CENTAUR GLOBAL NETWORK MINIREVIEWS
MYCOBACTERIA AS A PUBLIC HEALTH RISK

COMMENTS – DISCUSSION – OPINIONS

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2013-09-15

I have two questions. First, is it possible to diagnose and/or detect MAP bacilli on acid fast stained smears with any new methods such as PCR, etc.? Second, when we use acid fast staining for paraTB suspicious feces, sometimes we see some red bacilli in clusters such as MAP, BUT they are much larger, in both width and length. What are they? We see these organisms in both MAP negative and positive smears.

Any comments or information would be appreciated.

Best regards

Masoud Haghkhah, DVM PhD

Re by K. Hruska

(1) PCR and FISH with specific DNA or PNA probes can be used for typing mycobacteria.
(2) Ziehl-Neelsen staining is not a specific method for MAP so more species can be detected in one smear.
All methods have to be used by experienced personnel both for the methodological and interpretation reasons.

For more information see:

Acid Fast Bacilli

More general information on acid fast staining and interpretation of results"

Ziehl–Neelsen stain
Acid-fast Ziehl-Neelsen Stain Reaction
Acid-Fast Stain Protocols
UG practicals: Ziehl-Neelsen staining
Frequently asked questions

Annex to a reply to Dr. Haghkhah (2013-09-15)

Cauwelaert, N.D., Ramarokoto, H. , Ravololonandriana, P., Richard, V., Rasolofo, V. (2011)
DNA Extracted from Stained Sputum Smears Can Be Used in the MTBDRplus Assay
Journal of Clinical Microbiology, 49, 3600-3603

We examined the feasibility of using DNA extracted from stained sputum smears for the detection of rifampin and isoniazid resistance with the commercial MTBDRplus assay from Hain Lifescience GmbH, Nehren, Germany. Overall sensitivity was initially low (70.0%) but increased to 96.7% when a multiplex PCR preamplification step was added. We then tested stored Mycobacterium tuberculosis-positive stained smears prepared from 297 patients' sputum samples. Species identification and drug susceptibility testing (DST) had been performed at the Institut Pasteur de Madagascar. Overall, the performance of the MTBDRplus assay applied to slide DNA was similar to that obtained in other studies with DNA extracted from clinical specimens. With the ready availability of stained smears in routine diagnostic laboratories and their easy transport and storage at room temperature, this approach should

be useful for optimizing the treatment of multidrug-resistant tuberculosis and for conducting resistance surveys aimed at identifying hot-spot regions and breaking chains of transmission

Coelho, A.C., Pinto, M.L., Miranda, A., Coelho, A.M., Pires, M.A., Matos, M. (2010)
Comparative evaluation of PCR in Ziehl-Neelsen stained smears and PCR in tissues for diagnosis of Mycobacterium avium subsp paratuberculosis
Indian Journal of Experimental Biology, 48, 948-950

Thirty six tissues from sheep, previously diagnosed with paratuberculosis, were tested by PCR in positive Ziehl-Neelsen staining smears of tissues, and PCR in tissues targeting IS900 specific for Mycobacterium avium subsp. paratuberculosis. DNA amplification was achieved in 33.3% Ziehl-Neelsen smears, and in 61.1% tissue samples. Combination of both techniques found 66.7% samples as positive. Combination of techniques would, therefore, increase the sensitivity of diagnosis

Khan, F.A., Chaudhry, Z.I., Ali, M.I., Khan, S., Mumtaz, N., Ahmad, I. (2010)
Detection of Mycobacterium avium subsp paratuberculosis in tissue samples of cattle and buffaloes
Tropical Animal Health and Production, 42, 633-638

