

Prevalence and risk factors associated with T.Equii in different District of Punjab, Pakistan

Ch Qasim Bilal

qasimbilal7860@gmail.com

Gomal University

Muhammad Shuaib Khan

Gomal University

Shakeeb Ullah

Gomal University

Faiqah Ramzan

Gomal University

Muhammad Irfan

University of Sargodha

Research Article

Keywords: Tick Borne Diseases, Spatiotemporal epidemiology, Diagnostic accuracy and climatic factors

Posted Date: October 14th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-7171557/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Equine theileriosis is an emerging tick-borne disease threatening equine health, causing anemia, immunosuppression, and economic losses in the equine industry. This study provides critical insights into the spatiotemporal dynamics of Theileria infection in horses to guide targeted surveillance and control. We analyzed 1,414 equine samples collected monthly over one year across multiple regions, assessing seasonal trends, geographical variations, environmental drivers, and diagnostic performance. Microscopy revealed peak transmission in March (43.3%, 95% CI: 34.5–52.6%) and October (37.3%, CI: 28.6–46.8%), with the lowest prevalence in April (6.5%, CI: 3.6–11.3%). Seasonal highs occurred in Fall (27.9%) and Spring (21.5%), significantly exceeding Winter (9.8%, $P < 0.001$). Geographically, MBD/Head Fagerian (30.0%, CI: 23.3–36.7%) and Sargodha (29.4%, CI: 22.8–36.0%) were high-burden hotspots, with 12–15% higher prevalence ($P < 0.001$) in high-density equine populations versus low-density regions (e.g., Sahiwal, 14.1%). Temperature was the strongest predictor (0.82% increase per 1°C , $P < 0.001$), while humidity $> 60\%$ added 0.31% risk ($P = 0.002$). Heatwaves (April–May) elevated risk by 2.45% ($P = 0.001$). Diagnostically, ELISA showed superior sensitivity (88.3%) for subclinical cases, whereas PCR had optimal specificity (92.1%) and positive predictive value (75.6%). Microscopy remained field-practical (sensitivity = 72.1%, specificity = 89.4%). Test agreement was strongest during peak transmission (March $\kappa = 0.54$ –0.61) but unreliable in low-prevalence months (April $\kappa = 0.22$). In Conclusions; Equine theileriosis is driven by climate-density interactions, with spring/fall peaks and overcrowding amplifying transmission. The study suggests that pre-peak acaricide use (March/October), density management in hotspots, and ELISA/PCR dual testing for accuracy may be adopted to mitigate the effects of this menace. Future studies should integrate tick surveillance, host mobility patterns, and genomic characterization of circulating Theileria strains.

Introduction

Since their domestication, horses (*Equus caballus*) have been integral to human civilization, serving roles in trade, warfare, agriculture, and recreation. In developing nations, horses remain essential for farming and transportation, while in developed countries, they are predominantly used for recreational activities. There are an estimated 116 million equines (donkeys, horses and mules) globally, with 36 million in the 38 lowest-income countries (2025). In Pakistan, the estimated horse population is around 0.4 million (2024). These horses are primarily utilized for racing, gaming, cart pulling, and riding (Gould et al. 2025). Pakistan's geographical position within the subtropical zone, along the plains of the Indus River in South Asia, creates diverse agro-ecological regions with varying climates and water resources (Qureshi et al. 2023). This agricultural landscape provides a favorable environment for ticks to thrive, potentially transmitting diseases to both livestock and humans (Hussain et al. 2023).

Theileria equi is an obligate hemoprotozoan parasite transmitted by ticks from the phylum Apicomplexa (Amiri et al. 2020; Bělková et al. 2021; Kalantari et al. 2022; Altay et al. 2024). It is a major causative agent of equine piroplasmiasis in tropical and subtropical regions. Theileriosis, recognized as a significant equine ailment, results in considerable economic impacts (Bělková et al. 2021; Altay et al.

2024). The life cycle of *T. equi* involves two developmental stages within the host's body. During the initial phase of the illness, *T. equi* infects white blood cells before red blood cells, leading to a persistent type of infection that is believed to be more aggressive than *Babesia caballi* (Ramadan et al. 2024).

The classification of *T. equi* is subject of debate since its detection. Earlier it was labeled as *Babesia equi*, later; reclassified on the basis of its infection to white blood cells instead of red blood cells (Mehlhorn and Schein 1998). Recent phylogenetic analyses have identified four distinct clades of *T. equi*, designated as A, B, C, and D genotypes based on the hypervariable region of the 18S rRNA gene (Kappmeyer et al. 2012).

