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Pathogen-specific incidence rate of clinical mastitis in Flemish dairy herds, severity, and association with herd hygiene

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ABSTRACT

A one-year survey on clinical mastitis was conducted on 50 randomly selected commercial Flemish dairy herds to estimate the pathogen-specific incidence rate of clinical mastitis (IRCM). The severity of the cases and the potential associations with herd hygiene were studied. Participating producers sampled 845 cases and 692 dairy cows. The mean and median IRCM was estimated at 7.4 and 5.3 quarter cases per 10,000 cow-days at risk, respectively. A large between-herd variation was observed (range of 0–21.3). In general, the IRCM was lower in heifers compared with multiparous cows (2.9 vs. 11.0 quarter cases per 10,000 cow-days at risk). However, the overall IRCM in the first week after calving was higher in heifers compared with cows (43.4 vs. 31.6 quarter cases per 10,000 cow-days at risk). *Streptococcus uberis* (18.2% of the cases) and *Escherichia coli* (15.5%) were the most frequently isolated pathogens and no growth was observed in 19.9% of the cases. The majority of the cases (63.1%) were mild (only clots in milk). Moderate (hard quarter without general signs) and severe symptoms (systemic illness) were observed in 29.9 and 7.0% of the cases, respectively. Isolation of *E. coli* (vs. any other culture result) was more likely in moderate and severe cases compared with mild cases. Overall IRCM and *E. coli* IRCM were higher in dirty compared with clean herds based on udder hygiene scores (9.0 and 1.7 vs. 6.0 and 0.6 quarter cases per 10,000 cow-days at risk, respectively). This study broadens the knowledge on clinical mastitis in Flemish dairy herds and underlines the high risk of CM in early-lactation heifers, the role of the so-called environmental pathogens, and herd hygiene.

Key words: clinical mastitis, incidence rate, severity, herd hygiene

INTRODUCTION

Mastitis is one of the most common diseases in dairy cattle. The inflammatory reaction primarily occurs in response to bacterial IMI and impairs milk quality (Ma et al., 2000; Santos et al., 2003). The disease is accompanied by clinical signs [clinical mastitis (**CM**)] or presents itself without observable signs (subclinical mastitis).

Several countries have reported incidence rate of CM (**IRCM**) data, ranging from 5.5 quarter cases per 10,000 cow-days at risk in French herds with a low bulk milk SCC (**BMSCC**) to 12.9 quarter cases per 10,000 cow-days at risk in randomly selected herds in England and Wales (Barkema et al., 1998; Barnouin et al., 2005; Bradley et al., 2007; Olde Riekerink et al., 2008; Wolff et al., 2012). In contrast to subclinical mastitis (Piepers et al., 2007), there is a general lack of information on the occurrence of CM in Flanders. As for most Flemish dairy herds cases are not recorded and rarely sampled for culture, the exact incidence of CM remains unknown. Yet, according to an internet questionnaire performed by P. Passchyn, S. Piepers, and S. De Vliegher (Ghent University, Ghent, Belgium, unpublished data), 300 dairy producers estimated that 46% of the Flemish dairy cows suffer at least once per year from CM. Because the majority of questioned producers admitted not to keep disease records, the latter estimate might differ substantially from the actual IRCM in Flanders.

In studies performed in the Netherlands, Canada, and Ireland, *Staphylococcus aureus* was the most frequently isolated pathogen isolated from CM cases (Barkema et al., 1998; Olde Riekerink et al., 2008; Keane et al., 2013), whereas *Streptococcus uberis* and *Escherichia coli* were the most frequently isolated pathogens in studies performed in the United Kingdom (Bradley et al., 2007) and the United States (Oliveira et al., 2013). Because management systems differ between regions (e.g., Olde Riekerink et al., 2008) and dairy farming and mastitis control evolve over time (Bradley, 2002), regular CM studies at regional or national level remain indispensable for adapted mastitis prevention programs and development of novel prevention and control tools.

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In 2012, *Strep. uberis* and *E. coli* were the most frequently isolated pathogens from samples of CM cases submitted to the Milk Control Centre (**MCC**) Flanders (MCC Vlaanderen, Lier, Belgium), the largest milk laboratory in Flanders (Milk Control Centre Flanders, 2012). Although the included herds and cases are not randomly selected, the results suggest these so-called environmental pathogens to be the main cause of CM in Flanders. Reducing exposure of teats with manure and organic materials is pivotal in the prevention of environmental mastitis (Smith and Hogan, 1993). Schreiner and Ruegg (2003) developed a 4-point scale to score udder hygiene and reported that cows with a higher udder hygiene score (**UHS**; dirtier) had higher SCC values and were more likely to have subclinical mastitis. Using the same scoring system, Breen et al. (2009) identified high UHS as a cow-level risk factor for CM in general and for *E. coli* CM specifically. It remains unclear whether hygiene issues can be detected as risk factors for CM in Flanders, Belgium, as well.

