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Genomic approach to high resolution typing of *Mycoplasma bovis*

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Background

Conventional MLST (cMLST) approach relying on variations within portions of few housekeeping genes has been extensively used to study structure and dynamics of bacterial populations. Although highly useful in identifying global population structure, its resolution power does not permit analysis of genetic variations within a single clonal line of descent over short time frames.

Objective

To examine genetic diversity within clonal lineages of *Mycoplasma bovis* during outbreak periods and within short geographical distances by using MLST based on partial (cMLST), full length sequences (eMLST) of housekeeping genes, a complete set of coding regions (wgMLST) in the *M. bovis* chromosome and genome wide single nucleotide polymorphism (SNP) analysis.

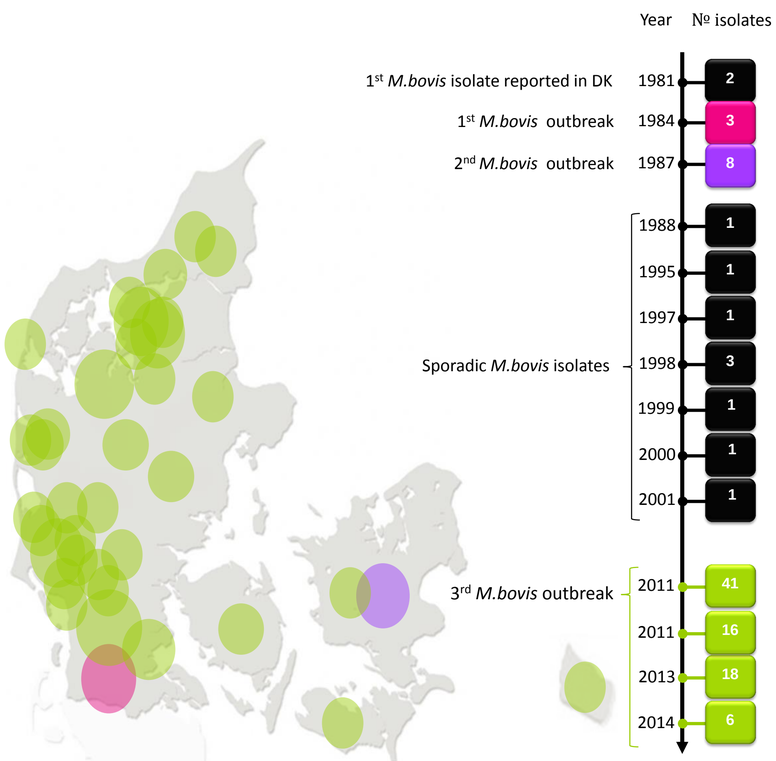


Fig. 1.: Spatiotemporal distribution of Danish Isolates

Materials & Methods

The examination was carried out by analysis of 104 Danish *M.bovis* isolates collected throughout Denmark (Fig. 1) over a period of 34 years. For cMLST and eMLST sequences of the housekeeping genes included in a recently published MLST scheme (1) were extracted from reference genome (PG45), and pre-processed sequencing reads were mapped on them using Burrows-Wheeler algorithm (Fig. 2).

