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Original article

Accuracy of the detection of intramammary infection using quarter somatic cell count when taking parity and stage of lactation of the dairy cow into account

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Abstract — The aim of this study was to determine if taking parity and stage of lactation of the dairy cow into account could improve the sensitivity (Se) and specificity (Sp) of a SCC-test to detect quarter intramammary infection (IMI). A total of 27 315 quarter milk samples were collected from 277 Holstein cows from one experimental herd from 1980 to 1994. Two analyses were carried out. In the first analysis (MIN+MAJ analysis), quarter samples showing at least a single isolation of a minor or a major pathogen were considered as infected. In the second analysis (MAJ analysis), only quarter samples showing at least a single isolation of a major pathogen were considered as infected. The SCC threshold value above which a quarter was defined positive was the one which maximised the Youden index (YI). Se, Sp and YI were calculated on (1) the whole study sample (herd level), and (2) 6 subsamples defined according to parity (primiparous versus multiparous) and stage of lactation (0 to 60 days, 61 to 120 days and 120 to 400 days). Se, Sp and YI were also recalculated at the herd level on the basis of Se and Sp obtained for each subsample. The SCC threshold selected on the whole study sample was 265 000 cells·mL^{-1} in the MIN+MAJ analysis and 420 000 cells·mL^{-1} in the MAJ analysis. At these thresholds, the Se and Sp values were 71.2% and 75.8% respectively (MIN+MAJ analysis) and 82.9% and 83.6% respectively (MAJ analysis). For both analyses, the SCC thresholds, Se and Sp varied according to parity and stage of lactation. Finally, the results showed that taking the cow characteristics into account had a very limited impact on the accuracy of IMI detection.

dairy cattle / somatic cell count / detection / intramammary infection

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Résumé — Qualité de la détection des infections intramammaires basée sur la concentration en cellules somatiques du lait de quartier en prenant en compte la parité et le stade de lactation des vaches laitières. L'objectif de cette étude était de déterminer si la prise en compte de la parité et du stade de lactation des vaches laitières pouvait améliorer la sensibilité (Se) et la spécificité (Sp) d'un test basé sur la concentration en cellules somatiques (CCS) du lait pour détecter les infections au niveau du quartier (IIM). Un total de 27 315 échantillons de lait de quartier ont été prélevés sur 277 vaches Holstein d'un troupeau expérimental entre 1980 et 1994. Deux analyses ont été effectuées. Dans la première (analyse MIN+MAJ), les échantillons présentant au moins un agent pathogène mineur ou majeur ont été considérés comme infectés. Dans la seconde (analyse MAJ), seuls les échantillons présentant au moins un agent pathogène majeur ont été considérés comme infectés. La valeur seuil de CCS retenue était celle maximisant l'index de Youden (IY). Les Se, Sp et IY ont été calculées (1) au niveau du troupeau, (2) sur 6 sous-ensembles définis selon la parité (primipares ou multipares) et le stade de lactation (0 à 60 jours, 61 à 120 jours et 120 à 400 jours), puis (3) au niveau du troupeau sur la base des Se et Sp obtenues dans chaque sous-ensemble. Au niveau du troupeau, le seuil de CCS était de 265 000 cellules·mL^{-1} pour l'analyse MIN+MAJ et de 420 000 cellules·mL^{-1} pour l'analyse MAJ. A ces seuils, Se et Sp étaient de 71,2 % et 75,8 % respectivement (analyse MIN+MAJ) et de 82,9 % et 83,6 % respectivement (analyse MAJ). Pour les deux analyses, les valeurs seuils, les Se et Sp variaient entre les sous-ensembles. Finalement, les résultats montrent que la prise en compte des caractéristiques des vaches a un impact limité sur la qualité de détection des IIM.

vache laitière / concentration en cellules somatiques / détection / infection intramammaire

1. INTRODUCTION

Somatic cell count (SCC) is now recognised as an indirect method which is able to detect intramammary infection (IMI) [15, 31]. This application requires the determination of an SCC threshold which gives a reliable discrimination between uninfected and infected quarters. This threshold should depend upon the economic consequences related to the failure in the detection of infected quarters (false-negative results) compared with those of the failure to detect uninfected quarters (false- positive results) [18].