Signs of CM range from abnormal milk to systemic illness, with the severity of cases depending on both cow and pathogen factors (Burvenich et al., 2003; Bannerman et al., 2004; Schukken et al., 2011). Oliveira et al. (2013) recently characterized CM on large dairy herds in Wisconsin and reported that systemic signs of illness were more likely to be observed in gram-negative cases. *Escherichia coli* was isolated in 12.5, 22.3, and 48.3% of the mild, moderate, and severe cases, respectively. To the best of our knowledge, no scientific information on signs of CM is available in Flanders, let alone a link with the associated pathogens. Yet, as in other regions, *E. coli* mastitis is often used as a synonym for severe CM.

The primary objective of this study was to estimate the pathogen-specific IRCM in Flanders, Belgium. Additionally, associations between the culture result and the reported severity and between (pathogen-specific) IRCM and herd hygiene were studied.

MATERIALS AND METHODS

Herds and Study Design

Clinical mastitis was monitored at the cow level (experimental unit) on randomly selected dairy herds (sampling unit) during 1 yr. The sample size for this study was estimated at 42 herds, using the following formula (Dohoo et al., 2003): $n = \frac{Z^2_{\alpha/2} pq}{L^2} = \frac{1.96^2 \times 0.46 \times 0.54}{0.15^2}$, where n = number of herds needed to estimate the incidence rate, $Z_{\alpha/2}$ = 95th percentile of a standard normal distribution, p = a priori estimate of the proportion set at 0.46 (P. Passchyn, S. Piepers, and S. De Vliegher, Ghent University, Ghent, Belgium, unpublished data), $q = 1 - p$, and L = margin of error set at 0.15.

To allow for noncompliance, 67 herds were randomly selected from the database of the MCC Flanders comprising all Flemish dairy producers (n = 5,261) using the Excel RAND function (Excel 2010; Microsoft Corp., Redmond, WA). Thirteen, 4, 7, 19, and 24 herds were contacted in the provinces Antwerp, Flemish Brabant, Limburg, East Flanders, and West Flanders, respectively, matching the distribution of dairy herds over the 5 Flemish provinces (19, 6, 10, 28, and 37%, respectively; Federal Public Service Economy, Small and Medium Enterprises, Self-Employed, and Energy, 2011). No inclusion criteria were applied. Fifty-three producers agreed to participate in this study (response rate of 79%). The main argument for noncompliance was lack of time (n = 7), followed by planned retirement (n = 3). The 4 remaining producers gave no specific argument for not participating.

The selected dairy herds were visited at the beginning of the study between September and October 2012. At that time, the study details were discussed with the producers and herd veterinarians, a questionnaire was filled out, and observations were made (see further). From then on, producers were asked to take a single sample from each quarter showing signs of CM during the 12-mo study period. Signs of CM were defined as visible abnormalities in the udder or milk, indicating udder inflammation, and were detected by examination of foremilk and the udder before milking. In herds with an automated milking system (**AMS**), producers examined foremilk and the udder of cows with changes in sensor data (electrical conductivity, color, and yield), reduced milking frequency, or visual abnormalities (e.g., swelling or redness of the udder). Thresholds were set by the producer. Dry cows were monitored by visual observation. Sample materials were provided and sampling procedures were explained as well as the importance of an aseptic sampling procedure. Sampling date, cow identification, quarter position, and clinical signs (absence or presence of clots in milk, a hard quarter, or systemic illness) were recorded by the producer as well. Cases were categorized as mild (only clots in milk), moderate (hard quarter but no general signs), or severe (signs of systemic illness) similar to the categorizations of Pinzón-Sánchez and Ruegg (2011). Samples were frozen on farm for one to several weeks and collected by the herd veterinarian. A courier of the Flemish Animal Health Service (DGZ) transported the samples from the veterinary practice to the MCC Flanders where bacteriological culture was performed. Three herds were omitted from the analysis during the study period because producers admitted

halfway through the study that they were not sampling all the cases, resulting in 50 herds and 4,133 cows to be included in the final data set.

Producers were motivated to sample each single case by making the culture results available to them as soon as possible, by paying an incentive of €3 per collected sample, by performing a secondary herd visit between February and March 2013 to discuss the preliminary results, and by contacting them at least once every 2 mo by phone. We kept herd veterinarians actively involved in the project by sharing all culture results from all herds they were associated with, inviting them to join both herd visits, and financially supporting them to keep track of the herd administration. Both producers and herd veterinarians were invited to a meeting in October 2013, after completion of the survey, to discuss the results and receive a summary with herd-specific results, as was promised at the onset of the study. Both producers and veterinarians were aware of this initiative at the start of the study.

