DOI: 10.22055/ivj.2026.566326.2875

Innovative semi-quantitative multiplex single primer pair PCR method for simultaneous detecting some obligate intracellular piroplasms

Zahra Alizadeh Sarabi[1], Sedighe Nabian[2], Parviz Shayan[2*], Said Bokaei[3] and Hamid Reza Shokrani[4]

[1] PhD Student of Parasitology, Research Center for Ticks and Tick-borne Diseases, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
[2] Professor, Department of Parasitology, Research Center for Ticks and Tick-borne Diseases, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
[3] Professor, Departeman of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
[4] Associate Professor, Department of Pathobiology, Lorestan University, Khorramabad, Iran

Received: 15.12.2025 Accepted: 20.05.2026

Abstract

The causative agents of piroplasmosis, *Babesia* spp. and *Theileria* spp. infect ruminants in the livestock industry with high economical loses worldwide. The early diagnosis of the disease can help manage the successful treatment. Giemsa staining is a common method used worldwide for diagnosis of piroplasmosis which unfortunately can be accompanied with some serious problems like suitable blood smears and quality of staining and needs high experience. Serological methods like ELISA or IFAT was also used in some laboratories. The molecular methods based on DNA analysis preferably used in many laboratories are accurate and reliable. The aim of the present study was to introduce a simple and innovative semi-quantitative multiplex PCR method which can be helpful for simultaneously detection of *Babesia spp.* and *Theileria spp*. For this aim, we used primers derived from 18S rRNA genes which can amplify simultaneously the DNA from hosts (ruminants and ticks) and also from *Babesia* spp., and *Theileria* spp with different easily distinguishable PCR products. Our results showed that, as was expected, the different length of ampilicons generated from *Theileria spp*. and *Babesia spp*. make it possible to differentiate these parasites from each other on the same agarose gel. Interestingly, since the same primer pair can also amplify the 18S rRNA from host cells with larger amplicon compared to the parasites, this property can be used for semi-quantitative analysis of the parasite burden in the samples. It is necessary to mention that the introduced primer pair (VetUTPiro) can be used for differential diagnosis of babesiosis and theileriosis as well as for semi-quantitative multiplex PCR analysis of the parasite burden consequently for controlled therapy management.

Key words: *Theileria spp., Babesia spp*., Semi-quantitative multiplex PCR, Therapy management, Sheep

[*] **Corresponding Author**: Parviz Shayan, Professor, Department of Parasitology, Research Center for Ticks and Tick-borne Diseases, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
E-mail: pshayan@ut.ac.ir

Refrences

Abdallah, M. O., Niu, Q., Yang, J., Hassan, M. A., Yu, P., Guan, G., ... & Yin, H. (2017). Identification of 12 piroplasms infecting ten tick species in China using reverse line blot hybridization. *The Journal of Parasitology*, *103*(3), 221-227.

El-Ashker, M., Hotzel, H., Gwida, M., El-Beskawy, M., Silaghi, C., & Tomaso, H. (2015). Molecular biological identification of Babesia, Theileria, and Anaplasma species in cattle in Egypt using PCR assays, gene sequence analysis and a novel DNA microarray. *Veterinary parasitology*, *207*(3-4), 329-334.

Estrada-Peña, A., Mihalca, A. D., & Petney, T. N. (Eds.). (2018). *Ticks of Europe and North Africa: a guide to species identification*. Springer.

Gubbels, J. M., De Vos, A. P., Van der Weide, M., Viseras, J., Schouls, L. M., De Vries, E., & Jongejan, F. (1999). Simultaneous detection of bovine Theileria and Babesia species by reverse line blot hybridization. *Journal of clinical microbiology*, *37*(6), 1782-1789.

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.

Jaffer, O., Abdishakur, F., Hakimuddin, F., Riya, A., Wernery, U., & Schuster, R. K. (2010). A comparative study of serological tests and PCR for the diagnosis of equine piroplasmosis. *Parasitology Research*, *106*(3), 709-713.

