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# Association of California Mastitis Test Scores with Intramammary Infection Status in Lactating Dairy Cows Admitted to a Veterinary Teaching Hospital

S.A. Kandeel (D, D.E. Morin, C.D. Calloway, and P.D. Constable

**Background:** Subclinical mastitis is of concern in veterinary hospitals because contagious mastitis pathogens might be unknowingly transmitted to susceptible cows and then back to their farm of origin.

**Objectives:** To evaluate the California mastitis test (CMT) as an indicator of intramammary infection (IMI) in lactating dairy cows admitted to a veterinary hospital.

Animals: A total of 139 admissions of 128 lactating dairy cows admitted to the University of Illinois Veterinary Teaching Hospital over a 2-year period.

**Methods:** A retrospective study with a convenience sample was conducted. Medical records of cows with CMT results and milk culture results for the day of admission were reviewed. Breed, age, season, maximum CMT score for the 4 quarters, maximum CMT score difference, and clinical diagnosis were evaluated as predictors of IMI by the chi-square test and stepwise logistic regression.

**Results:** An IMI was identified in 51% of quarters. For cows admitted without evidence of clinical mastitis, the sensitivity of a CMT score  $\geq$ trace in predicting an IMI on a quarter or cow basis was 0.45 and 0.68, respectively. The distributions of maximal quarter CMT score and the maximum difference in quarter CMT score for cows without evidence of clinical mastitis did not differ (*P* = 0.28, *P* = 0.84, respectively) for cows with and without IMI. Stepwise logistic regression did not identify significant predictors of IMI in cows without clinical mastitis.

**Conclusions:** Lactating dairy cattle admitted to a veterinary hospital should be managed as if they have an IMI, even in the absence of clinical mastitis.

Key words: Cow-side test; Intramammary infection; Mastitis; Somatic cell count.

Mastitis is a common and economically important disease of dairy cows<sup>1,2</sup> and is clinical or subclinical in nature.<sup>3,4</sup> Clinical mastitis results in visible abnormalities in milk and is readily detected during routine physical examination. A useful classification system for cows with clinical mastitis utilizes 3 categories<sup>5</sup>: (1) abnormal secretion (visible abnormalities in milk); (2) abnormal secretion and gland (clinical evidence of udder inflammation, including the presence of heat, redness, swelling, pain, and decreased milk production); (3) abnormal secretion, gland, and cow (clinical evidence of systemic illness, including fever, decreased appetite, and rumen fill). Subclinical mastitis is not detectable during routine physical examination but is identified by detecting the presence of inflammatory biomarkers or mastitis pathogens in glandular

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#### Abbreviations:

cfu	colony-forming unit
CMT	California Mastitis Test
IMI	intramammary infection
SCC	somatic cell count

secretions when an intramammary infection (IMI) is present.  $^{6-8}$ 

Veterinary Teaching Hospitals and veterinary practices with haul-in facilities for dairy cattle must take precautions to prevent the spread of infectious disease among animals from different farms. Lactating dairy cows must continue to be milked during hospitalization to maintain milk production, but mastitis pathogens can be spread from cow to cow by the milking cluster, milkers' hands, or contaminated milking utensils such as teat dip cups.<sup>9,10</sup> In teaching hospitals, veterinary students might also spread mastitis pathogens in the course of their daily examinations. The outcome of exposure to mastitis pathogens will depend on the pathogen, the extent of exposure, and the cow's defense mechanisms.<sup>11</sup> Hospitalized cows are more likely to be stressed as a result of medical or surgical conditions, recent transportation to the hospital, and nutritional or environmental changes, and consequently be more susceptible to IMI, than healthy cows remaining on a farm. Subclinical mastitis is of particular concern in a hospital setting as contagious mastitis pathogens might be unknowingly transmitted from infected cows to susceptible cows and then back to the farm of origin.

Microbiological culture of milk is the gold standard method for detecting IMI.<sup>12</sup> Unfortunately, culture results are not available for at least 24–48 hours,

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meaning that bacterial pathogens could be spread among hospitalized lactating dairy cows before culture results are known. The California Mastitis Test (CMT) is a practical, inexpensive, cow-side test used to estimate the number of inflammatory (somatic) cells in the milk of healthy cows<sup>13</sup> with higher scores being associated with an increased probability<sup>14–17</sup> and severity of IMI.<sup>18</sup> To our knowledge, the CMT has not been evaluated as a predictor of IMI in a population of dairy cows admitted to a veterinary hospital. Such cows often have low milk production and might have compromised immune defenses. Accurate and rapid identification of IMI on admission would permit implementation of additional biosecurity precautions to prevent spread of the IMI to susceptible cows from other farms. The main objective of the study reported here was therefore to evaluate the CMT as an indicator of IMI in lactating dairy cows admitted to a veterinary hospital. Additional objectives were to characterize the pathogens isolated from the milk of hospitalized cows and to identify cow factors associated with IMI on admission.

