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Review

Association of Milk Somatic Cell Count with Bacteriological Cure of Intramammary Infection—A Review

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Abstract: Mastitis is a costly disease in dairy cattle as a result of decreased milk production, discarded milk, and other economic drivers such as treatment costs. Although it can be costly, effective antibiotic therapy is useful to ensure the health and productivity of dairy cattle. Antibiotic usage to treat mastitis can be implemented after diagnosis based upon detection of increased milk somatic cell counts (SCC). Previous work demonstrated antibiotic treatment tends to be more effective when milk SCC are lower prior to treatment. An approach to increasing the cure rates of mastitis may be evaluating milk SCC prior to administering treatment. In order to investigate this potential tool, an effective and reliable method to enumerate SCC is critical. In this review, we (a) dissect the different definitions of cure, (b) review the methods available for enumerating SCC, and (c) discuss factors that are associated with intramammary infection cure with an emphasis on SCC.

Keywords: somatic cell count; mastitis; antibiotic treatment; cure; intramammary infection

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1. Introduction

Milk somatic cells, reported as somatic cell count (SCC), include all body cells but are predominately composed of macrophages, neutrophils, and lymphocytes (white blood cells), and a small fraction (<7%) of mammary epithelial cells [1–3]. A healthy mammary gland maintains a low or basal level of SCC, with reports supporting that up to 200,000 cells/mL is considered healthy, though the International Dairy Federation (IDF) suggests up to 250,000 cells/mL [4,5]. However, when the mammary gland is faced with trauma or intramammary infection (IMI), such as by bacteria, the resulting innate immune response and inflammation leads to a significant increase in SCC. In the field, IMI is commonly referred to as mastitis and is one of the most prevalent diseases in dairy production. According to the IDF, mastitis is inflammation of the mammary gland (one or more quarters) that is almost always caused by an infecting organism [5]. When caused by an infectious agent, mastitis is a costly disease due to decreased milk yield and quality, a result of the infecting pathogen itself and the immune response with an influx of SCC.

The majority of mastitis cases identified on farm are clinical, as they can be observed by changes to the mammary gland (heat, redness, swelling, pain) or milk (ropey, stringy, clots, flakes, blood, discoloration). Many of the physical changes in milk appearance are a result of increased immune cells in milk [6]. However, most cases of mastitis on dairies actually go clinically undetected as subclinical mastitis (SM) [7], where typical diagnosis depends on identification of elevated milk SCC and should also incorporate a measure to confirm pathogen presence. Even so, the detection of elevated SCC is a critical management strategy to monitor herd and individual cow health for subclinical mastitis. Petzer et al. [8] reported that utilization of SCC thresholds for IMI detection was effective whether composite or quarter milk samples were tested. Indeed, SCC is identified as a diagnostic tool which needs to be combined with microbiological results for an accurate mastitis diagnosis, according to the IDF [9].

Although antibiotic usage is costly for producers, is heavily scrutinized by consumers, and represents a reactive response rather than a proactive approach, effective antibiotic treatment is critical to ensure the productivity and health of the dairy herd. In fact, mastitis represents the top reason for antibiotic usage on dairy operations [10]. Reports suggest antibiotic treatment accounts for almost 30% of direct costs for mastitis [11]. In a study examining overall antibiotic usage on U.S. dairy farms by Sawant et al., [12], antibiotics were used to treat mastitis in 5% of heifers, 8% of cows in the dry period, and 14% of lactating cows in a single year. However, the basis for treatment of an infected quarter is variable between herds. In a survey examining the reasoning for antibiotic treatment of mastitis on farms, 66% of responders 'sometimes' cultured milk from the infected quarter or used another on-farm test for mastitis; although most producers based the decision for treatment upon visible signs rather than culture results [13]. Still, other operations utilized SCC as a deciding factor as to whether antibiotic treatment should be administered, as is the case for SM and in programs implementing selective dry cow therapy. In addition to the utilization of SCC as a diagnostic tool, previous research also suggests that SCC may impact the clinical and/or bacteriological cure following infection and could be a potential tool in combination with microbiological results for decision-making regarding the administration of antibiotics. Importantly, accurate and reliable SCC enumeration is critical, should this be a viable approach. Therefore, in this review, we (a) dissect definitions of IMI 'cure', (b) consider techniques for enumerating SCC and highlight advantages and limitations, and (c) discuss factors that impact SCC with an emphasis on the relationship, if known, with bacteriological cure.

2. Definitions of IMI 'Cure'

2.1. Clinical Cure

Historically, an IMI "cure" is typically defined as clinical or bacteriological. Clinical and bacteriological cures can be antibiotic-assisted or occur after spontaneous cure. Spontaneous cure is elimination of the infecting pathogen by the host's own immune response, without the need for antibiotic treatment. A clinical cure is generally defined as the resolution of symptoms post-treatment, but definitions vary depending on the study. For example, Roberson et al. [14] considered a clinical cure to occur if milk was normal in appearance for 2 weeks in a row, whereas Wraight [15] defined clinical cure as the appearance of normal milk at the end of the milk withhold period as determined by the antibiotic treatment label. Though producers desire a clinical cure to avoid discarding abnormal milk, the gland may not in fact be recovered and free from pathogenic bacteria. In fact, Pinzón-Sánchez and Ruegg [16] reported that the clinical appearance of the milk will return within 7 days of initial clinical signs regardless of the bacteriological outcome. Therefore, clinical cure is not considered a reliable indicator of antibiotic success, nor is it an indicator of successful spontaneous cure [17]. Bacteriological cure, on the other hand, provides a more effective means of evaluating mammary health, and, especially, antibiotic success.

2.2. Bacteriological Cure

Bacteriological cure is defined as having occurred when the causative pathogen identified is no longer isolated from the gland. In many cases of mastitis, especially those caused by Gram-negative pathogens such as *Escherichia coli*, bacteriological cure may occur in advance of clinical cure [6,18], or even in advance of clinical presentation. Although bacteriological cure is a more accurate measure of 'cure' compared to clinical cure, precision may fail when considering the differences in definitions of bacteriological cure. Persson et al. [19] defined bacteriological cure as the absence of causative pathogen at 3 and 28 days post-treatment. In contrast, McDougall [20] defined bacteriological cure as the absence of causative pathogen at 21 days post-treatment. Consecutive samples are most useful due to the nature of some bacteria, such as *Staphylococcus aureus*, that shed from infected mammary glands in cycles [21]. While bacteriological cure should be considered rather than clinical cure alone, especially when evaluating the success or failure of antibiotic

treatment, an acceptable SCC may not yet be achieved, thus providing another facet of 'cure' to assess.

2.3. Cytological Cure

A third type of 'cure', cytological cure, is a lesser discussed form and can be considered the point at which the SCC in the quarter or gland has returned to either preinfection levels or below 200,000 cells/mL [22]. Reports suggest that the SCC may remain elevated for up to 21 days post-treatment [17]. In fact, Schmenger and Kromker [22] reported that although a high bacteriological cure rate (73.3%) was identified after 21 days (samples collected at time of treatment for clinical mastitis (CM) and 14 and 21 days later), the cytological cure rate was only 22.3%, as the SCC of infected quarters failed to return to below 200,000 cells/mL. The authors suggested that mastitis therapies and plans should focus not only on eliminating the infection pathogen, but also on more quickly ameliorating the influx and presence of SCC. Ruegg [17] noted, also, that the rate of return to "healthy" SCC is impacted by causative pathogens. Published work does elicit a word of caution as to when SCC should be evaluated after antibiotic therapy. Ruegg and Tabone [23] discussed the increased SCC in samples which tested positive for antibiotic residues. Though this is more than likely due to the presence of subclinical mastitis and the associated antibiotic usage to attempt clinical and bacteriological cure, other studies reveal the possibility of short-term elevated SCC after antibiotic therapy [24]. The authors posited that a short-term elevated SCC after therapy indirectly or directly elicits additional white blood cells, such as neutrophils, to aide in pathogen clearance [24]. Decades-old work suggest that components of intramammary antibiotic preparations could cause irritation, resulting in SCC increases [25]. Though conjecture, these increases in SCC may aide in more rapid clearance of mastitis-causing pathogens. Nonetheless, the few studies that have evaluated SCC immediately following intramammary antibiotic administration report short-term elevation. Therefore, cytological cures assessed 14–21 days following detection and treatment as described in previous work [17,22] would be reasonable.

Assessing SCC serves not only as an indication of infection severity (i.e., postinfection), but previous work indicates the association of pretreatment SCC or SCC at time of mastitis diagnosis with 'cure'. Owens et al. [26] reported a higher SCC in quarters that failed to cure compared to quarters that successfully cured after intramammary treatment and intramammary plus intramuscular antibiotic treatment (4,047,000 cells/mL vs. 2,501,000 cells/mL). Sol et al. [27,28] similarly found that bacteriological cure significantly decreased as log SCC increased at time of infection diagnosis (dry off), and there was decreased probability of cure when SCC was greater than 1,000,000 cells/mL. For these reasons, various studies have proposed adding pretreatment SCC consideration into mastitis treatment plans aimed at achieving high bacteriological cure [29]. Therefore, the subsequent sections will discuss methods for enumerating SCC followed by investigating factors that impact SCC and bacteriological cure.

3. Methods of Enumerating SCC

There are various cow-side and laboratory-based methods for SCC enumeration in dairy cattle. Each technique possesses both advantages and disadvantages, with potentially critical pitfalls should methods be inaccurate or results interpreted incorrectly. One would argue that in the context of mastitis detection and decision-making for antibiotic treatment, cow-side evaluation of SCC would be particularly valuable. Note that there are other methods of assessing presence of inflammation, and potentially IMI, in the mammary gland, and they include, but are not limited to: conductivity, pH, lactate dehydrogenase, and infrared thermography; but they are outside the scope of this review [30–32].

3.1. Cow-Side SCC Assessment

One of the most common cow-side methods for assessment of SCC is the California mastitis test (CMT), developed over 50 years ago [33]. A CMT reagent (ImmuCell, Portland,

ME, USA) is added to individual quarter milk samples collected from the cow. The CMT reagent reacts with the DNA of cells in milk, resulting in gelling of the solution with increased SCC. Cows are identified as positive for mastitis if they have readings of T, 1, 2, or 3 depicted in Table 1 [34]. The CMT test is rapid, easy to use, inexpensive, and when performed correctly, precise [35]. Previous research suggested CMT to be an accurate tool to diagnose IMI in cows due to its high specificity (86.2%) and sensitivity (88.5%) [34]. Another advantage of the CMT test is that it allows for individual quarter testing. Though the CMT is useful in mastitis control and prevention programs and a numerical score is usually obtained during moderate to severe mastitis, the test is considered qualitative and subjective [35,36]. Consequently, past researchers have found the sensitivity and the specificity of the CMT to be much lower, ranging from 61–69% and 65–68%, respectively [36,37]. Factors which may contribute to decreases in sensitivity and specificity include incorrect ratio of milk to CMT reagent, differences in score assignment based on gelling intensity, and inadequate swirling to induce gelling reaction [38]. In addition, the CMT test is not recommended for fresh cows and for late lactation cows nearing or at dry off, as the SCC elevation maybe unrelated to infection, though research suggests it could be a screening tool for further testing [35,39].

