Haematological findings in 158 dairy cows with toxic mastitis with a focus on the leukogram

Ueli Braun (ubraun@vetclinics.uzh.ch)
Department of Farm Animals, Vetsuisse Faculty, University of Zurich https://orcid.org/0000-0002-2573-687X

Christian Gerspach
Department of Farm Animals, Vetsuisse Faculty, University of Zurich

Barbara Riond
Diagnostic Services, Vetsuisse Faculty, University of Zurich

Carina Oschlies
Department of Farm Animals, Vetsuisse Faculty, University of Zurich

Sabrina Corti
National Institute of Safety and Hygiene, Vetsuisse Faculty, University of Zurich

Ulrich Bleul
Department of Farm Animals, Vetsuisse Faculty, University of Zurich

Research article

Keywords: Cattle, Toxic mastitis, Toxaemia, Leukocytes, Leukogram

Posted Date: November 11th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-103668/v1

Abstract

Background: Acute toxic mastitis is characterised by typical clinical findings and changes in the leukogram. The goal of our study was to compare the leukogram of 158 cows with toxic mastitis and 168 clinically healthy cows. The cows were examined clinically and underwent haematological and biochemical examination of blood and bacteriological culture of milk samples.

Results: All cows with toxic mastitis were ill and had a poor appetite or anorexia, and 34 cows (21.5%) were recumbent. A single quarter was affected in 119 cows (75.3%), two quarters in 37 cows (23.4%) and three quarters in two cows (1.3%). Bacteriological culture showed gram-negative pathogens in 100 cows (63.3%), gram-positive in 15 (9.5%) and yeast in 4 (2.5%). The median total leukocyte count of ill cows was 4,300 cells/µl, which was significantly lower than 8,000 cells/µl in controls. With the exception of band neutrophils and metamyelocytes, the counts of all components of the leukogram were lower in ill cows compared with controls. Significantly more cows with toxic mastitis had leukopenia (60.1 vs. 4.1%) or leukocytosis (10.1 vs. 3.0%) than controls. Ill cows had significantly lower segmented neutrophil counts than controls (860 vs. 2,598 cells/µl), and 69.5 and 17.3%, respectively, had counts below the reference interval. Ill cows had increased band (77.3%) and metamyelocyte (25.0%) counts compared with control cows (0.6 and 0%, respectively). In ill cows, eosinopenia occurred in 66.4% (controls, 1.8%), monocytopenia in 40.6% (controls, 4.2%) and lymphopenia in 60.2% (controls, 1.8%). Twenty-one ill cows (16.4%) had a regenerative and 57 (44.5%) had a degenerative left shift. The median neutrophil-to-lymphocyte ratio was 0.97 in ill cows and 0.63 in controls. Toxic changes in neutrophils including cytoplasmic basophilia and vacuolisation were seen in 101 (91.8%) of 110 blood smears of ill cows.

Conclusions: Acute toxic mastitis results in severe changes in the leukogram particularly leukopenia, lymphopenia and degenerative left shift. The leukogram has significant diagnostic value and may aid in the modification of treatment when needed.

Background

Acute toxic mastitis is characterised by typical clinical findings and changes in the leukogram [1] that include leukopenia, neutropenia, degenerative left shift and neutrophils with toxic changes. The clinical diagnosis of acute toxic mastitis is straightforward and therefore a leukogram is not required for an initial diagnosis. However, it aids in determining the severity of the disease. Cows with toxic mastitis commonly have leukopenia and neutropenia, which is in contrast to other domestic animal species such as the dog in which pronounced neutrophilia with a degenerative left shift occur in response to an inflammatory stimulus. This response typically occurs within six to eight hours after the insult and the degree of response is directly related to the severity of the inflammation [2]. A thorough knowledge of the process of leukopoiesis aids in the interpretation of changes in the leukogram of cattle with acute inflammation. Leukopoiesis involves the proliferation, maturation and storage of white blood cells [3, 4]. Pluripotent stem cells differentiate into myeloblasts, which mature through a series of stages that include promyelocytes, myelocytes, metamyelocytes, band cells and mature granulocytes; this so-called

proliferative pool accounts for approximately 10–30% and the maturation and storage pools for 65–90% of the granulocytes in the bone marrow [3]. The maturation pool includes metamyelocytes and band neutrophils, which have lost their mitotic potential, and the storage pool comprises band and segmented neutrophils ready to be released into the circulation. The normal transit time for maturation of a myeloblast is about 6–7 days. In several animal species, but not in cattle, the transit time can be reduced to 2–3 days when leukocyte consumption is high. Therefore, the supply of mature leukocytes from the bone marrow rapidly becomes insufficient in cows with high consumption of neutrophils at the site of inflammation. When interpreting a bovine leukogram, one must consider that bone marrow response is slower with increased demand for neutrophils [5] and the granulocyte storage pool is smaller compared with other species [6]. This often results in neutropenia, rather than neutrophilia, in the first 24–48 hours after the onset of acute inflammation because neutrophils move out of the circulation and into the tissues and the bone marrow cannot meet demand adequately. Approximately 24 hours after the start of the inflammatory process, immature leukocytes including band neutrophils, metamyelocytes and occasionally myelocytes are seen in blood smears. The neutrophils often have cytoplasmic basophilia due to staining of certain organelles that are present during early development [7]. Cytoplasmic basophilia is referred to as a *toxic change*, but this is an unfortunate term because the cells have not been damaged by the pathogen and in fact function normally.

The effects of intramammary infection on the total leukocyte and neutrophil counts in cows have been examined experimentally [8–12]. Gram-negative pathogens including *Escherichia coli*, *Klebsiella pneumoniae* and *Serratia marcescens* resulted in neutropenia within 16 hours, whereas neutropenia did not occur until 36 hours after infection with *Streptococcus uberis* and did not occur with *Staphylococcus aureus* infection. Transient neutropenia was observed 24 hours after infection with *Pseudomonas aeruginosa* [11] and from 48 to 168 hours period after infection with *Mycoplasma bovis*, which also included lymphopenia from 84 hours post infection until the end of the 10-day study [12]. Cows with acute mastitis caused by gram-negative bacteria had significantly lower leukocyte, segmented neutrophil, monocyte and lymphocyte counts than cows with mastitis caused by gram-positive bacteria [13]. Transient neutropenia, often followed by rebound neutrophilia with subsequent normalisation of the neutrophil count, is a good prognostic sign, whereas a continued decrease in neutrophil numbers on day 2 of infection or neutropenia that persists for more than 3–4 days is a poor sign indicating bone marrow suppression [5].

Lymphopenia and monocytopenia are also typical findings in acute mastitis [1]. Stress and exogenous administration of corticosteroids are the most common causes of leukopenia in cattle [5], but infection with viruses, *Anaplasma phagocytophilum*, mycoplasma and other microorganisms as well as septicaemia are other possible causes [14]. Monocytopenia is of lesser significance [15] and cell counts normalise more quickly because the transit time is only 3 days compared with 6 days for neutrophils. Therefore monitoring monocyte numbers can be useful for predicting the recovery in cases with bone marrow suppression. The goal of this study was to compare the leukograms of 158 cows with toxic mastitis and 168 clinically healthy cows and to discuss the differences.

Methods

Animals

One hundred and sixty-eight healthy controls and 158 cows with acute toxic mastitis were used. The controls consisted of 182 healthy cows that were the offspring of cows with BSE and therefore referred by the Federal Veterinary Office Switzerland (now Federal Food Safety and Veterinary Office) to our clinic for examination. Clinical and laboratory findings of these cows were published [16]; and the cows were previously used as controls [17]; all cows had a history of being healthy and the findings of daily clinical examinations during three consecutive days were normal. The 158 cows with toxic mastitis included 151 dairy cows and 7 beef cows that were referred to the Clinic for Ruminants, Vetsuisse Faculty, University of Zurich from 2004 to 2016. The dairy cows comprised Swiss Braunvieh (n = 55), Swiss Fleckvieh (n = 50) and Holstein Friesian cows (n = 46), and the beef cows belonged to different breeds or were cross bred. The cows were 2.0 to 15.1 years of age (mean ± sd = 6.4 ± 2.6 years), and 13 cows were dry. Of the remaining 145 cows, 91 were between 0.5 and 30 days postpartum and 54 were longer than 30 days postpartum or the lactation stage was not known. The duration of illness varied from 0.5 to 30 days (median = 2 days). One hundred and forty-four cows (91%) had received treatment from the herd veterinarian, but only 109 (69%) had been diagnosed as having mastitis. The reasons for referral included request for diagnostic workup (n = 62, 39.2%), downer cow syndrome (n = 29, 18.4%), tentative diagnosis of left or right displaced abomasum (n = 27, 17.1%), acute toxic mastitis (n = 12, 7.5%) and other disorders including fever, diarrhoea and suspicion of traumatic reticuloperitonitis, ileus, caecal dilatation, bronchopneumonia and endocarditis (n = 28, 17.7%).

Haematological and serum biochemical analyses, bacteriologic examination of milk

The following blood samples were collected from all cattle: 5 ml of EDTA blood for haematological analysis and 10 ml of whole blood for serum biochemistry. Haematological analysis included the determination of haematocrit, total leukocyte count and the concentrations of total protein and fibrinogen using an automated blood analyzer (CELL-Dyn 3500, Abbott Diagnostics Division, Baar). A differential leukocyte count was done in all control cows and in 128 cows with toxic mastitis. The concentrations of serum urea, sodium, potassium, chloride, calcium, inorganic phosphate and magnesium were determined at 37 °C using an automated analyzer (Cobas-Integra-800-Analyser, Roche Diagnostics, Basel) and the manufacturer's reagents (Roche-Reagents) according to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). Results of the leukocyte count were compared with published reference intervals [18]: Leukocytes (5,100 – 13,300/μl), segmented neutrophils (1,700-6,000/μl), band neutrophils (0-200/μl), metamyelocytes (0), eosinophils (100-1,200/μl), basophils (0-200/μl), monocytes (100–700/μl) and lymphocytes (1,800-8,100/μl).

Two blood smears were prepared for each cow and stained using a modified Wright's stain in an automated staining system (HemaTek, Siemens, Switzerland). A manual differential of 100 leukocytes per smear was done by two technicians each with ten years of experience in veterinary haematology. The absolute numbers of lymphocytes, monocytes and granulocytes was determined by multiplying the

percentage obtained in the 200-cell differential count with the total leukocyte count from the haematology analyzer. Additionally, the morphology of erythrocytes, leukocytes and platelets was evaluated. The microbiological examination of milk was done as described [19].

Statistical analysis

The program SPSS Version 24 was used for statistical calculations and analysis of the total leukocyte count, segmented, band and metamyelocyte neutrophils, eosinophils, basophils, monocytes and lymphocytes of both groups. Frequency distributions were determined for both groups and the median and the 5th and 95th percentiles were calculated because the distributions were not normal based on the Shapiro-Wilk test. Differences between medians and frequency distributions of both groups were analysed using ANOVA and the Mann-Whitney U test. To calculate the neutrophil-to-lymphocyte ratio, the segmented, band and metamyelocyte neutrophil counts were divided by the lymphocyte count. The distributions of the ratios were not normal and differences between the medians of both groups were analysed using the Mann-Whitney U test. Pearson correlation coefficients were calculated to assess the relationships between variables.

For the analysis of the leukograms in relation to mastitis pathogens, the latter were divided into 3 groups including gram-negative (*Escherichia coli*, *Klebsiella* spp., *Proteus* spp.) and gram-positive bacteria (*Staphylococcus aureus*, other *Staphylococcus* spp., *Streptococcus uberis*, other *Streptococcus* spp., *Trueperella pyogenes*) and yeast. Differences between medians and frequency distributions of the leukograms of the three groups of pathogens were analysed using ANOVA and the Bonferroni post hoc test.

Frequency distributions were determined for clinical variables including changes in the udder and the milk of the diseased cows, and the median and the 5th and 95th percentiles were calculated for rectal temperature, heart rate and respiratory rate.

Results

Clinical findings

All cows with toxic mastitis were ill, had a poor appetite or anorexia, and 34 cows (21.5%) were down and unable to rise. Many cows had signs of pain including bruxism (n = 29, 18.4%), weight shifting between hind limbs (n = 15, 9.5%), spontaneous grunting (n = 9, 5.7%) and muscle tremors (n = 6, 3.8%). Signs of shock included increased capillary refill time (n = 131, 82.9%), reduced skin turgor (n = 124, 78.5%), sunken eyes (n = 118, 74.7%), cool skin surface temperature (n = 102, 64.6%) and dry muzzle (n = 48, 30.4%). Scleral vessels were congested in 155 cows (98.1%), and the oral mucosa was discoloured in 46 cows (29.3%) (mostly pale and occasionally hyperaemic). The median heart rate was 92 beats per minute (bpm) (5th to 95th percentiles, 68 to 130 bpm), the respiratory rate was 32 breaths per minute (18 to 76 breaths per minute) and the rectal temperature was 38.9 °C (37.5 to 40.4 °C). Rumen motility was reduced in 61 cows (38.6%) and absent in 90 (57.0%); the respective frequencies for intestinal motility were 94 (59.5%) and 25 cows (16.0%). Percussion and simultaneous auscultation and ballottement and

simultaneous auscultation were positive in nine cows (5.7%) on the left side and in 53 cows (33.5%) on the right side. The amount of faeces in the rectum was decreased in 60 cows (38.0%), and 27 cows (17.1%) had no faeces in the rectum. Twenty-four cows had one and two cows had two comorbidities that included endometritis/metritis (n = 6), left displaced abomasum (n = 4), paralytic ileus (n = 3) and pleurisy, aspiration pneumonia, ketosis, abomasal ulcer, caecal dilatation, intertrigo (necrotic dermatitis), gastrocnemius tendon rupture, myopathy and retained placenta in one cow each.

