DOI: 10.22055/IVJ.2020.209168.2201 DOR: 20.1001.1.17356873.1400.17.4.3.0

## Molecular detection and prevalence of *Babesia bovis* in cattle of Shahreza city, the south region of Isfahan province, Iran

Mohammad-Mehdi Jaridehdar<sup>1</sup>, Vahid Noaman<sup>2\*</sup>, Yaser Pirali<sup>3</sup> and Hamid-Reza Azizi<sup>4</sup>

<sup>1</sup> MSc Graduated, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran
<sup>2</sup> Associate Professor, Razi Vaccine and Serum Research Institute, Agricultural Research Education and Extension Organization (AREEO), Karaj, Iran

Received: 24.11.2019

Accepted:29.04.2020

## **Abstract**

This study aimed to determie the variety of Babesia species among cattle of shahreza city in the south part of Isfahan Province. A total of 253 blood samples were collected via the jugular vein from healthy cattle, randomly. The extracted DNA from blood cells was amplified by Babesia-all primers, which amplify an approximately 400bp DNA fragment from the region of the 18S rRNA gene from various members of the genus Babesia. All cattle positive samples were further analysed for the presence of B. bigemina and B. bovis by specific semi-nested PCR. B. bigemina and B. bovis were identified by specific semi-nested PCR in 0% and 65.2% of cattle blood samples, respectively. Chi-square tests were used to compare molecular prevalence values relative to Season, Farm, Type, Hygiene, Vectors, Use a disposable needle, Age, and Milk Yield. Among these factors, seasons and vectors were found to have significantly different in the prevalence. The significant major risk factors of B. bovis in cattle were identified as season, hygiene, and vectors by the univariate analysis. Moreover, multivariable logistic regression analysis revealed a statistically significant association of the prevalence of B. bovis with the season. The examination of 50 microscopic fields showed 59.39% sensitivity and 100% specificity compared to molecular examination. The Kappa coefficient between molecular and microscopy (50 fields) techniques indicated a moderate level of agreement (Kappa= 0.504). This study is the first molecular detection of Babesia species from cattle in the south of Isfahan Province, Iran. Further researches are needed to determine the vectors, vector-host interactions and genotypic variants that may affect the presence and distribution of Babesia species in Iran.

**Key words**: Iran, Isfahan Province, *Babesia* species, Cattle, Molecular detection

E-mail: v.noaman@areeo.ac.ir



<sup>© 2020</sup> by the authors. Licensee SCU, Ahvaz, Iran. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution-NonCommercial 4.0 International (CC BY-NC 4.0 license) (http://creativecommons.org/licenses/by-nc/4.0/).

<sup>&</sup>lt;sup>3</sup> Professor, Department of Pathobiology, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran

<sup>&</sup>lt;sup>4</sup> Associate Professor, Department of Pathobiology, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran

<sup>\*</sup> Corresponding Author: Vahid Noaman, Associate Professor, Department of Animal Parasitic Disease, Razi Vaccine and Serum Research Institute, Agricultural Research Education and Extension Organization (AREEO), Karaj, Iran