Table 1. Interpretation of CMT Scores.

CMT Score	Estimated SCC
Negative	0–200,000 cells/mL
Trace	150,000–500,000 cells/mL
1	400,000–1,500,000 cells/mL
2	800,000–5,000,000 cells/mL
3	Over 5,000,000 cells/mL

A similar cow-side test to the CMT is PortaSCC® (PortaCheck, Moorestown, NJ, USA) [40]. The PortaSCC® requires additional materials and takes longer compared to the CMT test, but past data support agreement in results between CMT and laboratory-obtained SCC [32]. Similar to the CMT, SCC is estimated after a reaction between the cells in milk and the test reagent that is added to test strips. In high SCC samples, a color change (blue) on the test strip will occur rapidly, but results should not be read for 45 min. The darker the blue, the higher the SCC, with specific SCC on the test strip ranging from 100,000 to >3,000,000 cells/mL. The PortaSCC®, like the CMT, is a qualitative test and is subjective, though a digital reader is available for the test strips, which would reduce human error. In spite of concerns, previous reports showed high sensitivity (94.14%) and specificity (87.3%) with the PortaSCC® and substantial agreement (k = 0.7) with laboratory-based SCC [41], which is consistent with other work as well [42,43]. Further results reported by Ferronatto et al. [44] showed that CMT is an effective tool in diagnosing mastitis postpartum when compared to laboratory-based techniques. Though sensitivity and specificity are lower in comparison to more rigorous methods described in the next section, cow-side tests such as the CMT are readily available in the field and are rapid [44].

Perhaps the most expensive initially, and most quantitative cow-side assessment of SCC, is the utilization of an electronic cell counter such as the DeLaval Cell Counter (DCC) (DeLaval International AM, Tumba, Sweden) [45]. The DCC is a portable unit that employs electronic somatic cell counting, which is similar in principle to the IDF reference method of direct microscopic somatic cell counting (DMSCC) [46,47]. Moon et al. [47] reported that utilization of the C-reader, a system similar to the DCC but not in production, for SCC enumeration resulted in greater or comparable results when compared to DMSCC and flow cytometry-based methods, which will be discussed in greater detail in the following section. Using the DCC and corresponding sample cassettes (DeLaval International AM, Tumba, Sweden), the cellular DNA in milk is stained with a fluorescent dye, an image is captured within the unit, and the individual nuclei are counted. Kawai et al. [48] reported a strong positive correlation (r = 0.963, $p < 0.001$) between the DCC SCC and SCC obtained

by a laboratory-based method. Similarly, Ruegg et al. [45] reported an agreement with laboratory-based methods of 95.6% and a Kappa coefficient of 0.9 when setting a diagnostic threshold of 250,000 cells/mL.

3.2. Laboratory-Based SCC Assessment

The reference standard of SCC enumeration is DMSCC [9], whereby milk cells are stained with methylene blue and microscopically counted by trained individuals. This method is tedious, requires trained professionals, and though most laboratory-based SCC assessments are not rapid in comparison to cow-side ones, DMSCC is by far the most time-consuming method [47]. Setup costs are high, though once established, costs are reasonable and not a barrier to utilization. Additionally, there are concerns that the methylene blue dye, which should primarily stain acidic components, i.e., the nuclear region, and it may also stain cytoplasmic particles shed from the gland resulting in artificially high SCC [46]. As an additional note in reference to DMSCC and previously discussed cow-side methods, the C-reader and DCC use ethidium bromide and propidium iodide, respectively, to stain DNA within cells rather than methylene blue, which is less specific to the nuclear material and may yield inaccurate results [49]. Though DMSCC is the reference method for SCC enumeration, the facilities, labor, and time which are required preclude it from use as a cost-effective, rapid method for use in on-farm decision-making for antibiotic use, especially in the context of CM.

By far the most widely used laboratory-based assessment of SCC utilizes flow cytometry to quantify SCC. In particular, the Fossomatic™ (FOSS, Hillerød, Denmark) machine has been standardized for use with cow's milk [50,51] and is an IDF-recommended method [51]. Similar to the C-reader, cells are stained with ethidium bromide and fluorescence is optically and electronically detected when reporting an actual SCC. The Fossamatic™ SCC (FSCC) was previously found to be highly correlated to DMSCC [52,53]. In fact, many studies evaluating different and novel assessments of SCC enumeration utilize FSCC as the comparison method [32,35,40,41,43,45,47,49]. However, there are still limitations for rapid, on-farm decision-making, which is similar to DMSCC, especially in the case of CM. Indeed, Owens et al. [26] commented that accurate, cow-side assessment of SCC in combination with culture results is necessary for maximal chance of cure following IMI.

3.3. Differential SCC (DSCC)

More recently, DSCC has gained attention as a potential tool in the evaluation of mammary health, and potentially in mastitis prevention and control programs [54–56]. The DSCC is an enumeration of the individual populations or ratios of the SCC to provide an indication of inflammation or IMI. In normal, healthy mammary glands, macrophages and lymphocytes typically comprise the majority of the SCC (49–69%), with the remainder being neutrophils (31–50%), followed by mammary epithelial cells (1–3%), then eosinophils and basophils (<1% each) [1–3]. After invasion of a mastitis-causing pathogen, an influx of neutrophils is elicited so as to phagocytose and kill the infecting pathogen. Therefore, during these early stages of the innate immune response and inflammation, neutrophil proportions can be as high as 95% [57]. Farschtschi et al. [58] recently published an exhaustive review, which is recommended for additional information.

Briefly, there are currently a handful of technologies which can be utilized for obtaining a DSCC. The QScout® Farm Lab (Advanced Animal Diagnostics, Morrisville, NC, USA) is a portable analyzer capable of differentiating between leukocyte populations in milk samples and is most useful as an on-farm method of assessing DSCC. The QScout® Milk Leukocyte Differential (MLD) test is read by the built-in microscope to give total cell counts and the percentages of neutrophils, macrophages, and lymphocytes. Godden et al. [59] reported excellent repeatability with the MLD test; however, there were concerns for sensitivity and specificity depending on definition of or cutoff for IMI, and whether the cow or quarter diagnosis was desired. Lozado-Soto et al. [60] further refined the use of MLD for individual parameters (total leukocytes, neutrophils, macrophages, and lymphocytes).

Interestingly, specific thresholds were found to be different between Holsteins and Jerseys, with Holsteins having a threshold for total leukocytes of 105,000 cells/mL and Jerseys at 207,000 cells/mL, though the authors commented that additional animals would be needed for robust thresholds [60].

Another commonly used method for DSCC is based on flow cytometry [3], such as the Fossomatic™ machine (FOSS, Hillerød, Denmark) [61]. Importantly, the European Commission Joint Research Centre released certified reference material for use in calibrating these fluoro-optic instruments, such as the Fossomatic™; cooperative guidelines have been released by IDF and the International Committee for Animal recording [62]. The new reference sample and guidelines aim to ensure accurate and comparable SCC counts within and between countries and laboratories. The differential DSCC (FDSCC), as defined by Damm et al. [61], utilizing the fluoro-optic method is the combined proportion of neutrophils and lymphocytes, with the calculation of milk macrophages performed by subtracting the FDSCC from 100%. A strong positive correlation was found between FDSCC and the DSCC obtained by the fluorescence microscopy [61]. The authors reported that this is the first method for routine analysis for FDSCC and SCC that can be utilized in central testing laboratories, though the minimum threshold is 50,000 cells/mL [61]. Schwarz et al. [63] further investigated the use of FDSCC and found that combining FDSCC with DSCC increased sensitivity for IMI and decreased specificity. Moreover, the negative predictive value was increased, but the positive predictive value decreased [63]. Though a higher sensitivity and negative predictive value for IMI diagnosis is desired when considering antibiotic treatment, the authors noted that all confidence intervals were overlapping, indicating further research needs to be conducted to refine the use of DSCC. Interestingly, Souza et al. [64] employed flow cytometric analysis of milk SCC during nonspecific mastitis (culture-negative but with elevated SCC) and revealed that not only was the percentage of neutrophils increased in elevated SCC similar to previous work [57], but a specific population of lymphocytes (CD4+ T cells) was elevated. The authors presented the conjecture that the increased presence of CD4+ T cells aided in reducing the recovery of viable bacteria following an infection [64], an activity which would expedite mammary recovery and perhaps even promote mammary adaptive immunity.

4. Factors That Affect SCC and Their Association with 'Cure'

Previous work demonstrates the complex contributions of the cow, the causative pathogen, and the treatment regimen to antibiotic success following antibiotic treatment [26–29,65–68]. Specifically, lower SCC is reported to be associated with a greater chance of success or 'cure' after antibiotic treatment. Models have shown that the probability of bacteriological cure after treatment decreased from 40% for cows with a pretreatment SCC of <200,000 cells/mL to 27% for cows with a pretreatment SCC of 200,000 cells/mL to 800,000 cells/mL [68,69], indicating pretreatment SCC could be a useful predictor of treatment outcome. Evaluation of the efficacy of mastitis treatment on cattle with high SCC on DHI reports (400,000 cells/mL) showed some interesting results as well [70]. Cows were separated into either a high SCC group or a clinical symptom group, and the results demonstrated no significant decreases in SCC post-treatment within the high SCC group [70]. Moreover, the bacteriological cure rate was low (23.3%). Ultimately, the authors posited that treating cows with high SCC values may only be desirable in cases occurring in young cows within early lactation. Shephard et al. [71] found similar results by examining cows with SCC > 500,000 cells/mL, with treatment offering almost no financial gain to producers due to low cure rates. One might wonder if pretreatment SCC should be included in mastitis treatment plans, as it seems uneconomical to administer antibiotic treatment with very little chance of success. Therefore, further investigations should focus on understanding whether this is a viable option, with a particular interest in which factors impact SCC. Those factors include infection status, age or parity, stage of lactation, and stress, all of which impact basal or 'healthy' SCC and thus could contribute to differences in pretreatment SCC.