Udder and milk abnormalities

One hundred and nineteen cows (75.3%) had toxic mastitis in a single quarter, 37 cows (23.4%) in two quarters and two cows (1.3%) in three quarters. The affected quarters were enlarged and firm in 157 (99.4%) cows. In seven cows (4.4%), the skin overlying the quarter was hyperaemic, and in 11 cows (7.0%) the teat was affected by the inflammation. The colour and consistency of the milk were markedly abnormal in 152 (96.2%) and 153 cows (96.8%), respectively, and clots were visible in the secretion of 128 cows (81.0%). A California mastitis test score of 3 positive (almost a solid gel) was seen in all affected quarters.

Culture of the milk samples showed gram-negative pathogens (*Escherichia coli*, n = 87; *Klebsiella* spp., n = 12; *Proteus*, n = 1) in 100 cows (63.3%) and gram-positive pathogens (*Streptococcus uberis*, n = 5; *Staphylococcus aureus*, n = 3; *Trueperella pyogenes*, n = 3; non-*aureus Staphylococcus* spp., n = 2; *Streptococcus* spp. other than *uberis, dysgalactiae, agalactiae*, n = 2) in 15 cows (9.5%). Yeast was recovered in 4 cows (2.5%). Culture yielded no growth in 29 cows (18.4%) that had been pre-treated with antibiotics, and in 10 cows (6.3%) culture results were not available or they were unclear.

White blood cells, medians and frequency distributions

The leukocyte count was significantly lower in cows with mastitis compared with controls (4,300 versus 8,000 cell/μl; $P < 0.01$) (Table 1); with the exception of band neutrophils and metamyelocytes, all components of the leukogram were significantly lower in the ill cows ($P < 0.01$) (Fig. 1). The frequency distributions also differed between the two groups ($P < 0.01$) (Fig. 2); cows with toxaemia had leukopenia (60.1%) or leucocytosis (10.1%) ($P < 0.01$) significantly more often than controls (4.1%, 3.0%, respectively). The number of segmented neutrophils was significantly lower in 69.5% of ill cows (860 cells/μl) compared with 17.3% of controls (2,598 cells/μl) (Fig. 3). Increased numbers of segmented neutrophils were seen in 16.4% of ill cows compared with 4.8% of controls. Band neutrophils were seen in 77.3% of ill cows (median, 530 cells/μl) and in 0.6% of controls (median, 0 cells/ μl) ($P < 0.01$) (Fig. 4). Metamyelocytes were seen in 25% of ill cows but did not occur in controls (Fig. 5). Eosinopenia was seen in 66.4% of ill cows and in 1.8% of controls ($P < 0.01$) (Fig. 6). Basophils were in the normal range (0 to 200 cells/μl) in all ill cows and in 97.0% of controls. Monocytopenia (< 100 cells/μl) was seen in 40.6% of ill cows (155 cells/μl) compared with 4.2% of controls (336 cells/μl) ($P < 0.01$) (Fig. 7). Lymphopenia occurred in 60.2% of ill cows (1,550 cells/μl) compared with 1.8% of controls (3,998 cells/μl, $P < 0.01$) (Fig. 8).

Table 1
Medians and frequency distributions of the components of the leukogram in cows with toxic mastitis and in clinically healthy controls

Variable	Finding	Controls	Toxic mastitis
Leukocytes (/µl)	Median	8,000 /µl (n = 168)	4,300 /µl (n = 158)
	Normal (5,100 – 13,300 (/µl)[1]	92.9% (n = 156)	29.8% (n = 47)
	Decreased (600-5,099)	4.1% (n = 7)	60.1% (n = 95)
	Increased (13,301 – 29,100)	3.0% (n = 5)	10.1% (n = 16)
Segmented	Median	2,598 (n = 168)	860 (n = 128)
neutrophils (/µl)	Normal (1,700-6,000)	77.9% (n = 131)	14.1% (n = 18)
	Decreased (20 – 1,699)	17.3% (n = 29)	69.5% (n = 89)
	Increased (6,001–18,070)	4.8% (n = 8)	16.4% (n = 21)
Band neutrophils	Median	0 (n = 168)	530 (n = 128)
(/µl)	Normal (0-200)	99.4% (n = 167)	22.7% (n = 29)
	Increased (201 – 12,670)	0.6% (n = 1)	77.3% (n = 99)
Metamyelocytes	Median	0 (n = 168)	0 (n = 128)
(/µl)	Normal (0)	100% (n = 168)	75% (n = 96)
	Increased (1–13,301)	0%	25% (n = 32)
Eosinophils (/µl)	Median	582 (n = 168)	50 (n = 128)
	Normal (100-1,200)	85.1% (n = 143)	32.8% (n = 42)
	Decreased (0–99)	1.8% (n = 3)	66.4% (n = 85)
	Increased (1,201-2,280)	13.1% (n = 22)	0.8% (n = 1)
Basophils (/µl)	Median	70 (n = 168)	0 (n = 128)
	Normal (0-200)	97% (n = 163)	100% (n = 128)
	Increased (201–339)	3% (n = 5)	0%
Monocytes (/µl)	Median	336 (n = 168)	155 (n = 128)

The medians and frequency distributions of all components of the leukogram differ significantly between the two groups ($P < 0.01$, ANOVA, Mann-Whitney U test)

[1] Reference intervals for all variables: Wood and Quiroz-Rocha [18]

Variable	Finding	Controls	Toxic mastitis
	Normal (100–700)	94% (n = 158)	44.6% (n = 57)
	Decreased (0–99)	4.2% (n = 7)	40.6% (n = 52)
	Increased (701-2,170)	1.8% (n = 3)	14.8% (n = 19)
Lymphocytes (/µl)	Median	3’998 (n = 168)	1’550 (n = 128)
	Normal (1,800-8,100)	97.0% (163)	39.8% (n = 51)
	Decreased (400-1,799)	1.8% (n = 3)	60.2% (n = 77)
	Increased (8,101-8,721)	1.2% (n = 2)	0%
The medians and frequency distributions of all components of the leukogram differ significantly between the two groups (P < 0.01, ANOVA, Mann-Whitney U test)			
[1] Reference intervals for all variables: Wood and Quiroz-Rocha [18]			

Frequency distributions of leukocytes relative to the total leukocyte count and left shift

Of the cows with leukopenia, 95.5% had decreased numbers of segmented neutrophils, 72.7% had increased numbers of band neutrophils (Table 2), 67.0% had eosinopenia, 56.8% had monocytopenia and 73.9% had lymphopenia. Of the cows with leukocytosis, 81.2% had increased numbers of segmented neutrophils, 87.5% had increased numbers of band neutrophils and 75.0% had monocytosis. Of the cows with leukocytosis, 56.2% had eosinopenia.

Table 2
Frequency distributions of the components of the leukogram in 128 cows with toxic mastitis and normal, decreased and increased total leukocyte counts[1]

Leukocytes				
Variable	Classification	Normal (n = 24)	Decreased (n = 88)[2]	Increased (n = 16)
Segmented neutrophils	Normal (n = 18)	50.0%	4.5%	12.5%
	Decreased (n = 89)	16.7%	95.5%	6.3%
	Increased (n = 21)	33.3%	0%	81.2%
Band neutrophils	Normal (n = 29)	12.5%	27.3%	12.5%
	Increased (n = 99)	87.5%	72.7%	87.5%
Metamyelocytes	Normal (n = 96)	87.5%	71.6%	75.0%
	Increased (n = 32)	12.5%	28.4%	25.0%
Eosinophils	Normal (n = 42)	29.2%	33.0%	37.5%
	Decreased (n = 85)	70.8%	67.0%	56.2%
	Increased (n = 1)	0%	0%	6.3%
Basophils	Normal (n = 128)	100%	100%	100%
Monocytes	Normal (n = 57)	66.7%	42.0%	25.0%
	Decreased (n = 52)	8.3%	56.8%	0%
	Increased (n = 19)	25.0%	1.1%	75.0%
Lymphocytes	Normal (n = 51)	50.0%	26.1%	100%
	Decreased (n = 77)	50.0%	73.9%	0%

[1] See Table 1 for definitions of normal, decreased and increased ranges

[2] Leukocyte differentials were done in 88 of the 95 cows with leukopenia

All 21 cows with an increased number of segmented neutrophils had a regenerative left shift, i.e., the number of segmented neutrophils exceeded the number of band neutrophils. Fifty-seven cows (44.5%) had a degenerative left shift and thus the number of immature neutrophils (bands and metamyelocytes) exceeded the number of segmented neutrophils.

Neutrophil-to-lymphocyte ratio

The median neutrophil-to-lymphocyte (NL) ratio in controls was 0.63 and ranged from 0.28 to 1.53 (5th to 95th percentiles), which was lower than that of ill cows (0.97, $P < 0.01$, Mann-Whitney U test). The 5th percentile (0.13) was lower and the 95th percentile was higher (7.46) than in controls. The frequency distributions of the NL ratio differed between the two groups ($P < 0.01$, chi-square test, Fig. 9).

Toxic changes in neutrophils

Toxic changes in neutrophils that included cytoplasmic basophilia and vacuolisation were detected in 101 (91.8%) of 110 blood smears. In 58 cows, the occurrence of toxic changes was quantified; 11 cows had changes in 5 to 10% of neutrophils, 9 had changes in 20 to 30% and 38 had changes in greater than 30% of the neutrophils.

Effect of gram-negative and gram-positive pathogens and yeast on the leukogram

Differences between the medians and frequency distributions of the total leukocyte counts and the numbers of the components of the leukogram associated with gram-negative (n = 100), gram-positive (n = 15) and yeast infection (n = 4) were not significant (Table 3), but there were several significant differences between cows of the different pathogen groups and controls. Lymphocyte counts were significantly lower in all three groups, and cows with gram-negative infections had significantly lower total leukocyte, segmented neutrophil and monocyte counts and significantly higher band neutrophil counts than controls. Cows with gram-positive infections had significantly greater band neutrophil and significantly lower eosinophil, basophil and monocyte counts.

Table 3
Leukogram of 119 cows with toxic mastitis caused by gram-positive, gram-negative or mycotic pathogens (medians and 5th to 95th percentiles)

		Toxic mastitis		
Variable	Controls (n = 168)	Gram nega-tive (n = 100)	Gram positive (n = 15)	Yeast (n = 4)
Leukocytes (/µl)	8,000 (5,345 – 11,910)	3,950[a] (1’100 – 13'570)	5,700 (2,300 – 14,880)	3,650 (2,800-4,350)
Segmented neutrophils (/µl)	2,598 (1,163-5,771)	560[a] (42 – 9,696)	1,160 (50 – 13,142)	1,200 420-2,663)
Band neutrophils (/µl)	0 (0–59)	520[a] (0–5,739)	890[a] (0–4,450	135 (40 – 1,133)
Metamyelocytes (/µl)	0 (0)	0[a] (0-284)	0 (0-112)	0 (0–38)
Eosinophils (/µl)	582 (174-1,666)	50[a] (0-397)	40[a] (0-270)	125 (110–170)
Basophils (/µl)	70 (0-179)	0[a] (0–20)	0[a] (0–36)	0 (0)
Monocytes (/µl)	336 (102–640)	100[a] (2 – 1,072)	320[a] (100-1,020)	110[a] (80–145)
Lymphocytes (/µl)	3998 (2,292-6,959)	1,525[a] (765-3,261)	1,730[a] (640-2,992)	1,545[a] (840-2,028)
[a] Different from control cows P < 0.01				
[b] Different from control cows P < 0.05				

Effect of duration of illness on leukogram

There were significant differences in total leukocyte and segmented neutrophil counts among cows with a duration of illness of one, two, three or more than three days (Table 4). Cows examined on the first day

of illness had significantly lower total leukocyte (3,250 vs. 9,200 cells/μl) and segmented neutrophil counts (610 vs. 6,170 cells/μl, $P < 0.05$) than cows that had been ill for more than three days. The counts of other components of the leukogram were not related to duration of illness.