# **Materials and Methods**

### **Study Population**

Medical records were retrieved for lactating dairy cows admitted to the University of Illinois Veterinary Teaching Hospital during a 2-year period (January 1, 1998, to December 31, 1999). During those 2 years, aseptic milk samples were routinely submitted for bacteriologic culture from dairy cows admitted as inpatients. Milk was also examined visually and, in most (99%) cases, CMT scores were recorded. Medical records containing milk culture results and CMT scores were selected for use in this study.

#### Data Retrieval

Data retrieved from each cow's medical record were as follows: admission date, breed, birth date, most recent parturition date, diagnosis, milk culture results for each mammary gland at admission, CMT score for each mammary gland at admission, and milk production on the morning after admission.

Admission dates were ordered by season as follows: winter = December to February; spring = March to May; summer = June to August; fall = September to November. Breed of cow was categorized as Holstein or non-Holstein. Days in milk at admission were calculated from the admission date and most recent parturition date. Age at admission was used to categorize animals as heifers (<36 months old) or mature cows (≥36 months old). Diagnoses were categorized as clinical mastitis, metritis, displaced abomasum, lameness, or other condition. Clinical mastitis was diagnosed on the basis of visible abnormalities in the milk, with or without a swollen mammary gland or systemic signs of illness. Metritis included cows <14 days in milk with abnormal vaginal discharge or retained fetal membranes. Displaced abomasum included cows with left displaced abomasum, right displaced abomasum, or abomasal volvulus confirmed at surgery. Lameness included cows with hoof or limb lesions and abnormal gait. Any other diseases present at admission were listed as other conditions. If a condition developed after admission, that condition was not included in the analysis.

Cows were milked during hospitalization with a portable bucket milking machine twice daily (at 06:00 and 14:00 hours) and milk weights recorded on a stall card. Milk weights for the morning after admission were taken from the medical record as this milking reflected a standardized period (16 hours) since the previous milking.

#### Milk Sampling and Bacteriologic Methods

At admission, milk from all mammary glands was expressed onto a black plate for detection of gross abnormalities. The CMT was performed and interpreted as described.<sup>19</sup>

Briefly, 2 mL of fresh foremilk sample from each quarter was placed in the appropriate chamber of the CMT plastic paddle and mixed with 2 mL of CMT reagent at ambient temperature by gently moving the paddle in a circular motion. A change in viscosity indicated an increase in quarter SCC, with the CMT reaction being visually scored by 1 investigator at 45 seconds after adding the reagent by a 5-point scale as follows: negative, mixture remains liquid with no evidence of formation of precipitate; trace, a slight precipitate evident which tends to disappear with continued movement of the paddle; 1 positive (1), a distinct precipitate but no tendency toward gel formation; 2 positive (2), the mixture thickens immediately with some gel formation, and with motion, the mixtures tend to move in toward the center leaving the bottom of the outer edge of the cup exposed, and out again covering the bottom of the cup if the motion stopped; 3 positive (3), a distinct gel forms which tends to adhere to the bottom of the paddle and a distinct central peak forms during swirling.

Milk samples were collected aseptically from each teat after scrubbing the teat ends with 70% isopropyl alcohol. Samples were plated immediately or refrigerated at 4°C for up to 48 hours. Ten microliters of milk was plated onto 5% sheep blood agar and MacConkey agar and incubated in a 5% CO<sub>2</sub> incubator at 37°C. Plates were examined after 24 and 48 hours of incubation, and isolates were identified in accordance with National Mastitis Council recommendations.<sup>20</sup> Results were reported as colony-forming units (cfu) per mL of milk. For this study, 1 colony on a plate (equivalent to 100 cfu/mL) was considered to be indicative of an IMI.

