The Research of Medical Science Review
ISSN: 3007-1208 & 3007-1216 Volume 3, Issue 7, 2025

FUNGAL-BASED INTERVENTION AGAINST TICK-BORNE PARASITIC THREATS IN CATTLE: FOCUS ON *RHIPICEPHALUS MICROPLUS*

Shamim Khan[1], Shah Zeb[*2], Sadaf[3], Bushra Yasin[*4], Sidra Tul Muntaha Iqbal[*5], Nazir Ullah[6], Shehzad Zareen[7]

[1,2,7]*Department of Zoology, Kohat University of Science and Technology, Kohat, KP Pakistan*
[3]*Department of Zoology University of Science and technology Bannu, KP Pakistan*
[4]*Institute of Biochemistry and Biotechnology, University of Veterinary and Animal Sciences, Lahore, Punjab, Pakistan*
[5]*Department of Zoology, Abdul Wali Khan University Mardan*
[6]*Department of Zoology University of Science and technology Bannu, KP Pakistan*

[*1]zo420211006@kust.edu.pk, [2]shahzebwazir554@gmail.com, [3]saddaf903khan@gmail.com, [4]bushrayasin520@gmail.com, [5]si0427333@gmail.com, [6]nazirullahkhan814@gmail.com, [7]shehzadzareen1@kust.edu.pk

DOI: https://doi.org/10.5281/zenodo.16632020

Keywords
Rhipicephalus microplus, Beauveria bassiana, Metarhizium anisopliae, Biological control

Article History
Received on 25 April 2025
Accepted on 03 July 2025
Published on 29 July 2025

Corresponding Authors: *
Shah Zeb
Sidra Tul Muntaha Iqbal
Bushra Yasin

Abstract

Ticks are a serious threat to both humans and animals, spreading diseases babesiosis anaplasmosis, theileriosis, borreliosis, Crimean-Congo haemorrhagic fever (CCHF) and Lyme disease globally including Pakistan. They also cause enormous financial losses, particularly in in Khyber Pakhtunkhwa Pakistan's livestock sector. Resistance and environmental issues make traditional chemical control approaches difficult to use. In order to investigate environmentally acceptable biopesticides, entomopathogenic fungus Beauveria bassiana and Metarhizium anisopliae that were isolated from soil and tested against Rhipicephalus microplus ticks were used. The results demonstrated that higher fungal concentrations increased ticks death, with the mixed fungus having a 96% efficacy at 10^9 conidia/mL. The LC50 values for B. bassiana and M. anisopliae were 1 x 10^{15} and 4.68 x 10^{16} conidia/mL, respectively, indicating significant pathogenicity. This entailed experimenting with various application methods, including as sprays, baits, and formulations, to improve efficacy while reducing environmental impact

INTRODUCTION

Ticks are blood-feeding arthropods that infests vertebrate's hosts including mammals, birds, reptiles and amphibians all over the world but are more common in tropical and subtropical regions including Pakistan (Hussain et al., 2023). They directly damage their hosts by causing weight loss, reduced milk supply, blood loss, and skin damage (Yadav & Upadhyay. 2024).While indirectly ticks are regarded as the second most significant carriers of animals and humans illnesses, after mosquitos (Baneth, 2014). The global yearly cost of ticks and tick-borne diseases (TTBDs) control is projected to be between $13.9 billion and $18.7 billion (Riaz et al., 2024). The ticks fauna of Pakistan consists of 48 tick species in seven genera of family Ixodidae (*Nosomma*, *Amblyomma*, *Ixodes*, *Dermacentor*, *Haemaphysalis*, *Rhipicephalus*, *Hyalomma*) and four genera of family Argasidae (*Otobius*, *Carios*, *Ornithodorus* and *Argus*) reported from various hosts such as cattle, buffaloes, sheep, donkeys, dogs, cats, camels, goats, fowls, snakes, and wild animals . (Hussain et al., 2021, Aiman et al., 2022).Tick species in Pakistan are quite diverse, according to studies like in Khyber Pakhtunkhwa, 17 species from six genera were found, including *R microplus*, *Hae. anatolicum*, and *A. persicus* (Ali et al., 2019; Hussain et al., 2024). Another investigation on dogs in the same area discovered six tick species from the *Hyalomma* and *Rhipicephalus* genera (Zeb et al., 2023). Similarly, a study revealed in Punjab nine tick species infecting agricultural animals, such as *Hya. anatolicum*, *Rh. sanguineus*, and *Rh. microplus* (Batool et al., 2019).