Table 4
Leukogram of cows with toxic mastitis by duration of illness

	Controls	Duration of illness			
Variable	(n = 168)	1 day	2 days	3 days	> 3 days
Leukocytes (/µl)	8,000 (5,345 – 11,910)	3,250 (n = 70) (900 – 15,625)	4,250 (n = 28) (945 – 19,920)	5,300 (n = 33)[a] (1,500 – 16,600)	9,200 (n = 23)[b,c] (1'560 – 19'580)
Segmented neutrophils (/µl)	2,598 (1,163-5,771)	610 (n = 63) (50 – 8,492)	480 (n = 23) (24 – 16,424)	660 (n = 21) (91 – 9,163)	6,170 (n = 18)[c,d] (150 – 14,522)
Band neutrophils (/µl)	0 (0–59)	460 (n = 63) (0–6,948)	490 (n = 23) (0–6,924)	1,140 (n = 21) (0–6,604)	1,025 (n = 18) (0–3,737)
Metamyelo-cytes (/µl)	0 (0)	0 (n = 63) (0-180)	0 (n = 23) (0-762)	0 (n = 21) (0-318)	0 (n = 18) (0-125)
Eosinophils (/µl)	582 (174-1,665)	40 (n = 63) (0-408)	100 (n = 23) (0–1,650)	100 (n = 21) (0-469)	50 (n = 18) (0-325)
Basophils (/µl)	70 (0-179)	0 (n = 63) (0–20)	0 (n = 23) (0–42)	0 (n = 21) (0–74)	0 (n = 18) (0–8)
Monocytes (/µl)	336 (102–640)	120 (n = 63) (10 – 1,292)	150 (n = 23) (20 – 1,340)	190 (n = 21) (2-810)	255 (n = 18) (0–1,325)
Lymphocytes (/µl)	3,998 (2,292-6,959)	1,430 (n = 63) (654-3,334)	1,690 (n = 23) (770-4,438)	1,630 (n = 21) (977-3,430)	1,820 (n = 18) (840-3,164)

[a] Different from day 1 P < 0.05, Kruskal-Wallis-test

[b] Different from day 1 P < 0.01, Kruskal-Wallis-test

[c] Different from day 2 P < 0.05, Kruskal-Wallis-test

[d] Different from day 3 P < 0.05, Kruskal-Wallis-test

Other laboratory variables

Cows with toxic mastitis had a significantly higher haematocrit (34 vs. 33%) and significantly higher concentrations of fibrinogen (6.0 vs. 5.0 g/l), urea (7.9 vs. 4.5 mmol/l) and sodium (146 vs. 145 mmol/l) and a significantly lower total erythrocyte count (6.5 vs. 7.3×10^6 cells/µl) and lower concentrations of total protein (74 vs. 79 g/l), potassium (3.7 vs. 4.2 mmol/l) and calcium (2.3 vs. 2.4 mmol/l) than controls (Table 5).

Table 5

Haematocrit, total erythrocyte count and total protein, fibrinogen, urea and serum electrolyte concentrations in 158 cattle with toxic mastitis and in 168 control cows

Variable	Mean ± sd or median (5th to 95th percentiles)	Controls	Toxic mastitis
Haematocrit (%)	Mean ± sd	33 ± 3.6	34 ± 0.4*
Erythrocytes (10^6/µl)	Mean ± sd	7.3 ± 0.79	6.5 ± 1.08**
Total protein (g/l)	Mean ± sd	79 ± 8.0	74 ± 10.0**
Fibrinogen (g/l)	Median	5.0	6.0**
	(5th to 95th percentiles)	(3.0–8.0)	(2.0-10.2)
Urea (mmol/l)	Mean ± sd	4.5 ± 1.49	7.9 ± 4.95**
Sodium (mmol/l)	Median	145	146**
	(5th to 95th percentiles)	(142–149)	(138–153)
Chloride (mmol/l)	Median	100	101
	(5th to 95th percentiles)	(92–107)	(88–112)
Potassium (mmol/l)	Median	4.2	3.7**
	(5th to 95th percentiles)	(3.5–4.8)	(2.4–5.3)
Calcium (mmol/l)	Median	2.4	2.3*
	(5th to 95th percentiles)	(2.2–2.7)	(1.6–3.3)
Inorganic phosphate	Median	1.9	1.6
(mmol/l)	(5th to 95th percentiles)	(1.2–2.6)	(0.6–3.4)
Magnesium (mmol/l)	Median	1.1	0.9
	(5th to 95th percentiles)	(0.8–1.3)	(0.6–1.7)

* Different from control cows $P < 0.05$, ANOVA

** Different from control cows $P < 0.01$, ANOVA

Discussion

This study has shown that the total leukocyte count in cows with toxic mastitis is considerably lower than in healthy cows. Approximately 60.1% of the cows with mastitis had leukopenia and only 10.1% had leukocytosis. Segmented neutrophil counts were similar and were decreased in about 69.5% and increased in about 16.4% of the cows. Acute inflammation is generally accompanied by leukocytosis rather than leukopenia, but our findings are in agreement with published reports of clinical [1, 13, 19] as well as experimental acute mastitis [8–12]. The reason for the leukopenia is the low bone marrow reserve of neutrophils in cattle as opposed to other domestic animal species such as the dog [6] and the fact that the transit time from myeloblast to complete maturation is not reduced in the face of high neutrophil demand [5]. In the dog the transit time can be reduced from 6 to 2 to 3 days with increased demand of segmented neutrophils but not in calves [3] and in all likelihood not in adult cattle either. With acute mastitis, large numbers of segmented neutrophils are moved from the circulation into the infected mammary tissue causing leukopenia. This is followed by an increase in leukocyte numbers after about 48 hours, often associated with a rebound neutrophilia [5]. For these reasons, the leukocyte count and particularly the segmented neutrophil count were lower in the cows that were examined on the first day of illness compared with cows that were referred and sampled at a later stage of illness. Severe leukocytosis with cell counts exceeding 20,000 cells/µl occurred in only two cows (1.3%), which was in agreement with the observation that cattle as well as sheep and goats with acute inflammation have much lower leukocyte peaks than other domestic animal species [14]. A leukocyte count between 20,000 and 30,000 cells/µl is considered extremely high in cattle [14] and only 16.4% of ill cows had neutrophilia in the present study. Stress is another cause of neutrophilia. Stress leukograms caused by endogenous or exogenous corticosteroids are common in cattle [14] and are characterised by neutrophilia, lymphopenia, eosinopenia and monocytosis [14, 20]. However, a stress leukogram is not associated with a left shift and therefore band neutrophils and metamyelocytes are not seen. In the present study, neutrophilia was always accompanied by a regenerative left shift in affected cows, which means that neutrophilia was due to inflammation and not stress. Degenerative left shift, characterised by an abundance of band neutrophils and metamyelocytes relative to segmented neutrophils, was more common than regenerative left shift and occurred in 44.5% of ill cows. Degenerative left shift with increased numbers of metamyelocytes is an alarming haematological finding and a poor prognostic sign when it persists for more than 3 to 4 days [5].

Lymphopenia was detected in 60.4% of all cows with toxic mastitis. Lymphopenia in cattle is most commonly caused by endogenous or exogenous corticosteroids [5], and in the present study was due to toxic mastitis. This was also observed after experimental intramammary infection with *Pseudomonas aeruginosa* [11] and *Mycoplasma bovis* [12]. Interestingly, lymphopenia occurred as early as 24 hours after *Pseudomonas aeruginosa* inoculation but only 84 hours after *Mycoplasma bovis* inoculation. Lymphopenia is also seen in viral infections and diseases caused by *Anaplasma* spp. and other bacteria [14].

Metamyelocytes were not detected in blood smears from healthy cows, which was in agreement with the veterinary literature [21]. The occurrence of metamyelocytes should therefore be considered pathological and indicative of a massive demand for leukocytes.

In 104 cows (62%), toxic mastitis occurred during late pregnancy or during the puerperal period, which most likely was related to impaired neutrophil function in the periparturient period [22, 23] described previously [24, 25]. It is believed that this is related to increased stress and thus increased glucocorticoid concentrations at this lactation stage [26]. Furthermore, recruitment of neutrophils into the mammary gland and neutrophil function are altered in the periparturient period, which may also contribute to the occurrence of mastitis in early-lactation cows [22, 24, 27]. This is supported by the observation that experimental intramammary infusion of endotoxin results in more severe clinical signs in early lactation compared with late lactation in cows [28].

In contrast to other domestic animal species, cows have more lymphocytes than neutrophils in the circulation and therefore a relatively low NL ratio of about 0.5 [6, 14]; in the present study, the controls had a median NL ratio of 0.63. The significantly larger NL ratio of 0.97 in the cows with toxic mastitis was due primarily to lymphopenia (in 60.2%) rather than neutrophilia (only 16.4%). Similarly, cows with sole ulcer had a NL ratio of 1.04, which was significantly greater than that of healthy cows even though neutrophil and lymphocyte counts did not differ significantly between the groups [29]. It was therefore recommended to include the NL ratio in the assessment of a blood cell count to emphasise the difference in absolute neutrophil and lymphocyte numbers [5].

Cytoplasmic basophilia combined with vacuolisation in all but 8.2% of the examined blood smears was indicative of toxic change [5]. This confirms that the cows with mastitis had toxaemia because the changes are generated when maturation of neutrophils in the bone marrow is hindered because of acute and severe inflammation [14].

Cows with mastitis caused by gram-negative and gram-positive bacteria did not differ significantly with respect to total leukocyte, segmented and band neutrophil, monocyte and lymphocyte counts. Differences in cell counts between cows with gram-negative and gram-positive infections were significant in an earlier study but the number of cows was larger in that study [13]. The observation that cows with gram-positive infection tended to have lower total leukocyte and segmented neutrophil counts and significantly lower eosinophil, basophil, monocyte and lymphocyte counts than controls indicates that mastitis caused by gram-positive pathogens constitutes a severe strain on the bone marrow. It should be remembered that differentiation of mastitis caused by gram-negative and gram-positive pathogens requires bacteriological examination and is not possible based on clinical signs alone [1].

Increased haematocrit and azotaemia in cows with toxic mastitis was in all likelihood attributable to prerenal causes. The increased fibrinogen concentration points to the role of fibrinogen as a positive acute-phase protein [18, 30]. Similar to haptoglobin, it increases rapidly in response to inflammation and was therefore higher in cows that were ill longer than three days compared with cows on the first day of illness. In cattle, hyperfibrinogenaemia is considered as good as or better than the neutrophil count for

determining inflammation [18]. As a general rule, the degree of increase in concentration of positive acute-phase proteins parallels the severity of inflammation. Fibrinogen is preferred by many because it is easy to measure.

We were surprised that only about 69% of all toxic mastitis cases were diagnosed by the primary care veterinarian. This may have been due to other clinical signs, such as poor general health status, poor rumen motility, low faecal output and positive percussion and/or ballottement and simultaneous auscultation, which were considered diagnostic of other disorders. This emphasises the importance of a comprehensive clinical examination that includes the assessment of the udder and the milk.

Conclusions

Acute toxic mastitis is accompanied by severe changes in the leukogram, chiefly leukopenia and degenerative left shift. The leukogram has significant diagnostic value and may aid in the modification of treatment when needed. Leukograms similar to those presented in this study strongly suggest toxic mastitis or toxaemia of another aetiology when mastitis can be ruled out clinically.

Abbreviations

ANOVA: Analysis of variance; EDTA: Ethylenediaminetetraacetic acid; Fig.: Figure; NL ratio: Neutrophil-to-lymphocyte ratio.

Declarations

Ethics approval and consent to participate

The study was not submitted to our institutional ethics committee, because it was a retrospective study using medical records of cows that had been referred to our clinic for examination and treatment. No experimental animals were used. Under these circumstances, ethical approval is not required in Switzerland. Written consent was obtained from all owners to participate.

Consent for publication

All owners signed a consent form allowing us to use the animals and all the associated medical data for scientific analysis and publication.

Availability of data and materials

The datasets used and analysed for this study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Fundings

Not applicable since it was a retrospective analysis of medical records.

Author's contributions

UBr initiated, planned and supervised the study and prepared the manuscript. CG, SB and UBl were involved in the clinical examination and treatment of the cows. BR was responsible for the haematologic examination and SC for the bacteriologic examination of milk. All authors made contributions to acquisition and interpretation of data and were involved in drafting the manuscript. All authors read and approved the final manuscript.

Acknowledgements

The authors thank the technicians of the Medial Laboratory for the haematological and biochemical analysis, the technicians of the Institute of Food Safety and Hygiene for the bacteriologic examination of the milk samples and the agricultural assistants for their help with the clinical examinations.

Author details

[1]Department of Farm Animals, Vetsuisse-Faculty, University of Zurich, Winterthurerstrasse 260, CH-8057 Zurich, Switzerland, [2]Department of Clinical Diagnostic and Services, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 260, CH-8057 Zurich, Switzerland, [3]Institute of Food Safety and Hygiene, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 260, CH-8057 Zurich.