In Pakistan, *T. equi* infection is prevalent among working horses, adversely affecting their health and productivity (Hussain et al. 2014; Afridi et al. 2017). Given the economic and social importance of horses in Pakistan, understanding the epidemiology of *T. equi* is crucial for developing effective control and prevention strategies.

Materials and methods

Sampling strategy

The time period of the study was one year (Jan 2024 to December 2024). A cross-sectional study was designed to detect positive cases of theileriosis and to identify various risk factors associated with the disease. A total of 1414 animal samples were collected from study area by assuming 50% prevalence of unknown disease with 95% confidence interval in equine population. Samples were collected from those animals but one of the priority criteria for the sampled horse were; the presence of ticks on their body. All precautionary measures were adopted to avoid any accidental injury to horse and person during blood collection. Blood samples were obtained from jugular vein and collected in two different vacutainers: one for DNA extraction and haematological analysis and the other for serum/ biochemical analysis. After collection, the blood samples were stored in iceboxes and dispatched to laboratory immediately for examination. The blood samples were collected to check the disease status of the animal. Every individual sample was also accompanied by a questionnaire to collect information related to risk factors for theileriosis in horses consisting of month, area, sex, age, breed, density of equine holding, body condition, nutrition, concentrate feeding, management and tick infestation. Detail of study area, its coordinate, elevation and sample size is mentioned in Table 1.

Table 1
Study Area, Coordinates, elevation and sample Processed

Area	Latitude	Longitude	Elevation from Sea	Samples Collected
Sargodha	32.082	72.6691	190	180
Kot Momin	32.1892	73.0286	205	173
Bhalwal	32.2723	72.9043	187	177
Shahpur	32.2676	72.4685	193	178
Sillanwali	31.8260	72.5396	184	177
Sahiwal	31.9735	72.3301	189	177
Bhera	32.4833	72.9167	192	172
Mandi Bahaudin / Head Faqeerian	32.1614	73.0500	200	180

Blood smear examination

Blood smears are a valuable diagnostic test when parasitaemia is very low (Harman 2025), thin and thick blood smears were prepared and stained with field and Giemsa stain, air-dried and then fixed in methanol (OIE 2018). Blood smears were examined for protozoa under the oil immersion (100x) lens by three experienced observers (Zajac and Conboy 2012). The examination fields were chosen at the periphery of the stained blood smears and at least 6 fields were examined for the presence of *T. equi* parasite or other parasites.

ELISA TEST

All positive, doubtful and random negative samples in protozoology blood smear examination were subjected for commercially available ELISA kit (BioStone Animal Health-AsurDx™ *Theileria equi* Antibody Test Kit, USA). Kit was used according to manufacturer's instructions. These assays detected *T. equi* horse antibodies of IgG in horse serum/ plasma by enzyme based immunoassay screening method. The optical density values were measured through an ELISA plate reader at 450 nm and the test results were obtained using the following formula:

$$\text{Percent Positivity (PP)} = \frac{\text{OD450 Test Sample}}{\text{Mean OD450 Positive Control}} \times 100$$

All samples producing $\geq 60\%$ PP were regarded as positive whereas those with values $< 60\%$ were considered negative.

MOLECULAR DETECTION

A DNA extraction kit (GeneAll₊, ExgeneTM, Catalog # 105 – 101)¹, was used for extraction of DNA from all blood samples according to the manufacturer's instructions and sample for subjected to qPCR using 5'-CATCGTTGCGGCTTGGTTGG-3' and 5'- CCAAGTCTCACACCCTATTT-3' primers. PCR cycling conditions were 50°C for 2 min, followed by 95°C for 10 min and then 40 cycles of 95°C for 15 s and 60°C for 1 min as shown in Fig. 1.

Haemato-biochemical analysis

A total of 1414 equine (n = 286 positive for blood protozoa; n = 1128 negative for blood protozoa) selected for haematologic and biochemical analyses. The blood and serum samples were collected in EDTA coated Vacutainer-5K2EDTA tubes and plain vacutainers respectively for haematologic analysis and biochemical analysis. Haematologic analysis including white blood cells (WBC), lymphocytes (LYM), erythrocytes (RBC), haemoglobin (HGB), monocytes (MON), granulocytes (GRA), mean corpuscular volume (MCV), haematocrit (HCT), mean corpuscular haemoglobin (MCH), mean corpuscular, haemoglobin concentration (MCHC), platelets (PLT) and mean platelet volume (MPV) was performed using haematology analyser biobased haematology analyzer. Biochemical analysis including glucose, calcium, phosphorus, iron, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was performed using a biochemistry analyser.