The predictability of SCC for diagnosis of IMI relies on two criteria: the predictive value of a positive test is the likelihood of infected quarters among the positive SCC-test (≥ SCC threshold) results, whereas the predictive value of a negative test is the likelihood of uninfected quarters among the negative SCC-test (< SCC threshold) results. These predictive values depend on three factors: the sensitivity and specificity of the SCC test and the prevalence of IMI in the population considered [18]. Thus the predictability of SCC varies according to epidemiological situations, in contrast to sensitivity and specificity, which are IMI-prevalent independent. Assessing the intrinsic performances (sensitivity and specificity) of an SCC-test is then needed before any use in various contexts to detect IMI.

Several studies report variations of quarter SCC according to non infectious factors such as parity and stage of lactation. Quarter SCC is reported to increase with increasing parity with a larger effect in the bacteriologically-positive quarters than in the bacteriologically-negative ones [4, 9, 31]. In bacteriologically negative quarters, the SCC value varies by about 40 000 cells·mL^{-1} from the first to the fourth lactation [3, 4, 9, 16, 21, 31] while in the bacteriologically-positive ones variations reach about 200 000 cells·mL^{-1} [4, 9, 13, 31]. Similarly, quarter SCC is reported to be high at the beginning of lactation, to decrease until midlactation, and then to increase steadily until the end of lactation, with a larger effect in the bacteriologically-positive quarters. Throughout lactation, SCC varies by about 60 000 cells·mL^{-1}

in bacteriologically-negative quarters [4, 9, 23, 16, 28] and by about 300 000 cells·mL^{-1} in the bacteriologically-positive ones [4, 9, 13, 31]. Therefore a hypothesis is that parity and stage of lactation of cows influence the relationship between SCC and IMI.

The objective of this study was to determine whether taking parity and stage of lactation into account could improve the sensitivity and specificity of an SCC-test to detect quarter IMI.

2. MATERIALS AND METHODS

2.1. Study sample

This study was based on data collected from 1980 to 1994 in 277 Holstein cows from the experimental herd of the "Laboratoire de Pathologie Infectieuse et Immunologie INRA, France" (PII-herd).

2.2. Sampling procedures, bacteriological examination and somatic cell measurement

The sampling procedure and bacteriological examination have been previously described [26, 27]. Briefly, foremilk quarter samples were routinely obtained from each lactating cow at three week intervals throughout lactation until drying-off. In addition, foremilk samples were also collected during the five days after calving, at drying-off and before any treatment for clinical mastitis. Collection was done at the afternoon milking after teat cleaning and teat end disinfection with 70% alcohol. An aliquot (0.025 mL) from each sample was spread with a calibrated loop on esculin blood agar plates. Bacteria were identified after 24 and 48 h of incubation at 37 °C.

The Coulter counter (Coulter Counter, ZM model, Coulter Electronics, Margency, France) was used to measure the SCC value of each milk sample.

2.3. Preliminary exclusions from the initial dataset

To reach the objectives of this study, both SCC determination and bacteriological examination were done using identical milk samples. Samples lacking the SCC value or the bacteriological examination result, as well as the contaminated ones (when the bacteriological examination provided more than 3 bacteria in the same sample) were discarded from the initial dataset (30 347 available observations).

In this experimental herd, some cows experienced challenges (inoculation of bacteria and immunisation trials). Since this study only focused on natural infections, all data registered after a successful experimental challenge (that is, the inoculated bacterium was present in the sample following the challenge), as well as data from quarters taken within 50 days of an unsuccessful experimental challenge and within 70 days of immunisation were eliminated. Finally, all data coming from quarters previously treated with antibiotics were discarded. This procedure led to a 27 315 observation dataset.

2.4. Strategy of analysis

2.4.1. Samples considered

The analysis was carried out considering (1) the whole study sample, and (2) 6 subsamples defined according to parity (primiparous versus multiparous) and to stage of lactation (0 to 59 days, 61 to 119 days and 120 to 400 days). Observations registered after 400 days of lactation were not considered (247 observations were discarded).

2.4.2. Definition of infected and uninfected quarters

Bacteriological results were considered independently (without considering the previous or the subsequent result). Infection

was considered when at least a single pathogenic bacterium was isolated which means at least 40 cfu·mL^{-1} (1 colony out of 0.025 mL of milk). The infection was considered to be due to the major pathogens when samples simultaneously showed major and minor pathogens. Major pathogens included all streptococci, *Staphylococcus aureus* and Gram-negative bacteria (*Escherichia coli*, *Serratia* spp, *Klebsiella* spp, *Pseudomonas* spp and *Proteus* spp) whereas minor pathogens included staphylococci other than *S. aureus* and *Corynebacterium bovis*.

