Mycobacterium bovis antibiotic susceptibility test: a “new” liquid method

INTRODUCTION
Proportion method is the most widely used susceptibility test and is considered the reference method for *M.tuberculosis* (2). When adopted to slow growing strains, such as *M. bovis*, it couldn’t produce results on time. In addition, this method cannot be applied to pyrazinamidase activity test (PZAase test), which is used to differentiate between *M. bovis* and *M. bovis subsp. caprae* (1).

The aim of this study was to evaluate the liquid VersaTREK (VT) pyrazinamide (PZA) susceptibility test as an alternative method able to rule out the major errors reported with others broth-based systems (3) and to check if the results obtained with the VT method support the use of a reduced incubation time (from 35 to 13 days).

MATERIALS & METHODS
The susceptibility of 16 *M.bovis* and 5 *M. bovis subsp. caprae* strains versus first – line anti-tuberculosis drugs (Isoniazide -INH, Rifampicin - RIF, Streptomycin - STR and Ethambutol - EMB) was evaluated using the VT system. PZAase test was conducted with the aim to differentiate *M.bovis* to *M.bovis subsp.caprae*. Tested strains, belonging to the IZS PLV’s collection, were isolated from cattle in the period 2003-2013.

RESULTS
Three of the sixteen *M. bovis* strains tested showed to be resistant to at least one of the drugs used: one strain was resistant to STR at the concentration of 2.0 µg/ml and two strains were resistant to INH at the concentration of 0.1µg/ml. All the other *M. bovis* strains were sensitive to all drugs used. On the other hand, all *M. bovis subsp. caprae* strains tested were susceptible to all of the antibiotics at all of the concentrations tested.

All *M. bovis* strains (16/16) were found to be PZA-resistant, while all *M. bovis subsp. caprae* (5/5) were PZA-susceptible.

DISCUSSION & CONCLUSIONS
The VT antibiotic susceptibility test was proved to be:

- **simple to perform**: it does not require bacterial growth on solid culture media, as it directly detects the strain in the liquid VT medium;
- **able to significantly reduce the incubation time** when compared with the proportion method.

With regards to the PZA test, this has proved to be **100% reliable**. In conclusion, we found evidence of drug resistance in previously collected *M. bovis* strains of bovine origin. These results are most likely to be due to a selective pressure caused either by the routine use of antibiotics in veterinary medicine or by repetitive antibacterial treatments, both legal (e.g. streptomycin for cattle respiratory disease complex) and illegal (isoniazid), of animals misclassified as negative in vivo. However, results observed with resistant strains will need to be confirmed using standardized inoculum techniques as well as compared with those obtained using the proportion method, which is still considered the gold standard.

REFERENCES