4.1. Infection Status

The primary reason SCC is elevated beyond 'basal' levels is intramammary infection [72]. Challenge with mastitis-causing pathogens *E. coli*, *S. aureus*, *Serratia marcescens*, *Streptococcus uberis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Mycoplasma bovis* all contributed to a significant increase in milk SCC from as early as 16 h postchallenge to 66 h postchallenge, with peaks ranging from 32 h to 96 h postchallenge [73–77]. Peaks in SCC ranged from approximately 40,000,000 cells/mL to 120,000,000 cells/mL. Past studies from North American and U.S. dairy herds reviewed in Dohoo et al. [78] demonstrated that quarters free of IMI have an SCC ranging from 113,000 to 251,000 cells/mL, while quarters harboring bacteria had an SCC range of 190,000 to 519,000 cells/mL. Others conducting work in Pennsylvania, U.S., [79], report bulk tank SCC as high as 1,591,000 cells/mL corresponding to a possible infection prevalence of 48% and a 29% production loss. Further, researchers found significant increases in milk SCC as early as 6 h following challenge with *S. aureus* with a peak at 42 h, which are findings that were similar between Holsteins and Jerseys (approximately 28,000,000 cells/mL to 34,000,000 cells/mL) [77]. Petzer et al. [80] reported that milk samples positive for bacterial presence had SCC greater than 200,000 cells/mL. It must be noted that greater than 30% of samples with elevated SCC were culture-negative, reiterating the importance of multiple diagnostic methods for IMI. Interestingly, Petzer et al. [80] found that the highest SCC measured were positive for *S. aureus*, *S. dysgalactiae*, and *S. agalactiae*.

Previous work reviewed in [78,81] showed that when the bacteria were separated into major (streptococci, *S. aureus*, and coliforms such as *E. coli* and *Klebsiella*) and minor (coryneforms and coagulase-negative staphylococci) pathogens, quarters infected with major pathogens produced, on average, a SCC > 600,000 cells/mL compared to that of minor pathogens with an SCC range of 100,000 to 300,000 cells/mL. A more recent study examined both major and minor pathogens, as well as other uncommon mastitis pathogens (other Gram-negative and Gram-positive species, mycoplasmas, *Candida tropicalis*, *Prototheca* sp.) in several herds [82]. In herd 1, major pathogens had a cow-level SCC of 327,000 cells/mL, minor pathogens averaged at 172,000 cells/mL, and other pathogens averaged at 497,000 cells/mL, while herd 2 had an average SCC of 373,000 cells/mL, 230,000 cells/mL, and 295,000 cells/mL, respectively [82]. Major pathogens cause a notably higher SCC than minor pathogens, while the less common pathogens, including *C. tropicalis* and *Prototheca*, can have variable SCC and need to be investigated more thoroughly.

Other researchers have focused on individual bacterial species rather than grouping into major and minor pathogens and have found similar results. Coagulase-negative staphylococci (CNS), also described in some research articles as non-aureus staphylococci (NAS), have proven to be associated with lower SCC at the time of treatment in both quarters that cure and quarters that fail to cure in comparison with *S. aureus* and environmental streptococci, with quarters that failed to cure having pretreatment SCC at least 2–3 times greater than quarters that successfully cured [83]. Not only does CNS typically cause less severe IMI than other mastitis pathogens, but infections are also usually associated with lower SCC, possibly explaining why the chances of antibiotic cure are much higher in comparison to other pathogens, though this is not always the case. For example, Jenkins et al. [84] reported that two CNS in particular appeared to be persistent in nature (*S. chromogenes* and *S. simulans*). In fact, after analyzing data over a 15-year period from the northeastern U.S., Schukken et al. [85] found that in herds with low bulk tank SCC, the contribution of CNS was greatest (17.9%) compared to herds with bulk tank SCC over 400,000 cells/mL (7.9%). It was further reported that after analyzing over 350,000 records that three apparent groups of data merged; culture-negative with the lowest reported SCC (<3 linear score), CNS and *C. bovis* samples with moderate increases in SCC (3–4 linear score), and major pathogens (*S. agalactiae*, *Streptococcus* spp., and *S. aureus*) with the greatest increase in SCC > 4 linear score). In a study evaluating all microorganisms identified in milk from cows in New York and Pennsylvania (including fungi and algae), the average SCC value was lowest for IMI caused by coryneforms, CNS, and mold, and was highest for

IMI caused by Gram-negative pathogens and *Streptococcus* spp. [86]. Yeasts, algae, and *S. aureus* had average SCC between the lowest and highest groups; however, *S. aureus* had the largest range of SCC values, from 191,000 cells/mL to 9,433,000 cells/mL. Over 75% of the IMI in this study were caused by *Streptococcus* spp., *S. aureus*, and NAS [86], although it must be reiterated that more recent studies have found reductions in IMI caused by *S. aureus* and increases in the prevalence of Gram-negative pathogens [72]. While NAS and Gram-negative pathogens are similar in terms of frequency, Gram-negative pathogens are typically associated with higher SCC values that may reflect upon the severity of the signs and symptoms of these infections in comparison to signs and symptoms of infections by NAS. However, a more recent study investigating SM found that SCC was highest in IMI caused by *Enterobacter* spp. (Gram-negative), followed by *Bacillus* spp. (Gram-positive), CNS, *S. aureus*, and then *E. coli* [87], indicating that some Gram-negative pathogens may cause higher SCC in comparison to others. Interestingly, several studies demonstrate the association of SCC with IMI 'cure' and indicate a possible place for SCC in decision-making for antibiotic treatment [26–29,66]. Sol et al. [88] found that *S. aureus*-infected cows with a lower pretreatment SCC had higher bacteriological cure rates in comparison to cows with a higher pretreatment SCC, although averages were not given. Similarly, in a review of antibiotic success of *S. aureus* by Barkema et al. [66], almost all studies reported that if pretreatment SCC was lower, bacteriological cure rate was higher, suggesting the potential for pretreatment SCC as a prognostic tool in mastitis-treatment discussions and strategies. Though it is clear from previous work that pretreatment SCC or SCC at time of diagnosis is related to 'cure', the variability in SCC as a result of a causative pathogen, presentation of symptoms (i.e., CM vs. SM), or length of infection (i.e., new vs. chronic) presents a barrier to implementation.

Association of Infection Status with IMI 'Cure'

The success of antibiotic treatment varies depending on the infecting pathogen due to differing physiology, virulence factors, and presence of antimicrobial resistance genes of mammary gland bacteria (i.e., penicillin-resistant vs. penicillin-susceptible) [17]. Researchers have reported a wide range of cure rates (38–100%) for Gram-negative pathogens, possibly due to Gram-negative pathogen *K. pneumoniae* having lower cure rates than *E. coli* (74% vs. 98%) [89,90]. *Klebsiella* spp. are more resistant to treatment than *E. coli*, with some treatment labels having efficacy statements for *E. coli* but not *Klebsiella* spp. [17]. However, the use of antibiotics to treat Gram-negative pathogens such as *E. coli* is up for debate due to the high rate of spontaneous cures (55.2–90%) [17,91]. Some Gram-positive pathogens such as *S. aureus* have a spontaneous cure rate close to 0%, making the value of antibiotic treatment much more significant [17]. Studies have reported bacteriological cure rates varying from 50.8–63.5% for Gram-positive pathogens, with higher cure rates attributed to *Streptococcus* spp. in comparison to *S. aureus* [22,89,92]; differences in *Streptococcus* spp. and *Staphylococcus* spp. will be discussed in further detail in the next classification of pathogens. Gram-negative bacteria and Gram-positive bacteria can be split into another mastitis pathogen classification, major vs. minor pathogens, that also demonstrate noteworthy differences in treatment outcome.

Mastitis caused by major pathogens, *S. aureus*, *E. coli*, and environmental streptococci (*Streptococcus dysgalactiae*, *S. uberis*), have been documented as causing the greatest loss in milk yield and reproductive efficiency [93,94]. *S. aureus* is one of the most prevalent mastitis pathogens and is frequently isolated from cases of CM and SM, with milk production loss almost equal in both cases [94]. Treatment success of mastitis caused by *S. aureus* varies significantly with antibiotic cure rates ranging from 38%–52% [88]. Control of *S. aureus* has been a significant challenge to farms due to the limited antibiotic efficacy, but if the right quarters are chosen for treatment, i.e., have a greater potential for cure, antibiotic treatment can be justified. The cure rates of mastitis caused by *E. coli* in the context of antibiotic treatment is less relevant due to the nature of the bacteria to not respond to most common, broad-spectrum antibiotics. Again, most published results do not promote the

use of antimicrobials for Gram-negative bacteria species, especially when antimicrobials may increase selection pressure for resistance [95]. Lastly, environmental streptococci have shown to be the most common pathogen responsible for CM, and when regarding severe mastitis cases, *S. uberis* is detected just as frequently as *E. coli* and more than *S. aureus* [22,86]. In a study evaluating streptococcal cure rates, 10 out of 15 (66.7%) streptococci infections that were treated successfully were cured, whereas only 6 out of 17 (35.3%) infections that were not treated cured spontaneously [67]. Similarly, researchers have found that environmental streptococci have a treatment cure rate of 52.8%, but when broken down into specific species in a later study, *S. dysgalactiae* had a cure rate of 82.9%, and *S. uberis* had a lower cure rate of 73.9% [22,94]. Mastitis caused by contagious *Streptococcus agalactiae* will be investigated further when discussing environmental vs. contagious pathogens.

There are several pathogens that are classified as minor mastitis pathogens, such as NAS or CNS and coryneforms (i.e., *Corynebacterium bovis*). The duration and chronicity of these minor pathogens may be similar to those of major pathogens, but IMI by NAS and *C. bovis* are usually less severe. In a study evaluating different types of NAS, 80.5% of NAS mastitis cases indicated persistence of the same infection for at least 10 months [96]. Similarly, NAS had a 90 d recurrence rate of 25.7% in comparison to *E. coli* (26.9%); however, NAS only caused 4.4% of severe infections, while 30.5% were caused by *E. coli* [22,86]. As discussed, minor pathogens may occur just as frequently or more than the major pathogens, but the likelihood of antibiotic success is much greater. When examining antibiotic success across five different treatment products at both short-duration and long-duration treatment times, it was determined that NAS had the highest cure rate (85.7%), followed by environmental streptococci (36.4%) and then *S. aureus* (25%) [83]. While minor pathogens may have higher cure rates than the major pathogens, recent studies concluded that the losses associated with minor pathogens have been vastly underestimated, with NAS resulting in 5.7% of the loss of the 305 d milk yield in comparison to 10.6% loss caused by *E. coli*. [94]. It is pertinent that producers know what pathogen is causing mastitis in each individual cow to effectively treat the IMI, as antibiotic success varies with each pathogen.

