

1 **Between-herd prevalence of *Mycoplasma bovis* in bulk milk in Flanders, Belgium**

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13 **ABSTRACT**

14 ***Mycoplasma bovis* (*M. bovis*) is a highly infectious pathogen of cattle causing pneumonia,**

15 **polyarthritis, otitis, and less frequently, subcutaneous abscesses, abortions and**

16 **meningitis.**

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18 **Ineffective drugs treatments, culling of infected cows and loss of milk production can**

19 **lead to significant economic loss on dairy farms. The early detection of cows excreting**

20 ***M. bovis* bacteria to prevent mastitis outbreaks is warranted. Reports suggest that the**

21 **risk of *M. bovis* mastitis is higher in larger dairy herds.**

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23 **The objective of this study is to estimate the herd-level prevalence of *M. bovis* in**

24 **Flanders, Belgium by culturing bulk tank milk samples taken from dairy farms.**

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26 **Three bulk tank milk samples per dairy herd were taken over four weeks, with**
27 **collection intervals of two weeks. Culturing was done after pre-incubation using**
28 **modified Hayflicks media to increase the chances of recovery of bacteria. For the**
29 **identification of *M. bovis*, tDNA intergenic spacer PCR was used.**

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31 **In three herds (1.5%) of the 200 herds sampled, *M. bovis* was isolated from one of the**
32 **three consecutive bulk tank milk samples. We conclude that in Flanders in 2009 at least**
33 **1.5% of the dairy herds had one or more cows excreting *M. bovis* in the milk.**

34

35 **The frequent monitoring of bulk tank milk to detect the presence of *M. bovis*, especially**
36 **in expanding herds on farms that often purchase replacement animals, should be**
37 **encouraged in order to detect the presence of *M. bovis* and to monitor the success of**
38 **control procedures following an outbreak of mycoplasmal mastitis in the herd.**

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40 **KEYWORDS**

41 Mycoplasma – Mastitis – Dairy Cattle – Herd prevalence

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43 *Mycoplasma bovis* is a highly infectious pathogen of cattle, causing pneumonia,
44 polyarthritis, otitis, and, less frequently, subcutaneous abscesses, abortions, and meningitis
45 (Nicholas and Ayling, 2003). In addition, it is the most important agent of outbreaks of
46 mycoplasmal mastitis in dairy cows (Gonzalez, 2003). *Mycoplasma* spp. lack a typical cell
47 wall, and so are not affected by many of the commercially available antimicrobial drugs,
48 which act by interfering with cell wall synthesis (Bushnell, 1984).

49 Over the last decade, *M. bovis* isolates have developed an acquired resistance to a wide
50 range of commonly used antibiotics such as macrolides and tetracyclines (Nicholas et al.,

51 2008). Intramammary infections with *M. bovis* are difficult to treat successfully even if the
52 antimicrobials used show good *in vitro* activity against the agent (Ayling et al., 2000).
53 Unsuccessful therapy, culling of infected cows and loss of milk production can lead to
54 significant economic loss on a dairy farm (Nicholas and Ayling, 2003). The early detection of
55 cows excreting *M.bovis* to prevent mastitis outbreaks is warranted.

56 Recently, several cases of clinical and subclinical mastitis caused by *Mycoplasma* spp.
57 in Belgian dairy herds have been reported (personal communication, Milk Control Centre
58 Flanders, Lier, Belgium). Also, the number of milk samples submitted to the central milk
59 quality laboratory (Milk Control Centre Flanders, Lier, Belgium) for bacteriological culturing
60 for *M. bovis* increased from zero in 2007 to 553 in 2008, (a combination of bulk milk samples
61 and individual cow milk samples). Nearly 9% (n=48) of all samples were culture-positive
62 (Annual Report 2008, Milk Control Centre Flanders, Lier, Belgium). No information is
63 available on the between-herd prevalence of cows excreting *M. bovis* bacteria in Flanders,
64 Belgium.

65 The culture of bulk tank milk samples is a valuable procedure for screening and
66 surveillance of mastitis-causing pathogens at the herd level, in particular for the detection of
67 cows excreting *Streptococcus agalactiae* and *M. bovis* (Jasper, 1979; Bushnell, 1984;
68 Gonzalez, 1986; Gonzalez, 1992; Jayarao, 2003).

