Abstract title: Prevalence of *Mycoplasma bovis* antigens/antibodies in bulk tank milk and associated risk factors

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**Objective**

*Mycoplasma bovis* is a highly contagious bacterium, worldwide identified as an emerging pathogen in cattle in the last decade. The objectives of this study were to determine the prevalence of *M. bovis* in dairy herds in Flanders (Belgium) by determining antigens and antibodies in bulk tank milk samples and to identify risk factors for a positive bulk tank milk sample.

**Methods**

A cross-sectional study was conducted on 100 dairy farms in Flanders in 2016, randomly selected stratified on province. Bulk tank milk was collected during routine milk sampling. Real-time PCR (PathoProof Mastitis Complete 16 PCR assay, Thermo Fisher Scientific, Finland) and *M. bovis* antibody ELISA (BIO K 302, Bio-X Diagnostics S.A., Belgium) were performed on the samples. A questionnaire containing 44 questions on internal and external biosecurity and general herd management was distributed to all farms by email. Non-responders were interviewed by telephone. Risk factors associated with positive bulk tank milk results (either PCR or antibody ELISA positive) were identified using multivariable logistic regression.

**Results**

Of all farms, 7% tested *M. bovis* PCR positive (95% CI: 2.06 – 11.49% and estimated True Prevalence 7.1% (95% CI: 2.06-11.49%) and 17% (95% CI: 9.72 – 24.28% and estimated True Prevalence 24.8% (95% CI: 16.42-33.15%) was ELISA positive (when using the recommended optical density value of 37% (Spec: 60.4%, Sens: 97.3%)). No farms that tested positive on PCR had detectable antibodies present in the bulk tank milk. Of the farms, 55% returned the questionnaire. Two risk factors for an antigen/antibody positive bulk tank milk result were identified. Farms which used a breeding bull had 4.7 (1.1-19.8) higher odds to test positive. The use of a separate calving pen was a protective factor (OR= 0.27 (0.08-0.94)).

**Conclusions**

A considerable proportion of the Flemish dairy herds is in contact with *M. bovis*. The reported prevalence is much higher than the 1.5% prevalence found in 2008 in bulk tank milk for the same region, although the use of different test methods might play a role in this apparent increase in prevalence (antigen PCR/antibody ELISA versus repeated bacteriology in 2008). Further attention should be given to the role of the breeding bull and calving pen in the spread of *M. bovis* in a herd.