Chemical acaricides, the most widely used technique of tick control, have various drawbacks, including the emergence of acaricide-resistant ticks, food contamination, environmental pollution, harm to non-target creatures, and escalating prices (Obaid et al., 2022). These issues are prompting academics to investigate other, long-term tick management solutions (Eisen & Paddock, 2021). One possible strategy is to utilize entomopathogenic fungi to infect ticks by penetrating their cuticles, which could help manage sucking arthropod pests (Islam et al., 2021). These fungi are regarded as suitable biological control agents due to their widespread distribution, low risk to humans, animals, and ecosystems, high efficiency against ticks, and ease of commercial production (Rajput et al., 2024).

Recent research has shown that *Beauveria bassiana* and *Metarhizium anisopliae* are effective tick control agents having capacity to pierce arthropod cuticles (Rajput et al., 2024). Several strains of *B. bassiana and M. anisopliae* have shown virulence against multiple tick species (Wadaan et al., 2023). While several research have looked at their individual impacts, few have looked at how different fungi can work together while to close this gap, researchers investigated the virulence of *B. bassiana* and *M. anisopliae*, as well as their combined impact on *Rh. microplus*, with the goal of developing a novel and successful tick management technique (Leemon & Jonsson, 2008).

Aim of study

The aim of this study is to use Entomopathogenic fungus (*Beauveria bassiana and Metarhizium anisopliae*) against *Rhipicephalus microplus* tick populations. Additionally, the development of novel biopesticides or integrated pest management strategies for tick control in livestock and agricultural. *Beauveria bassiana* and *Metarhizium anisopliae* combine to show significant effects on *Rhipicephalus microplus* ticks. Biological control agents offer environment friendly alternatives to chemical pesticides, reducing reliance on synthetic chemicals by minimizing their impacts on ecosystems, non-target organisms, and human health.

Materials and Methods

Ethical Approval

Before the collection of samples, the ethical certificate was approved by the Kohat University of Science and Technology's Ethical Research Committee.

Study Area

The current study was conducted in the district of Bannu Khyber Pakhtunkhwa, Pakistan. Bannu is around 192 km south of Peshawar and is situated at 32° 59' 22" North and 70° 36' 21" East. It shares borders with South Waziristan to the southwest, Lakki Marwat and Bettani to the southeast, North Waziristan to the northwest, and Karak to the northeast. It is administratively divided in six tehsils such as Wazir Tehsil, Bakki Tehsil, Domel Tehsil, Bannu Tehsil, Miryan Tehsil and Bakkhel Tehsil.