## Refrences

- Abdela, N., Ibrahim, N. and Begna, F. (2018). Prevalence, risk factors and vectors identification of bovine anaplasmosis and babesiosis in and around Jimma town, Southwestern Ethiopia. Acta Tropica, 177: 9-18.
- Aktas, M. and Ozubek, S. (2015). Molecular and parasitological survey of bovine piroplasms in the Black Sea region, including the first report of babesiosis associated with *Babesia divergens* in Turkey. Journal of Medical Entomology, 52(6): 1344-1350.
- Amorim, L.S., Wenceslau, A.A., Carvalho, F.S., Carneiro, P.L.S. and Albuquerque, G.R. (2014). Bovine babesiosis and anaplasmosis complex: diagnosis and evaluation of the risk factors from Bahia, Brazil. Revista Brasileira de Parasitologia Veterinária, 23(3): 328-336.
- Bhat, S.A., Singh, H., Singh, N.K. and Rath, S.S. (2015). Molecular detection of *Babesia bigemina* infection in apparently healthy cattle of central plain zone of Punjab. Journal of Parasitic Diseases, 39(4): 649-653.
- Bloch, E.M, Lee, T.H., Krause, P.J., Telford, S.R., Montalvo, L. and Chafets, D. (2013). Development of a real-time polymerase chain reaction assay for sensitive detection and quantitation of *Babesia microti* infection. Transfusion, 53: 2299-2306.
- Chaudhry, Z., Suleman, M., Younus, M. and Aslim, A. (2010). Molecular detection of *Babesia bigemina* and *Babesia bovis* in crossbred carrier cattle through PCR. Pakistan Journal of Zoology, 42: 201-204.
- Cheshti, B., Razmi, G.R. and Naghibi, A. (2013). A Comparative Study on Haemoprotozoa Infection in Apparently Healthy Cattle in Different Geographical Areas of Iran Using PCR Method. Journal of Veterinary Microbiology, 9(2):139-145
- Dantas-Torres, F., Alves, L.C. and Uilenberg, G. (2017). Babesiosis. In Arthropod Borne Diseases. 2<sup>nd</sup> ed. Springer, Cham, Pp. 347-354.
- Fakhar, M., Hajihasani, A., Maroufi, S., Alizadeh, H., Shirzad, H., Piri, F. and Pagheh, A. S. (2012). An epidemiological survey on bovine and ovine babesiosis in Kurdistan Province, western Iran. Tropical animal health and production, 44(2): 319-322.
- Farooqi, S.H., Ijaz, M., Rashid, M.I., Aqib, A.I., Ahmad, Z., Saleem, M.H. and Khan, A. (2017). Molecular epidemiology of *Babesia bovis* in bovine of Khyber Pakhtunkhwa, Pakistan. Pakistan Veterinary Journal, 37: 275-80.
- Georges, K., Loria, G.R., Riili, S., Greco, A., Caracappa, S., Jongejan, F. and Sparagano, O. (2001). Detection of haemoparasites in cattle by reverse line blot hybridisation with a note on the distribution of ticks in Sicily. Veterinary Parasitology, 99(4): 273-286.
- Gubbels, J.M., De Vos, A.P., Van der Weide, M., Viseras, J., Schouls, L.M., De Vries, E. and Jongejan, F. (1999). Simultaneous detection of bovine theileria and babesia species by reverse line blot hybridization. Journal of Clinical Microbiology, 37(6): 1782-1789.
- Hashemi-Fesharaki, R. and Amjad, A.R. (1977). An outbreak of Babesia bovis infection in cattle and its control. Archives of Razi Institute, 29(1): 83-86.
- Hasheminasab, S.S., Moradi, P. and Wright, I. (2018). A four year epidemiological and chemotherapy survey of babesiosis and theileriosis, and tick vectors in sheep, cattle and goats in Dehgolan, Iran. Annals of Parasitology, 64(1): 43-48.
- Hussain, S., Ashraf, K., Anwar, N., Jamal, M.A., Naeem, H., Ahmad, N. and Rahman A.U. (2017). Diagnosis of *babesia bovis* infection in indigenous and crossbred cattlewith comparison between conventional and molecular diagnostic techniques. Journal of Information of Molecular Biology, 5(1): 1-6.
- Ibrahim, O., Taha, Z. and Jassim, S. (2012). Prevalence of *Babesia bovis* in cattle in Tikreet city and its surroundings with hematological study. Tikrit Journal of Pure Science, 17: 32-34.
- Jirapattharasate, C., Moumouni, P.F.A., Cao, S., Iguchi, A., Liu, M., Wang, G. and Ratanakorn, P. (2016). Molecular epidemiology of bovine *Babesia spp.* and *Theileria orientalis* parasites in beef cattle from northern and northeastern Thailand. Parasitology International, 65(1): 62-69.
- Kalani, H., Fakhar, M. and Pagheh, A. (2012). An overview on present situation babesiosis and their distribution of ticks in Iran. Iranian Journal of Medical Microbiology, 5(4):59-71. (In Persian)