Herd Hygiene

Udder hygiene was scored for 20 randomly selected lactating cows during both herd visits performed by the first author as described by Schreiner and Ruegg (2003). In herds with less than 20 lactating cows, all lactating cows were scored. The proportion of cows having UHS 3 or 4 was calculated for each visit. Herds having an average proportion >50% over the 2 visits were categorized as dirty; other herds were categorized as clean.

Cow Data

Cow-level records [calving date(s), parity, and culling date] were retrieved from DHI records for herds participating in the DHI program and from the identification and registration system of the Animal Health Service Flanders (Drongen, Belgium) for other herds.

Bacteriological Culture

Bacteriological culture was based on National Mastitis Council guidelines (NMC, 1999) and performed at the MCC Flanders. From each thawed sample, 10 μL of milk was spread on blood-esculin and MacConkey agar and incubated aerobically for 24 to 48 h at 37°C. Samples were considered to be culture positive if one or more colonies were observed (≥100 cfu/mL). Identification of bacteria was done by Gram staining, inspection of the colony morphology, and biochemical testing. Catalase tests were performed to differentiate gram-positive cocci as catalase-positive or catalase-negative cocci. Staphylococci were identified as *Staph. aureus* or non-*aureus* staphylococci, referred to as *Staphylococcus* spp. throughout this paper, by colony morphology, hemolysis patterns, and DNase tests. Isolates of the *Streptococcus-Enterococcus* group were differentiated as esculin-positive or esculin-negative cocci. *Streptococcus uberis* was distinguished from other esculin-positive cocci by incubation in NaCl 6.5% medium and bile esculin agar. Christie, Atkins, and Munch-Petersen (CAMP) tests were used to differentiate esculin-negative cocci as *Streptococcus agalactiae* or *Streptococcus dysgalactiae*. Gram-negative bacteria were identified by colony morphology, lactose fermentation on MacConkey agar, incubation in sulfide-indole-motility (SIM) medium, and oxidase, triple sugar iron (TSI), citrate and urease testing. The API 20 E system (bioMérieux SA, Marcy-l'Étoile, France) was used if the abovementioned tests failed to identify the gram-negative bacterium. Samples yielding 2 different bacterial species were grouped as "mixed culture," whereas samples yielding 3 or more different bacterial species were considered to be contaminated.

Outcome Variables

The cow IRCM was calculated by dividing the number of quarter cases by the days at risk (**DAR**) during the study and expressed as cases per 10,000 cow-days at risk. Samples taken from the same cow within 2 wk from a previous case (n = 22) were not considered new cases and, therefore, excluded from the analysis (Barkema et al., 1998). The at-risk period for a cow started at the beginning of the survey or at the date of first calving and ended at the end of the survey or at the culling date. As dry cows can suffer from CM (Scherpenzeel et al., 2014) and CM in early lactation may originate from IMI established in the nonlactating period (Bradley and Green, 2004), dry periods were included in the at-risk period. Overall IRCM (independent of the culture results), as well as pathogen-specific IRCM (*Staph. aureus*, *Strep. uberis*, *Strep. dysgalactiae*, and *E. coli*, specifically) were calculated.

Data Analyses

The association between pathogen isolation and severity was tested on a data set including all CM cases (n = 845). Five different logistic regression models (PROC LOGISTIC, SAS 9.4; SAS Institute Inc., Cary, NC) were fit with isolation as the outcome variable [(1) no growth vs. growth, (2) *Staph. aureus* isolation vs. any other culture result, (3) *Strep. uberis* isolation vs. any other culture result, (4) *Strep. dysgalactiae* isolation vs. any other culture result, and (5) *E. coli* isolation vs. any other culture result)] and severity (mild, moderate,

or severe) as categorical fixed effect. Odds ratios (**OR**) with 95% confidence intervals were calculated.