Isolates were classified as major or minor mastitis pathogens in accordance with National Mastitis Council recommendations.<sup>20</sup> Minor mastitis pathogens included *Staphylococcus* spp. other than *S. aureus* and *Corynebacterium* spp.,<sup>21,22</sup> with the remainder considered to be major mastitis pathogens. *Bacillus* spp, yeast, and fungi were typically isolated in very low numbers and included in the minor mastitis pathogen classification scheme. Milk samples with 2 major mastitis pathogen isolates were classified as containing a major pathogen infection. Milk samples with 2 minor mastitis pathogen isolates were classified as containing a minor pathogen infection. Milk samples with 1 major mastitis pathogen isolated and 1 minor mastitis pathogen infection. A milk sample was considered contaminated when 3 or more colony types were present on a plate.

Isolates were also classified as contagious or environmental mastitis pathogens. Contagious mastitis pathogens included *S. agalactiae, S. aureus,* and *Corynebacterium* spp.<sup>23</sup> Environmental mastitis pathogens included *coliform* and other gram-negative bacteria, *Streptococcus* spp. other than *S. agalactiae, Staphylococcus* spp. other than *S. aureus, Enterococcus* spp., and *Trueperella pyogenes* (formally *Arcanobacterium pyogenes*).<sup>24</sup>

#### Statistical Analysis

The quarter with the highest CMT score was determined for each admission. If 2 or more quarters had the highest CMT score, then the infection status was assigned to the quarter based on the following prioritization scheme that was designed to retain information related to the presence of an IMI: major pathogen isolated, then mixed pathogens isolated, then minor pathogen isolated. The maximum difference in CMT score was calculated as 0, 1, 2, or 3 by expressing a CMT score of trace as 1 and determining the maximum difference between the highest CMT score and lowest CMT score among functional mammary quarters on the same cow. The chi-square test was used to compare the distributions of CMT scores on a gland basis for IMI or pathogen group, and the distributions of maximum CMT score and maximum difference in CMT score on a cow basis for IMI or pathogen group, with P < 0.05 being considered as significant. Fisher's exact test was used whenever the expected count in more than 25% of the cells was <5. Sensitivity and specificity were calculated to determine the suitability of CMT score cut-points for predicting IMI.

Stepwise forward multivariable logistic regression was used to identify the most important predictive variable(s) associated with IMI in cows with and without clinical mastitis. Because the cow should be considered as the experimental unit in mastitis studies, infection in any quarter constituted an IMI for that admission. The logistic regression models contained the independent variable (IMI) and the maximum CMT score (0, trace, 1, 2, or 3), maximum difference in CMT score (0, 1, 2, or 3), breed (Holstein, non-Holstein), age (heifer, mature cow), and season (winter, spring, summer, fall) as dependent variables. The *P* values for entry into or removal from the logistic regression models were <0.05. A statistical software program<sup>a</sup> was used for all analyses.

#### Results

#### Animals

The final data set included 139 admissions of 128 cows, with 9 cows being admitted twice and 1 cow being admitted 3 times. The number of days between admissions for the 9 cows admitted twice was 7, 12, 90, 94, 105, 184, 372, 405, and 427, and for the cow admitted 3 times were 15 and 358 days. The 139 admissions contributed 546 glands to the data set, with 10 cows having 1 nonfunctional quarter.

Population characteristics are summarized in Table 1. The median number of days in milk was 14 with a range of 1–367. The median milk production on the morning after admission was 17 lbs (7.7 kg) with a range of 0-52 lbs (23.6 kg).

Organisms were isolated in 270 of 528 (51%) quarter samples excluding contaminated samples (18 quarter samples, Table 2). Of the 110 quarter samples with a major pathogen isolated, 2 major pathogens were isolated from 22 quarters and 1 major pathogen was isolated from 75 quarters. Mixed infections (containing both a major and minor pathogen) were identified in only 9 quarters in cows with clinical mastitis and 4 quarters in cows without clinical evidence of mastitis. Minor pathogens were isolated from an additional 160 quarters: 131 quarters had only 1 minor pathogen isolated and 29 quarters had 2 minor pathogens isolated.

Contagious mastitis pathogens, primarily *Corynebacterium* spp., were isolated from 13% (69) of the quarter samples.

# Intramammary Infection and CMT Scores for Cows with Clinical Mastitis

Clinical mastitis was identified in at least 1 quarter at 67 admissions of 57 lactating dairy cows. Four cows

Table 1. Characteristics of the 139 admissions of 128
lactating dairy cattle that had 546 quarters sampled and
cultured on admission to a veterinary hospital over a 2-
year period. Numbers in the diagnosis section sum to
more than 139 because cattle could have 2 or more
diagnoses.