Two definitions of infection were considered in the present study. In the first analysis (MIN+MAJ analysis), all quarter samples infected with either minor or major pathogens were regarded as infected. Bacteriologically negative samples (that is in which no bacterium was isolated) were regarded as uninfected. To study the ability of the SCC-test to detect major pathogen infections, a second analysis (MAJ analysis) was performed. In this analysis, only samples infected with major pathogens were regarded as infected and were opposed to both quarter samples infected with minor pathogens and bacteriologically negative samples.

2.4.3. Assessment of sensitivity and specificity of the SCC-test to detect the quarter IMI status

Sensitivity is defined as the likelihood of a positive SCC-test result (observed SCC ≥ SCC threshold) in the population of quarters known to be infected. Specificity is defined as the likelihood of a negative SCC-test result (observed SCC < SCC threshold) in the population of quarters known to be uninfected. Sensitivity and specificity are a function of the SCC threshold chosen to discriminate between two states of quarters (positive-tested and negative-tested).

The SCC threshold was determined by the mean of the Receiver Operating Characteristic (ROC) analysis to achieve the best combination between sensitivity (Se) and specificity (Sp) [11]. Crude SCC values were first transformed into a natural logarithm (LnSCC). LnSCC threshold values were selected based on the maximisation of the Youden index (YI = Se + Sp–1) [10], under the condition of $|Se - Sp| \leq 0.05$. In addition, the accuracy of detection of infection using the SCC-test was assessed in each analysis (MIN+MAJ analysis and MAJ analysis) based on the area under the ROC curve (AUC) which is a global summary statistic of diagnostic accuracy. The ROC curves in the two analyses were plotted and the AUCs and their standard error were calculated. A test with an AUC of 1 is perfectly accurate for all possible thresholds, whereas a test with an AUC of 0.5 is non informative. Finally, the two AUCs (MIN+MAJ analysis and MAJ analysis) were compared according to the method described in Hanley and McNeil [12].

2.4.4. Accounting for parity and stage of lactation

The interest of accounting for parity and stage of lactation was evaluated in two steps.

At the subsample i level, the sensitivity (Se_i), specificity (Sp_i) and Youden index (YI_i) obtained from the ROC analysis for each selected LnSCC threshold (ST_i) were compared to those obtained (Se_{WS}, Sp_{WS}, YI_{WS}) when considering for their calculation the selected LnSCC threshold in the whole study sample (ST_{WS}).

For the whole study sample, the sensitivity, specificity and Youden index (Se_{WS}, Sp_{WS}, YI_{WS}) calculated when considering the ST_{WS} were compared to a recalculated sensitivity (R-Se_{WS}), specificity (R-Sp_{WS}) and Youden index (R-YI_{WS}) which were estimated as follows:

$$\text{R-Se}_{WS} = \frac{\sum (TP_i)}{\sum (TP_i) + \sum (FN_i)}$$

$$R\text{-}Sp_{WS} = \frac{\sum(TN_i)}{\sum(TN_i) + \sum(FP_i)}$$

$$R\text{-}YI_{WS} = R\text{-}Se_{WS} + R\text{-}Sp_{WS} - 1$$

where:

$R\text{-}Se_{WS}$ = recalculated sensitivity,

$R\text{-}Sp_{WS}$ = recalculated specificity,

$R\text{-}YI_{WS}$ = recalculated Youden index,

TP_i = number of true positive samples in the subsample i (i=1 to 6),

FN_i = number of false negative samples in the subsample i (i=1 to 6),

TN_i = number of true negative samples in the subsample i (i=1 to 6),

FP_i = number of false positive samples in the subsample i (i=1 to 6).

The method used to compare the different Se and Sp values was a test of comparison of two proportions (paired case), separately considering the infected quarters (comparison of Se) and the uninfected quarters (comparison of Sp).

The threshold selection and the determination of Se and Sp were made using SAS® 8.1 [28]. The plot of the ROC curves, the estimations of the AUC and the comparison between them were made using GraphROC software downloaded from (http: //www.netti.fi/~maxiw/index.html).

