

Genotype profiling of *Mycobacterium avium* subspecies *paratuberculosis* isolates recovered from feces and milk of Iranian bovine, ovine and caprine hosts, are the sheep (S) type strains mysteriously overlooked in Iran

Lida Abdolmohammadi Khiav¹, Masoud Haghkhah², Nader Mosavari³ and Keyvan Tadayon^{3*}

¹ PhD Graduated of bacteriology, Faculty of Veterinary Medicine, Shiraz University, Shiraz, Iran

² Associate Professor, Department of Pathobiology, Faculty of Veterinary Medicine, Shiraz University, Shiraz, Iran

³ Associate Professor, Bovine Tuberculosis Reference Laboratory, Razi Vaccine & Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran

Received: 07.06.2019

Accepted: 0.9.10.2019

Abstract

Mycobacterium avium subspecies *paratuberculosis* (MAP) is known worldwide for its role in causing paratuberculosis (Ptb), a chronic incurable intestinal tract infection of ruminants. Concerns remain in place as the elusive MAP epidemiology and its potential zoonotic nature continue to challenge veterinarians across the world. Almost half a century since its initial reports, Ptb is still not a notifiable disease in Iran leading to the unfortunate lack of a public-funded control scheme. We undertook this study to extend our knowledge of the MAP population genetic in diverse hosts in Iran. During seven months, 35 MAP isolates collected from milk and feces obtained from sheep, goat and cattle originating 8 provinces plus a further eleven archived MAP isolates were genetically interrogated. The Collins strategy was conducted on examined isolates to differentiate between cattle and sheep types of MAP bacteria. The study was further expanded by RFLP-IS900 analysis using *PstI* in search for genetic diversity. The findings of Collins strategy showed that all animal species covered by the study were exclusively infected by 'cattle' strains. In genotyping, on the other hand, RFLP-IS900 analysis yielded 10 largely similar genotypes with five of them infecting multiple hosts. Nevertheless, no trace of varying profiles was detected in individual farms and conspecifics. These data, taken together with earlier observations by others, raise the question of the mysterious absence of sheep-type strains in Iran. Circulation of highly similar strains in Iranian farm ruminants might be an indication of a homogenic MAP population with a tendency for diversification started only recently. The extent of temporal and spatial characteristics of such diversification is still unclear and left for future works.

Key words: Paratuberculosis, *Mycobacterium avium*, Sheep type strain

* **Corresponding Author:** Keyvan Tadayon, Associate Professor, Bovine Tuberculosis Reference Laboratory, Razi Vaccine & Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran, E-mail: mmb093@gmail.com



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References

- Abdolmohammadi Khiav, L., Haghkhal, M., Tadayon, K., & Mosavari, N. (2018). Isolation of *Mycobacterium avium* subsp. *paratuberculosis* and confirmation of cases by Nested-PCR. *Veterinary Researches & Biological Products*, 32(1), 41-47.
- Baharsefat, M., Amjadi, A. R., Ahourai, P., Yamini, B., Entessar, F., & Hedayati, H. (1972). Paratuberculosis in goats and sheep in Iran. Epidemiological, clinical, pathological features and laboratory diagnosis. *Archives of Razi Institute*, 24(1), 49-61.
- Bannantine, J. P., Wu, C. W., Hsu, C., Zhou, S., Schwartz, D. C., Bayles, D. O., et al. (2012). Genome sequencing of ovine isolates of *Mycobacterium avium* subspecies *paratuberculosis* offers insights into host association. *BioMed Central Genomics*, 13, 89.
- Bohrer, V. L. (1972). On the relation of harvest methods to early agriculture in the Near East. *Economic Botany*, 26(2), 145-155.
- Castellanos, E., Aranaz, A., & De Buck, J. (2010). Rapid identification and differentiation of *Mycobacterium avium* subspecies *paratuberculosis* types by use of real-time PCR and high-resolution melt analysis of the MAP1506 locus. *Journal of Clinical Microbiology*, 48(4), 1474-7.
- Castro, C., González, L., Rozo, J. C., Puerto, G., & Ribón, W. (2009). Biosafety evaluation of the DNA extraction protocol for *Mycobacterium tuberculosis* complex species, as implemented at the Instituto Nacional de Salud, Colombia. *Biomedica*, 29(4), 561-566.
- Collins, D. M., De Zoete, M., & Cavaignac, S. M. (2002). *Mycobacterium avium* subsp. *paratuberculosis* strains from cattle and sheep can be distinguished by a PCR test based on a novel DNA sequence difference. *Journal of Clinical Microbiology*, 40(12), 4760-4762.
- Collins, M. T., Kenefick, K. B., Sockett, D. C., Lambrecht, R. S., McDonald, J., & Jorgensen, J. B. (1990). Enhanced radiometric detection of *Mycobacterium paratuberculosis* by using filter-concentrated bovine fecal specimens. *Journal of Clinical Microbiology*, 28(11), 2514-2519.
- Cousins, D. V., Williams, S. N., Hope, A., & Eamens, G. J. (2000). DNA fingerprinting of Australian isolates of *Mycobacterium avium* subsp. *paratuberculosis* using IS900 RFLP. *Australian Veterinary Journal*, 78(3), 184-90.
- Dimareli-Malli, Z., Mazaraki, K., Stevenson, K., Tsakos, P., Zdragas, A., Giantzi, V. et al. (2013). Culture phenotypes and molecular characterization of *Mycobacterium avium* subsp. *paratuberculosis* isolates from small ruminants. *Research in Veterinary Science*, 95(1), 49-53.
- Djonne, B., Pavlik, I., Svastova, P., Bartos, M., & Holstad, G. (2005). IS900 restriction fragment length polymorphism (RFLP) analysis of *Mycobacterium avium* subsp. *paratuberculosis* isolates from goats and cattle in Norway. *Acta Veterinaria Scandinavica*, 46(1-2), 13-8.
- Dohmann, K., Strommenger, B., Stevenson, K., De Juan, L., Stratmann, J., Kapur, V. et al. (2003). Characterization of genetic differences between *Mycobacterium avium* subsp. *paratuberculosis* type I and type II isolates. *Journal of Clinical Microbiology*, 41(11), 5215-5223.
- Galiero, A., Fratini, F., Mataragka, A., Turchi, B., Nuvoloni, R., Ikononopoulos, J., & Cerri, D. (2016). Detection of *Mycobacterium avium* subsp. *paratuberculosis* in cheeses from small ruminants in Tuscany. *International Journal of Food Microbiology*, 217, 195-9.
- Mobius, P., Hotzel, H., Rassbach, A., & Kohler, H. (2008). Comparison of 13 single-round and nested PCR assays targeting IS900, ISMav2, F57 and locus 255 for detection of *Mycobacterium avium* subsp. *paratuberculosis*. *Veterinary Microbiology*, 126(4), 324-33.
- Mobius, P., Luyven, G., Hotzel, H., & Kohler, H. (2008). High genetic diversity among *Mycobacterium avium* subsp. *paratuberculosis* strains from German cattle herds shown by combination of IS900 restriction fragment length polymorphism analysis and mycobacterial interspersed repetitive unit-variable-number tandem-repeat typing. *Journal of Clinical Microbiology*, 46(3), 972-81.
- Mohrekeesh Haghghat, M., Shahmoradi, A. H., Tadayon, K., Keshavarz, R., Ghaderi, R., Sekhavati, M., & Mosavari, N. (2017). Molecular identification of *Mycobacterium avium* subspecies *paratuberculosis* 316 F and III & V strains by a multi-approach strategy. *Veterinary Researches and Biological Products*, 30(2), 89-100.