Tissue samples were collected at random from cattle (Bos taurus) and buffalo (Bubalus bubalis) from an abattoir of the district of Lahore and were analyzed for the presence of Mycobacterium avium subsp. paratuberculosis and Mycobacterium bovis through acid-fast staining and polymerase chain reaction (PCR). Body condition of animals and diarrhea were recorded. Most of the animals were emaciated. Diarrhea was noticed in 15.6% of buffaloes and 19.2% of cattle. Intestinal pathology was observed in 29% of buffaloes and 32.8% of cattle. Number of mesenteric lymph node (MLN) showing gross lesions was a bit higher (35.6%) in cattle than buffalo (31.2%). Acid-fast staining of tissue scraping smears revealed the presence of acid-fast bacilli (AFB) in 17.4% intestinal and 16.4% MLN tissue samples in buffalo, while in cattle 19.2% intestinal and 17.8% MLN were found positive for AFB. In buffaloes, PCR confirmed 12.8% intestinal and 12.4% MLN positive samples for M. avium subsp. paratuberculosis. However, in cattle, PCR analysis demonstrated 14.2% positive results for M. avium subsp. paratuberculosis in both MLN and intestinal tissue samples. PCR also confirmed M. bovis in 5.8% of cattle and 5% of buffalo MLN and intestinal tissues. PCR positive tissue samples for M. avium subsp. paratuberculosis were from those animals which were emaciated, having diarrhea, and severe gross lesions. AFB were also detected in tissue scraping smears of these animals. It is concluded that infection by various mycobacterium species can be differentiated by PCR, which is not possible by acid-fast staining technique

Mota, R.A., Peixoto, P.V., Yamasaki, E.M., de Medeiros, E.S. , da Costa, M.M., Peixoto, R.M., Brito, M.F. (2010)
Occurrence of paratuberculosis in buffaloes (Bubalus bubalis) in Pernambuco
Pesquisa Veterinaria Brasileira, 30, 237-242

Mota R.A., Peixoto P.V., Yamasaki E.M., Medeiros E.S., Costa M.M., Peixoto R.M. & Brito M.F. 2010.[Occurrence of paratuberculosis in buffaloes (Bubalus bubalis) in Pernambuco.] Ocorrencia de paratuberculose em bufalos (Bubalus bubalis) em Pernambuco. Pesquisa Veterinaria Brasileira 30(3):237-242. Departamento de Medicina Veterinaria, Universidade Federal Rural de Pernambuco, Recife, PE 52171-900, Brazil.E-mail: rinaldo.mota@hotmail.com Paratuberculosis (PTB) is a disease of great economical importance for ruminant in several countries and represents a threat to the development of Brazilian livestock. The contagious disease caused by chronic PTB leads to incurable granulomatous enterocolitis of difficult control. PTB is caused by the Mycobacterium avium subsp. paratuberculosis (MAP). No record on the occurrence of paratuberculosis in buffaloes in Brazil could be found. Five of 100 buffaloes in a herd in Pernambuco-Brazil showed clinical signs characteristic of PTB. At necropsy, of two animals the lesions were restricted to the small intestine with thickening and corrugation of the mucosa, increase of mesenteric lymph nodes and prominent lymph vessels. Histopathology revealed granulomatous inflammation infiltrated with numerous epithelioid macrophages, Langhans type giant cells, and clusters of

Ziehl-Neelsen (ZN) positive organisms within the intestinal mucosa. In the mesenteric lymph nodes there was thickening of the capsule and marked granulomatous inflammation. Smears of feces and scrapping smears were prepared from intestinal mucosa and cut surface of mesenteric lymph nodes and, stained by the Ziehl-Neelsen method for research of acid fast bacilli, with positive results. Lymph nodes and intestinal mucosa revealed at IS900 specific polymerase chain reaction amplification of a fragment of about 110pb, confirmed by the comparison with other sequences of M. avium subsp. paratuberculosis available in GenBank

Munjal, S.K., Tripathi, B.N., Paliwal, O.P., Boehmer, J., Homuth, M. (2007)
Application of different methods for the diagnosis of experimental paratuberculosis in goats
Zoonoses and Public Health, 54, 140-146