3. RESULTS

3.1. Descriptive results

The nature and frequency of isolated bacteria are shown in Table I. A total of 27 315 bacteriologically examined samples were considered. Bacteriologically negative quarters accounted for 76.7%. The most frequently isolated bacteria were staphylococci other than *Staphylococcus aureus* (64.0% of positive samples). Streptococci, *Staphylococcus aureus, Corynebacterium bovis* and Gram negative bacteria were isolated in 13.7, 13.7, 4.7 and 3.9% of positive samples respectively.

LnSCC varied widely according to the infectious status of the quarters (Tab. I, Fig. 1). A marked increase in mean LnSCC was observed in the group of the infected (with minor or major pathogens) quarters compared to the uninfected ones. Mean LnSCC were 5.31 (203 000 cells·mL^{-1}),

Table I. Frequency and quarter somatic cell count (expressed as natural logarithms) according to infectious status (n = 27 315).

Infectious status	Frequency (%)	SCC value			
		Mean	SD[1]	Minimum	Maximum
Whole study sample		5.57	0.95	2.30	10.98
Uninfected	76.7	5.31	0.64	2.71	10.10
Infected	23.3	6.44	1.25	2.30	10.98
Major pathogens	7.3	7.42	1.41	3.47	10.98
Staphylococcus aureus	3.2	7.92	1.35	3.47	10.98
streptococci	3.2	6.92	1.29	3.74	10.97
Gram-negative bacteria	0.9	7.46	1.40	4.57	10.76
Minor pathogens	16.0	6.00	0.86	2.30	10.18
staphylococci other than *S. aureus*	14.9	6.02	0.86	2.40	10.18
Corynebacterium bovis	1.1	5.68	0.79	2.30	8.63

[1] SD = standard deviation

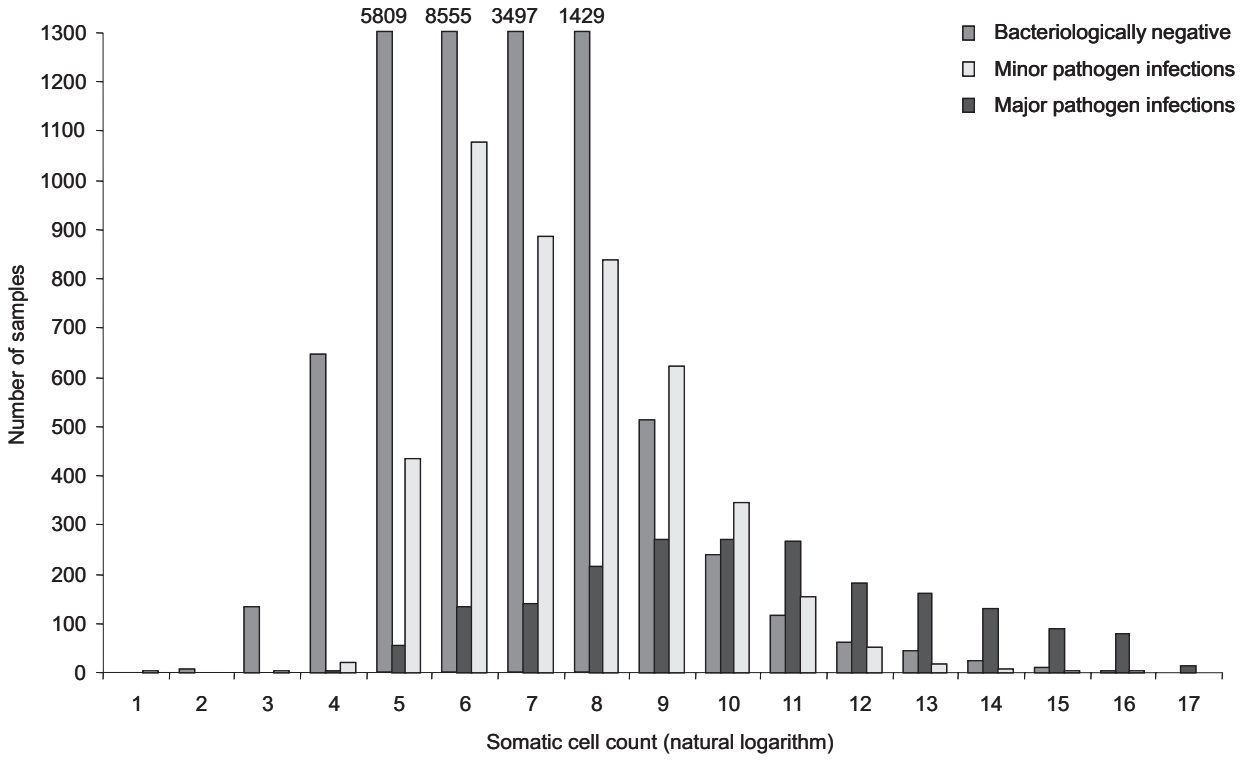

Figure 1. Distribution of the somatic cell count values (natural logarithm) according to infectious status.