Results

Prevalence of *Theileria equi*

A total of 1414 samples were processed in this study and 29.8% of sampled animal were positive for blood protozoan including *Theileria*, *Babesia*, *Anaplasma* and *Trypanosoma* species (286/ 1414) (Fig. 2). The overall prevalence of *Theileria equi*, piroplasmosis and others blood protozoa were 8.42%, 9.48% and 2.3% (119, 134 and 33 samples respectively) on microscopic examination (Table 2). However, more sensitive and specific diagnostic tests revealed higher rates, ELISA detected *Theileria equi* from 19.3% (273/1414) samples and PCR confirmed active infection of 14.4% (203/1414) animals (Table 3). The study revealed the presence of 9.48% cases of piroplasmosis (mixed infections of *babesia* and *thelera*) and 2.3% cases were attributable by other protozoan species by microscopy. These results demonstrate that *T. equi* is endemic in the area and combined diagnostic approaches (microscopy, serology, and molecular methods) are needed for accurate surveillance and control programs.

Table 3 demonstrate the monthly variation in *Theileria* prevalence was significant ($\chi^2 = [XX]$, $P < 0.0001$). March showed highest infection/ prevalence rates (Microscopy: 43.3%, 95% CI: 34.5–52.6%; PCR: 30.0%, CI: 22.6–38.6%). October (37.3%, CI: 28.6–46.8%) was only month analogous to March (* $P = 0.18$), while April had the lowest incidence (6.5%, CI: 3.6–11.3%; $P < 0.0001$ vs. March).

Furthermore, The comparison of diagnostic methods produced important understandings about test performance characteristics. cELISA consistently identified the highest prevalence rates (e.g., 40.0% in March), reflecting its sensitivity in detecting both active and past exposures. Whereas, PCR results are

more specific than microscopy, showed intermediate positivity rates, serving as a valuable confirmatory tool. These diagnostic differences were statistically significant ($P < 0.0001$ for monthly variations).

Diagnostic Test Performance

The diagnostic performance analysis revealed the differences in test characteristics for *Theileria* detection (table 4). ELISA revealed the highest sensitivity (88.3%), being the most effective test for screening and ruling out of infection, while PCR demonstrated the best overall accuracy with both high sensitivity (79.4%) and the highest specificity (92.1%). Microscopy offered moderate sensitivity (72.1%) but maintained good specificity (89.4%). In terms of predictive values, PCR showed superior positive predictive value (75.6%) compared to ELISA (59.1%) and microscopy (68.3%), indicating it is the most reliable test for confirming true infections when positive. All tests exhibited strong negative predictive values ($> 90\%$), with ELISA performing best (94.7%), making negative results from any method highly trustworthy for ruling out disease.

Seasonal Prevalence of *Theileria equi*

The seasonal analysis of *Theileria* prevalence revealed significant variations in infection rates around the year ($P < 0.001$ for all seasonal comparisons). Fall showed the highest prevalence of 27.9% positivity by microscopy (95% CI: 23.0–32.8%), followed by Spring at 21.5% (CI: 17.6–25.4%) and Summer at 19.3% (CI: 15.8–22.8%). Winter had lower prevalence of 9.8% positivity (CI: 6.2–13.4%). This pattern held across all diagnostic methods, with Fall consistently showing the highest rates: 12.7% for *T. equi* (CI: 9.2–16.2%), 24.7% by ELISA (CI: 20.0–29.4%), and 20.1% by PCR (CI: 15.8–24.4%). The diagnostic concordance was strongest during high-transmission seasons, with Fall showing particularly elevated rates across all testing modalities. Winter showed uniformly low prevalence (3.8% *T. equi*, CI: 1.6–6.0%; 7.7% PCR, CI: 4.4–11.0%), suggesting substantially reduced transmission during colder months. These seasonal patterns were highly statistically significant (all $P < 0.001$) and demonstrate clear temporal trends in disease risk, with Fall and Spring representing critical periods for surveillance and control measures. The data supports the need for seasonal adaptation of prevention strategies, with particular emphasis on the high-risk Fall period when infection rates peak across all diagnostic methods.