The associations between herd hygiene and the different outcome variables [(1) overall IRCM, (2) *Staph. aureus* IRCM, (3) *Strep. uberis* IRCM, (4) *Strep. dysgalactiae* IRCM, and (5) *E. coli* IRCM] were determined using 5 mixed Poisson regression models (PROC GLIMMIX, SAS 9.4). All models contained herd as a random effect to correct for clustering of cows within herds, the natural logarithm of the number of DAR as an offset variable (Barkema et al., 1999), and herd hygiene (dirty vs. clean) as a categorical fixed effect. For each outcome, variable rate ratios (**RR**) with 95% confidence intervals were calculated. Confounding by parity distribution in the herd was tested by adding the proportion of heifers in the herd (heifer DAR/total DAR) to the model as a continuous fixed effect. Parity distribution was considered to act as a confounder if the regression coefficients of herd hygiene underwent a relative change >25%. No confounding was detected. Overdispersion was evaluated for each model by calculating a dispersion parameter (Pearson χ^2/df; Dohoo et al., 2003). The power to demonstrate a significant difference between dirty and clean herds ($1 - \beta$, where β is the type II error rate) was estimated for each model using the following formula (Dohoo et al., 2003): $P(z > Z_\beta)$, where z = the standard normal variable and Z_β = the calculated boundary value, and $Z_\beta = \sqrt{\frac{n(\lambda_2 - \lambda_1)^2}{\lambda_1 + \lambda_2}} - Z_{\alpha/2}$, where $n = 25$ (herds per group), λ_1 = average count of cases in clean herds, λ_2 = average count of cases in dirty herds and $Z_{\alpha/2}$ = 95th percentile of a standard normal distribution = 1.96.

Because CM detection in AMS herds is different, all analyses were repeated on a subset of data excluding the 3 AMS herds. Because of the limited proportion of AMS herds, only changes in regression coefficients were calculated and reported.

RESULTS

Herd Characteristics

Thirty-two (64%) herds participated in the DHI program, whereas 18 did not. The average herd size was 60 lactating cows (range of 16 to 240). All producers milked Holstein-Friesian cows. Cows were housed in freestalls with cubicles in 34 herds (68%), in freestalls with deep litter bedding in 7 herds (14%), and in tiestalls in 9 herds (18%). Zero grazing was practiced in 10 herds (20%). Cows were milked using an AMS in 3 herds (6%). Forty-one producers (82%) used individual paper towels for premilking treatment, whereas 6 producers used a cotton towel (12%). Eight producers (16%) used a foaming predip. Cow teats were sprayed in 12 herds (24%) and dipped in 29 herds (48%) after milking. Postmilking teat disinfection was not practiced in 9 herds (18%). In all herds, blanket dry cow therapy was applied. Dry cow therapy with long-acting antimicrobial agents was combined with an internal teat sealant for all cows in 8 herds (16%) and for some cows in 10 (20%) herds. Twenty-seven (54%) producers declared never to purchase cows/young stock, whereas 23 did. During the survey, participating herds had an average BMSCC of 236,000 cells/mL (range of 85,000 to 453,000 cells/mL). On average, 47.9% of the cows per herd had an UHS of 3 or 4 (range of 15 to 77.5%). Twenty-seven herds (54%) were categorized as clean (≤50% of cows having UHS 3 or 4) and 23 as dirty (>50% of cows having UHS 3 or 4). The average proportion of heifers in a herd (heifer DAR/total DAR) was 23.8% (range of 6.0 to 45.9%).

Clinical Mastitis

In total, 845 CM cases from 692 cows were sampled by the producers. During the survey, 490 (77.7%), 111 (17.4%), 30 (4.7%), 5 (0.8%), and 3 (0.5%) cows suffered from 1, 2, 3, 4, and 5 cases, respectively. The population was 1,192,800 cow-days at risk. In total, 1,032 heifers calved during the survey and 722 animals were culled.

The IRCM was estimated at 7.1 quarter cases per 10,000 cow-days at risk for the whole population and was lower in heifers (2.9 quarter cases per 10,000 cow-days at risk) compared with multiparous cows (11.0 quarter cases per 10,000 cow-days at risk). However, the IRCM in the first week after calving was higher in heifers compared with cows (Figure 1).

Forty-eight out of 50 participating producers (96%) submitted CM samples during the study period. The number of cases per herd ranged from 0 to 107. The producers of the herds without submitted samples declared not to have observed CM cases during the study and typically milked a relatively low number of cows (20 and 40, respectively) in conventional milking systems. The average and median herd IRCM was 7.4 and 5.3 quarter cases per 10,000 cow-days at risk, respectively. The IRCM per herd is presented in Figure 2, showing a wide between-herd variation (range: 0–21.3 quarter cases per 10,000 cow-days at risk). Herds with an AMS had a lower average and median IRCM (4.7 and 2.6 quarter cases per 10,000 cow-days at risk, respectively; Figure 2).