6.00 (403 000 cells·mL^{-1}) and 7.42 (1 670 000 cells·mL^{-1}) in bacteriologically negative samples, and bacteriologically positive samples with minor and major pathogens respectively.

LnSCC also varied widely according to parity and stage of lactation both in uninfected and infected quarters (Tab. II). For a given stage of lactation, mean LnSCC was higher in multiparous cows than in primiparous cows. For a given lactation number, the mean LnSCC was high at the beginning of lactation, decreased from 60 to 120 dpp, and then increased in the last months of lactation. A higher percentage of infection with major pathogens was observed in multiparous cows whatever the stage of lactation.

3.2. Selected threshold, sensitivity and specificity of the SCC-test to detect a quarter IMI with minor or major pathogens (MIN+MAJ analysis)

The ROC curve summarising the diagnostic performance of the SCC-test is shown in Figure 2. AUC was estimated at 0.80 (SE = 0.0034).

The selected LnSCC threshold (ST_{WS}) was 5.58 (265 000 cells·mL^{-1}). This resulted in a sensitivity (Se_{WS}) of 71.2% and a specificity (Sp_{WS}) of 75.8% (Tab. III, Fig. 2).

The selected LnSCC thresholds, ST_i, estimated for each subsample varied from 5.27 to 5.79 (195 000 to 327 000 cells·mL^{-1}), resulting in Se_i varying from 64.2 to 72.4% and Sp_i varying from 68.9 to 76.1%. For a given stage of lactation, ST_i was found to be higher in multiparous cows than in primiparous cows. For a given lactation number, ST_i was found to be high at the beginning of lactation, the lowest from 60 to 120 dpp, and then increased in the last months of lactation (Tab. III). Sensitivity and specificity were the lowest in the subsamples of primiparous cows between 0 and 59 dpp and between 60 to 119 dpp.

3.3. Selected threshold, sensitivity and specificity of the SCC-test to detect a quarter IMI with major pathogens (MAJ analysis)

The ROC curve is shown in Figure 2. The AUC was 0.89 (SE = 0.0042).

Table II. Quarter somatic cell count in milk (expressed as natural logarithms) according to infectious status, parity and stage of lactation.

Parity	Stage of lactation	Infectious status							
		Uninfected			Infected				
		Number	Mean	SD[1]	Number	Mean	SD	MajPI[2] (%)	MinPI[3] (%)
Primiparous	0 to 59 days	1 377	5.27	0.70	271	6.06	1.25	21.4	78.6
Primiparous	60 to 119 days	1 319	5.09	0.45	288	5.80	0.85	11.5	88.5
Primiparous	120 to 400 days	3 783	5.14	0.46	1 025	6.00	0.94	8.4	91.6
Multiparous	0 to 59 days	3 164	5.36	0.74	839	6.65	1.36	48.2	51.8
Multiparous	60 to 119 days	3 112	5.18	0.56	854	6.42	0.34	40.5	59.5
Multiparous	120 to 400 days	8 209	5.46	0.68	3 074	6.63	1.25	34.4	65.6

[1]SD = standard deviation
[2]MajPI = infections with major pathogen
[3]MinPI = infections with minor pathogen