Jongejan, F., Du, C., Papadopoulos, E., Blanda, V., Di Bella, S., Cannella, V., ... & Wang, X. (2024). Diagnostic performance of a rapid immunochromatographic test for the simultaneous detection of antibodies to Theileria equi and Babesia caballi in horses and donkeys. *Parasites & vectors*, *17*(1), 160.

Jouglin, M., Bonsergent, C., de La Cotte, N., Mege, M., Bizon, C., Courouce, A., ... & Malandrin, L. (2025). Equine piroplasmosis in different geographical areas in France: Prevalence heterogeneity of asymptomatic carriers and low genetic diversity of Theileria equi and Babesia caballi. *Ticks and Tick-borne Diseases*, *16*(1), 102434.

Malekifard, F., Tavassoli, M., Yakhchali, M., & Darvishzadeh, R. (2014). Detection of Theileria equi and Babesia caballi using microscopic and molecular methods in horses in suburb of Urmia, Iran. In *Veterinary research forum: an international quarterly journal*, *5*(2), 129.

Mendoza, F. J., Pérez-Écija, A., Kappmeyer, L. S., Suarez, C. E., & Bastos, R. G. (2024). New insights in the diagnosis and treatment of equine piroplasmosis: pitfalls, idiosyncrasies, and myths. *Frontiers in Veterinary Science*, *11*, 1459989.

Modirrousta, H., Habibi, G., Shayan, P., Afshari, A., Mirjalili, A., & Abdigoudarzi, M. (2019). Determination of the Infectivity of Cryopreserved Theileria annulata Sporozoites in Tick Derived Stabilates Iran Ak-93 Strain, by in vivo and in vitro Methods. *Iranian Journal of Parasitology*, *14*(4), 572.

Onyiche, T. E., Suganuma, K., Igarashi, I., Yokoyama, N., Xuan, X., & Thekisoe, O. (2019). A review on equine piroplasmosis: epidemiology, vector ecology, risk factors, host immunity, diagnosis and control. *International journal of environmental research and public health*, *16*(10), 1736.

Onyiche, T. E., Taioe, M. O., Molefe, N. I., Biu, A. A., Luka, J., Omeh, I. J., ... & Thekisoe, O. (2020). Equine piroplasmosis: An insight into global exposure of equids from 1990 to 2019 by systematic review and meta-analysis. *Parasitology*, *147*(13), 1411-1424

Ranjbar-Bahadori, S., Eckert, B., Omidian, Z., Shirazi, N. S., & Shayan, P. (2012). Babesia ovis as the main causative agent of sheep babesiosis in Iran. *Parasitology Research*, *110*(4), 1531-1536.

Riaz, M., Nasreen, N., Khan, A., & Said, M. B. (2023). Differential diagnosis of theileriosis through blood smear examination and polymerase chain reaction in small ruminants from Pakistan. *Open Veterinary Journal*, *13*(6), 697-704.

Rosenblatt, J. E., Reller, L. B., & Weinstein, M. P. (2009). Laboratory diagnosis of infections due to blood and tissue parasites. *Clinical infectious diseases*, *49*(7), 1103-1108.

Shayan, P., & Rahbari, S. (2005). Simultaneous differentiation between Theileria spp. and Babesia spp. on stained blood smear using PCR. *Parasitology Research*, *97*(4), 281-286.

Shayan, P., Hooshmand, E., Rahbari, S., & Nabian, S. (2007). Determination of Rhipicephalus spp. as vectors for Babesia ovis in Iran. *Parasitology research*, *101*(4), 1029-1033.

Tian, Z., Du, J., Yang, J., Liu, A., Liu, X., Liu, G., & Yin, H. (2015). A PCR-RFLP Assay targeting RPS8 gene for the discrimination between bovine Babesia and Theileria species in China. *Parasites & Vectors*, *8*(1), 475.

Tirosh-Levy, S., Gottlieb, Y., Fry, L. M., Knowles, D. P., & Steinman, A. (2020). Twenty years of equine piroplasmosis research: Global distribution, molecular diagnosis, and phylogeny. *Pathogens*, *9*(11), 926.

Walker, A. R. (2003). *Ticks of domestic animals in Africa: a guide to identification of species* (Vol. 74). Edinburgh: Bioscience Reports.