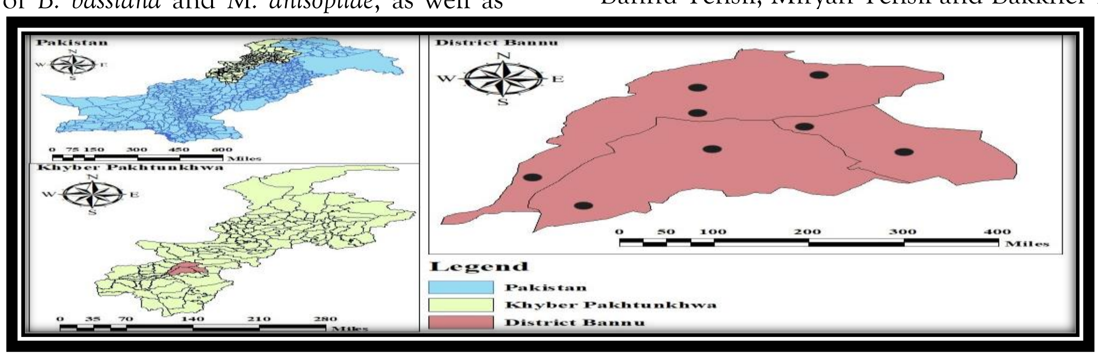

Figure 2.1: Show Study area map of district Bannu.

Ticks Sample Collection

Ticks samples were randomly from January 2024 to December 2024 from various animals' hosts such as sheeps, goats, cattles, and buffaloes. Collected ticks were kept into eppendorf tubes and properly labeled along with other relevant information including type of organism, age, gender, and body region of the host animal were recorded in predesigned pro-forma. The geographical coordinates (Latitude and Longitude) of sample locations were obtained using Global Positioning System (GPS) and added attribute table of ArcGIS software tagging on study area map.

Collected ticks samples were carried to Entomology and Parasitology Laboratory, Department of Zoology, KUST for further process.

Figure 2.2: Show ticks sampling from animal hosts in district Bannu.

Morphological identification of ticks

Collected ticks were morphologically identified by using stereomicroscope Olympus Corporation, Tokyo, Japan), according to their morphological characteristics using the valid identification keys (Walker et al., 2005).

Growth of Fungi and Making Conidial Suspensions

Using fungus isolation methods soil fungal samples were gathered from the study area which includes strains of *Beauveria bassiana* and *Metarhizium anisopliae*. Ten grams of fresh potato dextrose agar (PDA) media were dissolved in distal water and put in Petri plates with a minor adjustment in order to cultivate fungal isolation and then incubate at core temperature (26–28 C) and 75–85% RH. A vortex mixer is used to homogenize the suspension. The serial dilution technique was employed, along with the preparation of cocktail suspensions for both *Beauveria bassiana* and *Metarhizium anisopliae* strains, cocktail suspension is make to check the effect of both stains, to achieve concentrations ranging from 10^5 and 10^9 because at 10^5 have small conidia concentrations while at10^9 have high conidial concentration with minor adjustments (Ren et al., 2016).

Bioassays in a laboratory

Conidial suspensions were prepared from *Beauveria bassiana, Metarhizium anisopliae,* and *Beauveria bassiana* + *Metarhizium anisopliae* (Bb + Ma) at concentrations ranging from 10^5 to 10^9 conidia per milliliter, with minor modifications. For each treatment, ten *R. microplus* were immersed for 30 seconds in their respective conidial suspensions, followed by the removal of excess suspension. As a control, ticks were placed in separate petri dishes. Each experiment was replicated twice times. Afterward, the treated ticks were individually housed in Petri dishes and kept within an incubator at a temperature of 28 ± 2°C and a relative humidity of 80 ± 5%. Mortality assessments were conducted every 72 hours post-treatment by checking each tick in each treatment then we found the mortality of tick miner modification (Ren et al., 2016).

Statistical Analysis

Data will be statistically analyzed through different statistical software including MS Excel, SPPS, Graph Pad Prism and Minitab.

Result

A total of 500 ticks were recorded from total 68 infested hosts such as cows, buffaloes, sheeps, goats, during the sampling period (January 2024-February 2025) from selected areas in four tehsils of District Bannu. Collected tick samples represent genus *Rhipicephalus* of the family Ixodidae based on morpho-taxonomic characters.

Fungal isolation and growth techniques were used to isolate *Beauveria bassiana* and *Metarhizium anisopliae* strains from soil samples collected in the research region. For this procedure, 10 grams of fresh potato dextrose agar (PDA) medium was dissolved in distilled water and placed in Petri plates for fungal culture. The samples were then incubated at 26-28°C with 75-85% relative humidity. Following growth, the fungal strains were identified by morphology using a microscope, and biochemical tests were done to validate their identity.

