**Identifying co-infections of *Mycobacterium avium* subspecies *paratuberculosis***

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*Mycobacterium avium paratuberculosis* (MAP) causes chronic wasting of domestic and wild ruminants. Animals are assumed to acquire infection once, despite high lifelong infection pressure. The aim of this study was to evaluate the utility of single genome analysis (SGA) for the detection of multiple strains that coexist in clinical samples and thereby in animals. The methodology of SGA was standardized using MAP isolates from bovine fecal samples previously considered to include at least two different strains evidenced as overlaps in short sequence repeat loci used for strain typing. Control isolates were selected from animals that had consistent lifelong MLSSR profiles. DNA was extracted, diluted and used as template for PCR amplification using specific primers for each of 5 short sequence repeat of interest. Dilutions were titrated to allow an average of one genomic template copy per amplicon generated. Amplicons were purified, sequenced and analyzed with the SeqMan software. The isolates identified as mixed by short sequence repeat typing each two distinct strains (15-50% of replicates representing the minor bacterial strain). SGA also detected low prevalence point mutations which had not been detected by bulk typing methods. SGA allows the detection and molecular characterization of the different strains that coexist in some MAP isolates evidenced by different repeat profiles and point mutations, even if these are present as minor strain types in a host. With the predominance of liquid culture techniques and the poor growth of many MAP strains on solid media, SGA allows determination of whether animals carry multiple concurrent infections. Given the highly contaminated environment around dairy animals and the methods available to identify MAP strains, models of transmission dynamics need to consider the effect of co-infections on dynamics.