Mastitis pathogens can be further divided into environmental and contagious pathogens. Management practices have a large impact in determining which pathogen is causing IMI. *S. aureus* and *S. agalactiae* are both contagious pathogens that spread from cow to cow, and presence is highly influenced by improper management inside the milking parlor. Dirty towels and hands, as well as the milking machine itself, can act as fomites in cow-to-cow transmission. While the success rate of antibiotic treatment of these pathogens is not always high, some indirect benefits of antibiotic treatment can occur. When treatment of contagious IMI is successful, the number of infectious animals in the herd is reduced, thereby reducing infectious pressure and new cases [29]. The prevalence of IMI caused by *S. agalactiae* and *S. aureus* has declined with advances in milking machines and the elimination of improper milking techniques [72]. As contagious pathogen-caused IMI occurrence has dwindled over the years, Gram-negative pathogens and environmental streptococci have become the prevalent diagnosis of mastitis studies [22,72,89]. Environmental pathogens that come from the cow's environment, such as *S. uberis* and *S. dysgalactiae*, NAS, and coliforms, are influenced by the management of soil, bedding, and other factors outside the parlor. While originally described as environmental, there have been recent debates that *S. dysgalactiae* and *S. uberis* may behave as both contagious and environmental pathogens [97,98]. In either classification, contagious or environmental, prevention is the key to controlling these pathogens, as treatment is costly and not always successful. A clean environment, proper milking parlor hygiene, and appropriate milking machine function are required to reduce the risks of mastitis and lower bulk tank SCC.

The type of mastitis, i.e., CM vs. SM, may potentially impact the rate of success of any antibiotic regimen. Many studies have evaluated the efficacy of antibiotic treatment on cases of CM. In a study evaluating CM in large dairy herds by Oliveira et al. [89], treatment cure was determined by the assessment of bacterial cultures pretreatment and post-treatment, with bacteria identified pretreatment being culture-negative on post-treatment samples.

The results showed an overall CM treatment cure of 64.6% but differed depending on pathogen, with Gram-negative pathogens having a much higher cure rate (75%) than Gram-positive pathogens (50.8%) [89]. A more recent study that defined bacteriological cure in the same way as the previous study found similar results: an overall CM cure rate of 73.3%, with Gram-negative pathogens having the highest cure rate [22].

The basis for treatment of SM infections has proven to be more difficult to rationalize. While CM poses an urgent threat to mammary gland function and cow health, SM does not [99], which has led to many debates on the value of antibiotic treatment of SM. Avoiding SM issues on farms may not be economically feasible if controlling pathogens and reducing bulk milk SCC by treating SM can save producers money. Previous researchers have suggested that treatment of SM should be accompanied with additional preventative measures to reduce the transmission of contagious IMI, especially in terms of economic benefit [100,101]. For example, treatment of contagious *Staphylococcus* and *Streptococcus* species that cause SM is advised [102,103] to reduce the number of CM infections and spread of contagious pathogens [104]. On the other hand, past studies determined that the cure rates in SM cases that were treated vs. those not treated were 75% and 68%, respectively [94], a small difference in cure rates when considering the treatment costs. Additionally, Lavon et al. [67] found no significant differences in the antibiotic recovery and spontaneous recovery rates of SM, although recovery rates were much lower, ranging from 25–30%. This indicates that using antibiotics to treat SM in which spontaneous cure rates are similar may be uneconomical, but if any existing IMI were not cleared from the mammary gland before the next lactation, it is possible that SM incidence will be higher in the next lactation [102] or that the IMI will become chronic.

As indicated, duration of infection could impact cure rates after clinical presentation of mastitis without clarity as to whether this clinical presentation is evidence of a new IMI or could in fact be a result of a chronic infection with a clinical flare-up. Studies have shown that 64% of cows that have had two clinical cases of IMI within a lactation will have a third case before the end of that lactation [105]. Recurrent cases could also be dependent on degree of permanent damage, which increases susceptibility to repeated or chronic infections [106]. In some cases, mammary palpation may reveal evidence of damage in the form of nodes, fibrosis, or persistent edema [107,108]. If IMI persists for several months and is caused by the same pathogen or a recurrent IMI, it is deemed chronic [109]. The industry standard cure rate of antibiotic treatment normally settles around 50% but drops to 35% in chronic infections [66,106]. Most SM infections develop into chronic infections, sometimes considered so by having elevated SCC over 4-week intervals by the time they are diagnosed [100,104], but other studies may not agree that this period is long enough to deem an infection to be chronic. However, it has been demonstrated that cows with two or more consecutive culture-positive milk samples have a lower chance of bacteriological cure in comparison to cows with a single culture-positive sample [101]. In addition, Erskine et al. [99] suggests that since many SM cases are chronic and caused by *S. aureus*, antibiotic treatment would not be cost-effective. Researchers have concurred that control of chronic IMI should rely more heavily on prevention of new infections than antibiotic treatment [110,111], but still recommend antibiotic intervention for optimal bacteriological cure [111].

4.2. Age or Parity

There are conflicting studies regarding the impact of age and/or parity on SCC [112–115]. With the exception of cows greater than 7 years of age, SCC rose from an average of 126,000 cells/mL in healthy glands at 2 years of age to 251,000 cells/mL at 7 years of age [78]. In studies denouncing the significant, positive association of SCC with age, the causative factor for an observed increase in SCC as cows progressed through multiple lactations was identified as infection status or previous IMI history. Indeed, Eberhart et al. [79] demonstrated that glands infected with minor and major mastitis pathogens had a greater magnitude of increase, from 190,000 cells/mL to 320,000 cells/mL for minor pathogens and

614,000 cells/mL and 986,000 cells/mL for major pathogens. However, Sumon et al. [84] reported a positive correlation of age and parity with SCC, whether or not cows and quarters were infected. An important difference in previous studies mentioned and those more recent is the utilization of quarter samples. In Eberhart et al. [79], the results were milk SCC from composite samples, whereas Sumon et al. [87] tested quarter milk samples. As the number of quarters infected increases, so too does the composite milk SCC [81], a finding that is believed to be a primary cause of elevated SCC as age and parity increase. A primary question is whether these characteristics (multiple quarters infected, previous history of infections, etc.) impact SCC and cure rates simultaneously or if they are mutually exclusive.

Association of Age or Parity with IMI 'Cure'

Unfortunately, the probability of cure using antibiotic treatment has been noted to decrease with an increase in both SCC and cow age [27]. As the age of the cow increases, SCC tends to increase, possibly due to the increased number of infected quarters over time and the development of tissue damage from chronic infections [116]. Correspondingly, the parity of the cow is also a risk factor for antibiotic cure. Barkema et al. [117] evaluated the incidence of CM in several herds. First-calf heifers had the lowest incidence rate, whereas cows that calved eight times had the highest incidence rate, with rates increasing linearly as parity increased. Similarly, Deluyker et al. [118] found a significant interaction between SCC levels pre- and post-treatment and calving parity. Higher SCC levels prior to treatment resulted in larger differences in SCC post-treatment between parities [118]. This was suggested to be impacted by cure rates decreasing with cow age, and that the magnitude in SCC reduction that can be achieved is less in older cows than younger cows, in agreement with a more recent review by Alhussien and Dang [116] on factors influencing SCC. Issues may arise with older cows being prone to more IMI because their SCC typically remains higher overall, potentially increasing the probability of developing chronic infections [116]. Likewise, previous work shows that when cows were separated into groups by age and IMI status, milk from uninfected quarters displays little change in SCC as the number of lactations increase/as the cow ages; however, there is a significant increase in SCC when the quarter is infected with either minor or major pathogens [81,115]. This suggests that while parity/age has an impact on SCC, the inclusion of minor and major pathogens can significantly change the extensiveness of that impact.

4.3. Stage of Lactation

Not only has SCC been evaluated across lactations or parities, but within individual lactations as well. Immediately following parturition, SCC levels are usually elevated due to changes coinciding with calving and colostrum production, or even dysfunctions as a result of stresses, but decreases to a normal level a few days after calving in a healthy mammary quarter [116]. It has been shown that SCC remains high in infected quarters after these first few days, thus indicating that SCC should be useful in detecting new IMI early in lactation [6,79]. When looking at lactation curves based on SCC, SCC is high after parturition, decreases to a minimum value around 50 days in milk, and slowly increases again toward the end of lactation [119]. Sheldrake et al. [114] demonstrated that milk from uninfected quarters had an average SCC of 83,000 cells/mL at 35 days in milk and increased to 160,000 cells/mL at 285 days in milk.

The effect of CM on the lactation curve and SCC was large but differed based upon the causative pathogen and the parity of the cow; multiparous cows had higher SCC at each day in milk than did first-lactation heifers [119], providing more evidence that older cows have higher SCC overall. The SCC was much lower in lactations without a case of both CM and SM in comparison to all lactations and lactations without a case of CM, and the difference between the SCC of each lactation curve was much smaller in primiparous than multiparous cows [119]. These data demonstrate that SCC may have a larger association with CM than SM, but the size of that effect likely depends on the infecting pathogen.

Association of Stage of Lactation with IMI 'Cure'

There have been conflicting findings when discussing IMI cures in the context of stage of lactation or days in milk (DIM). Past work demonstrates a decrease in IMI cure as DIM increases [67,120]; however, the authors presented the conjecture that infections treated in late lactation could have been chronic, and thus, cure rates were lower. Sol et al. [88] reported no associations with cure and stage of lactation, though this was not the primary focus of the study. Schmenger and Kromker [22] reported conversely that as DIM increased beyond 200 DIM, the bacteriological cure rate increased. Similarly, Sol et al. [28] demonstrated that early- and mid-lactation cows had lower cure rates following antibiotic treatment in comparison to late lactation cows. Deluyker [118] reported analogous findings, with early-lactation cows <100 DIM demonstrating the lowest cure rates, and mid-lactation cows having the highest cure rates. Decreased cure during early lactation has been theorized to be a result of antibiotic elimination at a higher and faster rate in comparison to mid- and late-lactation, when milk production is decreased [118]. Early lactation is also a metabolically and immunologically challenging time for dairy cattle, potentially contributing to differences in cure observed in previous work. Interestingly, as noted in the previous section, SCC trends during lactation follow many of the trends reported for cure rates. Specifically, SCC may be elevated just after parturition and reach a nadir near peak lactation, with a slight increase as DIM increase. Thus, the differences in cure rates identified are associated with SCC. At this point in time, there are not concrete findings answering the questions of: are cure rates correlated with and caused by the higher or lower SCC alone, or are physiological events which impact cure rates similarly impacting SCC?