Microscopy of fungus and ticks samples.

The Fungus *Beauveria bassiana and Metarhizium anisopliae* were identified using a microscope and GMS stain. The following features were observed.

Table 3.1: Features of *Beauveria bassiana* and *Metarhizium anisopliae*

Feature	*Beauveria bassiana*	*Metarhizium anisopliae*
Colonial Morphology	Gray-green to olive-brown, fluffy colonies.	Green to dark green, powdery or fluffy colonies.
Conidiophores	Branched, arising from hyphae.	Branched, often forming clusters.
Conidia (spores)	Oval to cylindrical, smooth, or slightly roughened.	Fusiform to cylindrical, often with distinct ornamentation.
Hyphal Characteristics	Septate, pigmented.	Septate, often darkened.
Sporulation Conditions	High humidity, moderate temperatures.	Humid environments, often on insect cadavers.
Cultural Characteristics	Rapid growth on fungal media.	Fast-growing

Biochemical test

- **Inoculation**: *Beauveria bassiana* and *Metarhizium anisopliae* are inoculated into a urea-containing medium. The culture is then incubated under the appropriate conditions.
- **Observation**: After incubation, a color change or pH change in the media indicates urease activity.
- ***Metarhizium anisopliae***: Typically shows positive urease activity. It hydrolyzes urea, creating ammonia and raising the pH of the medium, causing a hue shift to pink or purple.
- ***Beauveria bassiana***: In general, no urease activity is detected, yielding a negative result. In the urease test. The medium remains yellow, indicating no hydrolysis of urea into ammonia.

Table 3.2 Show Key Morphological Characters *R. microplus.*

Species Name	Key Morphological Characters	
	Male	**Female**
R. microplus	• 4+4 teeth row on hypostome, narrow caudal appendages. • Ventral plate spurs are not observable dorsally and Coxae 1 is long.	• Similar mouth parts as male • U shaped genital aperture • Peer shaped scutum,

Figure 3.1: *R. microplus* (Male: A & B, Female C &D dorsal & ventral side)

Entomopathogenic fungus against *Rhipicephalus microplus.*

The *Rhipicephalus microplus* mortality rate in different fungal strains are shown in ***Table 4.4*** amd ***4.5 and 4.5.*** There was a statistically significant similarity in the mortalities caused by *Beauveria bassiana*, *Metarhizium anisopliae*, and their combination (*Beauveria bassiana* + *Metarhizium anisopliae*). All fungal-treated groups, however, displayed noticeably greater fatalities in comparison to the control. The *Beauveria bassiana*, *Metarhizium anisopliae*, and their combination were pathogenic to *Rhipicephalus microplus* at doses of 10^5 and 10^9 conidia mL-. Interestingly, the 10^9 conidia mL-1 concentration showed the highest death rates (92–100%), although the 10^5 concentration produced fewer mortalities. however, no mortality was seen in the control groups, whereas the Bb + Ma combination showed a 96% efficacy at the higher dose.