Regional Prevalence of *Theileria equi*

The regional analysis revealed significant variations in *Theileria* prevalence across different study locations ($P < 0.001$ for most comparisons). The highest infection rates were observed in Mandi Bahaudin/ Head Fagerian (30.0% microscopy positivity, 95% CI: 23.3–36.7%) and Sargodha (29.4%, CI: 22.8–36.0%), which were significantly greater than all other regions ($P < 0.001$). These trends were also observed in all diagnostic methods like MBD/Head Fagerian demonstrating 12.8% *T. equi* (CI: 8.5–17.1%), 22.2% ELISA positivity (CI: 16.4–28.0%), and 28.3% PCR confirmation (CI: 21.9–34.7%). In contrast, Shahpur (15.2%, CI: 10.1–20.3%) and Sahiwal (14.1%, CI: 9.1–19.1%) showed the lowest microscopy positivity rates, with all diagnostic methods consistently yielding lower percentages in these regions (6.2–6.8% *T. equi*, 11.2–14.1% ELISA, 12.9–15.8% PCR). Intermediate prevalence was observed

in Kot Momin (17.9%), Bhalwal (18.1%), Sillanwali (18.1%), and Bhera (18.6%), with overlapping confidence intervals suggesting similar transmission intensity in these areas.

Seasonal Pairwise Post Hoc Comparisons

The pairwise seasonal comparisons revealed significant differences in *Theileria* prevalence across all diagnostic methods. Fall showed markedly higher infection rates than Winter, with an 18.1% greater microscopy positivity ($P < 0.0001$), 8.9% higher *T. equi* detection ($P = 0.0002$), 9.0% increased ELISA positivity ($P = 0.0003$), and 12.4% greater PCR confirmation ($P < 0.0001$). Spring also demonstrated significantly elevated prevalence compared to Winter across all tests, though with smaller differences: +11.7% microscopy ($P = 0.0001$), + 6.2% *T. equi* ($P = 0.003$), + 5.6% ELISA ($P = 0.012$), and + 5.1% PCR ($P = 0.029$).

When comparing Fall to Summer, Fall maintained significantly higher rates: +8.6% microscopy ($P = 0.004$), + 5.7% *T. equi* ($P = 0.011$), + 8.8% ELISA ($P = 0.002$), and + 6.1% PCR ($P = 0.018$). However, Spring and Summer showed minimal differences, with only ELISA reaching significance (+ 5.4%, $P = 0.022$) while other methods showed non-significant variations (microscopy + 2.2%, $P = 0.42$; *T. equi* + 3.0%, $P = 0.12$; PCR – 1.2%, $P = 0.65$).

Multivariable analysis for the prevalence of *Theileria equi*

The multivariate regression analysis revealed that average temperature, animal density, and humidity were the strongest predictors of *Theileria* prevalence, collectively explaining 73% of the observed variation in infection rates. Higher temperatures showed a particularly strong effect, with each 1°C increase in average monthly temperature associated with a 0.82% rise in prevalence ($P < 0.001$). This explains why May (33.5°C) and June (36.0°C) had such high infection rates, while cooler months like January (13.1°C) and December (13.6°C) showed significantly lower transmission. Heatwaves ($\geq 40^\circ\text{C}$) further amplified risk, adding an extra 2.45% to prevalence ($P = 0.001$), which aligns with the spikes seen in April and May.

Animal density emerged as another critical factor; regions with high stocking densities (MBD and Sargodha) had 12–15% higher prevalence than low-density areas ($P < 0.001$), even after controlling for climate. This suggests that overcrowding facilitates tick transmission, independent of weather conditions. Humidity also played a role, with higher humidity ($> 60\%$) increasing risk by 0.31% per 1% rise ($P = 0.002$), particularly during July-September monsoons, when warm, moist conditions likely prolonged tick survival.

Interestingly, precipitation alone did not significantly affect prevalence ($P = 0.18$), possibly because heavy rains both wash away ticks and create humid microclimates, producing counteracting effects. The model's high explanatory power ($R^2 = 0.73$) confirms that temperature-driven transmission, animal density, and humidity are the dominant drivers of *Theileria* outbreaks.

Discussion

The aim of this research was to determine the prevalence Theileriosis in equines, assuming high prevalence of this infection in study area. Though number of studies have been published regarding the epidemiology in this region, but results are contrasting due to variations in the experimental design, sample population, and diagnostic testing (Hussain et al. 2014; Nadal et al. 2022) (Hussain et al. 2023). Equine theileriosis is globally prevalent disease and often infected animals does not exhibit clinical manifestations being asymptomatic carriers spread infection among population. Due to low parasitemia in carrier animals, conventional methods (microscopic examination) often fail to precisely identify those carriers. However, tests that rely on identify the genomic fingerprints or antibodies of pathogens have greater precision for diagnosing equine theileriosis (Soliman et al. 2021). Therefore, this study investigated the prevalence of *Theileria equi* using multiple diagnostic test and wider sample data from all the regions of study area and found overall 20.23% prevalence of protozoal infection having 8.42% share *T. equi* on microscopic examination and 14.4%, 19.3% on PCR and cELISA share respectively among studied population. This divergence between tests advocates that microscopy alone could underestimate true prevalence and may miss subclinical or chronic cases. These findings also highlight that *T. equi* is an important pathogen in the studied population, with nearly one out of every fifth animal had exposure of *T. equi* (ELISA) and about 14% having active infections (PCR). These results are similar to the finding of many published studies (Bhagwan et al. 2015) (Ali et al. 2021), (Zanet et al. 2017), (Sant et al. 2016), (Abedi et al. 2014) but significantly not match with the finding of study conducted by (Soliman et al. 2021), (Khurram et al. 2013), (Coluccia et al. 2024).