Pathogen Distribution and Severity

In total, 677 CM samples (80.1%) were culture positive, including 87 contaminated samples (10.3%), where-

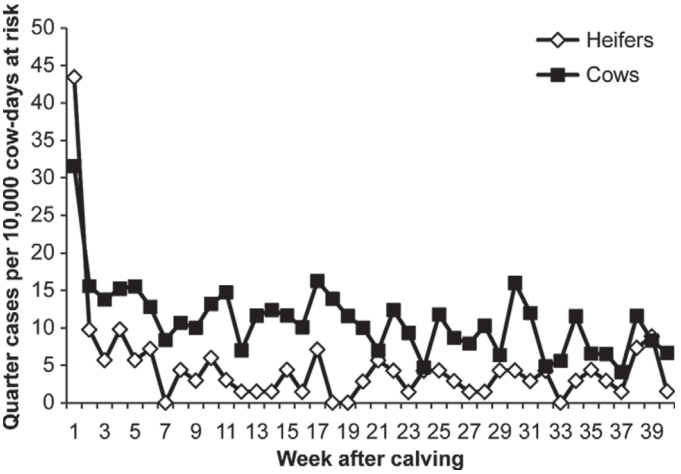

Figure 1. Incidence rate of clinical mastitis per week after calving for heifers (n = 2,168) and multiparous cows (n = 2,753).

as 168 CM samples (19.9%) yielded no growth. *Streptococcus uberis* was most frequently isolated (18.2%), followed by *E. coli* (15.5%), *Staph. aureus* (7.3%), and *Strep. dysgalactiae* (7.2%). Non-*aureus* staphylococci, *Corynebacterium bovis*, and other esculin-positive cocci besides *Strep. uberis* were isolated in 5.0, 3.0, and 2.1% of the samples, respectively (Table 1). Mixed cultures were isolated in 35 CM samples (4.1%). Yeasts were the most frequently isolated nonbacterial pathogens (2.0%). In 5.4% of the samples, other pathogens were isolated, including *Prototheca* spp. (1.4%), *Klebsiella* spp. (0.8%), *Trueperella pyogenes* (0.7%), *Bacillus* spp. (0.6%), *Pasteurella* spp. (0.5%), *Streptococcus agalactiae* (0.4%), *Streptococcus canis* (0.4%), *Pseudomonas aeruginosa* (0.4%), *Serratia* spp. (0.2%), and molds (0.1%). The pathogen-specific IRCM are shown in Table 1.

The majority of the CM cases were mild (63.1% of the cases; Table 1). Moderate severity was noticed in 29.9% of the cases and in 7.0% of the cases, producers observed severe clinical signs. In AMS herds, 30.8, 39.2, and 40.0% of the cases were, respectively, mild, moderate, and severe. Severity was associated only with the likelihood of *E. coli* isolation ($P < 0.0001$; Table 2). The likelihood of *E. coli* isolation (vs. any other culture result) was higher in severe and moderate cases compared with mild cases [OR = 1.85 (95% CI = 1.22–2.79) and OR = 5.04 (95% CI = 2.80–9.07), respectively]. *Escherichia coli* was isolated in 11, 19, and 39% of the mild, moderate, and severe cases, respectively. Regression coefficients changed little when AMS herds were excluded from the data set (<10% change, data not shown).

Association Between Pathogen-Specific IRCM and Herd Hygiene

Overall IRCM, *Staph. aureus* IRCM, *Strep. uberis* IRCM, *Strep. dysgalactiae* IRCM, and *E. coli* IRCM were higher in dirty herds compared with clean herds. Differences were significant for *E. coli* IRCM (RR = 2.57; 95% CI = 1.36–4.85) and tended to be significant for overall IRCM (RR = 1.49; 95% CI = 0.95–2.33). Depending on the model, the dispersion parameter and estimated power ranged from 0.71 to 1.59 and from 0.03 to 0.99, respectively (Table 3). Regression coefficients changed little when AMS herds were excluded from the data set (<10% change; data not shown).