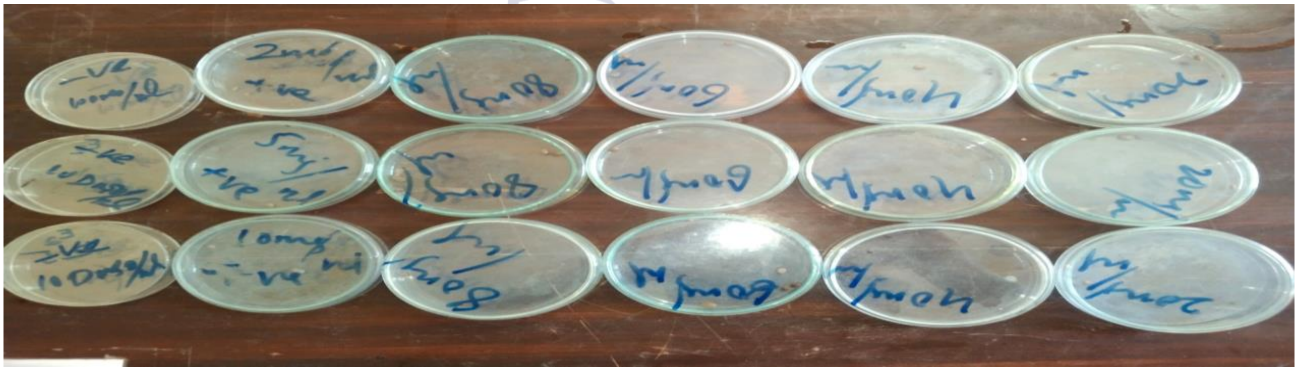

Figure 3.2: Entomopathogenic fungus against *R. microplus*.

Table 4.4: **Mortality rate of *Rhipicephalus microplus* at *Beauveria bassiana.***

Fungi	Concentration	Day	Mortality%	P valve	average	variance	LC50
Beauveria bassiana	10^5	1	0	0.03288	5	5	1 x10^{15}
		2	20				
		3	30				
	10^9	1	0				
		2	30				
		3	50				
Control			0	0	0	0	0

(P-value 0.05 < 0.03288)

***Table 4.4* (*Beauveria bassiana*):**
Shows *R. microplus* mortality rates with varying *Beauveria bassiana* fungal concentrations. Higher fungal concentrations resulted in higher average death rates, with significant differences observed ($p < 0.05$).

Table 4.5: **Mortality rate of *Rhipicephalus microplus* at *Metarhizium anisopliae***

Fungi	Concentration	Day	Mortality %	P valve	Average	Variance	LC50
Metarhizium anisopliae	10^5	1	0				
		2	10				
		3	30				
	10^9	1	0				
		2	20				
		3	40	0.033183	5	5	4.68×1016
Control			0	0	0	0	0

(P valve 0.05 < 0.033183)

***Table 4.5* (*Metarhizium anisopliae*):**
Shows the *R. microplus* death rates at various *Metarhizium anisopliae* fungal concentrations. As demonstrated by Table 1, mortality rose as fungal concentrations increased, with statistically significant differences ($p < 0.05$) in outcome.

Table 4.6: **Mortality rate of *Rhipicephalus microplus* at *Beauveria bassiana* + *Metarhizium anisopliae***

Fungi	Concentration	Day	Mortality %	P valve	Average	Variance	Lc50
Beauveria bassiana + Metarhizium anisopliae	10^5	1	0				
		2	30				
		3	50				
	10^9	1	0				
		2	40				
		3	50	0.033183	5	5	$1x10^{15.83}$
Control			0	0	0	0	0

(P value 0.05 < 0.033183)

***Table 4.6* (B. bassiana, M. anisopliae):**
Combines both fungi (*B. bassiana, M. anisopliae*) and examines their combined influence on *R. microplus* mortality rates. Higher concentrations of mixed fungus resulted in increased mortality rates, which were statistically significant ($p < 0.05$).

The LC50 values for *B. bassiana, M. anisopliae,* and their combination (*B. bassiana, M. anisopliae*) against *Rhipicephalus microplus* are shown in **Tables 4.4, 4.5, and 4.6.** Ticks exposed to *B. bassiana* had an LC50 of 1×10^{15} conidia mL^{-1}, while *M. anisopliae* had an LC50 of 4.68×10^{16} conidia mL^{-1}. The combination of *B. bassiana* and M. *anisopliae* (Bb + Ma) revealed an LC50 of 1×10^{15}.83 conidia mL^{-1}. These results indicate that the LC50 values for *M. anisopliae* and the *B. bassiana* + *M. anisopliae* combination are relatively high.