Characteristic	Ν	Percentage (%)
Breed		
Holstein	109	78
Non-Holstein	30	22
Age		
<36 months	39	28
≥36 months	100	72
Season of admission		
Winter	49	35
Spring	27	19
Summer	37	27
Fall	26	19
Diagnosis		
Clinical mastitis	67	48
Displaced abomasum	47	34
Metritis	20	14
Lameness	11	8
Other	29	21

**Table 2.** Prevalence of pathogens isolated from 546 quarters of 128 cows with 139 admissions to a veterinary hospital over a 2-year period. Ten cows had 1 missing quarter, 9 cows were admitted twice, and 1 cow was admitted 3 times. Eighteen quarters were contaminated and consequently their infection status could not be identified.

	Prevaler	Prevalence on Quarter Basis		
Pathogen	n <sup>a</sup>	Percentage (%)		
Major pathogens	115	21.1		
Staphylococcus aureus	6	1.1		
Streptococcus agalactiae	1	0.2		
Streptococcus uberis	8	1.5		
Streptococcus dysgalactiae	3	0.5		
Streptococcus bovis	11	2.0		
Enterococcus spp.	11	2.0		
Other streptococci spp.	27	4.9		
Trueperella pyogenes	6	1.1		
Escherichia coli	33	6.0		
Klebsiella spp.	6	1.1		
Other gram-negative	3	0.5		
Minor pathogens	219	40.1		
Corynebacterium spp.	69	12.6		
Other staphylococci	105	19.2		
Yeast	8	1.5		
Fungus	2	0.4		
Bacillus spp.	32	5.9		
Other gram-positive	3	0.5		
Contaminated	18	3.3		
No growth	258	47.3		

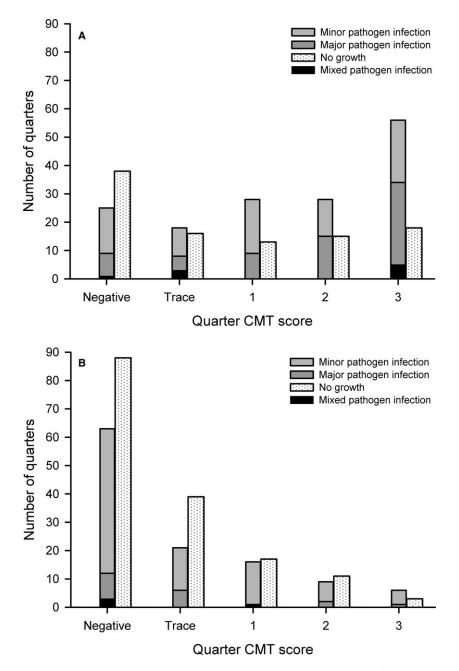
<sup>a</sup>Includes 64 quarter samples that contained more than 1 type of organism (mixed infections); therefore, the percentage column totals more than 100.

with clinical mastitis had 1 nonfunctional quarter, and 9 cows with clinical mastitis had a contaminated milk sample, providing 255 quarter samples for analysis from cows with clinical mastitis. For cows with clinical mastitis, IMI was present in 25, 18, 28, 28, and 56 quarters with a CMT reaction of negative, trace, 1, 2, or 3, respectively (Fig 1A). Only 9 quarters had a mixed infection. The distribution of CMT scores differed (P = 0.0003) for quarters with and without an IMI. Higher CMT scores in a quarter were associated with

an increased probability of IMI and the presence of a major pathogen.

The sensitivity and specificity for various CMT thresholds in predicting an IMI on a quarter basis from cows with clinical mastitis is summarized in Table 3; the sensitivity (0.84) was highest for quarters with a CMT score of trace or greater, and specificity (0.82) was highest for quarters with a CMT score = 3.

The distribution of maximal quarter CMT score for cows with clinical mastitis did not differ (P = 0.076) for



**Fig 1.** Panel **A**. Associations between the California Mastitis Test (CMT) score and the presence of an intramammary infection for 255 quarter samples obtained from cows with clinical mastitis in 1 or more quarters. Data were obtained from 67 admissions to a veterinary hospital of 57 lactating dairy cows. Panel **B**. Associations between the CMT score and the presence of an intramammary infection for 273 quarter samples obtained from cows without clinical evidence of mastitis. Data were obtained from 72 admissions to a veterinary hospital of 71 lactating dairy cows.

cows with and without an IMI (Fig 2A). The distribution of the maximum difference in quarter CMT score for cows with clinical mastitis did not differ (P = 0.25) for cows with and without an IMI (Fig 3A).