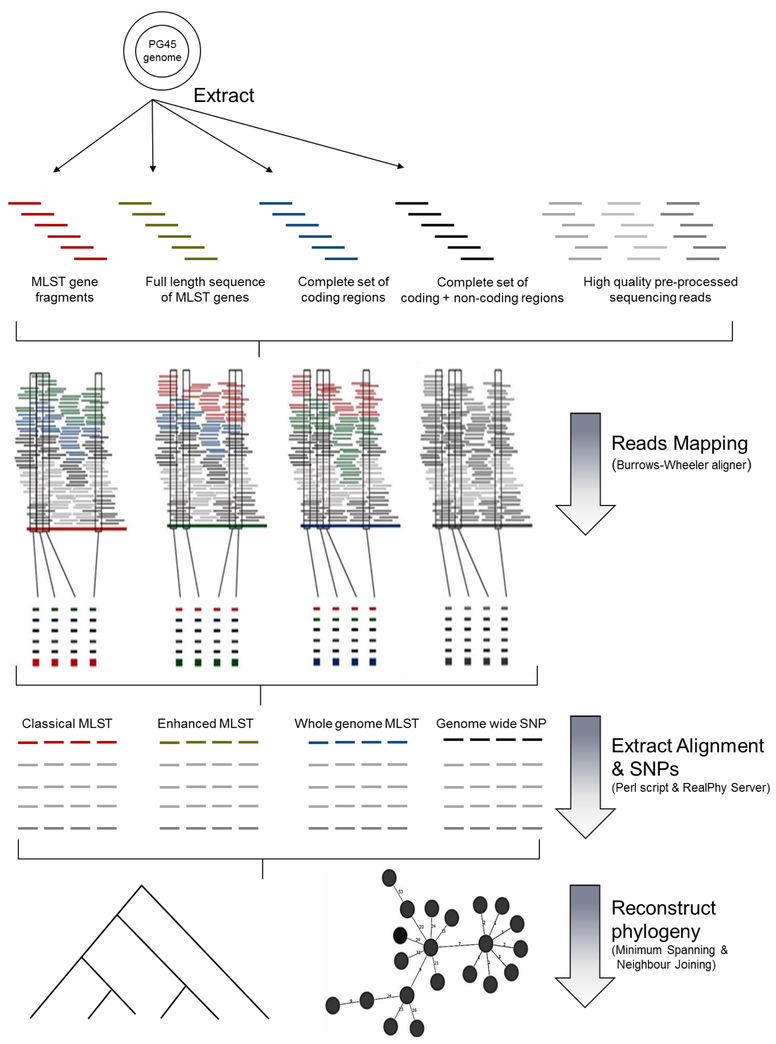


Fig. 2.: Bioinformatics Worklfow

Fig 4.: atpA gene sequence with bars indicating polymorphisms in the test population. Partial gene sequence used in classical MLST scheme is indicated by red rectangle. Additional mutations facilitating further differentiation of the strains were found in the flanking region and are indicated by asterisk.