Discussion

Entomopathogenic fungi isolated from soil, notably *Beauveria bassiana and Metarhizium anisopliae,* were tested against *R. microplus.* Both fungi and their combination resulted in significantly greater mortality rates than controls. At higher doses, the combination achieved 96% efficacy, indicating its potential application in biological control efforts against *R. microplus* ticks. Furthermore, *M. anisopliae* is regarded as one of the most promising tick biological control agents (Frazzon et al., 2000).

Previous research has shown that *M. anisopliae* is highly effective in vitro in reducing engorged females and *R. microplus* larvae (Fernandes & Bittencourt, 2008). The findings of this study also demonstrate that entomopathogenic fungi isolated from soil, notably *Beauveria bassiana* and *Metarhizium anisopliae,* were tested against *R. microplus.* Both fungi, alone and combined, resulted in significantly greater mortality rates than controls. At larger doses, the combo proved 96% effective.

In this study, previous findings demonstrate the efficacy of *M. anisopliae* in the field. The Ma34 strain showed the highest efficacy against engorged females at a concentration of 1×10^8 conidia/ml (100% mortality at 12 days post-treatment). Conversely, the Ma14 strain was more effective against larvae at the same concentration (62% mortality at 20 days post-treatment). A blend of both strains (Ma34 + Ma14) matched the efficacy of Ma34 on engorged females (100% mortality at 14 days post-treatment) and exhibited higher efficacy on larvae (90% mortality at 20 days post-treatment (Frazzon et al., 2000).In my research, the 10^9 conidia/ml concentration showed the highest mortality rates (92–100%), while the 10^5 concentration resulted in lower mortalities. No mortality was observed in the control groups, whereas the Bb + Ma combination showed 96% efficacy at the higher dose.

Conclusion and **Recommendation:**

The study shows that both *Beauveria bassiana* and *Metarhizium anisopliae*, either administered alone or in combination, have promising potential for biological control of *Rhipicephalus microplus* ticks. Significant mortality rates were reported, especially at higher concentrations (10^9 conidia mL^{-1}). While both fungal strains were efficient individually against *Rhipicephalus microplus,* their combination (*Beauveria bassiana* + *Metarhizium anisopliae*) significantly increased mortality rates, particularly at the highest dose tested. This demonstrates that two fungus (*Beauveria bassiana and Metarhizium anisopliae*) are beneficial in tick management**.** The current study recommended that further studied must be takes place on ticks and tick-borne pathogens control strategies through other fungicides.

References

Aiman, O., Ullah, S., Chitimia-Dobler, L., Nijhof, A. M., & Ali, A. (2022). First report of Nosomma monstrosum ticks infesting Asian water buffaloes (Bubalus bubalis) in Pakistan. Ticks and Tick-borne Diseases, 13(2), 101899.

Ali, A., Khan, M. A., Zahid, H., Yaseen, P. M., Qayash Khan, M., Nawab, J., ... & Ibrahim, M. (2019). Seasonal dynamics, record of ticks infesting humans, wild and domestic animals and molecular phylogeny of Rhipicephalus microplus in Khyber Pakhtunkhwa Pakistan. Frontiers in physiology, 10, 793.

Baneth, G. (2014). Tick-borne infections of animals and humans: a common ground. International Journal for Parasitology, 44(9), 591–596.

Batool, M., Nasir, S., Rafique, A., Yousaf, I., & Yousaf, M. (2019). Prevalence of tick infestation in farm animals from Punjab, Pakistan. Pakistan Veterinary Journal, 39(3).

Eisen, R. J., & Paddock, C. D. (2021). Tick and tickborne pathogen surveillance as a public health tool in the United States. Journal of Medical Entomology, 58(4), 1490–1502.

Fernandes, E. K. K., & Bittencourt, V. R. E. P. (2008). Entomopathogenic fungi against South American tick species. Experimental and Applied Acarology, 46, 71–93.