The sensitivity and specificity for various CMT thresholds in predicting an IMI on a cow basis from the maximal quarter CMT score of cows with clinical mastitis are summarized in Table 3; the sensitivity (0.93) was highest for a CMT score of trace or greater in any quarter and specificity (0.64) was highest if there was at least 1 quarter with a CMT score = 3.

Stepwise forward logistic regression utilizing data from the 67 episodes of clinical mastitis did not identify any significant predictors of IMI.

# Intramammary Infection and CMT Scores for Cows Without Clinical Mastitis

Clinical mastitis was not evident in 72 admissions of 71 cows, comprising 273 quarters (9 quarters were defined as contaminated and 6 cows had 1 nonfunctional quarter). For cows without clinical mastitis, IMI was present in 63, 21, 16, 9, and 6 quarters with a CMT reaction of negative, trace, 1, 2, or 3, respectively (Fig 1B). Only 4 quarters had a mixed infection. The distribution of CMT scores did not differ (P = 0.39) for quarters with and without an IMI.

The sensitivity and specificity for various CMT thresholds in predicting an IMI on a quarter basis from cows without clinical mastitis are summarized in Table 3; the sensitivity (0.45) was highest for quarters with a CMT score of trace or greater, and specificity (0.98) was highest for quarters with a CMT score = 3.

The distribution of maximal quarter CMT score for cows without clinical mastitis did not differ (P = 0.30) for cows with and without an IMI (Fig 2B). The distribution of the maximum difference in quarter CMT

**Table 3.** Sensitivity and specificity of California Mastitis Test (CMT) score cut-points on admission for detecting quarters or cows with an intramammary infection based on bacterial culture. Data were obtained from 67 admissions of 57 lactating dairy cows with clinical mastitis (total of 255 quarters) and 72 admissions of 71 lactating dairy cows without clinical mastitis (total of 273 quarters).

CMT Cut-point	Cows with Clinical Mastitis		Cows Without Clinical Mastitis				
	Sensitivity	Specificity	Sensitivity	Specificity			
Quarter bas	sis						
≥trace	0.84	0.38	0.45	0.56			
$\geq 1$	0.72	0.54	0.27	0.80			
≥2	0.54	0.67	0.13	0.91			
=3	0.36	0.82	0.05	0.98			
Cow basis (maximal quarter CMT value)							
≥trace	0.93	0.00	0.68	0.22			
$\geq 1$	0.91	0.00	0.42	0.63			
≥2	0.86	0.27	0.22	0.78			
=3	0.66	0.64	0.09	0.96			

score for cows without clinical mastitis also did not differ (P = 0.84) for cows with and without an IMI (Fig 3B).

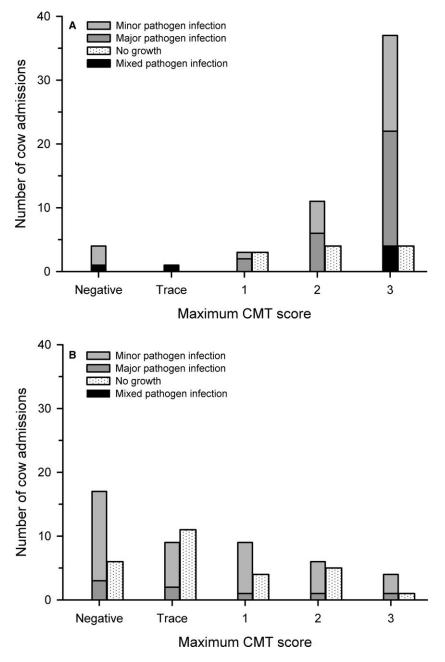
The sensitivity and specificity for various CMT thresholds in predicting an IMI on a cow basis from the maximal quarter CMT score of cows with clinical mastitis are summarized in Table 3; the sensitivity (0.68) was highest for a CMT score of trace or greater in any quarter, and specificity (0.96) was highest if there was at least 1 quarter with a CMT score = 3.