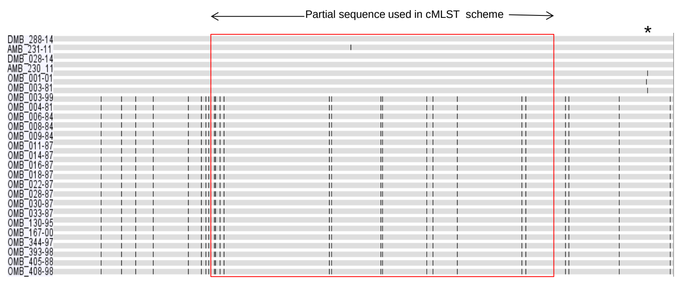
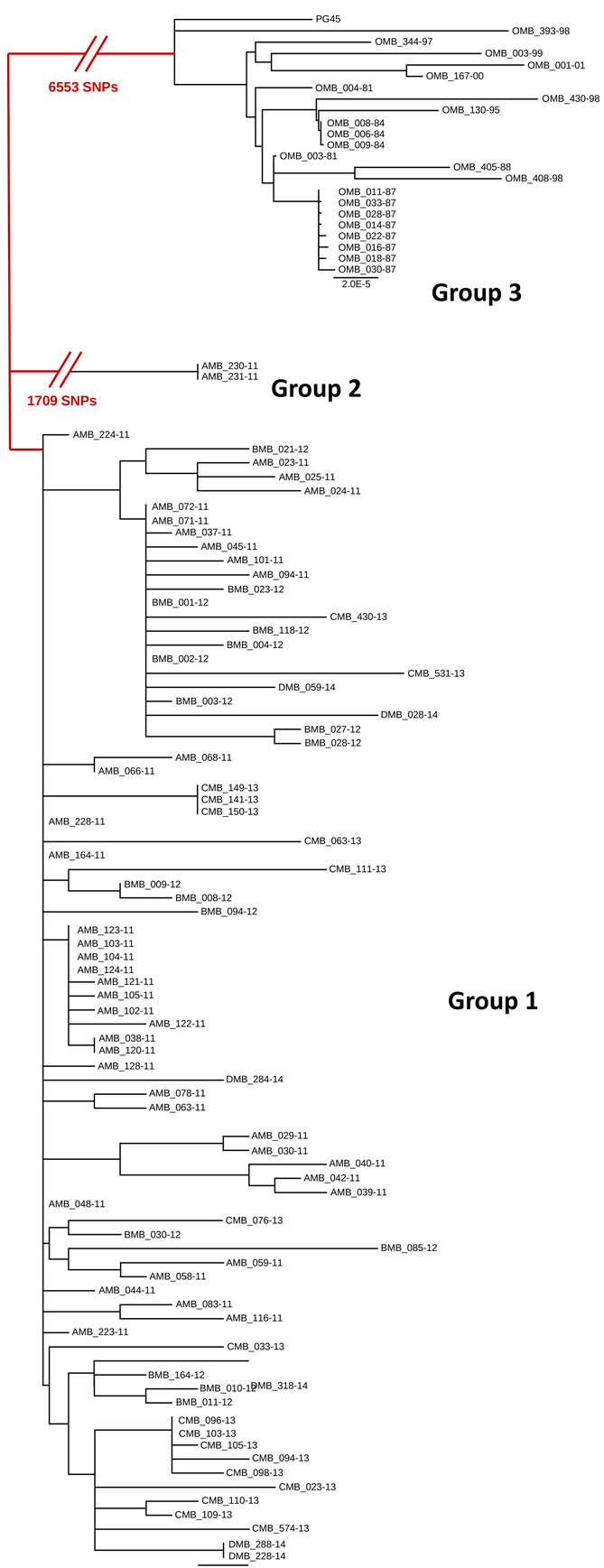


Fig 5.: Genome wide SNP tree representing 3 distinct groups based on coding and non-coding regions



Results & Discussion

Classical MLST based on partial gene sequences (cMLST) divided the 104 Danish *M.bovis* isolates into two distinct types (Fig. 3a), which differed only differed at *tkt* locus. Including full length housekeeping gene sequences (eMLST) of the used MLST scheme resulted in differentiation of the test population in 7 types (Fig. 3b) based on additional 4 loci (*atp*A, *dna*A, *tuf*A and *rpo*D). Substantial number of variations was detected in the regions flanking partial sequences used in cMLST scheme, some of which contributed to further discrimination within the group (Fig. 4). wgMLST based on analysis of 365 full length coding sequences spanning more than 400 kb, which were common for all strains in the test population revealed three distinct groups comprising 80 different types. Genome wide SNP based on analysis of coding and non-coding regions spanning more than 600 kb clearly identified outbreak strains and separated them from sporadic isolates in contrast to cMLST, eMLST and wgMLST .

Conclusions

Both cMLST and eMLST methods were able to divide the test population in two distinct types based on variations in partial and full sequences of housekeeping genes used in the cMLST scheme (1). Extending the analysis to include full length sequences (eMLST) of the housekeeping genes have only marginally improved the actual resolution of the cMLST method. wgMLST improved the resolution and revealed three distinct groups, however it did not completely resolve outbreak strains from 1984. The highest level of resolution was attained by genome wide SNP analysis which clearly distguished outbreak strains including outbreak strains from 1984, which allowed for detailed analysis of outbreaks and microevolution of a given clonal line.