References

1. Constable PD, Hinchcliff KW, Done SH, Grünberg W. Diseases of the mammary gland. In: Constable PD, Hinchcliff KW, Done SH, Grünberg W, editors. Veterinary Medicine. A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs, and Goats. St. Louis, Missouri: Elsevier; 2017. p. 1904–2001.
2. Schultze AE. Interpretation of canine leukocyte responses. In: Weiss DJ, Wardrop KJ, editors. Schalm's Veterinary Hematology. Ames, Iowa: Wiley-Blackwell; 2010. p. 321–34.
3. Radin MJ, Wellman ML. Granulopoiesis. In: Weiss DJ, Wardrop KJ, editors. Schalm's Veterinary Hematology. Ames, Iowa: Wiley-Blackwell; 2010. p. 43–9.
4. Weiser G. Neutrophil production, trafficking, and kinetics. In: Thrall MA, Weiser G, Allison RW, Campell TW, editors. Veterinary Hematology and Clinical Chemistry. Ames, Iowa: Wiley-Blackwell; 2012. p. 123–6.
5. Sharkey LC, Heinrich DA. Alterations in the leukogram. In: Smith BP, Van Metre DC, Pusterla N, editors. Large Animal Internal Medicine. St. Louis, Missouri: Elsevier; 2020. p. 429–34.
6. Roland L, Drillich M, Iwersen M. Hematology as a diagnostic tool in bovine medicine. J Vet Diagn Invest. 2014;26:592–8.

7. Weiser G. Interpretation of leukocyte responses in disease. In: Thrall MA, Weiser G, Allison RW, Campell TW, editors. Veterinary Hematology and Clinical Chemistry. Ames, Iowa: Wiley-Blackwell; 2012. p. 127–39.
8. Bannerman DD, Paape MJ, Goff JP, Kimura K, Lippolis JD, Hope JC. Innate immune response to intramammary infection with *Serratia marcescens* and *Streptococcus uberis*. Vet Res. 2004;35:681–700.
9. Bannerman DD, Paape MJ, Hare WR, Hope JC. Characterization of the bovine innate immune response to intramammary infection with *Klebsiella pneumoniae*. J Dairy Sci. 2004;87:2420–32.
10. Bannerman DD, Paape MJ, Lee JW, Zhao X, Hope JC, Rainard P. *Escherichia coli* and *Staphylococcus aureus* elicit differential innate immune responses following intramammary infection. Clin Diagn Lab Immunol. 2004;11:463–72.
11. Bannerman DD, Chockalingam A, Paape MJ, Hope JC. The bovine innate immune response during experimentally-induced *Pseudomonas aeruginosa* mastitis. Vet Immunol Immunopathol. 2005;107:201–15.
12. Kauf ACW, Rosenbusch RF, Paape MJ, Bannerman DD. Innate immune response to intramammary *Mycoplasma bovis* infection. J Dairy Sci. 2007;90:3336–48.
13. Smith GW, Constable PD, Morin DE. Ability of hematologic and serum biochemical variables to differentiate gram-negative and gram-positive mastitis in dairy cows. J Vet Intern Med. 2001;15:394–400.
14. Tornquist SJ, Rigas J. Interpretation of ruminant leukocyte responses. In: Weiss DJ, Wardrop KJ, editors. Schalm's Veterinary Hematology. Ames, Iowa: Wiley-Blackwell; 2010. p. 307–13.
15. Weiss DJ, Souza CD. Monocytes and macrophages and their disorders. In: Weiss DJ, Wardrop KJ, editors. Schalm's Veterinary Hematology. Ames, Iowa: Wiley-Blackwell; 2010. p. 298–306.
16. Braun U, Amrein E, Estermann U, Egli J, Schweizer T, Lutz H, Ehrensperger F, Vandevelde M, Kihm U. Untersuchungen an 182 Nachkommen von an boviner spongiformer Enzephalopathie (BSE) erkrankten Kühen in der Schweiz. Teil 1: Klinische Befunde. Schweiz Arch Tierheilk. 1998;140:240–9.
17. Braun U, Nuss K, Warislohner S, Reif C, Oschlies C, Gerspach C. Frequency and diagnostic reliability of clinical signs in cows with traumatic reticuloperitonitis and abomasal ulcers. BMC Vet Res. 2020;16:359.
18. Wood D, Quiroz-Rocha GF. Normal hematology of cattle. In: Weiss DJ, Wardrop KJ, editors. Schalm's Veterinary Hematology. Ames, Iowa: Wiley-Blackwell; 2010. p. 829–35.
19. Bleul U, Sacher K, Corti S, Braun U. Clinical findings in 56 cows with toxic mastitis. Vet Rec. 2006;159:677–80.
20. Anderson BH, Watson DL, Colditz IG. The effect of dexamethasone on some immunological parameters in cattle. Vet Res Commun. 1999; 23:399–413.
21. Weiser G. Introduction to leukocytes and the leukogram. In: Thrall MA, Weiser G, Allison RW, Campell TW, editors. Veterinary Hematology and Clinical Chemistry. Ames, Iowa: Wiley-Blackwell; 2012. p. 118–22.

22. Weiss DJ. Neutrophil function disorders. In: Weiss DJ, Wardrop KJ, editors. Schalm's Veterinary Hematology. Ames, Iowa: Wiley-Blackwell; 2010. p. 275–80.
23. LeBlanc SJ. Review: Relationships between metabolism and neutrophil function in dairy cows in the peripartum period. Animal. 2020;14:S1,s44–54.
24. Kehrli ME, Nonneke BJ, Roth JA. Alterations in bovine neutrophil function during the periparturient period. Am J Vet Res. 1989;50:207–14.
25. Dosogne H, Burvenich C, Freeman AE, Kehrli ME, Detilleux JC, Sulon J, Beckers JF, Hoeben D. Pregnancy-associated glycoprotein and decreased polymorphnuclear leukocyte function in early post-partum dairy cows. Vet Immunol Immunopathol. 1999;67:47–54.
26. Burton JL, Madsen SA, Chang LC, Weber PSD, Buckham KR, Van Dorp R, Hickey MC, Earley B. Gene expression signatures in neutrophils exposed to glucocorticoids: a new paradigm to help explain "neutrophil dysfunction" in parturient dairy cows. Vet Immunol Immunopathol. 2005;105:197–219.
27. Paape MJ, Bannerman DD, Zhao X, Lee JW. The bovine neutrophil: Structure and function in blood and milk. Vet Res. 2003;34:597–627.
28. Lehtolainen T, Suominen S, Kutila T, Pyörälä S. Effect of intramammary *Escherichia coli* endotoxin in early- vs. late-lactating dairy cows. J Dairy Sci. 2003;86:2327–33.
29. O'Driscoll K, McCabe M, Earley B. Differences in leukocyte profile, gene expression, and metabolite status of dairy cows with or without sole ulcers. J Dairy Sci. 2015;98:1685–95.
30. Constable PD, Hinchcliff KW, Done SH, Grünberg W. General systemic states. In: Constable PD, Hinchcliff KW, Done SH, Grünberg W, editors. Veterinary Medicine. A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs, and Goats. St. Louis, Missouri: Elsevier; 2017. 2017. p. 43–112.

Figures

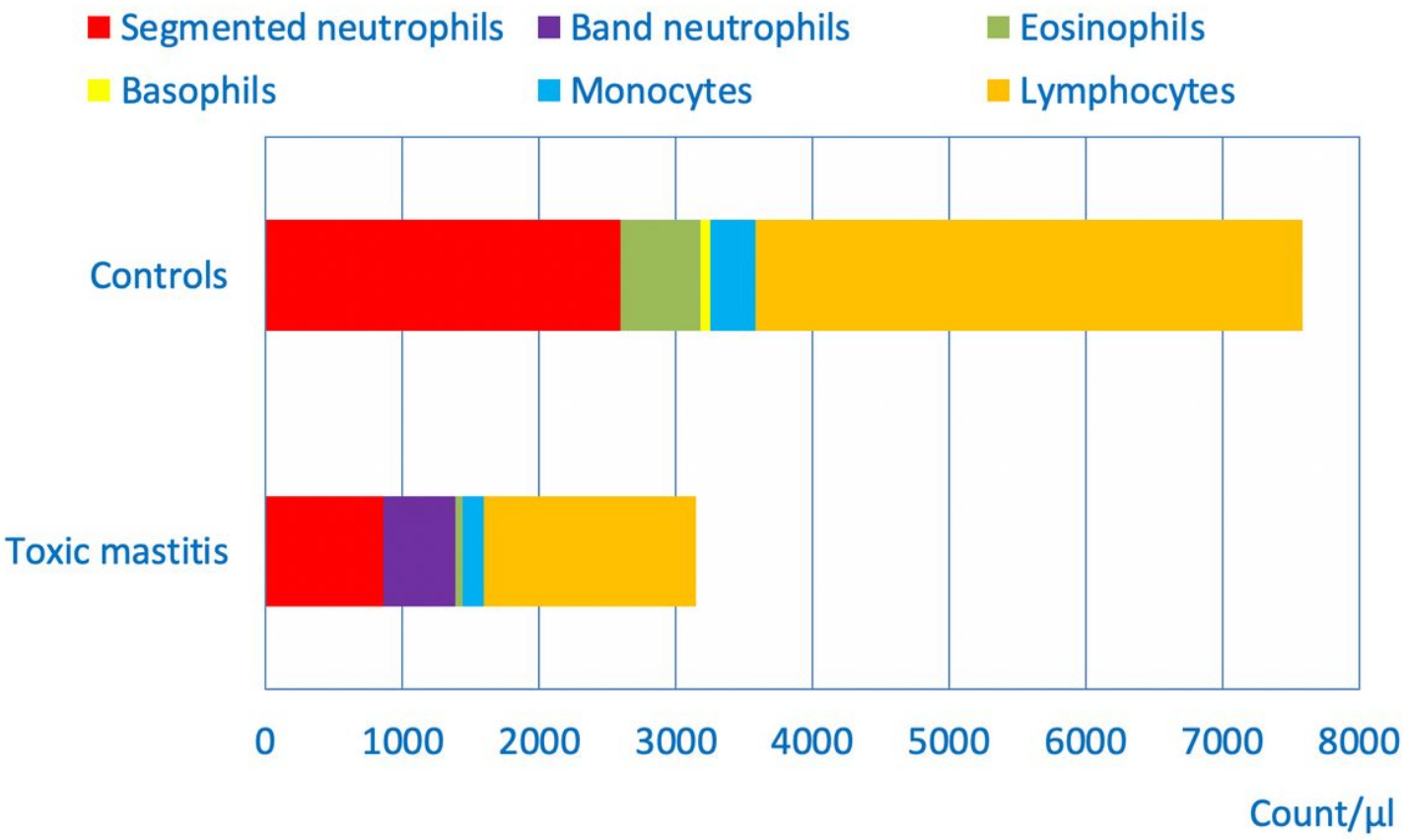

Figure 1

Frequency distributions of the components of the leukogram in 168 healthy control cows and in 158 cows with toxic mastitis (medians).

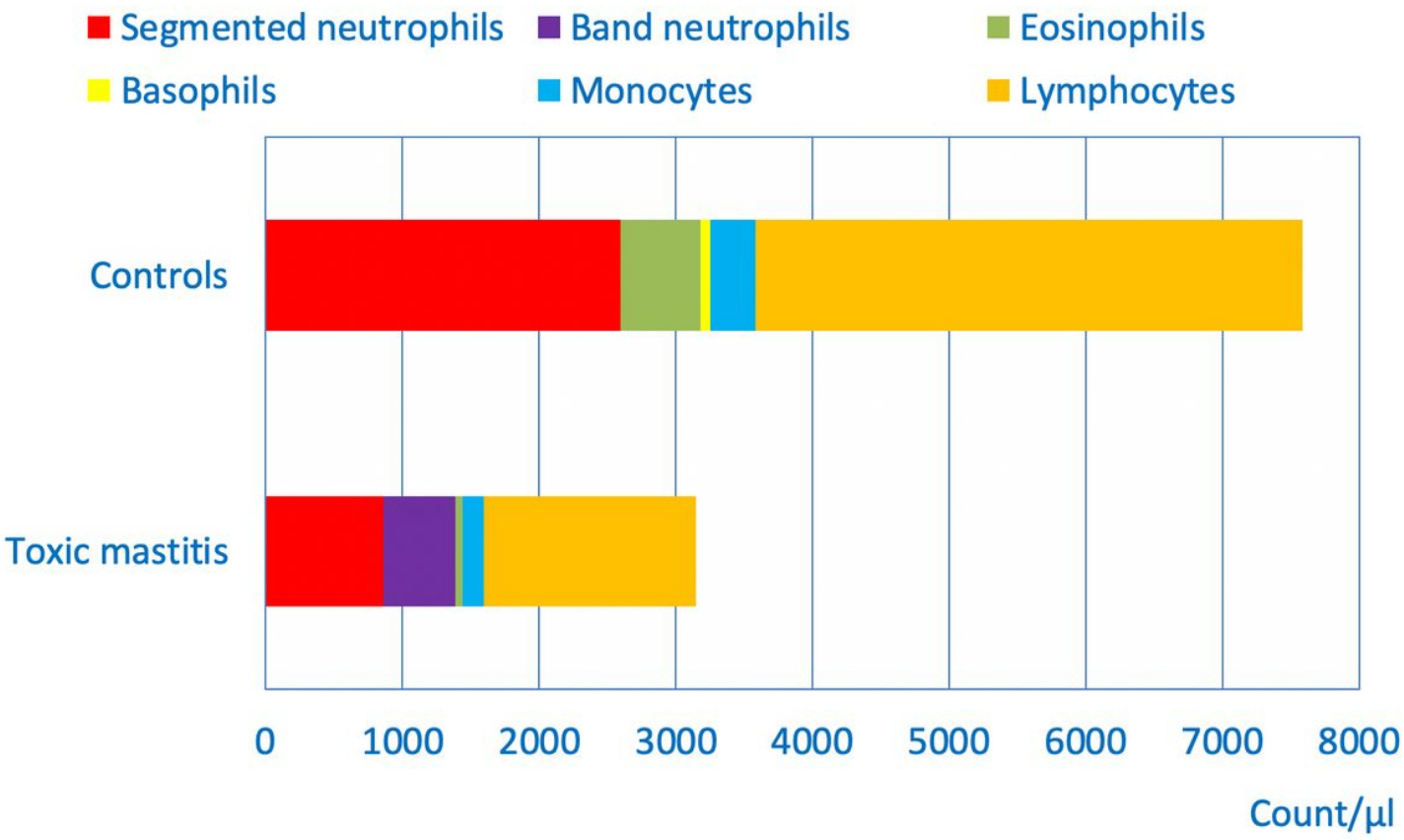

Figure 1

Frequency distributions of the components of the leukogram in 168 healthy control cows and in 158 cows with toxic mastitis (medians).