The diagnosis of subclinical paratuberculosis is still considered a major prob-lem worldwide. As part of investigating diagnostic strategies for the paratuberculosis infection, sequential results of various diagnostic methods in a progressive experimental infection in goats were evaluated. Twenty-three goat kids were divided into three groups: the infected, contact and control, comprising 10, five and eight goats respectively. Animals of the infected group were orally inoculated on seven occasions with 5 ml of inoculum containing 2 x 10(9) Mycobacterium avium ssp. paratuberculosis per ml. Lymphoycte proliferation test using johnin PPD detected paratuberculosis infection from 60 days post-infection (DPI) onwards. The johnin PPD was found to be a better antigen for the proliferative assays as compared with the sonicated antigen. The faecal smear examination with acid-fast staining detected more goats as positive than bacterial culture and polymerase chain reaction (PCR). Lipoarabinomannan enzyme-linked immunosorbent assay (ELISA) started detecting infected goats from 150 DPI onwards followed by indirect ELISA and agar gel immunodiffusion from 180 DPI onwards. Histological examination was confirmatory and detected five infected goats as positive

Bannantine, J.P., Li, L.L., Sreevatsan, S., Kapur, V. (2013)
How does a Mycobacterium change its spots? Applying molecular tools to track diverse strains of Mycobacterium avium subspecies paratuberculosis
Letters in Applied Microbiology, 57, 165-173

Defining genetic diversity in the wake of the release of several Mycobacterium avium subsp. paratuberculosis (MAP) genome sequences has become a major emphasis in the molecular biology and epidemiology of Johne's disease research. These data can now be used to define the extent of strain diversity on the farm. However, to perform these important tasks, researchers must have a way to distinguish the many MAP isolates/strains that are present in the environment or host to enable tracking over time. Recent studies have described genetic diversity of the Mycobacterium avium complex (MAC), of which MAP is a member, through pulsed-field gel electrophoresis, single sequence repeats, variable-number tandem repeats, genome rearrangements, single nucleotide polymorphisms and genomewide comparisons to identify insertions and deletions. Combinations of these methods can now provide discrimination sufficient for dependable strain tracking. These molecular epidemiology techniques are being applied to understand transmission of Johne's disease within dairy cattle herds as well as identify which strains predominate in wildlife

Biet, F., Sevilla, I.A., Cochard, T., Lefrancois, L.H., Garrido, J.M., Heron, I., Juste, R.A., McLuckie, J., Thibault, V.C., Supply, P., Collins, D.M., Behr, M.A., Stevenson, K. (2012)
Inter- and Intra-subtype genotypic differences that differentiate Mycobacterium avium subspecies paratuberculosis strains
BMC Microbiology, 12,

Background: Mycobacterium avium subspecies paratuberculosis (Map) is the aetiological agent of Johne's disease or paratuberculosis and is included within the Mycobacterium avium complex (MAC). Map strains are of two major types often referred to as 'Sheep' or 'S-type' and 'Cattle' or 'C-type'. With the advent of more discriminatory typing techniques it has been possible to further classify the S-type strains into two groups referred to as Type I and Type III.

This study was undertaken to genotype a large panel of S-type small ruminant isolates from different hosts and geographical origins and to compare them with a large panel of well documented C-type isolates to assess the genetic diversity of these strain types. Methods used included Mycobacterial Interspersed Repetitive Units - Variable-Number Tandem Repeat analysis (MIRU-VNTR), analysis of Large Sequence Polymorphisms by PCR (LSP analysis), Single Nucleotide Polymorphism (SNP) analysis of gyr genes, Pulsed-Field Gel Electrophoresis (PFGE) and Restriction Fragment Length Polymorphism analysis coupled with hybridization to IS900 (IS900-RFLP) analysis. Results: The presence of LSP(A)4 and absence of LSP(A)20 was confirmed in all 24 Map S-type strains analysed. SNPs within the gyr genes divided the S-type strains into types I and III. Twenty four PFGE multiplex profiles and eleven different IS900-RFLP profiles were identified among the S-type isolates, some of them not previously published. Both PFGE and IS900-RFLP segregated the S-type strains into types I and III and the results concurred with those of the gyr SNP analysis. Nine MIRU-VNTR genotypes were identified in these isolates. MIRU-VNTR analysis differentiated Map strains from other members of Mycobacterium avium Complex, and Map S-type from C-type but not type I from III. Pigmented Map isolates were found of type I or III. Conclusion: This is the largest panel of S-type strains investigated to date. The S-type strains could be further divided into two subtypes, I and III by some of the typing techniques (IS900-RFLP, PFGE and SNP analysis of the gyr genes). MIRU-VNTR did not divide the strains into the subtypes I and III but did detect genetic differences between isolates within each of the subtypes. Pigmentation is not exclusively associated with type I strains