- Momotani, E., Ozaki, H., Hori, M., Yamamoto, S., Kuribayashi, T., Eda, S., & Ikegami, M. (2012). *Mycobacterium avium* subsp. *paratuberculosis* lipophilic antigen causes Crohn's disease-type necrotizing colitis in mice. *SpringerPlus*, 1(47), 1-10.
- O'Mahony, J. & Hill, C. (2002). A real time PCR assay for the detection and quantitation of *Mycobacterium avium* subsp. *paratuberculosis* using SYBR Green and the Light Cycler. *Journal of Microbiological Methods*, 51(3), 283-293.
- Schonenbrucher, H., Abdulmawjood, A., Failing, K., & Bulte, M. (2008). New triplex real-time PCR assay for detection of *Mycobacterium avium* subsp. *paratuberculosis* in bovine feces. *Applied and Environmental Microbiology*, 74(9), 2751-2758.
- Singh, A. V., Chauhan, D. S., Singh, A., Singh, P. K., Sohal, J. S., & Singh, S. V. (2015). Application of IS1311 locus 2 PCR-REA assay for the specific detection of 'Bison type' *Mycobacterium avium* subspecies *paratuberculosis* isolates of Indian origin. *Indian Journal of Medical Research*, 141(1), 55-61.
- Sohal, J. S., Singh, S. V., Singh, A. V., & Singh, P. K. (2010). Strain diversity within *Mycobacterium avium* subspecies *paratuberculosis*--a review. *Indian Journal of Experimental Biology*, 48(1), 7-16.
- Sting, R., Hrubenja, M., Mandl, J., Seemann, G., Salditt, A., & Waibel, S. (2014). Detection of *Mycobacterium avium* subsp. *paratuberculosis* in faeces using different procedures of pre-treatment for real-time PCR in comparison to culture. *Veterinary Journal*, 199(1), 138-142.
- VLA. (2008). Johne's disease continues to be the most common cause of bovine enteric disease, *Veterinary Record*, 163(6), 171-174.
- Walton, C. L. (1917). Some geographical aspects of the sheep industry. *Scottish Geographical Magazine*, 33(7), 303-315.
- Whipple, D., Kapke, P., & Vary, C. (1990). Identification of restriction fragment length polymorphisms in DNA from *Mycobacterium paratuberculosis*. *Journal of Clinical Microbiology*, 28(11), 2561-2564.
- Windsor, P. A. (2015). Paratuberculosis in sheep and goats. *Veterinary Microbiology*, 181(1-2), 161-169.
- Wolf, R., Barkema, H. W., De Buck, J., & Orsel, K. (2015a). Factors affecting management changes on farms participating in a Johne's disease control program. *Journal of Dairy Sciences*, 98(11), 7784-96.
- Wolf, R., Barkema, H. W., De Buck, J., & Orsel, K. (2015b). Sampling location, herd size, and season influence *Mycobacterium avium* ssp. *paratuberculosis* environmental culture results. *Journal of Dairy Sciences*, 98(1), 275-87.
- Zikovitz, A. E., Stalis, I. H., Bicknese, E. J., Rideout, B. A., & Pye, G. W. (2018). Resolution of a Localized Granuloma Caused by *Mycobacterium avium-intracellulare* Complex on the Cere of a Bruce's Green Pigeon (Treron waalia). *Journal of Avian Medicine and Surgery*, 32(4), 322-327.