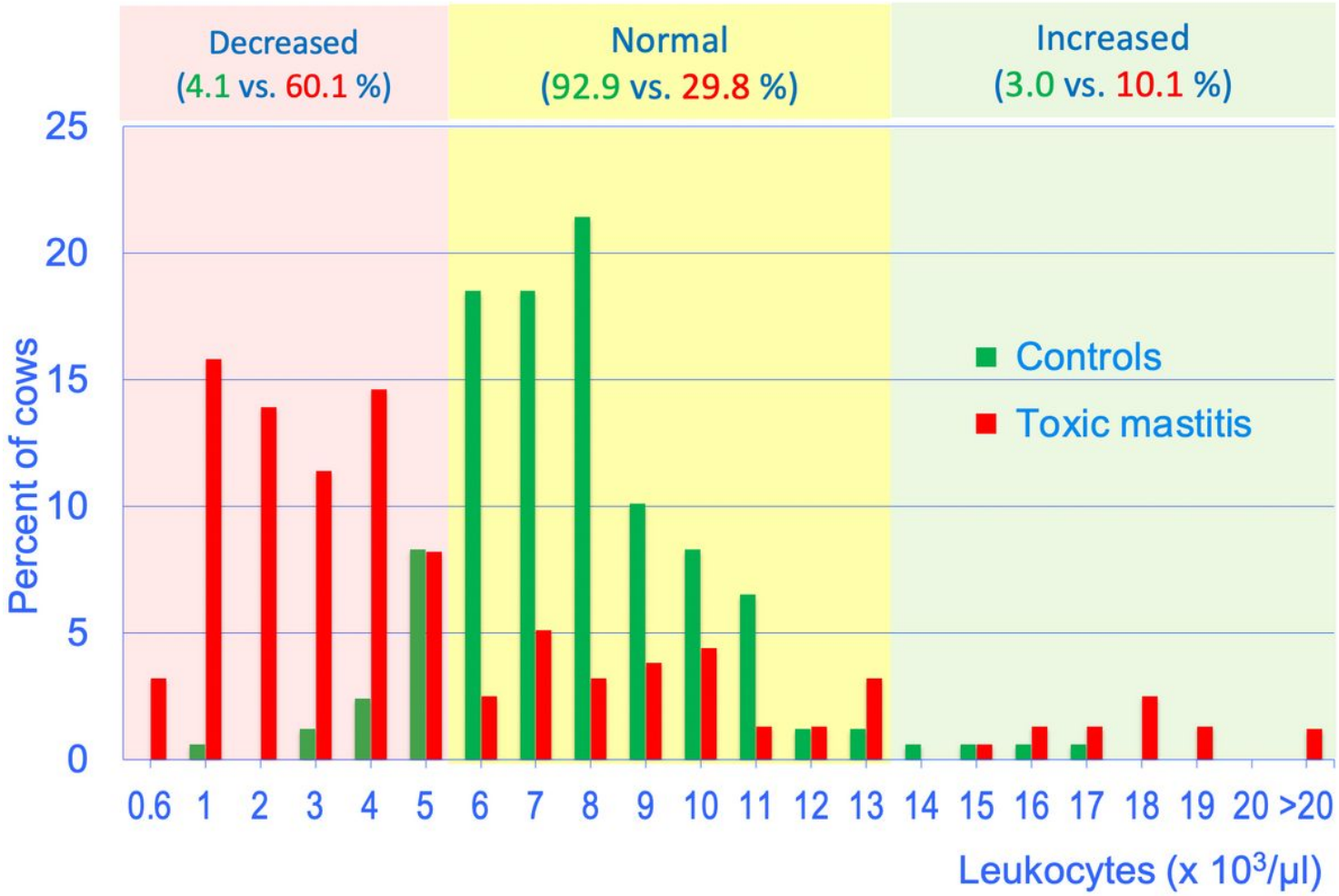

Figure 2

Frequency distributions of the total leukocyte count in 168 healthy control cows and in 158 cows with toxic mastitis.

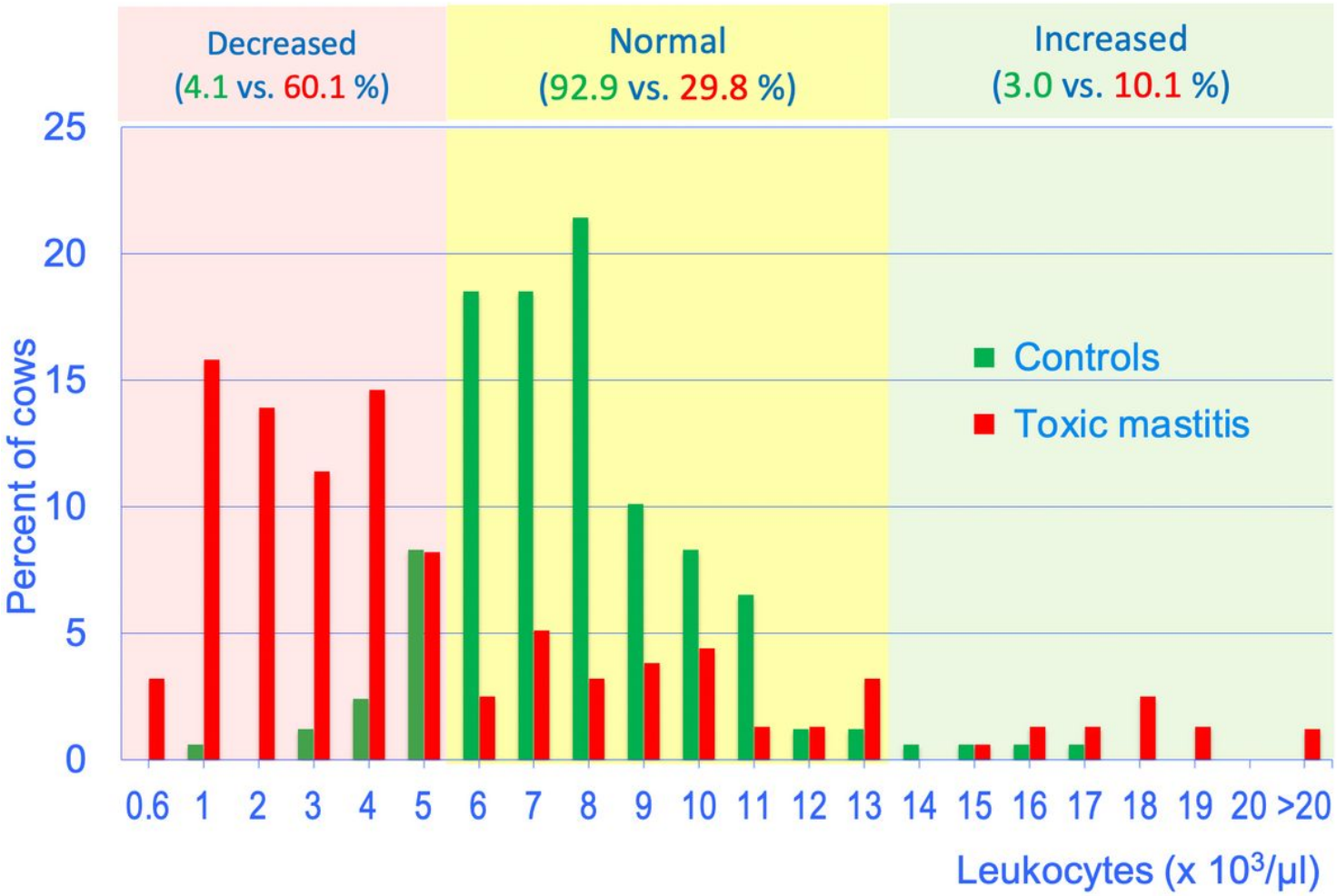

Figure 2

Frequency distributions of the total leukocyte count in 168 healthy control cows and in 158 cows with toxic mastitis.

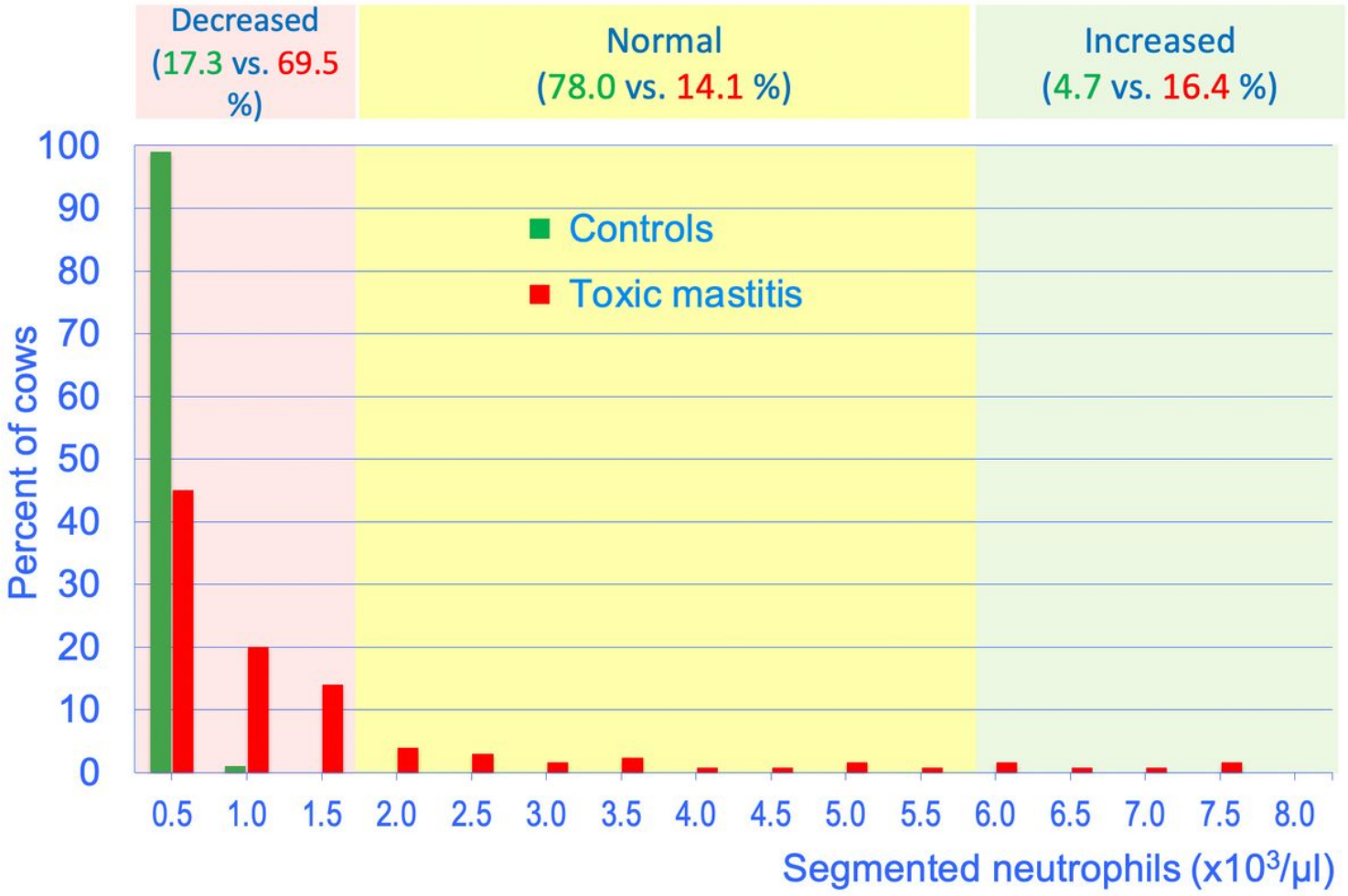

Figure 3

Frequency distributions of segmented neutrophils in 168 healthy control cows and in 128 cows with toxic mastitis.