Rawther, S.S., Saseendranath, M.R., Nair, G.P.K., Tresamol, P.V., Pillai, U.N., Abraham, J., Senthilkumar, T.M.A., Nagalakshmy, S., Nimisha, K.K.N. (2012)
Diagnosis of paratuberculosis in goats by cell mediated immune response, conventional and molecular diagnostic techniques
Tropical Animal Health and Production, 44, 911-914

In the present study efficacy of single intradermal Johnin test, acid fast staining of faecal smear and IS 900 faecal polymerase chain reaction tests was evaluated in 200 goats for detection of Mycobacterium avium subsp paratuberculosis. Two hundred goats comprising 150 goats from an organised farm in Trichur district and 50 goats reared under field condition at farmers premise from Malappuram district of Kerala state formed the study population. Faecal smear from all the 200 goats was stained by Ziehl-Neelsen acid fast stain and faecal polymerase chain reaction (PCR) specific for M. avium subsp paratuberculosis (MAP); IS 900 was performed on all samples. All the animals were subjected to single intradermal Johnin test. Out of 200 goats screened for paratuberculosis, six goats (3%), 11 goats (5.5%) and 42 goats (21%) were found positive by Ziehl-Neelsen acid fast staining of faecal smear, single intradermal Johnin test and IS 900 PCR respectively. Results of the present study indicate that amplification of IS 900 insertion element was the most specific and sensitive diagnostic detection method. Single intradermal Johnin test and Ziehl-Neelsen acid fast staining did not show any significant difference

Barani, R., Sarangan, G., Antony, T., Periyasamy, S., Kindo, A.J., Srikanth, P. (2012)
Improved detection of Mycobacterium tuberculosis using two independent PCR targets in a tertiary care centre in South India
Journal of Infection in Developing Countries, 6, 46-52

Introduction: Tuberculosis (TB) causes significant morbidity and mortality worldwide as one of the leading infectious diseases. In India, more than 1.8 million new cases occur every year. Rapid and accurate diagnosis of TB would improve patient care and limit its transmission. This study aimed to evaluate a dual target polymerase chain reaction (PCR) diagnostic assay to detect Mycobacterium tuberculosis from pulmonary and extra-pulmonary samples at a tertiary care centre in South India. Methodology: Samples were collected from patients with a low index of suspicion of TB. Acid-fast smears were performed by Auramine O fluorescent microscopy and PCR was performed by using two site-specific primer pairs targeting IS6110 by nested PCR and TRC4 by conventional PCR. Amplified products for IS6110 and/or TRC4 were indicative of M. tuberculosis. Results: Among 114 (19 pulmonary and 95 extra-

pulmonary) samples tested by PCR assay, 12 (11%) were positive for both IS6110 and TRC4, of which 11 (10%) were non-respiratory and one was (1%) respiratory in origin. PCR for TRC4 alone was positive for eight (7%) non-respiratory and two (2%) respiratory samples, while IS6110 alone tested positive for six (5%) non-respiratory samples and one (1%) respiratory sample. Of a total of 29 PCR positive samples, 17 (15 %) were acid-fast smear positive. Conclusion: Although the target site of IS6110 is specific for M. tuberculosis, some strains from South India may lack this region. Therefore, the use of an additional target site (TRC4) is required for improved detection of M. tuberculosis.

Go back to COMMENTS – DISCUSSION – OPINIONS