Zortman et aal., 2023, Chepkwonyet al., 2021). Strengthening diagnostic infrastructure and professional training in molecular diagnostics is essential to bridge current gaps in surveillance and clinical management (Chauhan et al., 2023, Ma et al 2024). The incorporation of integrated cattle health programs and targeted interventions based on ecological and epidemiological insights will enhance control efforts and reduce the impact of these pathogens on rural communities (Singh et al., 2024, Kappeset al., 2023).
The emergence of Neoehrlichia mikurensis and Candidatus Neoehrlichia spp. in Pakistan represents a wake-up call for the scientific and medical communities. It underscores the urgent need to prioritize TBD research and implement evidence-based policies that address the intricate interplay between ticks, pathogens, and their hosts. By embracing the One Health approach, Pakistan can better confront the challenges posed by TBDs and ensure improved outcomes for both public and veterinary health.

Conclusion

An important turning point in the study of tick-borne diseases has been reached with the discovery of Neoehrlichia mikurensis and Candidatus Neoehrlichia spp. in Pakistan, which highlights the complex interactions between ecological dynamics and newly developing pathogens. This finding emphasizes how urgently proactive, interdisciplinary efforts are needed to address the growing threat posed by tick-borne pathogens (TBPs). Collaboration between researchers, public health officials, and policymakers can help Pakistan create effective surveillance systems, put integrated tick management plans into place, and encourage

community education to reduce risks. This study contributes to the worldwide battle against tick-borne diseases by providing a crucial basis for comprehending the epidemiology of Neoehrlichia spp. and emphasizing the need for ongoing research and innovation to protect veterinary and public health.

ETHICAL STATEMENT

No ethical issues were raised during the course of study.

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AUTHORS` CONTRIBUTION

Concept: ZH, AK, SU. Plan: ZH, AK, SU. Data Analysis: AK, AA, RA. Writing, review and editing: AK, AM, RA. All authors have reviewed and consented to the final version of the manuscript for publication.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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