Stepwise forward logistic regression utilizing data from the 72 admissions of cows without clinical mastitis did not identify any significant predictors of IMI.

## Discussion

The major finding of this study was that the CMT does not provide sufficient test sensitivity to identify quarters and dairy cows with an IMI on admission to a veterinary hospital. A CMT cut-point ≥trace was the most sensitive for detecting an IMI in individual quarters (0.45) and cows (0.68). Our sensitivity estimates for the CMT cut-points were similar to those reported elsewhere on a quarter and cow basis for IMI based on bacterial culture.<sup>14,16,25,26</sup> High test sensitivity for detecting an IMI is required when admitting cows to a veterinary hospital because of the potential consequences of failing to identify an infected cow, particularly cattle harboring a major mastitis pathogen. The suboptimal sensitivity of the CMT means that some infected mammary glands will not be detected when a cut-point ≥trace is used. Our findings therefore suggest that all lactating dairy cattle admitted to a veterinary hospital should be treated as if they have an IMI. Consideration should be given to the routine application of biosecurity measures for all admitted lactating dairy cattle, including assigning veterinary students to the care of only 1 lactating dairy cow at a time, wearing disposable gloves and using disposable paper towels when handling the udder and teats and preparing the cow for milking, stripping of quarters into a bucket rather than onto the floor, hand-milking of low production cows, milking twice a day, use of a portable milking unit with disinfection of the cluster between cows, use of a sprayer or individual cup rather than a shared cup for postmilking teat disinfection, and thorough hand washing after handling teats or udder or milking a cow.

The IMI of greatest concern in a hospital population is that caused by contagious mastitis pathogens. Traditional contagious mastitis pathogens, *S. agalactiae* and *S. aureus*, were uncommon in hospitalized cows in this study. *Corynebacterium* spp. (presumably *C. bovis*), which is a contagious mastitis pathogen most commonly associated with subclinical infection of long duration,<sup>3,27</sup> was isolated frequently in our study. Although *C. bovis* is usually considered a minor mastitis pathogen with a relatively low impact on SCC or milk production,<sup>28</sup> its effect on mammary gland health is still an area of debate and it can be an important cause of clinical mastitis in some herds.<sup>5,29</sup> We did not

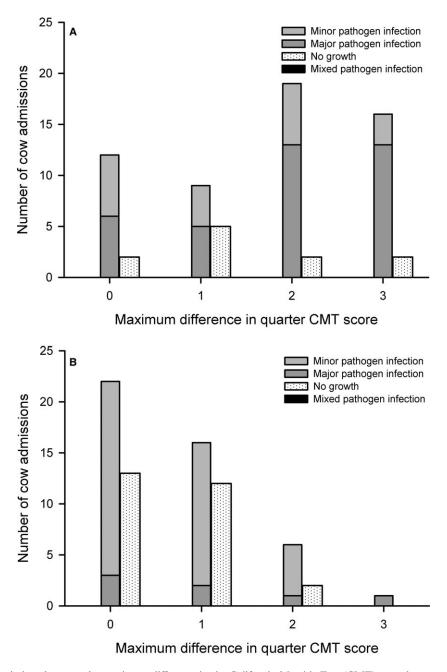


**Fig 2.** Panel **A**. Associations between the maximum California Mastitis Test (CMT) score and the presence of an intramammary infection on a cow basis for 67 admissions to a veterinary hospital of 57 lactating dairy cows with clinical mastitis. Panel **B**. Associations between the maximum CMT score and the presence of an intramammary infection on a cow basis for 72 admissions to a veterinary hospital of 71 lactating dairy cows that did not have clinical evidence of mastitis.

culture milk for *Mycoplasma bovis* or other *Mycoplasma* spp. that can cause mastitis and spread between cows.<sup>30</sup> However, during the time of this study, *Mycoplasma* mastitis was not recognized as a problem in Illinois and the likelihood of mycoplasmal IMI was considered too low to justify routine culture. In other geographical locations and cow populations, the pathogen profile for hospitalized cows might differ substantially. A potential limitation of the study reported here is the age of the data (~20 year old).

The mastitis pathogen profile might not necessarily reflect mastitis pathogens in lactating dairy cows admitted to veterinary hospitals in 2017. However, minor mastitis pathogens such as CNS and *Corynebacterium* spp. are currently the most commonly isolated mastitis pathogens in confined herds with good mastitis control programs,<sup>3,26</sup> similar to the data reported here.

