DOI: 10.22055/ivj.2020.222815.2237

Comparison of PCR and designed ELISA methods to detect avian tuberculosis in suspected pigeons

Mahsa Lari Baghal[1]; Mansour Mayahi[2*]; Nader Mosavari[3] and Zahra Boroomand[4]

[1] DVSc Graduated in Poultry Health and Diseases, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
[2] Professor, Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
[3] Associate Professor, Bovine Reference Laboratory, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran
[4] Associate Professor, Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

Received: 09.03.2020 Accepted:04.06.2020

Abstract

Avian tuberculosis is a chronic disease that generally affects the bird's gastrointestinal tract and it often results in bird death. *Mycobacterium avium* subsp. avium is the most important cause of disease in birds. From October 2018 to October 2019, one hundred one suspected pigeons were selected based on clinical signs and poor physical condition. For ELISA system design, a blood sample was collected via pigeon wing vein and serum was collected. For the PCR method, pigeons were euthanized and post mortem were performed, and samples from liver and spleen and each organ with gross lesions were collected and stored in a freezer at -40 °C. *Mycobacterium avium* subsp. avium strain D4 antigen in Microbial Bank of Vaccine Research Institute was used in the design of the ELISA system. 16S rRNA, IS901 and IS1245 primers were used for molecular testing. The results showed that 39 out of 101 suspected pigeons were positive for the IS901 and IS1245 genomic sequences in PCR. But, only 13 cases out of 101 pigeons, were positive by the designed ELISA system. These 13 cases also were positive in the PCR test. Correlation between PCR and designed ELISA methods results was significant (0.485). Clinical sensitivity and specificity of the PCR method were 100% and the sensitivity and specificity of the ELISA method were 33.33% and 100%, respectively. It was concluded that the rate of avian tuberculosis among pigeon flocks in Ahvaz is relatively high and prevention and control plans should be applied by pigeon keeper and veterinary organization. The sensitivity of PCR to detect avian tuberculosis is higher than the designed ELISA system and ELISA test could be used for primary screening pigeon flocks in the early stages of avian tuberculosis.

Keywords: Avian Tuberculosis, Mycobacterium, PCR, Designed, ELISA

[*] **Corresponding Author**: Mansour Mayahi, Professor, Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz; Ahvaz, Iran, E-mail: mansoormayahi@scu.ac.ir

Refrences

Asadollahi, P.K.; Mosavari, N. and Mayahi, M. (2015). Genotyping of *Mycobacterium avium* subsp. avium isolates from naturally infected lofts of domestic pigeons in Ahvaz by IS901 RFLP. Iranian Journal of Microbiology, 7 (5): 260-264.

Barberis, I.; Bragazzi, N.L.; Galluzzo, L. and Martini, M. (2017). The history of tuberculosis: from the first historical records to the isolation of Koch's bacillus, Journal of Preventive Medicine and Hygiene, 58 (1): E9-E12.

Christal, G. P. (2013). Implication of Mycobacteria in clinical disorders. In: Greg J. Harrison,Teresa L. Lightfoot (Eds). Clinical Avian Medicine, Volume 2. *Spix Publishing*, Inc. *Palm Beach*, *FL*, USA., Pp: 681- 689.

Cromie, R.L.; Ash, N.J.; Brown, M.J. and Stanford, J.L. (2000). Avian immune Responses to *Mycobacterium avium*: the wildfowl example. Developmental and Comparative Immunology, 24(2-3): 169-185.

Cromie, R.L.; Brown, M.J.; Forbes, N.A.; Morgan, J. and Stanford, J.L. (1993). A comparison and evaluation of techniques for diagnosis of avian tuberculosis in wildfowl. Avian Pathology, 22: 617- 630.

Dvorska, L.; Bull, T.J.; Bartos, M.; Matlova, L.; Svastova, P.; Weston, R.T. et al. (2003). A standardised restriction fragment length polymorphism (RFLP) method for typing *Mycobacterium avium* isolates links IS901 with virulence for birds. Jornal of Microbiological Methods, 55(1): 11-22.

Fulton, R. and Sanchez, S. (2008). Tuberculosis. In: Disease of poultry.; Saif, Y.M.; Fadly, A.M.; Glisson, J.R.; McDougald, L.R.; Nolan, L.K. and Swayne, D.E. 12th ed. Blackwel publishing, P: 940-941.

Fulton, R.M. and Thoen, C.O. (2003). Tuberculosis, in Diseases of Poultry.; Saif, Y.M.; Barnes, H.J.; Glisson, J.R.; Fadly, F.M.; McDougald, L.R. and Swayne, D.E., Iowa State University Press, Ames, IA, USA, P: 836-844.

Guerrero, C.; Bernasconi, C.; Burki, D.; Bodmer, T. and Telenti, A. (1995). A novel insertion element from *Mycobacterium avium*, IS1245, is a specific target for analysis of strain relatedness. Journal of Clinical Microbiology, 33(2): 304-307.

Hawkey, C.; Kock, R.A.; Henderson, G.M. and Cindery, R.N. (1990). Hematological changes in domestic fowl (Gallus gallus) and cranes (Gruiformes) with *Mycobacterium avium* infection. Avian Pathology, 19(2): 223-234.