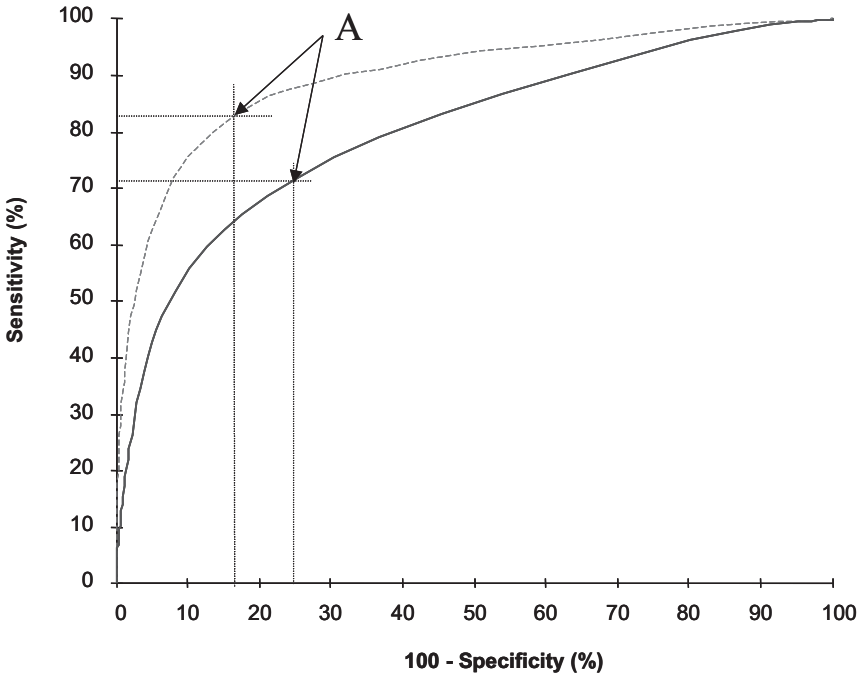

Figure 2. Receiver operator characteristics (ROC) curve for the SCC-test to detect IMI due to any pathogens (——) or only due to major pathogens (– – – – –). The selected operating point for each analysis is identified by the arrow A.

The selected LnSCC threshold ST_{WS} increased to 6.04 (420 000 cells·mL^{-1}). This resulted in a Se_{WS} of 82.9% and a Sp_{WS} of 83.6% (Tab. IV).

The selected LnSCC thresholds, ST_i estimated for each subsample varied from 5.54 to 6.28 (255 000 to 535 000 cells·mL^{-1}), resulting in Se_i varying from 75.8 to 83.3% and Sp_i varying from 79.9 to 87.6%. ST_i showed similar trends according to the lactation number and stage of lactation as those reported in the MIN+MAJ analysis. Sensitivity and specificity were the lowest in the subsample of primiparous cows, being between 60 and 119 dpp.

3.4. Accounting for parity and stage of lactation

In MIN+MAJ analysis, Se_{WS} and R-Se_{WS} were not significantly different, on the contrary to the specificities (R-Sp_{WS} was significantly lower than Sp_{WS}) (Tab. III). In MAJ analysis, either Se_{WS} or Sp_{WS} were not significantly different from the recalculated ones (R-Se_{WS} and R-Sp_{WS}) (Tab. IV).

In both analyses, considering each subsample separately, Se_i and Sp_i were significantly different from the ones obtained when considering the selected threshold ST_{WS} for their calculation (Tabs. III and IV).

Table III. Selected SCC thresholds and the corresponding sensitivities, specificities and Youden indexes in MIN+MAJ analysis[1].

Parity	Stage of lactation	Number of samples	Based on ROC analysis				ST_{WS}[2] applied to each subsample		
			SCC threshold (Ln)	Sensitivity (%)	Specificity (%)	Youden index (%)	Sensitivity (%)	Specificity (%)	Youden index (%)
Whole population		27 315	5.58	71.2[a]	75.8[a]	47.0			
Primiparous	0 to 59 days	1 648	5.40	64.2	68.9	33.1	56.8	77.7	34.5
Primiparous	60 to 119 days	1 607	5.27	66.3	70.6	36.9	50.0	88.2	38.2
Primiparous	120 to 400 days	4 808	5.35	70.8	74.8	45.6	62.5	86.0	48.5
Multiparous	0 to 59 days	4 003	5.63	72.4	75.9	48.3	74.3	74.0	48.3
Multiparous	60 to 119 days	3 966	5.42	71.8	75.3	47.1	65.5	82.6	48.1
Multiparous	120 to 400 days	11 283	5.79	72.2	76.1	48.3	78.2	66.9	45.1
Recalculated		27 315	–	71.3[a]	74.9[b]	46.2			

[1] = analysis in which quarter samples showing at least one isolation of a minor pathogen or a major pathogen were regarded as infected.
[2] = selected threshold in the whole study sample.
[a b] = values within columns with no common superscripts differ ($P < 0.05$).