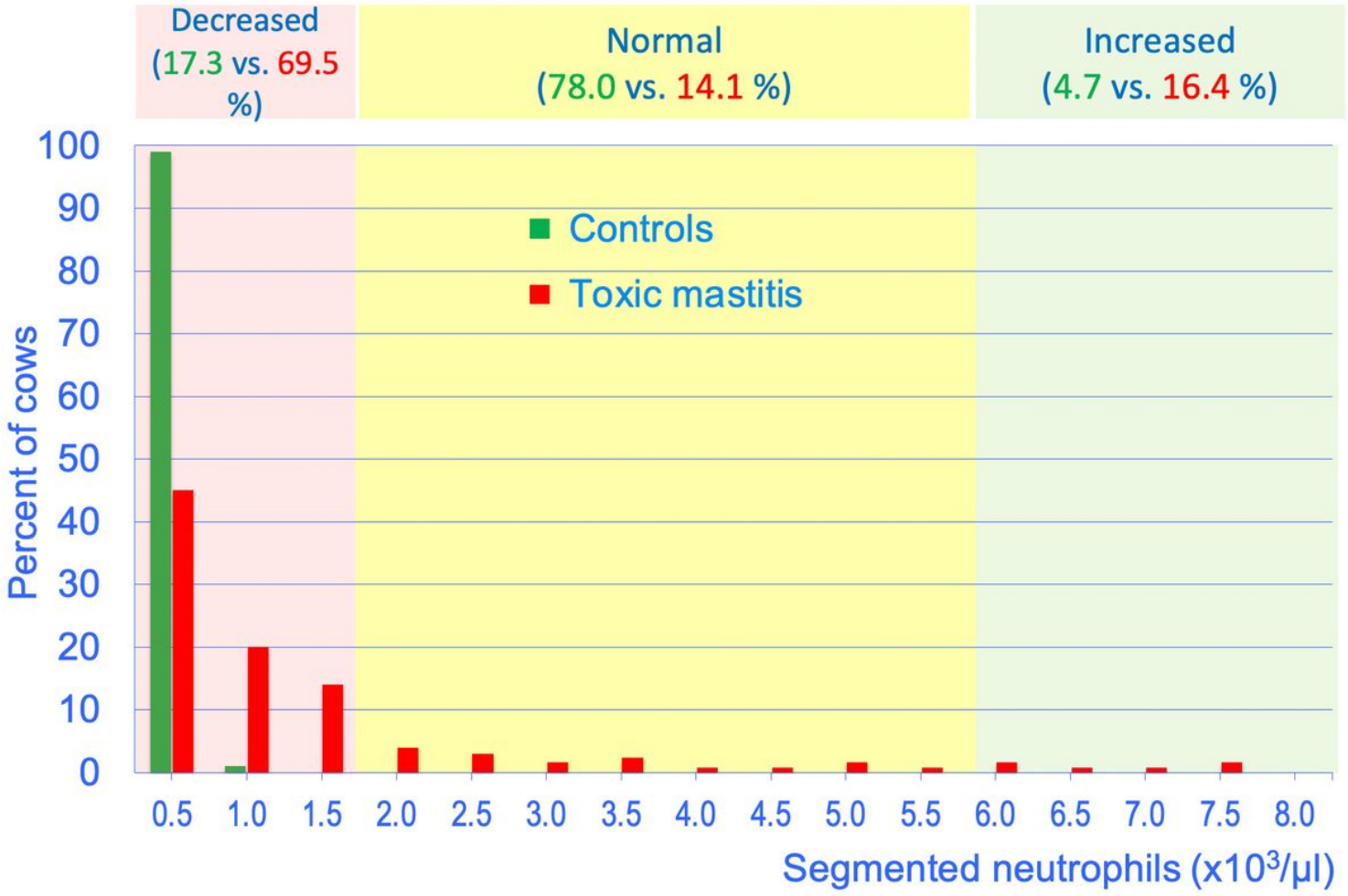

Figure 3

Frequency distributions of segmented neutrophils in 168 healthy control cows and in 128 cows with toxic mastitis.

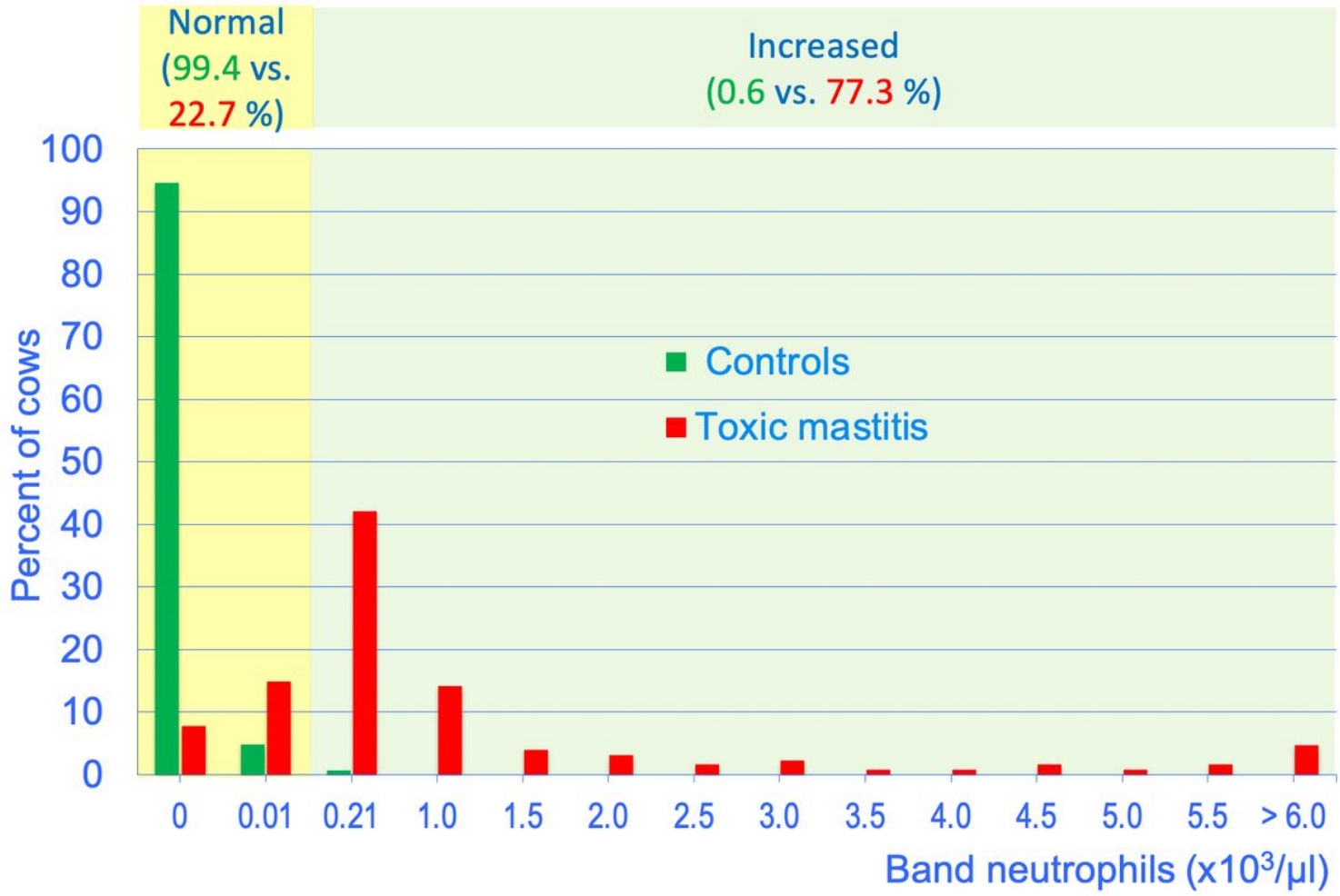

Figure 4

Frequency distributions of band neutrophils in 168 healthy control cows and in 128 cows with toxic mastitis.

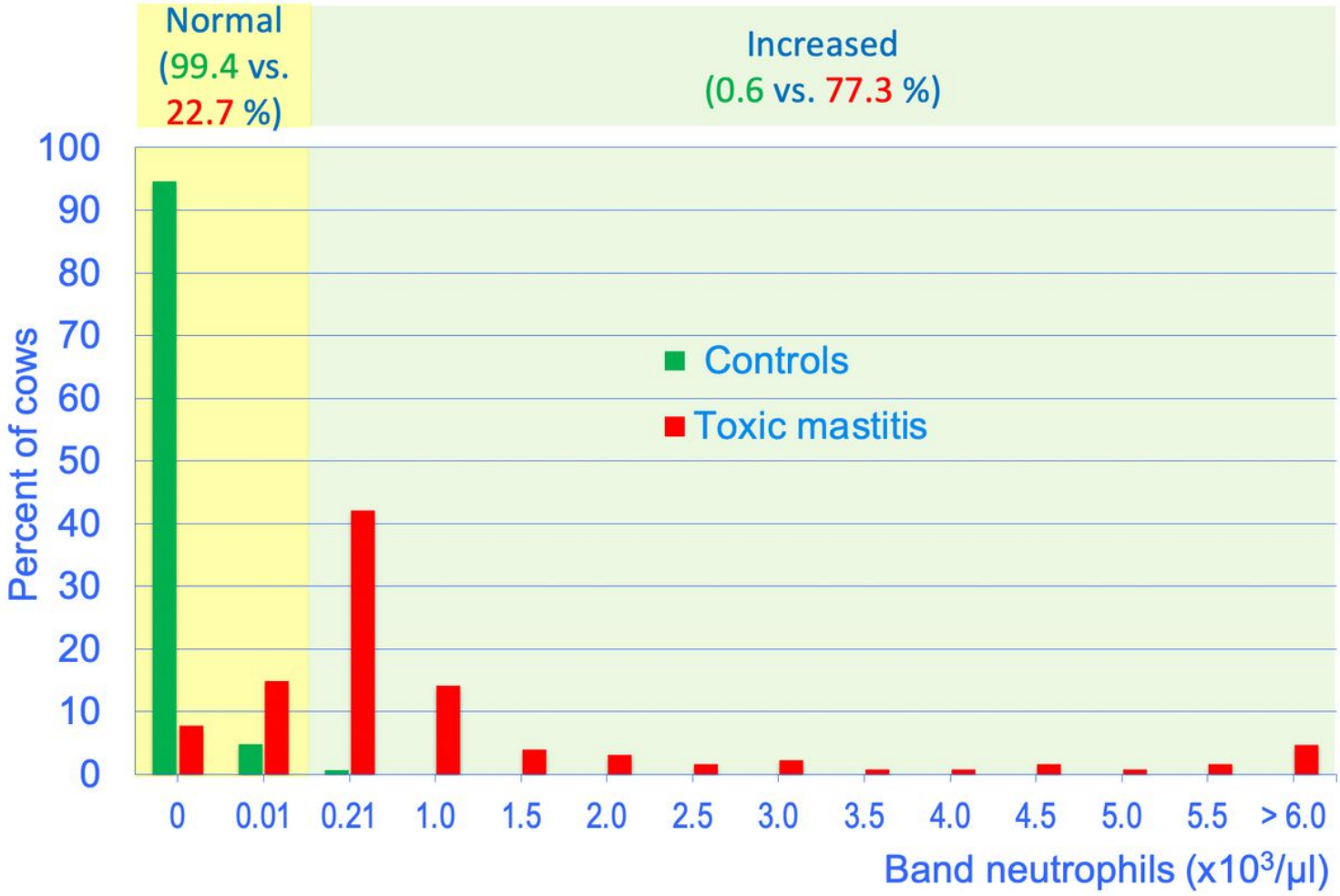

Figure 4

Frequency distributions of band neutrophils in 168 healthy control cows and in 128 cows with toxic mastitis.