- Khamesipour, F., Doosti, A., Koohi, A., Chehelgerdi, M., Mokhtari-Farsani, A. and Chengula, A. A. (2015). Determination of the presence of *Babesia DNA* in blood samples of cattle, camel and sheep in Iran by PCR. Archive of Biology Science of Belgrade, 63(1): 83-90.
- Maharana, B. R., Kumar, B., Prasad, A., Patbandha, T.K., Sudhakar, N.R., Joseph, J.P. and Patel, B.R. (2016). Prevalence and assessment of risk factors for haemoprotozoan infections in cattle and buffaloes of South-West Gujarat, India. Indian Journal of Animal Research, 50(5):733-739.
- Motavalli-Haghi, M., Etemadifar, F., Fakhar, M., Teshnizi, S.H., Soosaraei, M., Shokri, A., Hajihasani, A. and Mashhadi, H. (2017) Status of babesiosis among domestic herbivores in Iran: a systematic review and meta-analysis. Parasitology Research, 116:1101–1109.
- Nayel, M., El-Dakhly, K.M., Aboulaila, M., Elsify, A., Hassan, H., Ibrahim, E., Salama, A. and Yanai, T. (2012) The use of different diagnostic tools for Babesia and Theileria parasites in cattle inMenofia, Egypt. Parasitology Research, 111(3):1019–1024.
- Niu, Q., Liu, Z., Yu, P., Yang, J., Abdallah, M.O., Guan, G. and Yin, H. (2015). Genetic characterization and molecular survey of *Babesia bovis*, *Babesia bigemina* and *Babesia ovata* in cattle, dairy cattle and yaks in China. Parasites and Vectors, 8 (1): 1-13.
- Noaman, V. (2013). A molecular study on *Theileria* and *Babesia* in cattle from Isfahan province, Central Iran. Journal of Parasitic Diseases, 37(2): 208-210.
- Noaman, V. (2014). Comparison of molecular and microscopic technique for detection of *Theileria spp*. in carrier cattle. Journal of Parasitic Diseases, 38:64-67.
- Noaman, V., Abdigoudarzi, M. and Nabinejad, A. (2017). Abundance, diversity, and seasonal dynamics of hard ticks infesting cattle in Isfahan Province, Iran. Archives of Razi Institute, 72(1): 15-21.
- Noaman, V., Jahangirnejad, A.A. and Nabinejad, A.R. (2005). A study on prevalence and identification of Babesia spp. in immigrant and sheep & goats and nomadic people of Isfahan Province. Pajouhesh Va- sazandegi, 67: 35-41. (In Persian)
- Noaman, V. and Shayan, P. (2010). Comparison of Microscopy and PCR-RFLP for detection of *Anaplasma marginale* in carrier cattle. Iranian Journal of Microbiology, 2(2): 89-94.
- OIE (2013). Bovine Babesiosis: Aetiology Epidemiology Diagnosis Prevention and Control References.
- Rajabi, S., Esmaeilnejad, B. and Tavassoli, M. (2017). A molecular study on Babesia spp. in cattle and ticks in West-Azerbaijan province, Iran. Veterinary Research Forum, 8(4): 299-306.
- Romero-Salas, D., Mira, A., Mosqueda, J., García-Vázquez, Z., Hidalgo-Ruiz, M., Vela, N.A.O. and Schnittger, L. (2016). Molecular and serological detection of *Babesia bovis* and *Babesia bigemina* infection in bovines and water buffaloes raised jointly in an endemic field. Veterinary Parasitology, 217: 101-107.
- Schnittger, L., Rodriguez A.E., Florin-Christensen, M. and Morrison, D.A. (2012). Babesia: A world emerging. Infection, Genetics and Evolution, 12: 1788–1809.
- Sevgili, M., Cakmak, A., Atlas, M.G. and Ergun, G. (2010). Prevalence of *Theileria annulata* and *Babesia bigemina* in cattle in the vicinity of Sanliurfa. Journal of Animal and Veterinary Advances, 9:292-296.
- Silva, M., Henriques, G., Sanchez, C., Marques, P., Suarez, C. and Oliva, A. (2009). First survey for *Babesia bovis* and *Babesia bigemina* infection in cattle from Central and Southern regions of Portugal using serological and DNA detection methods. Veterinary Parasitology, 166:66-72.
- Simking, P., Yatbantoong, N., Saetiew, N., Saengow, S., Yodsri, W., Chaiyarat, R. and Jittapalapong, S. (2014). Prevalence and risk factors of *Babesia* infections in cattle trespassing natural forest areas in Salakpra Wildlife Sanctuary, Kanchanaburi Province. Journal of Tropical Medicine and Parasitology, 37(1): 10-9.
- Shayan, P. and Rahbari, S. (2005). Simultaneous differentiation between *Theileria spp.* and *Babesia spp.* on stained blood smear using PCR. Parasitology Research, 97(4): 281-286.
- Terkawi, M.A., Alhasan, H., Huyen, N.X., Sabagh, A., Awier, K., Cao, S. and Kalb-Allouz, A.K. (2012). Molecular and serological prevalence of *Babesia bovis* and *Babesia bigemina* in cattle from central region of Syria. Veterinary Parasitology, 187(1): 307-311.
- World Organization for Animal Health [OIE] (2008). Manual of Diagnostic Tests and Vaccines. Bovine babesiosis. http://www.oie.int/eng/normes/mmanual/2008/pdf/2.04.0 (online)
- Yusuf, J.J. (2017). Review on bovine babesiosis and its economical importance. Journal of Veterinary Medicine and Research, 4(5): 1090.