69 The objective of this study was to estimate the herd-level prevalence of *M. bovis* in
70 Flanders, Belgium, by means of culturing of bulk tank milk samples.

71 The sample size required to estimate the prevalence of *Mycoplasma* spp.-infected herds
72 accurately was calculated using Win Episcope 2.0 (Thrushfield et al., 2001). The target
73 population was 6,287 Flemish dairy producers with an expected prevalence of *M. bovis* of 5%
74 ($\pm 3\%$), and a 95% confidence interval. Based on this calculation, the suggested adjusted
75 sample size was 197 herds. In the 2009 year, 201 herds were selected randomly in Flanders in

76 proportion to the total number of dairy farmers in each of the five Provinces for this study.
77 (Table 1).

78 Bulk milk samples were collected through routine sampling as currently performed
79 when milk is collected as part of the legal requirements for milk quality control procedures
80 by the Milk Control Centre Flanders, Lier, Belgium. The milk samples were immediately
81 stored at 4°C and transported under cooled conditions (at 4°C) to the laboratory for
82 bacteriological analysis the next day. Three bulk milk samples per herd were collected and
83 analysed over four weeks, with collection intervals of two weeks. Culturing was performed as
84 described by the National Mastitis Council after pre-incubation using modified Hayflicks
85 media to increase the recovery rates of the bacteria (Hogan et al., 1999). For identification of
86 *M. bovis*, tDNA intergenic spacer PCR was used (Stakenborg et al., 2005).

87 One of the 201 selected farms stopped delivering milk during the study and was
88 sampled only once (with a negative culture result). In the remaining 200 herds, *M. bovis* was
89 isolated from one of the three consecutive bulk tank milk samples taken from 3 herds (1.5%).
90 All culture-positive samples were positive at the first reading (three days after the
91 commencement of incubation).

92

93 The between-herd prevalence of *M. bovis* in bulk milk ranges between 1% and 8% in
94 the USA (Fox et al., 2003); is 5.4% in Greece (Filioussis et al., 2007), and is nil in New
95 Zealand (McDonald et al., 2009). False-negatives may occur, suggesting the between-herd
96 prevalence in Flanders, Belgium may be higher than the 1.5% prevalence found in this study.

97 Infected cows may excrete the organisms in low numbers (Gonzalez, 1986) or
98 intermittently, and so may not be isolated on culture (Jasper, 1979; Bushnell, 1984; Biddle,
99 2003). Additionally, milk from *M. bovis* infected cows in large herds will be diluted in the

100 total herd milk. Dairy producers also often withhold abnormal milk from the milk tank
101 (Jasper, 1979; Thomas, 1981; Gonzalez, 1992).

102 Reports suggest that the risk of mastitis caused by *Mycoplasma spp.* is higher in large
103 herds, presumably because cows and heifers are purchased more frequently either to maintain
104 or expand the existing herd. (Thomas et al., 1981, Fox et al., 2003). During the last 20 years,
105 the size of the dairy herd increased in Flanders, Belgium as illustrated by the increasing
106 average volume of milk quota per farm (Annual Report 2010, Confederation of the Belgian
107 Dairy Industry, Belgium). This increase in herd size is mainly driven by the acquisition of
108 cows and heifers from other herds, a key risk factor for the introduction of *M. bovis* into a
109 dairy herd (Jasper, 1979; Gonzalez and Wilson, 2003).

110 As well, the average bulk milk somatic cell count in Flanders has increased since 1999,
111 indicating that more attention to udder health management by farmers is required. Both of
112 these observations indicate that the risk of *M. bovis* infection in a dairy herd has increased.

113 Our conclusion from the study is that in Flanders, Belgium in 2009, at least 1.5% of
114 dairy herds had one or more cows excreting *M. bovis* in the milk. Frequent monitoring of
115 bulk tank milk (especially on farms that purchase replacement animals) should be encouraged
116 in order to screen for and detect the presence of *M. bovis* and to monitor the success of
117 control procedures on the farm following an outbreak of mycoplasmal mastitis.

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