Huard, R.C.; Lazzarini, L.C.; Butler, W.R.; Van soolingen, D. and Ho, J.L. (2003). PCR-based method to differentiate the subspecies of the Mycobacterium tuberculosis complex on the basis of genomic deletions. Journal of Clinical Microbiology, 41(4): 1637-1650.

Izadi, H.; Sohrabi, N.; Tabyanian, M.; Mosavari, N. and Mahdavi, M. (2017). Evaluation of serum level of specific antibody against recombinant ESAT-6 antigen in patients with TB compared to healthy controls. Journal of Fasa University of Medical Sciences, 6(4): 474-480. (In Persian).

Jabbar zadeh, I. and Saifi, M. (2011). Rapid identification of different species of Mycobacteria in tuberculosis. Journal of Veterinary Clinical Research, 2(1): 5-13. (In Persian).

Jorgensen, J.P. (1978). Serological investigation of strains of *Mycobacterium avium* and *Mycobacterium intracellulare* isolated from animal and non-animal sources. Nordisk Veterinary Medicine, 30(4-5): 150-162.

Kul, O.; Tunca, R.; Haziroglu, R.; Diker, K.S. and Karahan, S. (2005). An outbreak of avian tuberculosis in peafowl (Pavo cristatus) and pheasants (phasianus colchicus) in a zoological aviary in Turkey. Veterinary Medicine Czech, 50(10): 446-450.

Kunze, Z.M.; Wall, S.; Appelberg, R.; Silva, M.T.; Portaels, F. and McFadden, J.J. (1991). *IS901*, a new member of a widespread class of atypical insertion sequences, is associated with pathogenicity in *Mycobacterium avium*. Molecular Microbiology, 5(9): 2265-2272.

O'Brien, A.; Whelan, C.; B. Clarke, J.; Hayton, A.; J. Watt, N. and D. Harkiss, G. (2017). Serological Analysis of Tuberculosis in Goats by Use of the Enferplex Caprine TB Multiplex Test. Clinical and Vaccine Immunology, 24 (2): e00518-16. doi: 10.1128/CVI.00518-16.

Pavlas, M.; Michalska, A. and Hunady, M. (1992). Diagnosis of avian tuberculosis-mycobacteriosis by rapid agglutination. Acta Veterinaria Brno, 62 (1): 63-69.

Qadiri, K.Q; Izadi, B.; Afsharian, M.; Rezaeii, M. and Namdari, S.A. (2007). Evaluation of the diagnostic value of serologic tests (IgA,IgG,IgM) in the diagnosis of TB in Kermanshah during 2002-2004. Journal of Infectious Diseases and Tropical Medicine Association of the Infectious Disease Specialists, 11(35): 55-58. (In Persian).

Shitaye, J.E.; Matlova, L.; Horvathova, A.; Moravkova, M.; Dvorska-Bartosova, L. et al. (2008). Diagnostic testing of different stages of avian tuberculosis in naturally infected hens (Gallus domesticus) by the tuberculin skin and rapid agglutination tests, faecal and egg examinations. Veterinarni Medicina, 53(2): 101-110.

Singhla, T.; Boonyayatra, S.; Chulakasian, S.; Lukkana, M.; Alvarez, J.; Sreevatsan, S. et al. (2019). Determination of the sensitivity and specificity of bovine tuberculosis screening tests in dairy herds in Thailand using a Bayesian approach. BMC Veterinary Research, 15: 149.

Taroudi zadeh, Y. and Imani fouladi, A.A. (2013). Evaluation of underlying and environmental factors causing TB. Journal of Medical System Organization of Islamic Republic of Iran, 30(4): 335-340. (In Persian).

Tell, L.A.; Woods, L. and Cromie, R.L. (2001). Mycobacteriosis in birds. "Avian tuberculosis in birds," Review Science and Technology office Internationale des Epizooties, 20 (1): 180-203.

Thoen, C.O.; Armbrust, A.L. and Hopkins, M.P. (1979). Enzyme- linked immunosorbent assay for detecting antibodies in swine infected with *Mycobacterium avium*. American Journal of Veterinary Research, 40(8): 1096-1099.

Van der Heyden, N. (1997). Mycobacterial infections: new strategies in the treatment of avian mycobacteriosis. Seminars in Avian Exotic Pet Medicine, 6(1): 25-33.

Van Soolingen, D.; de Haas, P.E.W. and Kremer, K. (2001). Restriction Fragment Length Polymorphism (RFLP) Typing of Mycobacteria. Methods in Molecular Medicine, 54: 165-203.

Velayati, A.A. (1988). Tuberculosis. Volume One, First Edition, University Publishing Center, Tehran, P: 20.

Wood, P.R.; Corner, L.A.; Rothel, J.S.; Ripper, J.L.; Fifis, T.; McCormick, B.S. et al. (1992). A field evaluation of serological and cellular diagnostic tests for bovine tuberculosis. Veterinary Microbiology, 31(1): 71-79.