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 spoligotypes subdivided the molecular types 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 profile of cases in 2016 was further investigated by molecular types 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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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 mRNASeq. 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.