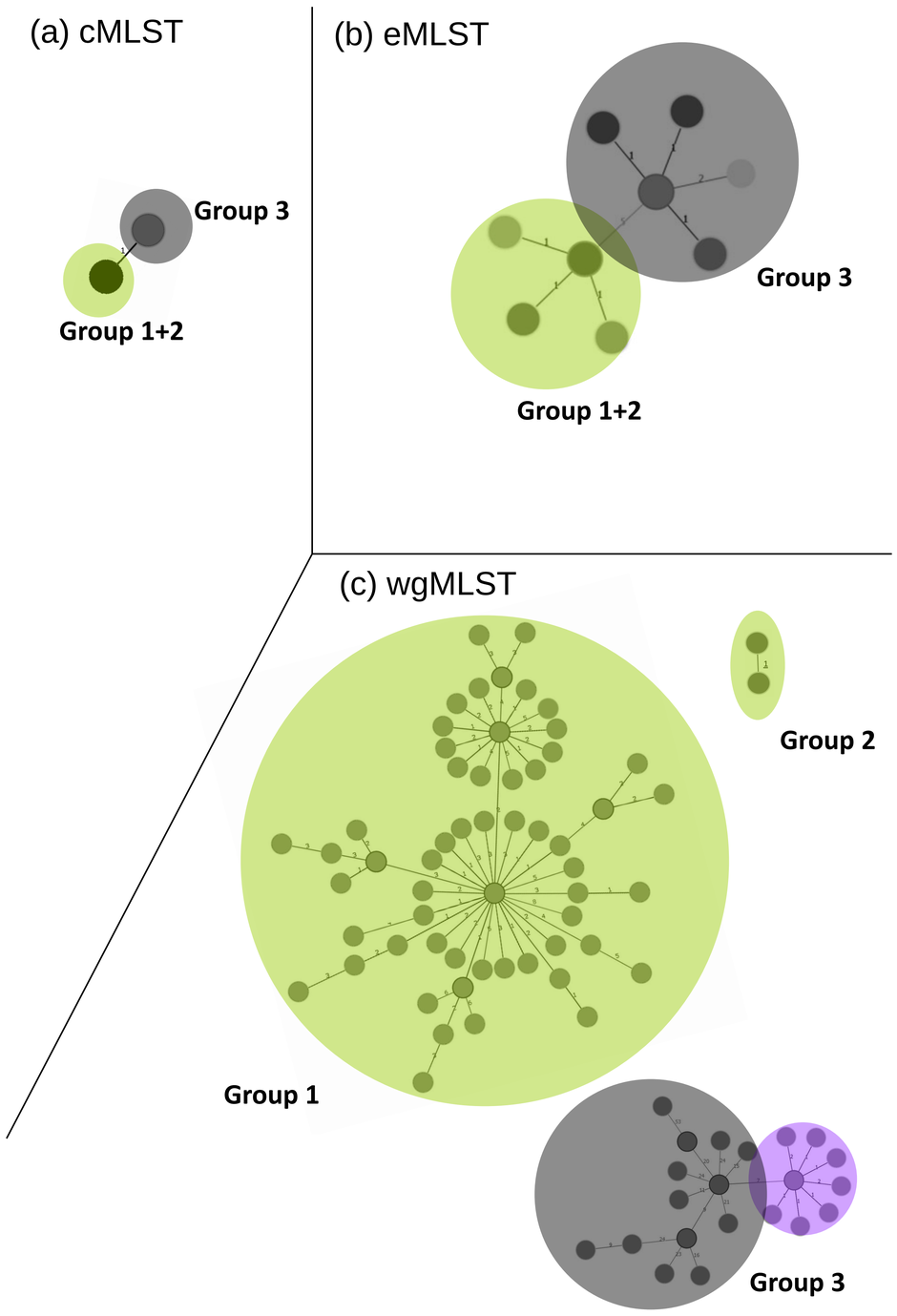


Fig. 3.: Minimum spanning tree based on (a) Classical MLST gene fragments (b) eMLST (c) whole genome MLST

References

1. Rosales et al. 2015. J. Clin. Microbiol., 53, 789-94

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