Table IV. Selected SCC thresholds and the corresponding sensitivities, specificities and Youden indexes in MAJ analysis[1].

Parity	Stage of lactation	Number of samples	Based on ROC analysis				ST_{WS}[2] applied to each subsample		
			SCC threshold (Ln)	Sensitivity (%)	Specificity (%)	Youden index (%)	Sensitivity (%)	Specificity (%)	Youden index (%)
Whole population		27 315	6.04	82.9[a]	83.6[a]	66.5			
Primiparous	0 to 59 days	1 648	5.93	81.0	85.3	66.3	75.9	88.1	64.0
Primiparous	60 to 119 days	1 607	5.54	75.8	80.6	56.4	51.5	92.2	43.8
Primiparous	120 to 400 days	4 808	5.66	82.6	79.9	62.5	65.1	88.3	53.4
Multiparous	0 to 59 days	4 003	5.97	83.3	82.6	65.8	80.6	84.3	64.9
Multiparous	60 to 119 days	3 966	5.90	82.8	87.6	70.5	80.9	90.5	71.3
Multiparous	120 to 400 days	11 283	6.28	81.4	84.0	65.5	87.0	76.7	63.8
Recalculated		27 315	–	81.9[a]	83.4[a]	65.4			

[1] = analysis in which quarter samples showing at least one isolation of a major pathogen were regarded as infected.
[2] = selected threshold in the whole study sample.
[a b] = values within columns with no common superscripts differ ($P < 0.05$).

4. DISCUSSION

The detection of a quarter IMI using SCC relies on the determination of an SCC threshold above which the quarter is presumed infected. In the present study, the SCC thresholds, together with the sensitivities and specificities of the SCC-test were determined by the Receiver Operating Characteristic analysis, which is recognised as an appropriate technique to assess the performances of diagnostic tests [11]. Each selected SCC threshold was chosen based (i) on the maximisation of the Youden index (ii) under the constraint $|Se - Sp| \leq 5\%$. Among all the available criteria to select a threshold, the Youden index, because it is prevalent-independent, provides SCC thresholds that may be extrapolated to other herds. In some cases, the maximisation of a Youden index results in distant sensitivity and specificity. Therefore an additional constraint ($|Se - Sp| \leq 5\%$) was added in order to limit the differential in weights given to false-negative and false-positive results. In some previous studies, the latter statement was the only condition to estimate the selected SCC threshold: it was defined as the point at which the proportion of false-positive and false-negative results were equal [2, 15, 31, 32]. Detilleux et al. [6] defined the selected SCC threshold as the point which maximises a "cost" function combining sensitivity, specificity and IMI prevalence. The strategy used in the present study accounts for both (i) the maximisation of a function of sensitivity and specificity (Youden index) and (ii) a quasi equal probability of testing positive an uninfected quarter, and negative an infected quarter.

In all studies, including the present one, the selected thresholds were chosen based on the assumption that the economic costs of false-positive and false-negative results were equivalent. In practice, the epidemiological situation in the herd and the relative consequences of false-negative and false-positive results need to be considered when choosing the SCC threshold [10, 15, 18]. If the cost given to false-positive results is deemed particularly heavy, it may be relevant to choose a higher SCC threshold (associated with an increased specificity and positive predictive value). On the contrary, a lower SCC threshold (associated with an increased sensitivity and negative predictive value) must be chosen for a very reliable detection of a true infected animal.

On the whole study sample basis, the selected SCC threshold (ST_{WS}) value (265 000 cells·mL^{-1}) obtained considering both milk quarters with minor and major pathogens as infected ones was, in most cases, slightly higher than the values reported elsewhere [2, 15, 29, 32]. This may be due to differences in the definition of the infectious status. In the current study, the latter was defined on the basis of a single sample. However, it was defined on the basis of two successive weekly samples in a study by Timms and Schultz [32] and on three successive samples (5 week intervals) in Schepers et al. [29]. This leads to discarding samples with divergent successive bacteriological results, and possibly to improving the accuracy of the SCC-test. However, in the current study, considering a single sample or two successive samples to define the infectious status (additional analysis not shown in the results part), had a small impact on the reported results. Using two successive samples to define the infectious status, the selected threshold (LnSCC = 5.57) and the corresponding values of sensitivity (71.2%) and specificity (75.7%) were almost the same as those reported in the current study (Tab. III).