The statistical analysis of monthly prevalence discovered significant variations in infection rates around the year, having marked surges during specific months. March emerged as the period of highest infestation, having a microscopic positivity 43.3% at 95% confidence interval it ranges between 34.5–52.6% and PCR detection was 30.0% at CI it ranges between 22.6–38.6%. A secondary, but significant, speak was observed in October, with 37.3% microscopic positivity at 95% CI: 28.6–46.8%. In contrast, April had lowest infestation rates at just 6.5% (95% CI: 3.6–11.3%), representing a 6–7 times difference compared to other peaks. This patterns of *Theileria* infestation stalwartly expressed a stratified diagnostic approach: staining for initial active cases, ELISA for surveillance/ screening, and PCR for confirmation of suspect cases. Moreover, finding also guides about surveillance systems timing for preventive interventions before/during/ after peak infection risk periods for both clinical management and research on Theileriosis.

However, findings of diagnostic tests (Staining, ELISA and PCR) mentioned in table 4 suggest an optimal testing strategy; initial screening with ELISA's harnessing its sensitivity and combining it with PCR's specificity for confirmation, whereas microscopy still remains a convenient test for screening/ diagnosis but have less accuracy being field diagnostic tool. The high Negative Predictive Values (NPV) across all methods are principally appropriate for surveillance.

The regional patterns are consistent irrespective of any diagnostic modality including microscopy, ELISA and PCR which shows the same prevalence in all regions. These findings reveal significant geographic heterogeneity for Theileriosis. These results determine that Fall constantly had highest theileria infestation and had significant statistical differences as compared to both summer and winter in all tests. This consistency of patterns in all diagnostic approaches reinforces the validity of the observed seasonal trends.

Conclusions

This study was designed to detect positive cases of theileriosis and to identify various risk factors associated with the disease. The overall prevalence of *Theileria equi*, piroplasmosis and others blood protozoa were 8.42%, 9.48% and 2.3% (119, 134 and 33 samples respectively) on microscopic examination. These findings suggest that targeted tick control in high-density areas before heatwaves and humidity spikes could effectively reduce infection rates. For precision planning, integrating tick surveillance data could further refine risk predictions.

References

1. Abedi V, Razmi G, Seifi H, Naghibi A (2014) Molecular and serological detection of *Theileria equi* and *Babesia caballi* infection in horses and ixodid ticks in Iran. *Ticks Tick-borne Dis* 5(3):239–244. <https://doi.org/10.1016/j.ttbdis.2013.11.008>
2. Afridi MJK, Mian AH, Saqib M, Abbas G, Ali J, Mansoor MK, Sial AUR, Rasheed I, Hussain MH (2017) Seroprevalence and Risk Factors for *Theileria equi* Infection in Equines from Khyber Pakhtunkhwa Province, Pakistan. *Iran J Parasitol* 12(4):597–605
3. Ali S, Ijaz M, Farooqi SH, Durrani AZ, Rashid MI, Ghaffar A, Ali A, Rehman A, Aslam S, Khan I, Masud A, Mehmood K (2021) Molecular characterisation of *Theileria equi* and risk factors associated with the occurrence of theileriosis in horses of Punjab (Pakistan). *Equine Veterinary Educ* 33(2):75–83. <https://doi.org/10.1111/eve.13161>
4. Altay K, Erol U, Sahin OF, Ulucesme MC, Aytmirzakizi A, Aktas M (2024) Survey of tick-borne pathogens in grazing horses in Kyrgyzstan: phylogenetic analysis, genetic diversity, and prevalence of *Theileria equi*. *Front Vet Sci* 11:1359974. <https://doi.org/10.3389/fvets.2024.1359974>
5. Amiri MS, Yaghfoori S, Razmi G (2020) Molecular detection of *Theileria annulata* in dairy cattle and vector ticks in the Herat area, Afghanistan. <https://doi.org/10.22092/ari.2019.128212.1407>. Archives of Razi Institute
6. Bělková T, Bártová E, Řičařová D, Jahn P, Jandová V, Modrý D, Hrazdilová K, Sedlák K (2021) *Theileria equi* and *Babesia caballi* in horses in the Czech Republic. *Acta Trop* 221:105993. <https://doi.org/10.1016/j.actatropica.2021.105993>
7. Bhagwan J, Kumar A, Kumar R, Goyal L, Goel P, Kumar S (2015) Molecular evidence of *Theileria equi* infection in *Hyalomma anatolicum* ticks infested on sero-positive Indian horses. *Acta Parasitol*