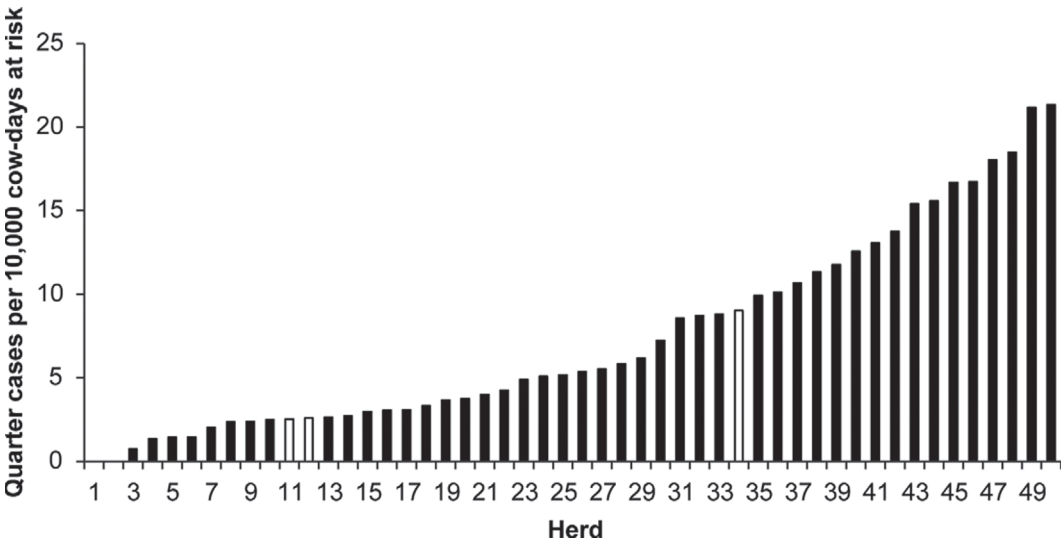

Figure 2. Incidence rate of clinical mastitis as determined through a 1-yr survey conducted on 50 randomly selected Flemish dairy herds. White bars represent automatic milking system (AMS) herds.

Table 1. Culture results, severity, and pathogen-specific incidence rates of clinical mastitis (IRCM) from a 1-yr survey conducted on 50 randomly selected Flemish dairy herds

Culture result	n	Cases[1] (%)	Severity[2]			IRCM[3]
			Mild[2]	Moderate	Severe	
Streptococcus uberis	154	18.2	62.3	33.8	3.9	1.3
Escherichia coli	131	15.5	45.8	36.6	17.6	1.1
Staphylococcus aureus	62	7.3	64.5	30.6	4.8	0.5
Streptococcus dysgalactiae	61	7.2	63.9	34.4	1.6	0.5
Non-*aureus* staphylococci	42	5.0	64.3	26.2	9.5	0.4
Corynebacterium bovis	25	3.0	72.0	28.0	0.0	0.2
Other esculin-positive cocci[4]	18	2.1	83.3	16.7	0.0	0.2
Yeast	17	2.0	58.8	23.5	17.6	0.1
Other pathogen	46	5.4	60.9	32.6	6.5	0.4
Mixed culture[5]	35	4.1	68.6	20.0	11.4	0.3
Contaminated sample[6]	87	10.3	76.7	19.8	3.5	0.7
Total culture positive	677	80.1	62.5	30.1	7.4	5.7
No growth	168	19.9	65.5	29.2	5.4	1.4
Total	845	100.0	63.1	29.9	7.0	7.1

[1]Number of cases with the specific culture result/total number of cases.

[2]Mild = only clots in milk; moderate = hard quarter without general signs; severe = systemic illness.

[3]Quarter cases per 10,000 cow-days at risk.

[4]Besides *Streptococcus uberis*.

[5]Isolation of 2 different pathogens.

[6]Isolation of 3 or more different pathogens.

DISCUSSION

To estimate the pathogen-specific IRCM in Flanders, 68 herds were randomly selected. A fair response rate of 79% was achieved. Three of the initial 53 herds were excluded from the analysis because producers admitted they did not sample all cases. Participation in the DHI program was comparable between the study herds (64%) and all Flemish dairy herds (60%; K. Huijps, CRV, Alken, the Netherlands, personal communication), suggesting randomization worked well. Still, the average herd size and BMSCC were slightly higher in the study herds (60 lactating cows and 236.000 cells/mL, respectively) compared with all Flemish dairy

Table 2. Logistic regression models describing the association between pathogen isolation and severity of clinical mastitis

Pathogen isolation[1]	Severity[2]	β[3]	SE	OR[4]	95% CI OR[5]	LSM[6]	*P*-value[7]
No growth	Mild	Ref.[8]	—	—	—	0.21	0.60
	Moderate	−0.08	0.19	0.92	0.63–1.34	0.19	
	Severe	−0.37	0.38	0.69	0.33–1.45	0.15	
Staphylococcus aureus	Mild	Ref.	—	—	—	0.08	0.79
	Moderate	0.00	0.29	1.00	0.57–1.77	0.08	
	Severe	−0.42	0.62	0.66	0.20–2.20	0.05	
Streptococcus uberis	Mild	Ref.	—	—	—	0.18	0.18
	Moderate	0.16	0.19	1.18	0.81–1.72	0.21	
	Severe	−0.66	0.45	0.52	0.22–1.23	0.10	
Streptococcus dysgalactiae	Mild	Ref.	—	—	—	0.07	0.27
	Moderate	0.14	0.28	1.15	0.66–1.99	0.08	
	Severe	−1.52	1.021	3.34	0.03–1.62	0.02	
Escherichia coli	Mild	Ref.	—	—	—	0.11	<0.0001
	Moderate	0.61	0.21	1.85	1.22–2.79	0.19	
	Severe	1.62	0.30	5.04	2.80–9.07	0.39	

[1]Outcome variable with 1 = isolation of the pathogen and 0 = any other culture result.

[2]Mild = only clots in milk; moderate = hard quarter without general signs; severe = systemic illness.