4.4. Environmental Stress

Various physiological and environmental stressors have reportedly been associated with changes, namely, increases, in SCC. In particular, season or weather, changes in social dynamics, and reproductive status have all been suggested. In healthy animals with proper housing and milking hygiene, little evidence exists to support a direct relationship with many physiological and environmental stressors [81,121]. In many cases, indirect effects may contribute to increased SCC. The most common discussion in the context of stress is related to season or weather. During hot, humid weather, SCC tends to be highest [80,122,123], though conflicting reports date back decades [124]. A primary hypothesis presented to explain this phenomenon in decades past was that the induction of cortisol during periods of stress, such as heat stress, contributed to dysregulated immune responses and therefore increase SCC. However, studies injecting dairy cattle with molecules to mimic this found that SCC were not increased in milk as a result [125], or SCC were increased moderately but not beyond a 'healthy' SCC [126]. However, also noted in many studies is that there is a similar increase in cases of clinical mastitis, suggesting that an increase in SCC during periods of heat stress may be a result of IMI, rather than simply heat stress directly impacting SCC in milk [91]. Further, the rates of clinical mastitis have been associated with the increased presence of and exposure to environmental bacteria such as *E. coli* and *S. uberis* [72]. Although, more recent studies reported that infections with *S. uberis* were highest during winter months [127]. In contrast, Hamel et al. [128] reported a correlation between increased temperature humidity index and shedding pattern of *S. uberis*, supporting not only the increased IMI and exposure to environmental pathogens during warm temperatures but also the potential reduced ability to control and eliminate infecting pathogens during periods of heat stress. Thus, another hypothesis to explain increased SCC during heat stress is that the immune system is suppressed such that susceptibility to infections increases. Indeed, Safa et al. [129] reported that heat stress decreased inflammatory cytokines, which coincided with increased SCC. To date, however, it is unclear if this potential immunosuppression as a result of heat stress is directly or indirectly associated with increased SCC observed during heat stress, and thus has an impact on cure rates following antibiotic treatment.

Association of Stress with IMI 'Cure'

To our knowledge, there is no available research to discuss antibiotic success during periods of stress, though one might argue that this discussion is covered in various sections of the current review. For example, early lactation animals experience metabolic and immunologic stress, which impact SCC and cure rates as previously discussed. With respect to heat stress in particular, as this is a primary topic in the context of mammary health and physiological stress, few studies exist. As reviewed by Ruegg [130], animals that are healthy enough to respond should be considered for antibiotic treatment. However, those animals that are experiencing events which prohibit them from mounting an appropriate immune response, as the purpose of antibiotic treatment is to support the immune response, should be viewed with caution. As heat stress may contribute to reduced immune responses, antibiotic treatment may not be advised, given the lowered chance of success [130]. It is unclear if there are direct seasonal effects on antibiotic success, and the industry would benefit greatly from additional investigations to enhance confidence in educated decisions for antibiotic usage.

5. Conclusions

As a field, we certainly acknowledge the tremendous association between SCC and health of the mammary gland, including the advantages of SCC as a diagnostic measure along with microbiological culture. Arriving at a homogenous definition of 'cure', or at least a more refined way to discuss either clinical, bacteriological, or cytological cure, will greatly enhance applicability and utilization of research findings on dairy operations. The authors recommended that a consistent definition, or at a minimum, clear descriptions of 'cure', are included in peer-reviewed literature. Moreover, selection of optimal methods to evaluate milk-quality parameters, including SCC, is critical. However, we have yet to fully elucidate the impact, whether direct or indirect, of SCC at the time of IMI diagnoses on cure rates given the pathogen, environmental, and cow-related factors which influence SCC. Given the increased interest in SCC and DSCC, future investigations should focus on understanding how to fully utilize SCC in a mastitis control, prevention, and treatment plan. These advances would be pivotal not only in reducing antimicrobial usage on dairy operations, but also enhancing animal health and well-being by addressing IMI at times in which cure is most probable.

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References

1. Sarikaya, H.; Prgomet, C.; Pfaffl, M.W.; Bruckmaier, R.M. Differentiation of Leukocytes in Bovine Milk. *Milchwissenschaft* **2004**, *59*, 586–589.
2. Koess, C.; Hamann, J. Detection of Mastitis in the Bovine Mammary Gland by Flow Cytometry at Early Stages. *J. Dairy Res.* **2008**, *75*, 225–232. [CrossRef] [PubMed]
3. De Matteis, G.; Grandoni, F.; Scatà, M.C.; Catillo, G.; Moioli, B.; Buttazzoni, L. Flow Cytometry-Detected Immunological Markers and on Farm Recorded Parameters in Composite Cow Milk as Related to Udder Health Status. *Vet. Sci.* **2020**, *7*, 114. [CrossRef] [PubMed]
4. Middleton, J.R.; Fox, L.K.; Pghetti, G.; Petersson-Wolfe, C. *Laboratory Handbook on Bovine Mastitis*, 3rd ed.; National Mastitis Council: New Prague, MN, USA, 2017.

5. IDF. Guidelines for the Use and Interpretation of Bovine Milk Somatic Cell Count. *Bull. DF* **2013**, *466*, 5118. Available online: https://www.fil-idf.org/wp-content/uploads/woocommerce_uploads/2013/03/B466-2013Guidelines-for-the-use-and-interpretation-of-bovine-milk-SCC-in-the-dairy-industry.CAT_-lmbdpy.pdf (accessed on 1 January 2022).
6. Pyörälä, S. Indicators of Inflammation in the Diagnosis of Mastitis. *Vet. Res.* **2003**, *34*, 565–578. [CrossRef]
7. Batavani, R.A.; Sakka, A. The Effect of Subclinical Mastitis on Milk Composition in Dairy Cows. *Iran. J. Vet. Res.* **2007**, *8*, 3.
8. Petzer, I.-M.; Karzis, J.; Donkin, E.F.; Webb, E.C.; Etter, E.M.C. Somatic Cell Count Thresholds in Composite and Quarter Milk Samples as Indicator of Bovine Intramammary Infection Status. *Onderstepoort J. Vet. Res.* **2017**, *84*, 1269. [CrossRef]
9. Smith, K.L. Suggested Interpretation of Mastitis Terminology. *Bull. Int. Dairy Fed.* **1999**, *338*, 3–26. Available online: https://fil-idf.org/wp-content/uploads/woocommerce_uploads/2011/03/Bulletin-of-the-IDF-No.-448_2011-Suggested-Interpretation-of-Mastitis-Terminology-revision-of-Bulletin-of-IDF-N%C2%B0-338_1999-1-fdvlh1.pdf (accessed on 1 January 2022).
10. Grave, K.; Greko, C.; Nilsson, L.; Odensvik, K.; Mørk, T.; Rønning, M. The Usage of Veterinary Antibacterial Drugs for Mastitis in Cattle in Norway and Sweden during 1990–1997. *Prev. Vet. Med.* **1999**, *42*, 45–55. [CrossRef]
11. Rollin, E.; Dhuyvetter, K.C.; Overton, M.W. The Cost of Clinical Mastitis in the First 30 Days of Lactation: An Economic Modeling Tool. *Prev. Vet. Med.* **2015**, *122*, 257–264. [CrossRef]
12. Sawant, A.A.; Sordillo, L.M.; Jayarao, B.M. A Survey on Antibiotic Usage in Dairy Herds in Pennsylvania. *J. Dairy Sci.* **2005**, *88*, 2991–2999. [CrossRef]
13. Raymond, M.J.; Wohrle, R.D.; Call, D.R. Assessment and Promotion of Judicious Antibiotic Use on Dairy Farms in Washington State. *J. Dairy Sci.* **2006**, *89*, 3228–3240. [CrossRef]
14. Roberson, J.R.; Warnick, L.D.; Moore, G. Mild to Moderate Clinical Mastitis: Efficacy of Intramammary Amoxicillin, Frequent Milk-out, a Combined Intramammary Amoxicillin, and Frequent Milk-out Treatment versus No Treatment. *J. Dairy Sci.* **2004**, *87*, 583–592. [CrossRef]
15. Wraight, M.D. A Comparative Efficacy Trial between Cefuroxime and Cloxacillin as Intramammary Treatments for Clinical Mastitis in Lactating Cows on Commercial Dairy Farms. *N. Z. Vet. J.* **2003**, *51*, 26–32. [CrossRef]
16. Pinzón-Sánchez, C.; Ruegg, P.L. Risk Factors Associated with Short-Term Post-Treatment Outcomes of Clinical Mastitis. *J. Dairy Sci.* **2011**, *94*, 3397–3410. [CrossRef] [PubMed]
17. Ruegg, P.L. What Is Success? A Narrative Review of Research Evaluating Outcomes of Antibiotics Used for Treatment of Clinical Mastitis. *Front. Vet. Sci.* **2021**, *8*, 639641. [CrossRef] [PubMed]
18. Erskine, R.J.; Wilson, R.C.; Riddell, M.G., Jr.; Tyler, J.W.; Spears, H.J.; Davis, B.S. Intramammary Administration of Gentamicin as Treatment for Experimentally Induced *Escherichia coli* Mastitis in Cows. *Am. J. Vet. Res.* **1992**, *53*, 375–381. [PubMed]
19. Persson, Y.; Katholm, J.; Landin, H.; Mörk, M.J. Efficacy of Enrofloxacin for the Treatment of Acute Clinical Mastitis Caused by *Escherichia coli* in Dairy Cows. *Vet. Rec.* **2015**, *176*, 673. [CrossRef]
20. McDougall, S. Intramammary Treatment of Clinical Mastitis of Dairy Cows with a Combination of Lincomycin and Neomycin, or Penicillin and Dihydrostreptomycin. *N. Z. Vet. J.* **2003**, *51*, 111–116. [CrossRef]
21. Sears, P.M.; Smith, B.S.; English, P.B.; Herer, P.S.; Gonzalez, R.N. Shedding Pattern of *Staphylococcus aureus* from Bovine Intramammary Infections. *J. Dairy Sci.* **1990**, *73*, 2785–2789. [CrossRef]
22. Schmenger, A.; Krömker, V. Characterization, Cure Rates and Associated Risks of Clinical Mastitis in Northern Germany. *Vet. Sci.* **2020**, *7*, 170. [CrossRef] [PubMed]
23. Ruegg, P.L.; Tabone, T.J. The Relationship Between Antibiotic Residue Violations and Somatic Cell Counts in Wisconsin Dairy Herds. *J. Dairy Sci.* **2000**, *83*, 2805–2809. [CrossRef]
24. Dosogne, H.; Meyer, E.; Sturk, A.; Van Loon, J.; Massart-Leën, A.M.; Burvenich, C. Effect of Enrofloxacin Treatment on Plasma Endotoxin During Bovine *Escherichia coli* Mastitis. *Inflamm. Res.* **2002**, *51*, 201–205. [CrossRef]
25. Pyörälä, S. Effect of Some Vehicles Used in Intramammary Mastitis Preparations on Inflammation Indicators in Milk. *J. Vet. Pharmacol. Ther.* **1987**, *10*, 248–253. [CrossRef] [PubMed]
26. Owens, W.E.; Watts, J.L.; Boddie, R.L.; Nickerson, S.C. Antibiotic Treatment of Mastitis: Comparison of Intramammary and Intramammary plus Intramuscular Therapies. *J. Dairy Sci.* **1988**, *71*, 3143–3147. [CrossRef]
27. Sol, J.; Sampimon, O.C.; Snoep, J.J.; Schukken, Y.H. Factors Associated with Bacteriological Cure After Dry Cow Treatment of Subclinical Staphylococcal Mastitis with Antibiotics. *J. Dairy Sci.* **1994**, *77*, 75–79. [CrossRef]
28. Sol, J.; Sampimon, O.C.; Snoep, J.J.; Schukken, Y.H. Factors Associated with Bacteriological Cure during Lactation after Therapy for Subclinical Mastitis Caused by *Staphylococcus aureus*. *J. Dairy Sci.* **1997**, *80*, 2803–2808. [CrossRef]
29. Barkema, H.W.; Green, M.J.; Bradley, A.J.; Zadoks, R.N. Invited Review: The Role of Contagious Disease in Udder Health. *J. Dairy Sci.* **2009**, *92*, 4717–4729. [CrossRef]
30. Polat, B.; Colak, A.; Cengiz, M.; Yanmaz, L.E.; Oral, H.; Bastan, A.; Kaya, S.; Hayirli, A. Sensitivity and Specificity of Infrared Thermography in Detection of Subclinical Mastitis in Dairy Cows. *J. Dairy Sci.* **2010**, *93*, 3525–3532. [CrossRef]
31. Langer, A.; Sharma, S.; Sharma, N.K.; Nauriyal, D.S. Comparative Efficacy of Different Mastitis Markers for Diagnosis of Sub-Clinical Mastitis in Cows. *Int. J. Appl. Sci. Biotechnol.* **2014**, *2*, 121–125. [CrossRef]
32. Iraguha, B.; Hamudikuwanda, H.; Mushonga, B.; Kandiwa, E.; Mpatswenumugabo, J.P. Comparison of Cow-Side Diagnostic Tests for Subclinical Mastitis of Dairy Cows in Musanze District, Rwanda. *J. S. Afr. Vet. Assoc.* **2017**, *88*, e1–e6. [CrossRef] [PubMed]

