

Shotgun genome sequence and population diversity of *Mannheimia haemolytica* isolates from sheep in South Africa

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INTRODUCTION

Respiratory disease caused by *Mannheimia haemolytica* is a major worldwide economic and welfare concern for ruminants. *M. haemolytica* is a facultative pathogen, Gram-negative coccus-bacilli and commensal of the upper respiratory tract and nasopharynx of ruminant hosts. During stress such as weaning, transportation, poor nutrition and various viral infections, the bacterium multiplies and often results in fatal fibrinous pleuropneumonia known as pneumonic pasteurellosis or shipping fever.

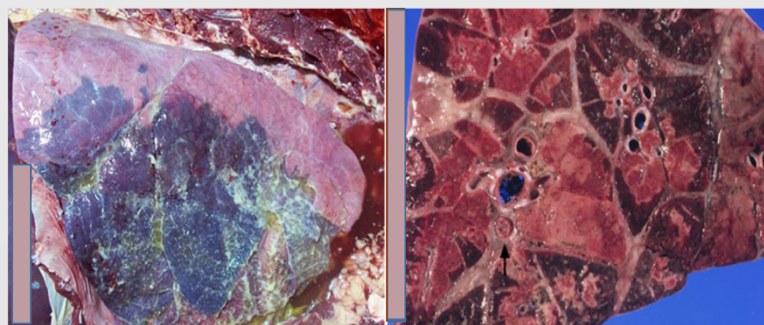


Fig.1 Lung pathology in pasteurellosis

AIM of STUDY

- To conduct whole genome sequence of *M. haemolytica* strain Mh10517 isolated from lung tissue of sheep with history of respiratory infections in South Africa.
- To perform MLST study to understand the evolutionary biology of *M. haemolytica* isolates from South Africa

METHODS

GENOME SEQUENCING:

Whole-genome sequencing of *M. haemolytica*, strain Mh10517 was performed using illumina® MiSeq sequencing platform. The initial De novo assembly performed using CLC Genomics Workbench 6.0.1 produced 91 contigs.

MULTI-LOCUS SEQUENCE TYPING (MLST):

MLST of seven housekeeping (HK) genes is described elsewhere (Petersen, et al., 2009). Amplicons of between 387bp to 671bp were assessed. Fragments of each unique sequence of a gene is assigned an allele number and each allelic combination, or profile for each isolate defines the sequence type (ST).

RESULTS.

Table 1. Basic features of *M. haemolytica* Mh10517 genome

Features	Mh10517	NZ_DS264611
Total length (Mb)	2,569,125	2,584,200
No. of contigs	91	114
G + C content (%)	40.9	40.7
No. of tRNAs	42	49
No. of CDS	2,817	2,682
No. (%) of CDS with assigned functions	2,303	1,649
No. (%) hypothetical proteins	514	575
Transposase genes	35	21
Phage genes	27	209

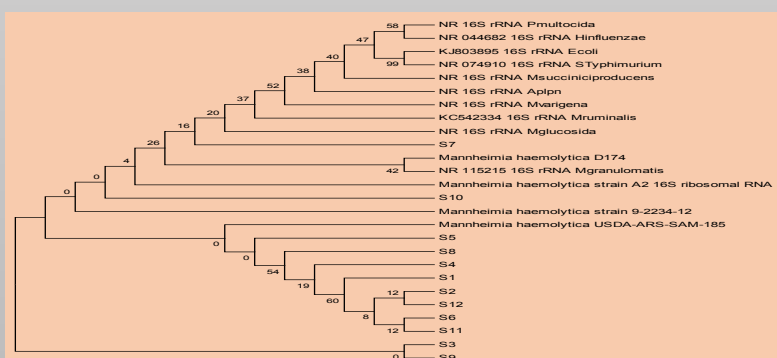


Fig 2. Phylogenetic tree based on 16s rRNA sequence

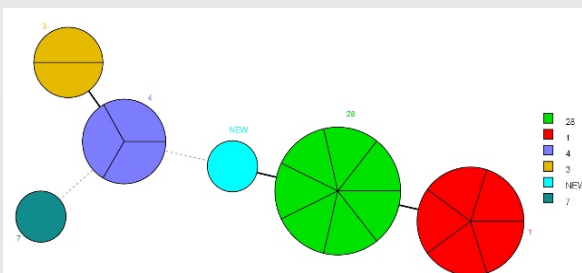


Fig 3. Minimum spanning tree based on MLST data

The 19 isolates belonged to six sequence types (ST) and ST 28 accounted for 33% of the isolates (Fig 4). Neighbour joining dendrograms of the seven loci in multilocus allelic profile in fig. 4. There was significant variation between the number of synonymous and non-synonymous substitutions between each sequence pairs ($p=0.018$) based on results from the Fisher's exact test of neutrality of sequence pairs

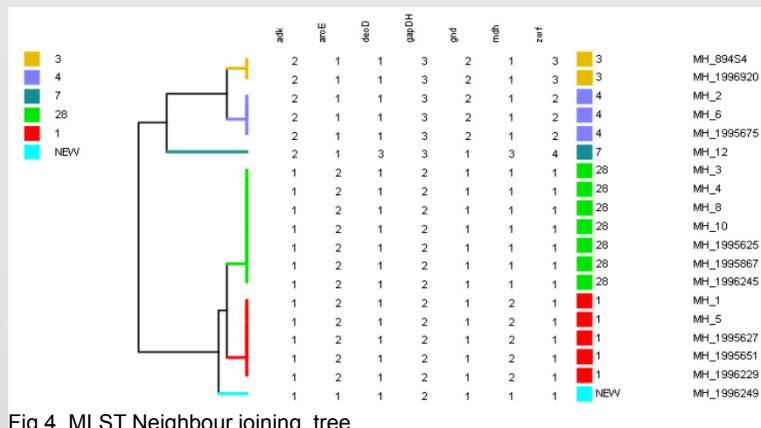


Fig 4. MLST Neighbour joining tree

CONCLUSION

16 s rRNA sequence analysis is a robust genotyping tool for taxonomical purposes. The 7 HK genes used in MLST are under high selective pressure and show real genetic variation. MLST profile is important for evolutionary and epidemiological relationships between isolates/strains & robust tool for genotyping. The 19 isolates were grouped into 6 ST; and an indication on the presence of diverse genotypes of *M. haemolytica* in South Africa. The isolate with "NEW" ST shall be further submitted to the MLST curator and ST assigned. MLST would clarify whether specific clone or lineage of *M. haemolytica* is involved in disease manifestations or outbreaks. These preliminary data show substantial sequence variations and this supports the hypothesis that ovine isolates of *M. haemolytica* are more diverse than what has been reported for isolates from other species. These results will advance studies on various aspects of the biology of *M. haemolytica* in Africa, and the world at large

REFERENCES

Petersen A, Christensen H, Kodjo A, Weiser GC, Bisgaard M. 2009. Development of a multilocus sequence typing (MLST) scheme for *Mannheimia haemolytica* and assessment of the population structure of isolates obtained from cattle and sheep. *Infection, Genetics and Evolution*, 9: 626-632.

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