an important role in the modern epidemiological investigations of tuberculosis in animals at the regional and international level. Bovine tuberculosis represents a significant economic burden to the agriculture of the affected countries. From 2000 to 2015 the disease shows cyclicity in private farms in different regions of Bulgaria. This study is a first molecular investigation of animal tuberculosis in the veterinary medicine in country. The macroscopic and microscopic observation of 35 diagnostic materials from slaughtered cattle, received in the National Reference Laboratory of animal tuberculosis were studied with the three molecular methods: RD4-PCR, spoligotyping and MIRU-VNTR. In 27 of the examined lymph nodes we found specific lesions for bovine tuberculosis. The findings were confirmed bacteriologically and by conventional PCR. To differentiate *M. bovis* from other *M. tuberculosis* complex subtypes, we used primers flanking specific deletion (RD4) in the genome of *M. bovis* and obtained the 446 bp DNA product. The spoligotyping subdivided the strains into 3 spoligotypes shared by two to 20 strains. Further molecular investigations of *M. bovis* strains are needed to characterize the genetic diversity and population structure of *M. bovis* strains isolated from cattle in Bulgaria. New information will be added to the global database in the field of molecular epidemiology of the prevalence of *M. bovis* strains in the cattle population in Bulgaria, which will allow comparative analysis with data from the Balkan region and Europe.

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INTERNATIONAL VALIDATION OF ANALYSIS PIPELINES FOR WHOLE GENOME SEQUENCING DATA OF *MYCOBACTERIUM TUBERCULOSIS* ISOLATES

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The aim of this multicenter study was to validate and compare different pipelines used for analysis of the Whole Genome Sequencing (WGS) data of *Mycobacterium tuberculosis* isolates.

All 535 *M. tuberculosis* isolates of culture positive cases in the Netherlands in 2016, were subjected to WGS, in addition to the routine application of VNTR typing. Transmission suggested on basis of identical VNTR profiles of cases in 2016 was further investigated by municipal health services and 41 epi-links were traced. Fastq.gz files of all 535 samples were analysed in four different WGS pipelines to facilitate international comparison: 1) SNP-based method at the RIVM/Bilthoven/The Netherlands; 2) SNP-based method at Oxford University/UK; 3) SNP-based method and 4) cgMLST at Borstel/Germany.

In all pipelines, shorter than 12 SNP distances between the 41 epi-linked cases was observed. One epi-linked pair revealed a higher genetic distance of 27 SNPs in the Bilthoven pipeline, due to poor sequence quality resulting in low coverage. In general, the genetic distances between isolates of the epi-linked cases were smaller in the Oxford and Borstel pipelines (0–3 SNPs), than in the Bilthoven pipeline (1–11 SNPs). All pipelines clustered roughly the same cases, more isolates without identified epi-links were clustered in the Oxford (n = 34) and both Borstel pipelines (n = 32 in the SNP pipeline and n = 39 in the cgMLST) than the Bilthoven pipeline (n = 29).

Also, some cases not clustered by VNTR were clustered by WGS. Patient characteristics revealed that in some of these pairs of cases an epi-link, missed by VNTR typing, was likely.

Several differences were observed among the pipelines with regard to the version of reference genome used, software used for mapping and SNP calling, (repetitive) regions excluded in the analysis, the minimum number of reads to support SNPs, and the minimum allele frequencies. The RIVM pipeline was adapted in the light of these results to function more in line with other international laboratories pipelines, facilitating the comparability of results.

International standardization on all these variables is necessary, and subsequently on the SNP cut-off to be applied to WGS clustering, to allow international-laboratory comparison of WGS data and reliable investigation of cross-border transmission.

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RNA-BASED DRUG SUSCEPTIBILITY TESTING OF *MYCOBACTERIUM TUBERCULOSIS*

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Multidrug resistant tuberculosis (MDR-TB) is one of the major WHO health concerns. One of the challenges that hampers the effective response to MDR-TB is the long turnaround time of phenotypic Drug Susceptibility Testing (DST) of *Mycobacterium tuberculosis*. To counter this, new fast and sensitive DNA-based methods were successfully introduced over the last years. However, these (a) are based on the knowledge on resistance mutations, (b) do not distinguish living from dead cells, (c) ignore all intrinsic resistance mechanisms, and (d) ignore the influence of compensatory mutations.

We introduce a next-generation diagnostic test based on quantification of drug-specific RNA biomarkers. The basic principle is that a brief antibiotic exposure triggers specific transcriptional responses in susceptible, but not in resistant, microbes within a few hours. This has the advantage that long culture-dependent steps are avoided, yet the resistance phenotype is detected independent of the specific cause of resistance.

First, the global transcriptional response of two *M. tuberculosis* strains to 10 anti-TB drugs was determined using RNAtaq-Seq. A set of highly responsive genes was selected for each drug and RNA-targeting probes were designed.

Next, the RNA-based DST was developed in 96 well format. In short, 200 µl of a positively flagged MGIT™ (BD) culture is spiked with a drug, while a replicate is incubated in absence of the drug. Multiplex mRNA quantification is performed directly on crude cell lysates using a combination of the bead-based MagPix™ (Luminex) and Quantigene™ Plex (Thermo Fisher) technology.