33. Schalm, O.W. Experiments and Observations Leading to Development of the California Mastitis Test. *J. Am. Vet. Med. Assoc.* **1957**, *130*, 199–204. [PubMed]
34. Leslie, K.E.; Jansen, J.T.; Lim, G.H. Opportunities and Implications for Improved On-Farm Cowside Diagnostics. In Proceedings of the De Laval Hygiene Symp, Kansas City, MO, USA, 20–23 January 2002; Volume 2002, pp. 147–160.
35. Dingwell, R.T.; Leslie, K.E.; Schukken, Y.H.; Sargeant, J.M.; Timms, L.L. Evaluation of the California Mastitis Test to Detect an Intramammary Infection with a Major Pathogen in Early Lactation Dairy Cows. *Can. Vet. J.* **2003**, *44*, 413. [PubMed]
36. Roy, J.-P.; Tremblay, D.; Descôteaux, L.; Messier, S.; Scholl, D.; Bouchard, E. Evaluation of the California Mastitis Test as a Precalving Treatment Selection Tool for Holstein Heifers. *Vet. Microbiol.* **2008**, *134*, 136–142. [CrossRef] [PubMed]
37. Mahmmod, Y.S.; Toft, N.; Katholm, J.; Grønbæk, C.; Klaas, I.C. Bayesian Estimation of Test Characteristics of Real-Time PCR, Bacteriological Culture and California Mastitis Test for Diagnosis of Intramammary Infections with *Staphylococcus aureus* in Dairy Cattle at Routine Milk Recordings. *Prev. Vet. Med.* **2013**, *112*, 309–317. [CrossRef]
38. Marshall, R.T.; Edmondson, J.E.; Steevens, B. Using the California Mastitis Test (1993). 1993. Available online: https://extension.missouri.edu/publications/g3653 (accessed on 1 January 2022).
39. Sargeant, J.M.; Leslie, K.E.; Shirley, J.E.; Pulkrabek, B.J.; Lim, G.H. Sensitivity and Specificity of Somatic Cell Count and California Mastitis Test for Identifying Intramammary Infection in Early Lactation. *J. Dairy Sci.* **2001**, *84*, 2018–2024. [CrossRef]
40. Barratt, K.; Leslie, K.; Bashiri, A. An Evaluation of the Porta SCCTM for Determining Udder Health Status in Dairy Cows at Drying Off. In Proceedings of the 42nd Annual Meeting, Fort Worth, TX, USA, 26–29 January 2003; pp. 280–281.
41. Salvador, R.T.; Soliven, R.L.; Balagan, E.J.Y.; Abes, N.S.; Gutierrez, C.A.; Mingala, C.N. Evaluation of a Portable Somatic Cell Counter in the Diagnosis of Bubaline Subclinical Mastitis. *Thai J. Agric. Sci.* **2014**, *47*, 205–209.
42. Lam, T.; Olde Riekerink, R.G.M.; Sampimon, O.C.; Smith, H. Mastitis Diagnostics and Performance Monitoring: A Practical Approach. *Ir. Vet. J.* **2009**, *62*, S34. [CrossRef]
43. Rodrigues, A.C.O.; Cassoli, L.D.; Machado, P.F.; Ruegg, P.L. Short Communication: Evaluation of an on-Farm Test to Estimate Somatic Cell Count. *J. Dairy Sci.* **2009**, *92*, 990–995. [CrossRef]
44. Ferronatto, J.A.; Ferronatto, T.C.; Schneider, M.; Pessoa, L.F.; Blagitz, M.G.; Heinemann, M.B.; Della Libera, A.M.; Souza, F.N. Diagnosing Mastitis in Early Lactation: Use of Somaticell®, California Mastitis Test and Somatic Cell Count. *Ital. J. Anim. Sci.* **2018**, *17*, 723–729. [CrossRef]
45. Ruegg, P.; Hulland, C.; Rieth, B. Performance of the Direct Cell Counter Used on Milk Samples Obtained from Fresh Cows. In Proceedings of the 44th National Mastitis Council Annual Meeting, Orlando, FL, USA, 16–19 January 2005; pp. 291–292.
46. No. 148 A; IDF Enumeration of Somatic Cells. 1995. Available online: https://shop.fil-idf.org/products/milk-enumeration-of-somatic-cells-part-2-guidance-on-the-operation-of-fluoro-opto-electronic-counters (accessed on 1 January 2022).
47. Moon, J.S.; Koo, H.C.; Joo, Y.S.; Jeon, S.H.; Hur, D.S.; Chung, C.I.; Jo, H.S.; Park, Y.H. Application of a New Portable Microscopic Somatic Cell Counter with Disposable Plastic Chip for Milk Analysis. *J. Dairy Sci.* **2007**, *90*, 2253–2259. [CrossRef]
48. Kawai, K.; Hayashi, T.; Kiku, Y.; Chiba, T.; Nagahata, H.; Higuchi, H.; Obayashi, T.; Itoh, S.; Onda, K.; Arai, S. Reliability in Somatic Cell Count Measurement of Clinical Mastitis Milk Using DeLaval Cell Counter. *Anim. Sci. J.* **2013**, *84*, 805–807. [CrossRef]
49. Gonzalo, C.; Linage, B.; Carriedo, J.A.; de la Fuente, F.; Primitivo, F.S. Evaluation of the Overall Accuracy of the DeLaval Cell Counter for Somatic Cell Counts in Ovine Milk. *J. Dairy Sci.* **2006**, *89*, 4613–4619. [CrossRef]
50. Heald, C.W.; Jones, G.M.; Nickerson, S.C.; Patterson, W.N.; Vinson, W.E. Preliminary Evaluation of the Fossomatic Somatic Cell Counter for Analysis of Individual Cow Samples in a Central Testing Laboratory. *J. Food Prot.* **1977**, *40*, 523–526. [CrossRef]
51. Schmidt-Madsen, P. Fluoro-Opto-Electronic Cell Counting in Milk. *J. Dairy Res.* **1975**, *42*, 227. [CrossRef]
52. *IDF Recommended Methods for Somatic Cell Counting in Milk*; International Dairy Federation: Brussels, Belgium, 1984.
53. Miller, R.H.; Paape, M.J.; Acton, J.C. Comparison of Milk Somatic Cell Counts by Coulter and Fossomatic Counters[1]. *J. Dairy Sci.* **1986**, *69*, 1942–1946. [CrossRef]
54. Rivas, A.L.; Quimby, F.W.; Blue, J.; Coksaygan, O. Longitudinal Evaluation of Bovine Mammary Gland Health Status by Somatic Cell Counting, Flow Cytometry, and Cytology. *J. Vet. Diagn. Investig.* **2001**, *13*, 399–407. [CrossRef]
55. Pilla, R.; Malvisi, M.; Snel, G.G.M.; Schwarz, D.; König, S.; Czerny, C.-P.; Piccinini, R. Differential Cell Count as an Alternative Method to Diagnose Dairy Cow Mastitis. *J. Dairy Sci.* **2013**, *96*, 1653–1660. [CrossRef]
56. Schwarz, D.; Diesterbeck, U.S.; König, S.; Brügemann, K.; Schlez, K.; Zschöck, M.; Wolter, W.; Czerny, C.-P. Microscopic Differential Cell Counts in Milk for the Evaluation of Inflammatory Reactions in Clinically Healthy and Subclinically Infected Bovine Mammary Glands. *J. Dairy Res.* **2011**, *78*, 448–455. [CrossRef]
57. Kehrli Jr, M.E.; Shuster, D.E. Factors Affecting Milk Somatic Cells and Their Role in Health of the Bovine Mammary Gland. *J. Dairy Sci.* **1994**, *77*, 619–627. [CrossRef]
58. Farschtschi, S.; Mattes, M.; Pfaffl, M.W. Advantages and Challenges of Differential Immune Cell Count Determination in Blood and Milk for Monitoring the Health and Well-Being of Dairy Cows. *Vet. Sci.* **2022**, *9*, 255. [CrossRef]
59. Godden, S.M.; Royster, E.; Timmerman, J.; Rapnicki, P.; Green, H. Evaluation of an Automated Milk Leukocyte Differential Test and the California Mastitis Test for Detecting Intramammary Infection in Early-and Late-Lactation Quarters and Cows. *J. Dairy Sci.* **2017**, *100*, 6527–6544. [CrossRef]
60. Lozada-Soto, E.; Maltecca, C.; Anderson, K.; Tiezzi, F. Analysis of Milk Leukocyte Differential Measures for Use in Management Practices for Decreased Mastitis Incidence. *J. Dairy Sci.* **2020**, *103*, 572–582. [CrossRef]