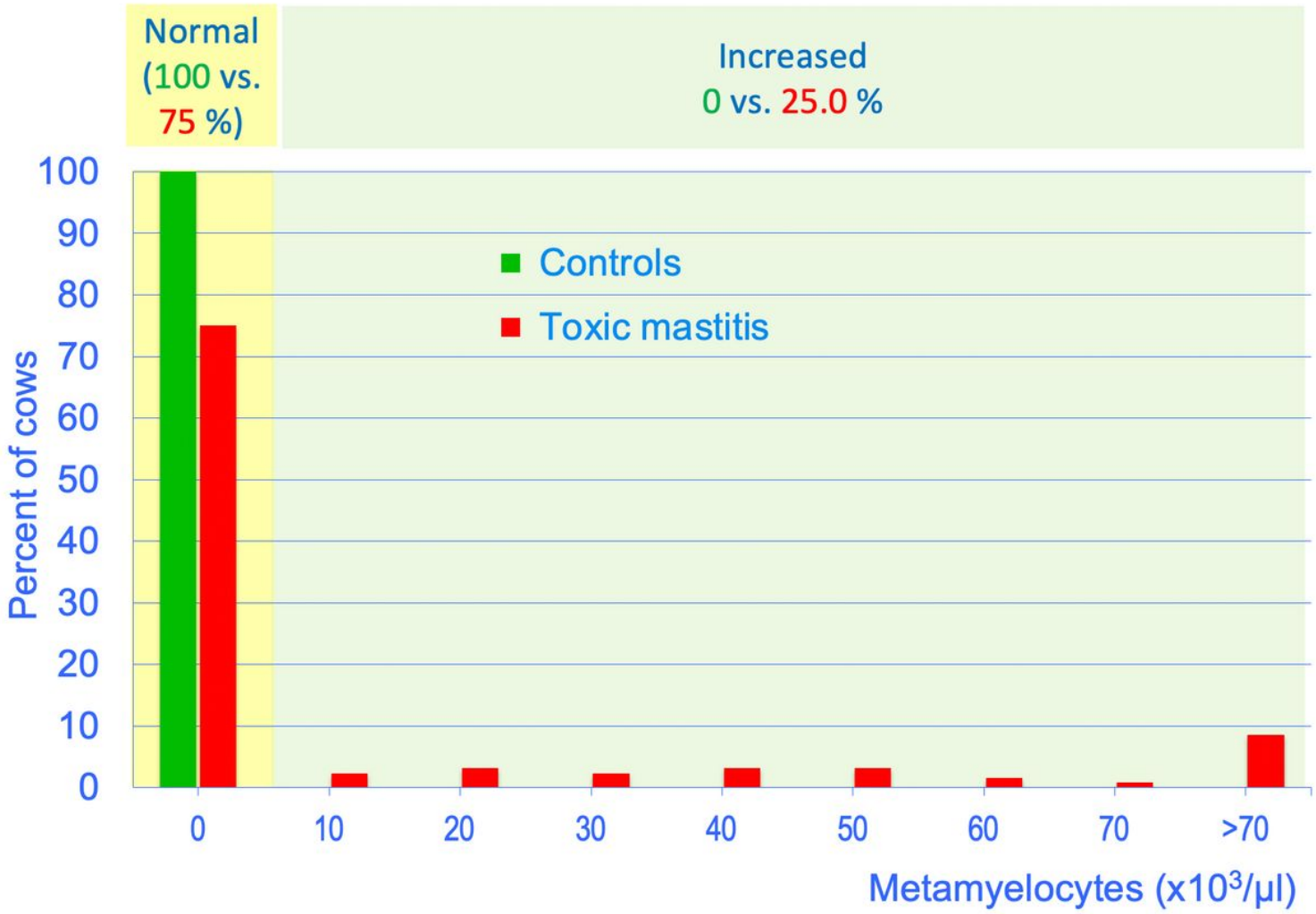

Figure 5

Frequency distributions of metamyelocytes in 168 healthy control cows and in 128 cows with toxic mastitis.

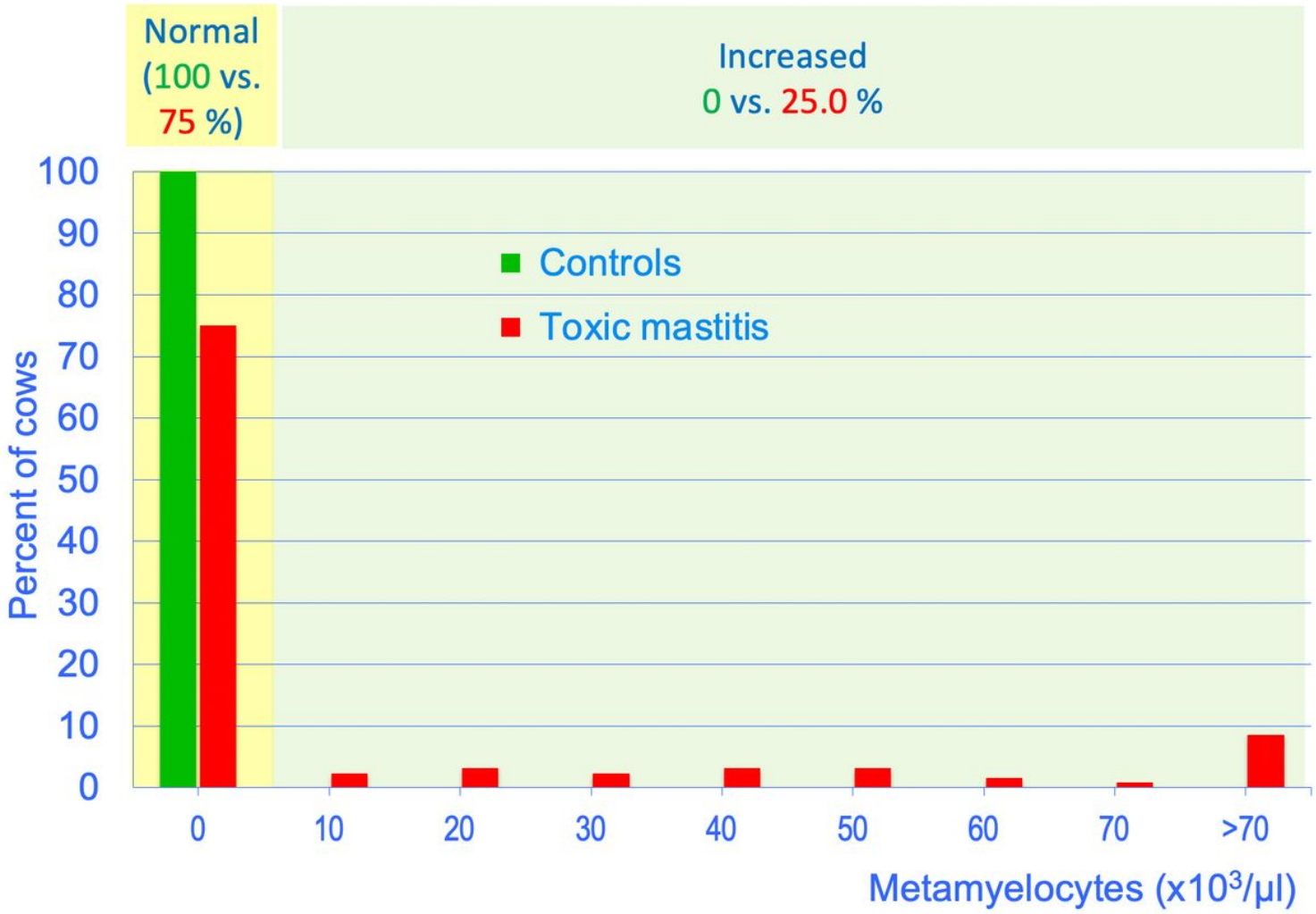

Figure 5

Frequency distributions of metamyelocytes in 168 healthy control cows and in 128 cows with toxic mastitis.

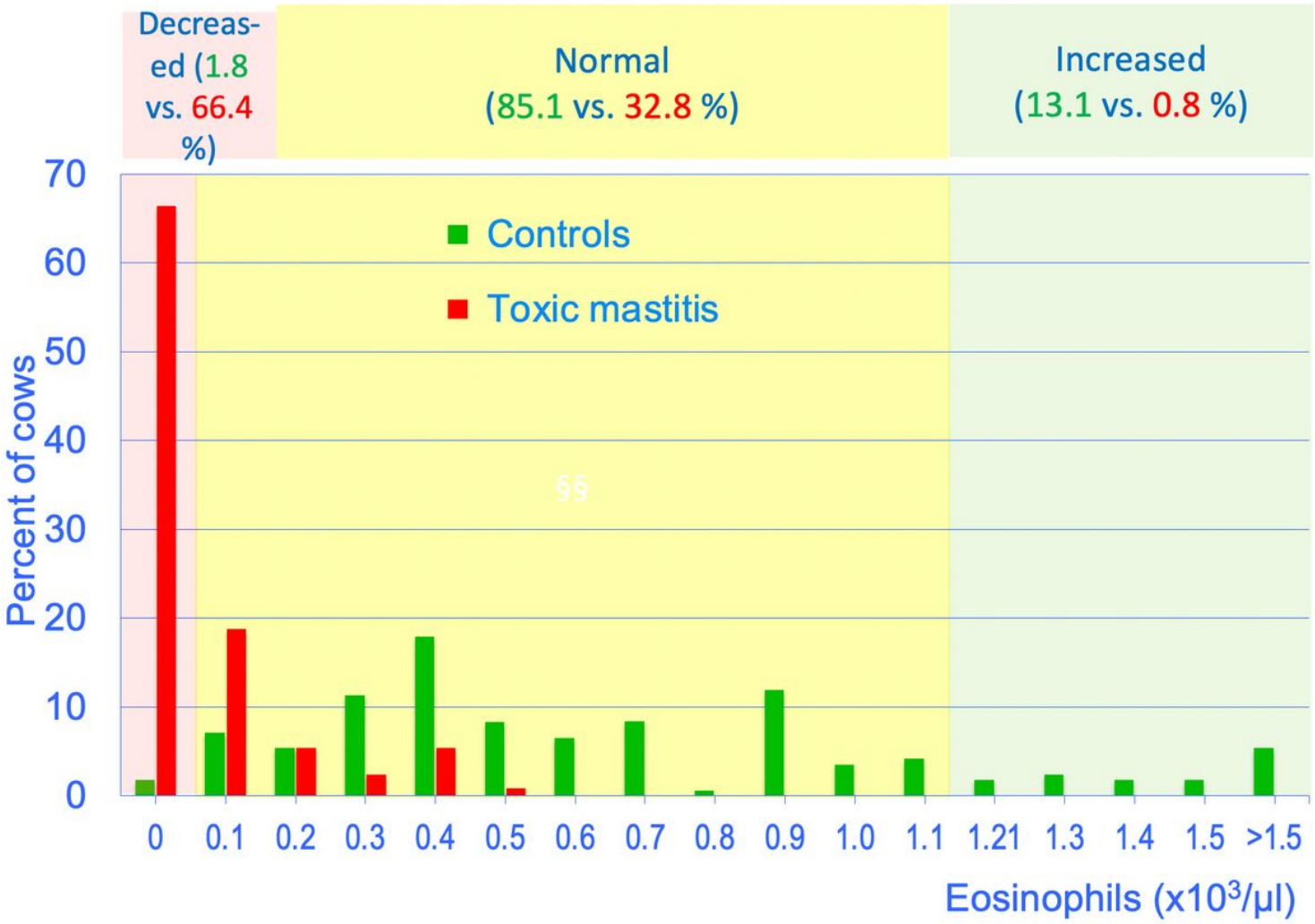

Figure 6

Frequency distributions of the eosinophils in 168 healthy control cows and in 128 cows with toxic mastitis.

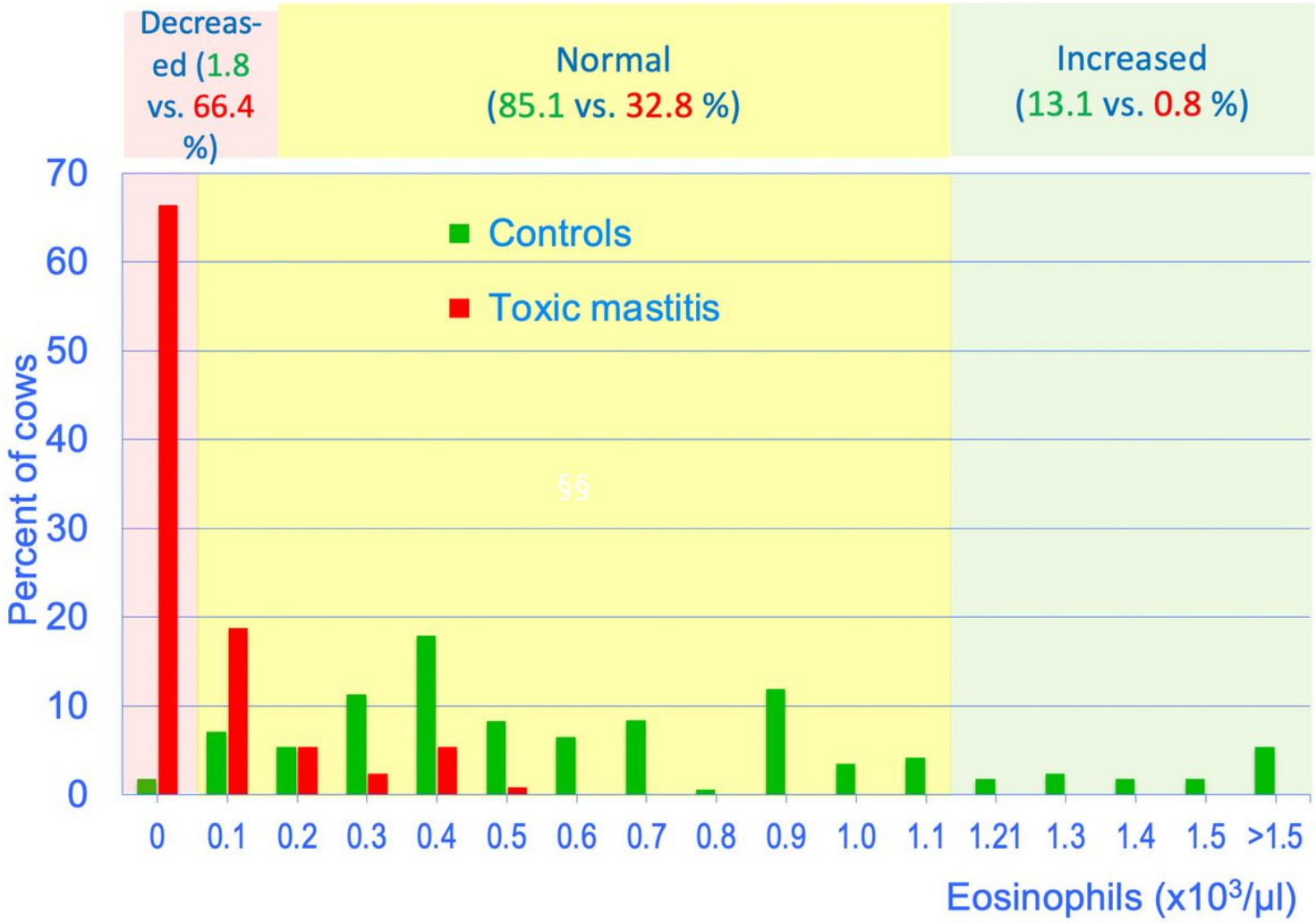

Figure 6

Frequency distributions of the eosinophils in 168 healthy control cows and in 128 cows with toxic mastitis.