- 60(2). <https://doi.org/10.1515/ap-2015-0045>
8. Coluccia P, Gizzarelli M, Scicluna MT, Manna G, Foglia Manzillo V, Buono F, Auletta L, Palumbo V, Pasolini MP (2024) A cross-sectional study on performance evaluation in Italian standardbred horses' real-time PCR-positive for *Theileria equi*. *BMC Vet Res* 20(1):79. <https://doi.org/10.1186/s12917-024-03908-0>
 9. FAO (2025) FAO Statistical Database Website. Food and Agriculture Organization, Rome, Italy
 10. Gould S, Roy IR, Gabriel FD, Lowe R, Lin CY (2025) Equestrian Sports Safety: An Update on Safety Equipment and Event Medical Coverage. *Curr Sports Med Rep* 24(1):11–17. <https://doi.org/10.1249/JSR.0000000000001222>
 11. Hussain MH, Saqib M, Raza F, Muhammad G, Asi MN, Mansoor MK, Saleem M, Jabbar A (2014) Seroprevalence of *Babesia caballi* and *Theileria equi* in five draught equine populated metropolises of Punjab. *Pakistan Veterinary Parasitol* 202(3–4):248–256. <https://doi.org/10.1016/j.vetpar.2014.01.026>
 12. Hussain N, Shabbir RMK, Ahmed H, Afzal MS, Ullah S, Ali A, Irum S, Naqvi SK-H, Yin J, Cao J (2023) Prevalence of different tick species on livestock and associated equines and canine from different agro-ecological zones of Pakistan. *Front Vet Sci* 9:1089999. <https://doi.org/10.3389/fvets.2022.1089999>
 13. Kalantari M, Sharifiyazdi H, Ghaemi M, Ghane M, Nazifi S (2022) *Theileria equi* in the horses of Iran: Molecular detection, genetic diversity, and hematological findings. *Veterinary Parasitology: Reg Stud Rep* 36:100792. <https://doi.org/10.1016/j.vprsr.2022.100792>
 14. Kappmeyer LS, Thiagarajan M, Herndon DR, Ramsay JD, Caler E, Djikeng A, Gillespie JJ, Lau AO, Roalson EH, Silva JC, Silva MG, Suarez CE, Ueti MW, Nene VM, Mealey RH, Knowles DP, Brayton KA (2012) Comparative genomic analysis and phylogenetic position of *Theileria equi*. *BMC Genomics* 13(1):603. <https://doi.org/10.1186/1471-2164-13-603>
 15. Khurram G, Iqbal Z, Muhammad Sohail S, Muhammad G (2013) Frequency Distribution of Equine Diseases in Three Metropolises of the Upper Punjab, Pakistan. *Int J Agric Biol* 15(6):1067–1074. <https://doi.org/10.1371/journal.pone.0071074>
 16. Mehlhorn H, Schein E (1998) Redescription of *Babesia equi* Laveran, 1901 as *Theileria equi* Mehlhorn, Schein 1998. *Parasitol Res* 84(6):467–475. <https://doi.org/10.1007/s004360050431>
 17. Nadal C, Marsot M, Le Metayer G, Boireau P, Guillot J, Bonnet SI (2022) Spatial and Temporal Circulation of *Babesia caballi* and *Theileria equi* in France Based on Seven Years of Serological Data. *Pathogens* 11(2):227. <https://doi.org/10.3390/pathogens11020227>
 18. Qureshi HU, Shah SMH, Teo FY (2023) Trend assessment of changing climate patterns over the major agro-climatic zones of Sindh and Punjab. *Front Water* 5:1194540. <https://doi.org/10.3389/frwa.2023.1194540>
 19. Ramadan RM, Taha NM, Auda HM, Elsamman EM, El-Bahy MM, Salem MA (2024) Molecular and immunological studies on *Theileria equi* and its vector in Egypt. *Exp Appl Acarol* 93(2):439–458. <https://doi.org/10.1007/s10493-024-00933-4>