61. Damm, M.; Holm, C.; Blaabjerg, M.; Bro, M.N.; Schwarz, D. Differential Somatic Cell Count-A Novel Method for Routine Mastitis Screening in the Frame of Dairy Herd Improvement Testing Programs. *J. Dairy Sci.* **2017**, *100*, 4926–4940. [CrossRef]
62. Bijgaart, H.; Orlandini, S.; Luginbühl, W. *Guidance on Application of EC JRC Certified Reference Material for Somatic Cell Coutning in Milk*; International Dairy Federation: Brussels, Belgium, 2021.
63. Schwarz, D.; Lipkens, Z.; Piepers, S.; De Vliegher, S. Investigation of Differential Somatic Cell Count as a Potential New Supplementary Indicator to Somatic Cell Count for Identification of Intramammary Infection in Dairy Cows at the End of the Lactation Period. *Prev. Vet. Med.* **2019**, *172*, 104803. [CrossRef]
64. Souza, F.N.; Blagitz, M.G.; Batista, C.F.; Takano, P.V.; Gargano, R.G.; Diniz, S.A.; Silva, M.X.; Ferronatto, J.A.; Santos, K.R.; Heinemann, M.B.; et al. Immune Response in Nonspecific Mastitis: What Can it Tell Us? *J. Dairy Sci.* **2020**, *103*, 5376–5386. [CrossRef]
65. Burvenich, C.; Merris, V.V.; Mehrzad, J.; Diez-Fraile, A.; Duchateau, L. Severity of *E. coli* Mastitis Is Mainly Determined by Cow Factors. *Vet. Res.* **2003**, *34*, 521–564. [CrossRef]
66. Barkema, H.W.; Schukken, Y.H.; Zadoks, R.N. Invited Review: The Role of Cow, Pathogen, and Treatment Regimen in the Therapeutic Success of Bovine *Staphylococcus aureus* Mastitis. *J. Dairy Sci.* **2006**, *89*, 1877–1895. [CrossRef]
67. Lavon, Y.; Gilad, D.; Leitner, G. Recovery Rates of Treated vs. Non-Treated Dairy Cows with Subclinical Mastitis. *Dairy* **2021**, *2*, 576–584. [CrossRef]
68. Breen, J.E.; Green, M.J.; Bradley, A.J. Quarter and Cow Risk Factors Associated with the Occurrence of Clinical Mastitis in Dairy Cows in the United Kingdom. *J. Dairy Sci.* **2009**, *92*, 2551–2561. [CrossRef]
69. Bradley, A.J.; Green, M.J. Factors Affecting Cure When Treating Bovine Clinical Mastitis with Cephalosporin-Based Intramammary Preparations. *J. Dairy Sci.* **2009**, *92*, 1941–1953. [CrossRef]
70. Timms, L.L.; Schultz, L.H. Mastitis Therapy for Cows with Elevated Somatic Cell Counts or Clinical Mastitis. *J. Dairy Sci.* **1984**, *67*, 367–371. [CrossRef]
71. Shephard, R.; Malmo, J.; Pfeiffer, D. A Clinical Trial to Evaluate the Effectiveness of Antibiotic Treatment of Lactating Cows with High Somatic Cell Counts in Their Milk. *Aust. Vet. J.* **2000**, *78*, 763–768. [CrossRef]
72. Ruegg, P.L. A 100-Year Review: Mastitis Detection, Management, and Prevention. *J. Dairy Sci.* **2017**, *100*, 10381–10397. [CrossRef]
73. Bannerman, D.D.; Paape, M.J.; Goff, J.P.; Kimura, K.; Lippolis, J.D.; Hope, J.C. Innate Immune Response to Intramammary Infection with *Serratia marcescens* and *Streptococcus uberis*. *Vet. Res.* **2004**, *35*, 681–700. [CrossRef]
74. Bannerman, D.D.; Paape, M.J.; Lee, J.-W.; Zhao, X.; Hope, J.C.; Rainard, P. *Escherichia coli* and *Staphylococcus aureus* Elicit Differential Innate Immune Responses Following Intramammary Infection. *Clin. Vaccine Immunol.* **2004**, *11*, 463–472. [CrossRef]
75. Bannerman, D.D.; Paape, M.J.; Hare, W.R.; Hope, J.C. Characterization of the Bovine Innate Immune Response to Intramammary Infection with *Klebsiella pneumoniae*. *J. Dairy Sci.* **2004**, *87*, 2420–2432. [CrossRef]
76. Kauf, A.C.; Rosenbusch, R.F.; Paape, M.J.; Bannerman, D.D. Innate Immune Response to Intramammary *Mycoplasma bovis* Infection. *J. Dairy Sci.* **2007**, *90*, 3336–3348. [CrossRef]
77. Bannerman, D.D.; Springer, H.R.; Paape, M.J.; Kauf, A.C.; Goff, J.P. Evaluation of Breed-Dependent Differences in the Innate Immune Responses of Holstein and Jersey Cows to *Staphylococcus aureus* Intramammary Infection. *J. Dairy Res.* **2008**, *75*, 291–301. [CrossRef]
78. Dohoo, I.R.; Meek, A.H. Somatic Cell Counts in Bovine Milk. *Can. Vet. J.* **1982**, *23*, 119–125.
79. Eberhart, R.J.; Hutchinson, L.J.; Spencer, S.B. Relationships of Bulk Tank Somatic Cell Counts to Prevalence of Intramammary Infection and to Indices of Herd Production (1). *J. Food Prot.* **1982**, *45*, 1125–1128. [CrossRef] [PubMed]
80. Petzer, I.-M.; Karzis, J.; Donkin, E.F.; Webb, E.C.; Etter, E.M.C. Validity of Somatic Cell Count as Indicator of Pathogen-Specific Intramammary Infections. *J. S. Afr. Vet. Assoc.* **2017**, *88*, a1465. [CrossRef] [PubMed]
81. Harmon, R.J. Physiology of Mastitis and Factors Affecting Somatic Cell Counts. *J. Dairy Sci.* **1994**, *77*, 2103–2112. [CrossRef]
82. Kirkeby, C.; Toft, N.; Schwarz, D.; Farre, M.; Nielsen, S.S.; Zervens, L.; Hechinger, S.; Halasa, T. Differential Somatic Cell Count as an Additional Indicator for Intramammary Infections in Dairy Cows. *J. Dairy Sci.* **2020**, *103*, 1759–1775. [CrossRef] [PubMed]
83. Nickerson, S.; Kautz, F.; Ely, L.; Resnic, G. Improve Your Chances for Curing Mastitis. 2018. Available online: https://hoards.com/article-24888-improve-your-chances-for-curing-mastitis.html (accessed on 1 January 2022).
84. Jenkins, S.N.; Okello, E.; Rossitto, P.V.; Lehenbauer, T.W.; Champagne, J.; Penedo, M.; Arruda, A.G.; Godden, S.; Rapnicki, P.; Gorden, P.J.; et al. Molecular Epidemiology of Coagulase-Negative *Staphylococcus* Species Isolated at Different Lactation Stages from Dairy Cattle in the United States. *PeerJ* **2019**, *7*, e6749. [CrossRef]
85. Schukken, Y.H.; González, R.N.; Tikofsky, L.L.; Schulte, H.F.; Santisteban, C.G.; Welcome, F.L.; Bennett, G.J.; Zurakowski, M.J.; Zadoks, R.N. CNS Mastitis: Nothing to Worry About? *Vet. Micro.* **2009**, *134*, 9–14. [CrossRef]
86. Wilson, D.J.; Gonzalez, R.N.; Das, H.H. Bovine Mastitis Pathogens in New York and Pennsylvania: Prevalence and Effects on Somatic Cell Count and Milk Production. *J. Dairy Sci.* **1997**, *80*, 2592–2598. [CrossRef]
87. Sumon, S.M.M.R.; Parvin, M.S.; Ehsan, M.A.; Islam, M.T. Dynamics of Somatic Cell Count and Intramammary Infection in Lactating Dairy Cows. *J. Adv. Vet. Anim. Res.* **2020**, *7*, 314–319. [CrossRef]
88. Sol, J.; Sampimon, O.C.; Barkema, H.W.; Schukken, Y.H. Factors Associated with Cure after Therapy of Clinical Mastitis Caused by *Staphylococcus aureus*. *J. Dairy Sci.* **2000**, *83*, 278–284. [CrossRef]
89. Oliveira, L.; Hulland, C.; Ruegg, P.L. Characterization of Clinical Mastitis Occurring in Cows on 50 Large Dairy Herds in Wisconsin. *J. Dairy Sci.* **2013**, *96*, 7538–7549. [CrossRef]