There is debate about the contagious nature of some mastitis pathogens traditionally classified as environmental



**Fig 3.** Panel **A**. Associations between the maximum difference in the California Mastitis Test (CMT) score between all 4 quarters and the presence of an intramammary infection on a cow basis for 67 admissions to a veterinary hospital of 57 lactating dairy cows with clinical mastitis. Panel **B**. Associations between the maximum difference in the CMT score between all 4 quarters and the presence of an intramammary infection on a cow basis for 72 admissions to a veterinary hospital of 71 lactating dairy cows that did not have clinical evidence of mastitis.

pathogens, particularly *streptococci*. Outbreaks of *S. uberis* mastitis after discontinuing teat dipping<sup>31</sup> or antibiotic treatment of clinical cases suggest that contagious spread is possible.<sup>32</sup> Even some strains of *Escherichia coli* have been shown to persist in the mammary gland and infect multiple cows on a farm or glands in a cow.<sup>33,34</sup> The concentration of microorganisms shed in the milk may therefore be more important in a hospital population than the traditional nature of the pathogen

(contagious versus environmental versus teat skin). Cows shedding high numbers of organisms in their milk presumably pose a greater risk for mastitis transmission than cows shedding few organisms if biosecurity measures are not perfect. Stressed cows in a hospital may also be more susceptible to IMI or suffer more severe disease; the latter has been demonstrated experimentally for ketotic cows<sup>35</sup> and periparturient cows.<sup>36</sup> Therefore, the potential for transmission of mastitis-causing

pathogens from cow to cow in a veterinary hospital should be minimized as much as possible.

We defined IMI as >100 cfu/mL in this study to include infected cows with low pathogen shedding. We also based our diagnosis on a single milk sample from each gland, rather than paired or consecutive samples, because the latter were considered uneconomical for dairy cattle owners. We may therefore have falsely classified some uninfected glands (contaminated samples) as infected, but this was considered unlikely to have biased out findings as only 3% of quarter samples were categorized as contaminated. We may also have falsely classified some infected glands as uninfected because the pathogen levels were below the limit of detection by conventional culture techniques. Recent studies utilizing multiplex real-time PCR and PCR amplification and sequencing of 16 rRNA gene fragments (16S metagenomics) have suggested that milk obtained from healthy mammary glands may be colonized by a variety of microbes.<sup>37–39</sup> An unresolved question is whether these observations reflect contamination of the milk sample during the collection process or localized colonization in the teat orifice, streak canal, and teat cisternal milk, instead of generalized microbial colonization of alveolar and gland cisternal milk.<sup>40-43</sup> A second unresolved question is whether PCR techniques primarily detect dead bacteria from transient resolved infections or the presence of viable bacteria. In the latter case, viable bacteria may be present at concentrations below the level that induces a detectable inflammatory response or below the level required for disease transmission at milking time.

Failure to isolate bacteria from quarter milk samples with a CMT score of 2 or 3 may be due to bacteria not being the cause of udder inflammation of these glands (unspecific mastitis), low concentration of the microorganism in milk, intermittent shedding of the pathogen from the infected gland, intracellular location of pathogens and the presence of some inhibitors in milk, or the spontaneous elimination of the infection from the udder.<sup>3</sup> Failure may also reflect delayed healing of infection in which the pathogens may be reduced or eliminated from the udder while the infiltration of leukocytes continues until complete healing has occurred.

Schalm and Noorlander<sup>19</sup> stated in 1957 that "there has been a need for a quick, reliable test for the detection of abnormal milk at the side of the cow. To be of value, the reaction should be instantaneous and sufficiently clear-cut to leave no doubt as to whether the milk is normal or abnormal." The CMT provides a quick and reliable test for increased SCC, particularly SCC >200,000 cells/mL, and this SCC cut-point is recommended for diagnosing the presence of subclinical mastitis (inflammation) with maximum sensitivity and specificity and minimal diagnostic error.8,44-46 Nevertheless, although the CMT is a sufficiently sensitive and specific test for diagnosing the presence of inflammation, we are still in need of a quick, reliable, and inexpensive cow-side test for diagnosing the presence of IMI.

### Footnote

<sup>a</sup> SAS 9.4, SAS Institute Inc, Cary, NC

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*Conflict of Interest Declaration*: Authors declare no conflict of interest.

*Off-label Antimicrobial Declaration*: Authors declare no off-label use of antimicrobials.

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