Another putative source of variation between our selected threshold and the values reported previously, is the SCC measurement equipment used. In recent studies [2, 15, 29], the Fossomatic was used as the SCC measurement equipment whereas in the present study (based on samples collected from 1980 to 1994), SCC were

measured using the Coulter Counter. Nowadays, quality procedures in laboratories ensure comparable SCC values between the Fossomatic and the Coulter Counter. However, in the 1980's, SCC measured using the Coulter Counter were reported to be higher than those measured using the Fossomatic device [14, 19]. In our study, the mean SCC values in bacteriologically negative samples were rather high in a single sample (201 000 cells·mL^{-1}) compared to studies using the Fossomatic device (46 000 in [3] and 81 000 cells·mL^{-1} in [6]). In addition, even when uninfected quarters were defined on the basis of two successive bacteriologically negative samples, the mean SCC value remained high (194 000 cells·mL^{-1}).

The variations in reported thresholds may also be due to the differences in samples studied in terms of predominant pathogens [2, 15, 25, 31]. In the present study, the proportion of minor pathogens among the infected quarters was 64%. In the literature it varied largely, from 48% [2] to 95% [29].

To assess the ability of the SCC-test to detect the IMI due to major pathogens, a separate analysis was carried out. In this analysis, infected quarters with major pathogens were compared to quarters with minor pathogen infections and bacteriologically negative ones. The SCC-test was more accurate for the detection of major pathogen infections than for the detection of minor plus major infections: the area under the curve was estimated to be 0.89 (test regarded as highly accurate [11]) in the first case and to be 0.80 (test regarded as moderately accurate [11]) in the second one. The difference between these two AUCs was highly significant. On the whole herd level or within a given category of cows, sensitivities and specificities were found to be higher when only quarters with major pathogens were considered as infected than when quarters involving both minor and major pathogens were considered as infected, in agreement with previous studies [8, 15, 25]. The more overlapping the SCC distributions in infected and uninfected quarters, the worse the sensitivities and specificities (Tabs. III and IV and Fig. 1). From Poutrel and Rainard [25], at an SCC threshold of 200 000 cells·mL^{-1}, the sensitivity rose from 61% when samples with either major or minor pathogens were considered as infected to 88% when only samples with major pathogens were considered as infected, whereas the specificity dropped from 76% to 66%.

The worth of considering additional information on the cow (parity number and stage of lactation) for detecting an IMI using SCC was assessed (1) for each category of the cows within the herd, and (2) considering the whole herd.

For a given category of cows (that is at the subsample level), considering, to define the quarters as positive or negative, the selected SCC threshold determined either (1) at the herd level (ST_{WS}) or (2) specifically (ST_i) resulted in highly significant difference in sensitivities and specificities. In 4 out of the 6 categories of cows, selected thresholds assessed within each category were lower than the one determined based on the whole sample. Consequently, sensitivities increased and specificities decreased (in comparison to those resulting from the application of the ST_{WS}), but finally the Youden indexes remained almost equal.

The sensitivities and specificities showed variations between categories, the Youden indexes being lower for primiparous than for multiparous cows, especially for the former in early lactation. This may be explained by the larger overlap in LnSCC distributions between infected and uninfected quarters in primiparous than in multiparous cows, probably in relation to the larger proportion of infections with minor pathogens in the former cows (Tab. II). A high prevalence of infections with minor pathogens in primiparous cows in early lactation was often reported [1, 5, 20, 22, 24, 30]. In addition, the mean increase in quarter SCC

associated with minor pathogens was found to be low (≈ 160 000 cells·mL^{-1}) [7].

At the PII-herd level, considering the selected SCC threshold determined specifically within each category of cows (ST_i) did not lead to increased sensitivities, specificities and Youden indexes. This suggests the poor interest of taking these cow characteristics into account to detect IMI.

The data used in this study came from a unique experimental herd that may limit the generalisation of the results to other herds, in particular to commercial herds. However, the collection of both infection status and SCC of quarter milk samples may be difficult to achieve in a large number of quarters and in different herds. Most previous studies having the same aim were carried out in a limited number of herds (< 10) of which a maximum of 5 000 milk quarter samples were considered [15, 18, 31, 32]. In the present study, the number of samples taken into account (> 20 000) was comparable to that of Schepers et al. [29]. Further research based on new or already available data collected in a large number of commercial herds could be performed to assess whether the results obtained from this herd may be conservative.

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