90. Fuenzalida, M.J.; Ruegg, P.L. Negatively Controlled, Randomized Clinical Trial to Evaluate Intramammary Treatment of Nonsevere, Gram-Negative Clinical Mastitis. *J. Dairy Sci.* **2019**, *102*, 5438–5457. [CrossRef] [PubMed]
91. Smith, K.L.; Todhunter, D.A.; Schoenberger, P.S. Environmental Mastitis: Cause, Prevalence, Prevention1, 2. *J. Dairy Sci.* **1985**, *68*, 1531–1553. [CrossRef]
92. Cattell, M.B.; Dinsmore, R.P.; Belschner, A.P.; Carmen, J.; Goodell, G. Environmental Gram-Positive Mastitis Treatment: In Vitro Sensitivity and Bacteriologic Cure. *J. Dairy Sci.* **2001**, *84*, 2036–2043. [CrossRef]
93. Dalanezi, F.M.; Joaquim, S.F.; Guimarães, F.F.; Guerra, S.T.; Lopes, B.C.; Schmidt, E.M.S.; Cerri, R.L.A.; Langoni, H. Influence of Pathogens Causing Clinical Mastitis on Reproductive Variables of Dairy Cows. *J. Dairy Sci.* **2020**, *103*, 3648–3655. [CrossRef] [PubMed]
94. Heikkilä, A.-M.; Liski, E.; Pyörälä, S.; Taponen, S. Pathogen-Specific Production Losses in Bovine Mastitis. *J. Dairy Sci.* **2018**, *101*, 9493–9504. [CrossRef]
95. Suojala, L.; Kaartinen, L.; Pyörälä, S. Treatment for Bovine *Escherichia coli* Mastitis—An Evidence-Based Approach. *J. Vet. Pharmacol. Ther.* **2013**, *36*, 521–531. [CrossRef]
96. Gillespie, B.E.; Headrick, S.I.; Boonyayatra, S.; Oliver, S.P. Prevalence and Persistence of Coagulase-Negative *Staphylococcus* Species in Three Dairy Research Herds. *Vet. Microbiol.* **2009**, *134*, 65–72. [CrossRef]
97. Wente, N.; Krömker, V. *Streptococcus dysgalactiae*-Contagious or Environmental? *Animals* **2020**, *10*, 2185. [CrossRef]
98. Zadoks, R.N.; Gillespie, B.E.; Barkema, H.W.; Sampimon, O.C.; Oliver, S.P.; Schukken, Y.H. Clinical, Epidemiological and Molecular Characteristics of *Streptococcus uberis* Infections in Dairy Herds. *Epidemiol. Infect.* **2003**, *130*, 335–349. [CrossRef]
99. Erskine, R.J.; Wagner, S.; DeGraves, F.J. Mastitis Therapy and Pharmacology. *Vet. Clin. N. Am. Food Anim. Pract.* **2003**, *19*, 109–138. [CrossRef]
100. Barlow, J.W.; White, L.J.; Zadoks, R.N.; Schukken, Y.H. A Mathematical Model Demonstrating Indirect and Overall Effects of Lactation Therapy Targeting Subclinical Mastitis in Dairy Herds. *Prev. Vet. Med.* **2009**, *90*, 31–42. [CrossRef] [PubMed]
101. van den Borne, B.H.P.; Halasa, T.; van Schaik, G.; Hogeveen, H.; Nielen, M. Bioeconomic Modeling of Lactational Antimicrobial Treatment of New Bovine Subclinical Intramammary Infections Caused by Contagious Pathogens. *J. Dairy Sci.* **2010**, *93*, 4034–4044. [CrossRef] [PubMed]
102. Pyörälä, S. Treatment of Mastitis during Lactation. *Ir. Vet. J.* **2009**, *62*, S40. [CrossRef]
103. StRose, S.G.; Swinkels, J.M.; Kremer, W.D.J.; Kruitwagen, C.L.J.J.; Zadoks, R.N. Effect of Penethamate Hydriodide Treatment on Bacteriological Cure, Somatic Cell Count and Milk Production of Cows and Quarters with Chronic Subclinical *Streptococcus uberis* or *Streptococcus dysgalactiae* Infection. *J. Dairy Res.* **2003**, *70*, 387–394. [CrossRef] [PubMed]
104. Wilson, D.J.; Gonzalez, R.N.; Case, K.L.; Garrison, L.L.; Gröhn, Y.T. Comparison of Seven Antibiotic Treatments with No Treatment for Bacteriological Efficacy against Bovine Mastitis Pathogens. *J. Dairy Sci.* **1999**, *82*, 1664–1670. [CrossRef]
105. Gruet, P.; Maincent, P.; Berthelot, X.; Kaltsatos, V. Bovine Mastitis and Intramammary Drug Delivery: Review and Perspectives. *Adv. Drug Deliv. Rev.* **2001**, *50*, 245–259. [CrossRef]
106. Nickerson, S.; Ryman, V. Antibiotic Therapy in Mastitis Control for Lactating and Dry Cows | UGA Cooperative Extension. *Dep. Anim. Dairy Sci.* **2019**.
107. Akers, R.M.; Nickerson, S.C. Mastitis and its Impact on Structure and Function in the Ruminant Mammary Gland. *J. Mammare Gland Biol. Neoplasia* **2011**, *16*, 275–289. [CrossRef]
108. Houe, H.; Vaarst, M.; Enevoldsen, C. Clinical Parameters for Assessment of Udder Health in Danish Herds. *Acta Vet. Scand.* **2002**, *43*, 173–184.
109. Cheng, W.N.; Han, S.G. Bovine Mastitis: Risk Factors, Therapeutic Strategies, and Alternative Treatments—A Review. *Asian-Australas. J. Anim. Sci.* **2020**, *33*, 1699–1713. [CrossRef]
110. Cobirka, M.; Tancin, V.; Slama, P. Epidemiology and Classification of Mastitis. *Animals* **2020**, *10*, 2212. [CrossRef] [PubMed]
111. Wente, N.; Grieger, A.S.; Klocke, D.; Paduch, J.-H.; Zhang, Y.; Leimbach, S.; tho Seeth, M.; Mansion-De Vries, E.M.; Mohr, E.; Krömker, V. Recurrent Mastitis–Persistent or New Infections? *Vet. Microbiol.* **2020**, *244*, 108682. [CrossRef] [PubMed]
112. Beckley, M.S.; Johnson, T. Five Year Study of a California Mastitis Test on a Commercial Dairy Herd. *J. Dairy Sci.* **1966**, *49*, 746.
113. Schultz, L.H. Somatic Cell Counting of Milk in Production Testing Programs as a Mastitis Control Technique. *J. Am. Vet. Med. Assoc.* **1977**, *170*, 1244–1246. [PubMed]
114. Sheldrake, R.F.; Hoare, R.J.T.; McGregor, G.D. Lactation Stage, Parity, and Infection Affecting Somatic Cells, Electrical Conductivity, and Serum Albumin in Milk. *J. Dairy Sci.* **1984**, *66*, 542–547. [CrossRef]
115. Eberhart, R.J.; Gdmore, H.C.; Hutchinson, L.J.; Spencer, S.B. Somatic Cell Counts in DHI Samples. In Proceedings of the 18th Annual Meeting, Louisville, KY, USA, 19–21 February 1979.
116. Alhussien, M.N.; Dang, A.K. Milk Somatic Cells, Factors Influencing Their Release, Future Prospects, and Practical Utility in Dairy Animals: An Overview. *Vet. World* **2018**, *11*, 562–577. [CrossRef] [PubMed]
117. Barkema, H.W.; Schukken, Y.H.; Lam, T.J.; Beiboer, M.L.; Wilmink, H.; Benedictus, G.; Brand, A. Incidence of Clinical Mastitis in Dairy Herds Grouped in Three Categories by Bulk Milk Somatic Cell Counts. *J. Dairy Sci.* **1998**, *81*, 411–419. [CrossRef]
118. Deluyker, H.A.; Van Oye, S.N.; Boucher, J.F. Factors Affecting Cure and Somatic Cell Count after Pirlimycin Treatment of Subclinical Mastitis in Lactating Cows. *J. Dairy Sci.* **2005**, *88*, 604–614. [CrossRef]
119. de Haas, Y.; Barkema, H.W.; Veerkamp, R.F. The Effect of Pathogen-Specific Clinical Mastitis on the Lactation Curve for Somatic Cell Count. *J. Dairy Sci.* **2002**, *85*, 1314–1323. [CrossRef]

120. McDougall, S.; Arthur, D.G.; Bryan, M.A.; Vermunt, J.J.; Weir, A.M. Clinical and Bacteriological Response to Treatment of Clinical Mastitis with One of Three Intramammary Antibiotics. *N. Z. Vet. J.* **2007**, *55*, 161–170. [CrossRef]
121. Reneau, J.K. Effective Use of Dairy Herd Improvement Somatic Cell Counts in Mastitis Control. *J. Dairy Sci.* **1986**, *69*, 1708–1720. [CrossRef]
122. Nelson, F.E.; Schuh, J.D.; Scott, G.H. Influence of Season on Leucocytes in Milk. *J. Dairy Sci.* **1967**, *50*, 978.
123. Bodoh, G.W.; Battista, W.J.; Schultz, L.H. Variation in Somatic Cell Counts in Dairy Herd Improvement Milk Samples. *J. Dairy Sci.* **1976**, *59*, 1119. [CrossRef]
124. Gill, M.S.; Holmes, C.W. Somatic Cell Counts, Mastitis and Milk Production in Dairy Herds. *N. Z. J. Dairy Sci.* **1978**, *13*, 157.
125. Paape, M.J.; Kral, A.J.; Desjardins, C.; Schultze, W.D.; Miller, R.H. Failure of Either Corticosteroids or ACTH to Increase the Leukocyte Concentration in Milk. *Am. J. Vet. Res.* **1973**, *34*, 353–356.
126. Elvinger, F.; Littell, R.C.; Natzke, R.P.; Hansen, P.J. Analysis of Somatic Cell Count Data by a Peak Evaluation Algorithm to Determine Inflammation Events1. *J. Dairy Sci.* **1991**, *74*, 3396–3406. [CrossRef]
127. Lundberg, Å.; Nyman, A.K.; Aspán, A.; Börjesson, S.; Unnerstad, H.E.; Waller, K.P. Udder Infections with *Staphylococcus aureus*, *Streptococcus dysgalactiae*, and *Streptococcus uberis* at Calving in Dairy Herds with Suboptimal Udder Health. *J. Dairy Sci.* **2016**, *99*, 2102–2117. [CrossRef]
128. Hamel, J.; Zhang, Y.; Wente, N.; Krömker, V. Heat Stress and Cow Factors Affect Bacteria Shedding Pattern from Naturally Infected Mammary Gland Quarters in Dairy Cattle. *J. Dairy Sci.* **2021**, *104*, 786–794. [CrossRef]
129. Safa, S.; Kargar, S.; Moghaddam, G.A.; Ciliberti, M.G.; Caroprese, M. Heat Stress Abatement during the Postpartum Period: Effects on Whole Lactation Milk Yield, Indicators of Metabolic Status, Inflammatory Cytokines, and Biomarkers of the Oxidative Stress. *J. Anim. Sci.* **2019**, *97*, 122–132. [CrossRef]
130. Ruegg, P.L. Making Antibiotic Treatment Decisions for Clinical Mastitis. *Vet. Clin. Food Anim. Pract.* **2018**, *34*, 413–425. [CrossRef]