20. Sant C, d’Abadie R, Pargass I, Basu AK, Asgarali Z, Charles RA, Georges KC (2016) Prospective study investigating transplacental transmission of equine piroplasmosis in thoroughbred foals in Trinidad. *Vet Parasitol* 226:132–137. <https://doi.org/10.1016/j.vetpar.2016.07.008>

21. Soliman AM, Amer MM, Memon FU (2021) Comparing the efficacy of three diagnostic tools (microscopic examination, conventional PCR, and Real-Time PCR) in detecting *Theileria equi* infection among Egyptian equines

22. Zanet S, Bassano M, Trisciuoglio A, Taricco I, Ferroglio E (2017) Horses infected by Piroplasms different from *Babesia caballi* and *Theileria equi*: species identification and risk factors analysis in Italy. *Veterinary Parasitology* 236:38–41. <https://doi.org/10.1016/j.vetpar.2017.01.003>

23. (2024) Economic Survey of Pakistan, Government of Pakistan, Finance division, Economic Advisor Wing, Islamabad

Table 2 To 6

Table 2 To 6 are available in the Supplementary Files section.

Figures

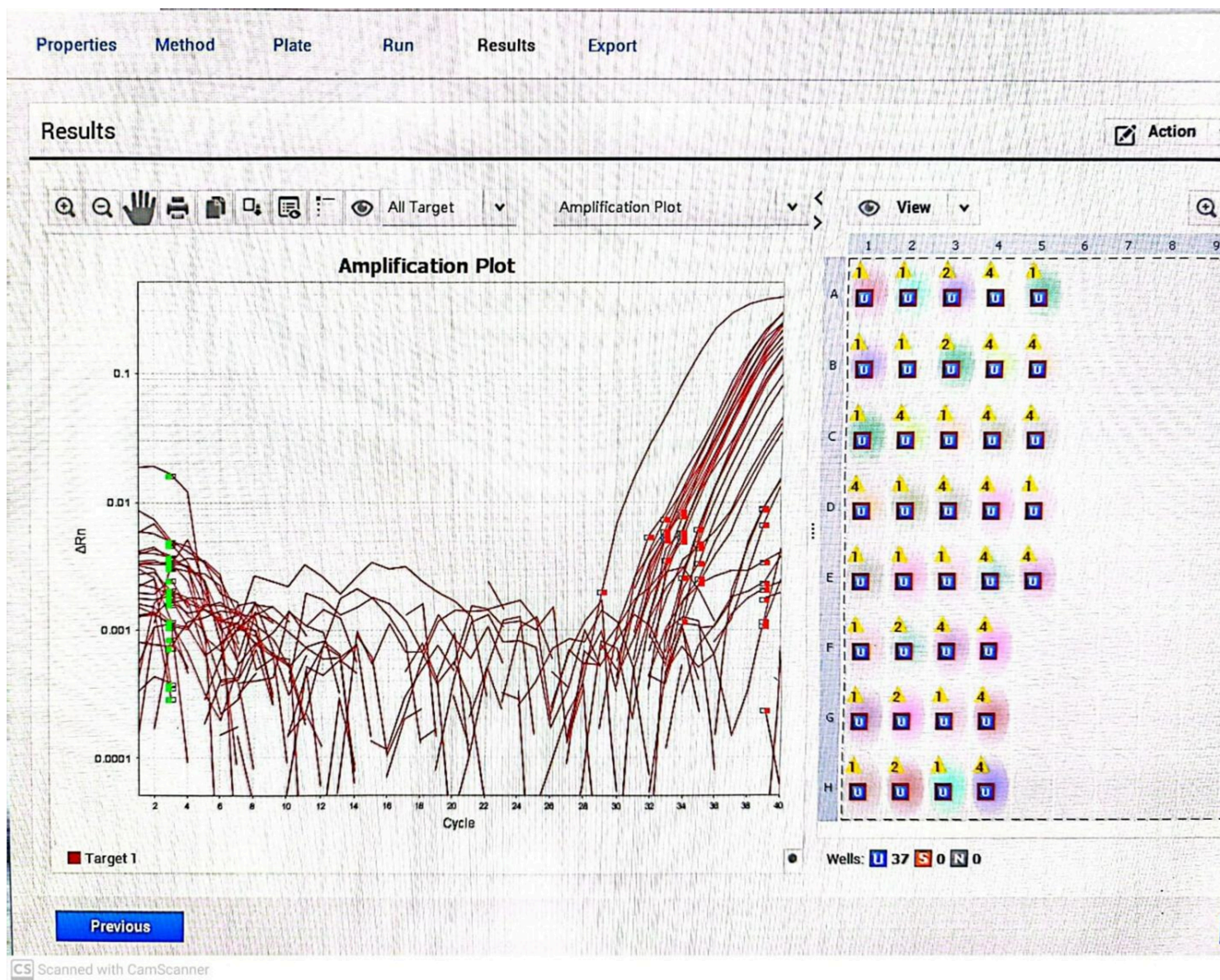


Figure 1

Graphical representation of qPCR of Equine samples

Figure 3

Graphical representation of Equine sample analyzed vs prevalence of protozoa

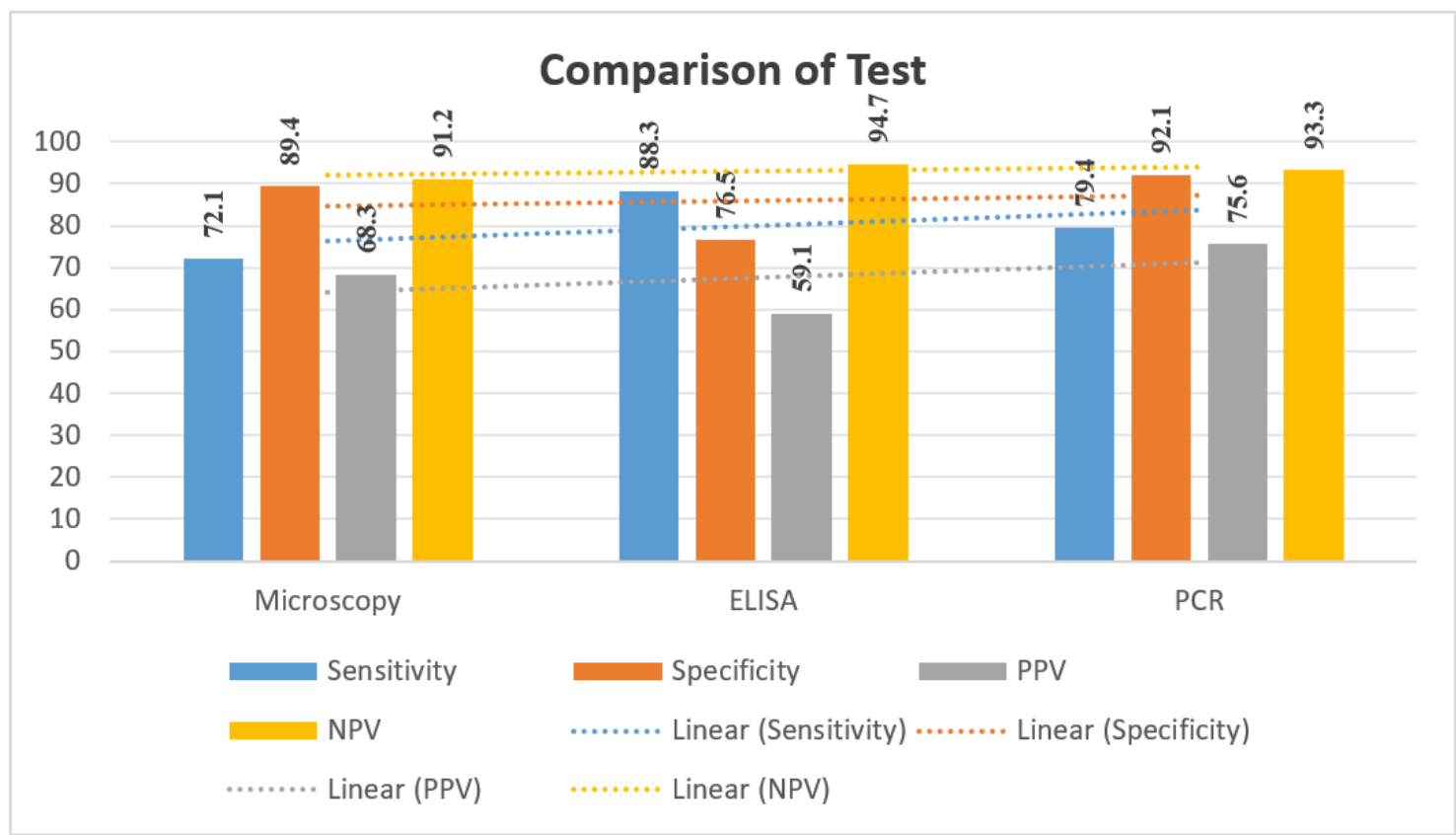


Figure 4

Graphical representation of comparison of different diagnostic test conduct for Equine theleriosis diagnosis

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [table2to6.docx](#)