[3]Regression coefficient.

[4]OR = odds ratio.

[5]95% CI around OR.

[6]Proportion of cases with the specific culture result compared with the total number of cases with the specific severity.

[7]Overall *P*-value.

[8]Ref. = reference.

Table 3. Poisson mixed regression models describing the association between pathogen-specific incidence rates of clinical mastitis (IRCM) and herd hygiene

Outcome variable	Herd hygiene[1]	β[2]	SE	RR[3]	95%CI RR[4]	LSM[5]	*P*-value	DP[6]	$1 - \beta$[7]
Overall IRCM	Clean	Ref.[8]	—	—	—	6.0	0.08	1.59	0.36
	Dirty	0.40	0.23	1.49	0.95–2.33	9.0			
Staphylococcus aureus IRCM	Clean	Ref.	—	—	—	0.4	0.10	1.01	0.17
	Dirty	0.57	0.34	1.76	0.90–3.46	0.7			
Streptococcus uberis IRCM	Clean	Ref.	—	—	—	1.4	0.56	1.58	0.04
	Dirty	0.22	0.38	1.25	0.59–2.62	1.7			
Streptococcus dysgalactiae IRCM	Clean	Ref.	—	—	—	0.4	0.25	0.79	0.03
	Dirty	0.38	0.33	1.46	0.77–2.79	0.6			
Escherichia coli IRCM	Clean	Ref.	—	—	—	0.6	<0.01	0.71	0.99
	Dirty	0.94	0.32	2.57	1.36–4.84	1.7			

[1]Determined by recording the udder hygiene score (UHS; scale 1 to 4; Schreiner and Ruegg, 2003) of cows during 2 herd visits. Herds with more than half of the cows having UHS 3 or 4 were categorized as dirty (n = 23); other herds were categorized as clean (n = 27).

[2]β = regression coefficient.

[3]Rate ratio.

[4]95% CI of RR.

[5]Quarter cases per 10,000 cow-days at risk.

[6]Dispersion parameter, calculated by dividing the Pearson χ^2 by its degrees of freedom (Dohoo et al., 2003).

[7]Estimated power to demonstrate a significant difference between dirty and clean herds.

[8]Ref. = reference.

herds [50 lactating cows (Federal Public Service Economy, Small and Medium Enterprises, Self-Employed, and Energy, 2011) and 214.000 cells/mL (Milk Control Centre Flanders, 2013), respectively], suggesting some selection bias. As high BMSCC was found to be associated with higher *Staph. aureus* and *Strep. dysgalactiae* IRCM and lower *E. coli* IRCM (Barkema et al., 1999; Olde Riekerink et al., 2008), we might have underestimated *E. coli* IRCM and overestimated *Staph. aureus* and *Strep. dysgalactiae* IRCM.

Similar to other research (Barkema et al., 1998; McDougall et al., 2007), heifers had a lower IRCM in general but a higher IRCM in early lactation compared with multiparous cows. The latter findings stress the importance of prevention and control of heifer mastitis (De Vliegher et al., 2012) and the need to understand differences between pathogens associated with this disease. The mean herd IRCM (expressed as quarter cases per 10,000 cow-days at risk) was estimated considerably lower (7.4) than what could have been expected from a recent internet questionnaire indicating that 46% of the Flemish dairy cows suffer at least once per year from CM (P. Passchyn, S. Piepers, and S. De Vliegher, Ghent University, Ghent, Belgium, unpublished data). Based on the internet questionnaire, one would expect at least 1,380 cases (46% × 60 lactating cows × 50 herds), whereas we collected 845 samples. Because methodology and especially selection criteria differ between studies, caution is required in comparing results between CM surveys (Olde Riekerink et al., 2008), especially as the producers participating in the aforementioned online questionnaire estimated the IRCM lacking accurate data, as the majority admitted they were not keeping disease records. Yet, our figure differed little from figures estimated by Barkema et al. (1998) in the Netherlands (7.6, 7.0, and 6.9 for herds with low, medium, and high BMSCC, respectively) and Wolff et al. (2012) in Sweden (7.2). The IRCM was estimated lower in France and Canada (5.5 and 6.4, respectively), but higher in England and Wales (12.9), Norway (8.6), Finland (10.6) and Denmark (12.8; Barnouin et al., 2005; Bradley et al., 2007; Olde Riekerink et al., 2008; Wolff et al., 2012). Herds were randomly selected in some studies (Barkema et al., 1998; Bradley et al., 2007; Wolff et al., 2012) and conveniently selected in others (Barnouin et al., 2005; Olde Riekerink et al., 2008). In contrast to the studies in which the animals were randomly selected as well, no inclusion criteria were applied in our study, underlining the external validity of our data.