Frazzon, A. P. G., Junior, I. da S. V., Masuda, A., Schrank, A., & Vainstein, M. H. (2000). In vitro assessment of Metarhizium anisopliae isolates to control the cattle tick Boophilus microplus. Veterinary Parasitology, 94(1–2), 117–125.

Hussain, I., Majeed, A., Imran, I., Ullah, M., Hashmi, F. K., Saeed, H., ... & Rasool, M. F. (2021). Knowledge, attitude, and practices toward COVID-19 in primary healthcare providers: a cross-sectional study from three tertiary care hospitals of Peshawar, Pakistan. Journal of community health, 46, 441-449.

Hussain, N., Shabbir, R. M. K., Ahmed, H., Afzal, M. S., Ullah, S., Ali, A., & Cao, J. (2023). Prevalence of different tick species on livestock and associated equines and canine from different agro-ecological zones of Pakistan. Frontiers in Veterinary Science, 9, 1089999.

Hussain, S., Hussain, A., Aziz, M. U., Song, B., Zeb, J., Moutailler, S., ... & Sparagano, O. (2024). Exploring the Coinfection and Genetic Diversity of Multiple Tick-Borne Pathogens in Livestock Population of Punjab, Pakistan. Transboundary and emerging diseases, 2024(1), 9958535.

Obaid, M. K., Islam, N., Alouffi, A., Khan, A. Z., da Silva Vaz Jr, I., Tanaka, T., & Ali, A. (2022). Acaricides resistance in ticks: selection, diagnosis, mechanisms, and mitigation. Frontiers in Cellular and Infection Microbiology, 12, 941831.

Rajput, M., Sajid, M. S., Rajput, N. A., George, D. R., Usman, M., Zeeshan, M., Iqbal, O., Bhutto, B., Atiq, M., & Rizwan, H. M. (2024). Entomopathogenic Fungi as Alternatives to Chemical Acaricides: Challenges, Opportunities and Prospects for Sustainable Tick Control. Insects, 15(12), 1017.

Ren, Q., Chen, Z., Luo, J., Liu, G., Guan, G., Liu, Z., Liu, A., Li, Y., Niu, Q., & Liu, J. (2016). Laboratory evaluation of Beauveria bassiana and Metarhizium anisopliae in the control of Haemaphysalis qinghaiensis in China. Experimental and Applied Acarology, 69, 233–238.

Riaz, M., Chang, S. C., Tasawar, Z., Sajid, M., Nasreen, N., Amaro-Estrada, I., ... & Ben Said, M. (2024). Molecular Epidemiology and Phylogeny of Theileria ovis and Theileria lestoquardi in Sheep and Goats from Southern Punjab, Pakistan. Vector-Borne and Zoonotic Diseases.

Wadaan, M. A., Khattak, B., Riaz, A., Hussain, M., Khan, M. J., Fozia, F., Iftikhar, A., Ahmad, I., Khan, M. F., & Baabbad, A. (2023). Biological Control of Hyalomma Ticks in Cattle by Fungal Isolates. Veterinary Sciences, 10(12), 684.

Walker, A. R., Matthews, J., & Preston, P. M. (2005). The development of electronic keys for the identification of ticks. International Journal of Tropical Insect Science, 25(1), 2–5.

Yadav, N., & Upadhyay, R. K. (2024). Status of Economic Losses and Health Impact of the Tick Bites on Farm, Field and Dairy Animals. Acta Sci. Med. Sci, 8.

Zeb, J., Song, B., Senbill, H., Aziz, M. U., Hussain, S., Khan, M. A., Qadri, I., Cabezas-Cruz, A., de la Fuente, J., & Sparagano, O. A. (2023). Ticks infesting dogs in Khyber Pakhtunkhwa, Pakistan: detailed epidemiological and molecular report. Pathogens, 12(1), 98.