The pathogen distribution in this survey corresponded with those of Flemish CM samples submitted to the laboratories of both MCC Flanders (n = 4,468; Milk Control Centre Flanders, 2012) and the M-team (Ghent University, Merelbeke, Belgium; n = 329). In the 3 data sets, *E. coli* and esculin-positive cocci, including *Strep. uberis*, were the most frequently isolated pathogens. Similar to other regions in the world (Bradley et al., 2007; Oliveira et al., 2013), environmental pathogens appear to be the most common cause of CM in Flanders. *Staphylococcus aureus* was less frequently isolated in our study (7.3% of the samples) compared with CM studies conducted in Canada (10.3% of the samples; Olde Riekerink et al., 2008) and Ireland (23% of the

samples; Keane et al., 2013), suggesting that a different focus in clinical mastitis prevention is required for each country. Both in this and in other studies (Bradley et al., 2007; Olde Riekerink et al., 2008; Keane et al., 2013; Oliveira et al., 2013), a relative large proportion of cases were culture negative. Spontaneous cure, low bacterial viability, and pathogens not growing in standard culture media (*Mycoplasma* spp.) might explain this high proportion (Taponen et al., 2009). Freezing and thawing of the samples might have decreased the culture sensitivity of *E. coli* (Schukken et al., 1989) but was required to allow convenient transportation of samples by the herd veterinarians. As the sensitivity of detecting IMI using a single milk sample was found to be higher for *Staph. aureus* compared with other pathogens (Dohoo et al., 2011), we might have overestimated the *Staph. aureus* IRCM compared with the IRCM by other pathogens.

Clinical signs were mostly mild. However, we should mention that the clinical signs were recorded by the producers who might have missed subtle signs of systemic illness (e.g., fever) in some cases. Compared with a CM study in large herds in Wisconsin (Oliveira et al., 2013), the proportion of moderate and severe cases was smaller (29.9 and 7% vs. 36.9 and 15.3%, respectively). Still, in the latter study, *E. coli* was more frequently isolated compared with our study (21.6 vs. 15.3% of the cases). Additionally, as producers were rewarded per collected milk sample to keep them motivated, it is not unlikely that very mild cases in our study were sampled. Nevertheless, cases might also have been missed. Especially in dry cows and AMS herds, where detection intensity could potentially have been less, resulting in some bias and an underestimation of the true IRCM. Isolation of *E. coli* was more likely in moderate and severe cases compared with mild cases. Still, *E. coli* could only be isolated in less than half of the severe cases. Using *E. coli* mastitis as synonym for severe mastitis should for that reason be discouraged. Instead, dairy producers and herd veterinarians should be informed that other pathogens besides *E. coli* can cause severe CM and that *E. coli* can also cause mild CM cases. Bacteriological culture of mild, moderate, and severe cases is required to estimate the herd pathogen distribution and a basis for an effective herd-specific mastitis treatment and control plan.

The accuracy of measuring herd hygiene by scoring the udder hygiene of a limited number of cows during 2 herd visits can be debated. Nevertheless, we observed a higher overall and *E. coli* IRCM in dirty herds compared with clean herds, which corresponds well with British research demonstrating a higher risk of CM in general and *E. coli* CM specifically in cows with dirty udders (Breen et al., 2009). However, and in contrast to the British study, we cannot claim that dirty cows were more likely to have CM because hygiene was measured at the herd level, potentially causing ecological bias (Dohoo et al., 2003). Yet, evaluating and improving udder and herd hygiene could reduce IRCM in Flanders. Interestingly, results in both studies demonstrated a much stronger association with *E. coli* CM compared with *Strep. uberis* CM. Although the power to detect a difference in *Strep. uberis* IRCM between dirty and clean herds in our study was very low and although *Strep. uberis* is considered as an environmental pathogen, cow-to-cow transmission might occur (Zadoks et al., 2003). We speculate that in several of the herds included in our study, cow-adapted *Strep. uberis* strains were causing CM. At the least, our findings indicate that improving hygiene will reduce the number of *E. coli* cases but will not have a large effect on *Strep. uberis* IRCM.

CONCLUSIONS

The mean and median IRCM in Flemish dairy herds was estimated at 7.4 and 5.3 quarter cases/10,000 cow-days at risk and showed high between-herd variation as indicated by the wide range (0–21.3). The IRCM of heifers compared with multiparous cows was lower throughout the entire lactation, yet higher in early lactation. *Streptococcus uberis* and *E. coli* were the most frequently isolated pathogens. Clinical signs were mild in most cases. Isolation of *E. coli* was more likely in moderate and severe cases compared with mild cases. Yet, less than half of the severe cases had *E. coli* as culture result. Overall and *E. coli* IRCM were higher in dirty compared with clean herds.

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