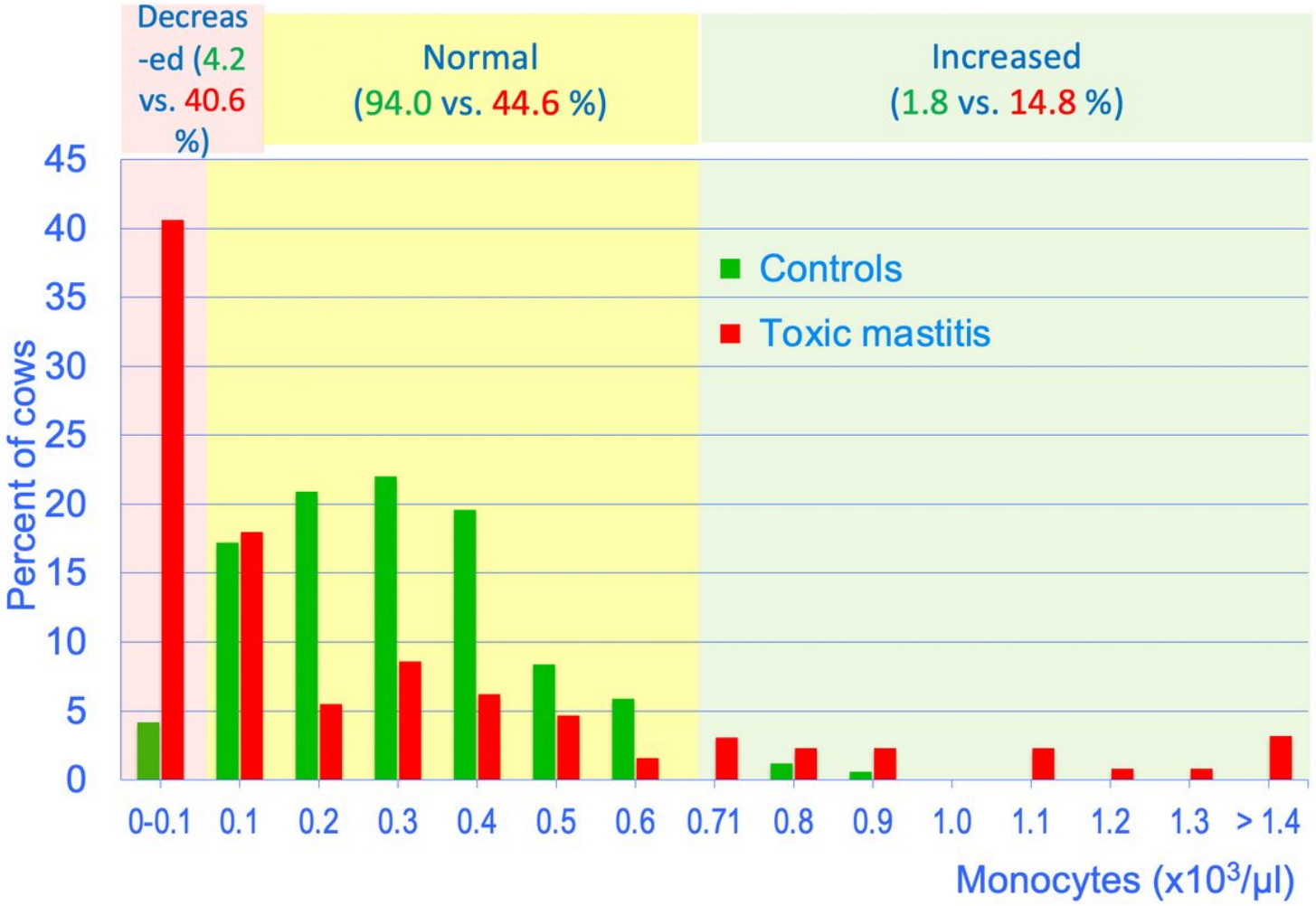

Figure 7

Frequency distributions of monocytes in 168 healthy control cows and in 128 cows with toxic mastitis.

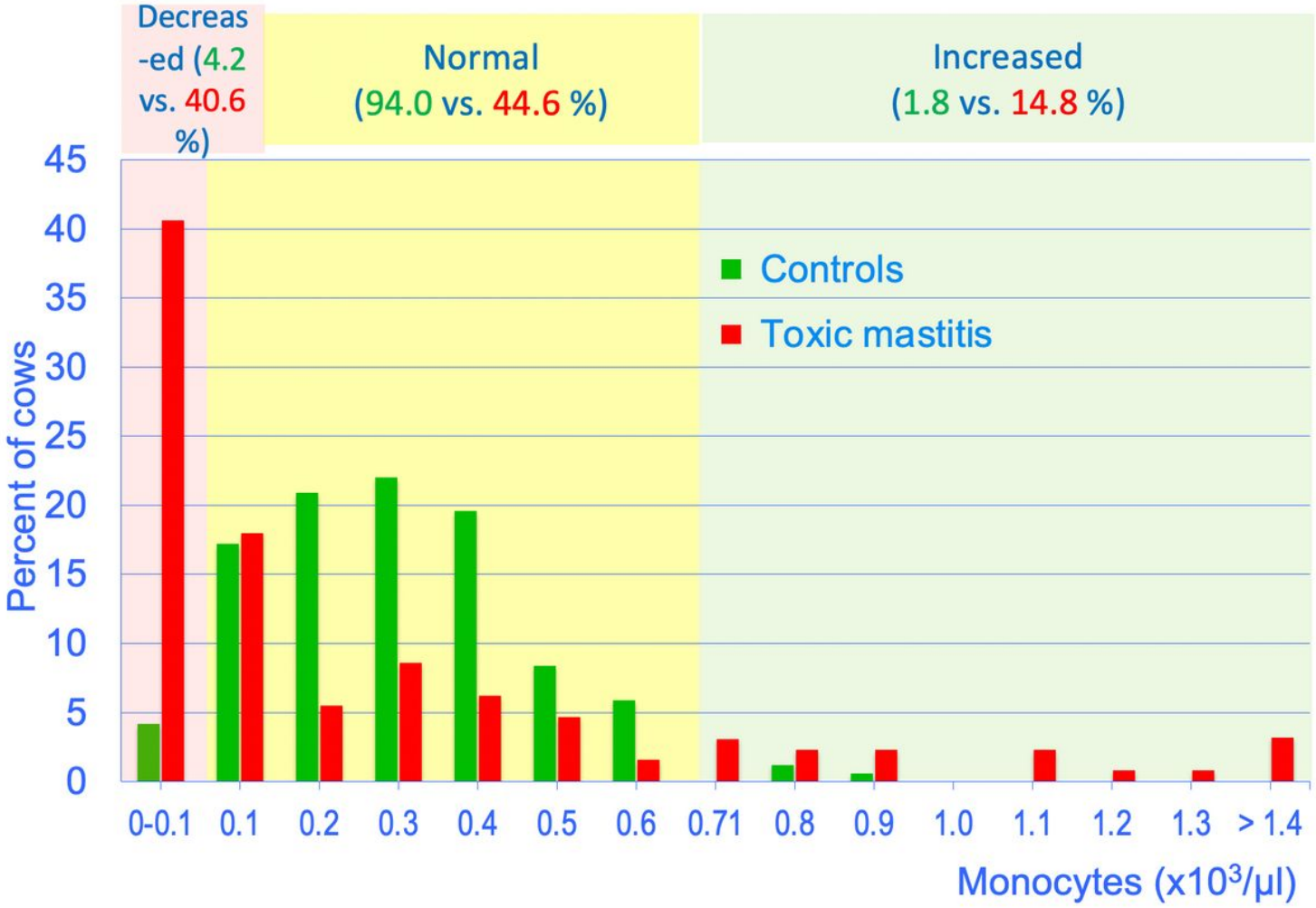

Figure 7

Frequency distributions of monocytes in 168 healthy control cows and in 128 cows with toxic mastitis.

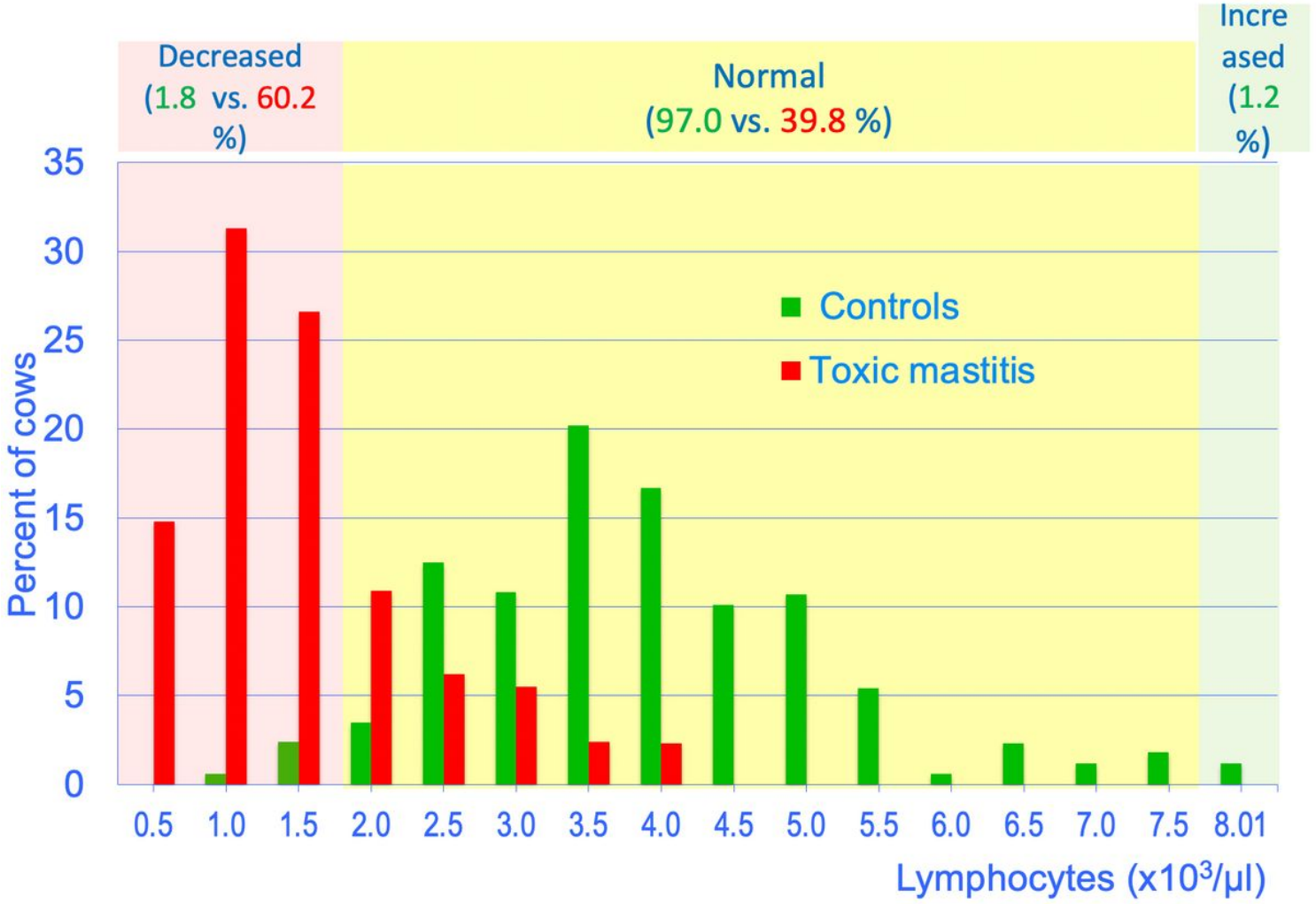

Figure 8

Frequency distributions of lymphocytes in 168 healthy control cows and in 128 cows with toxic mastitis.

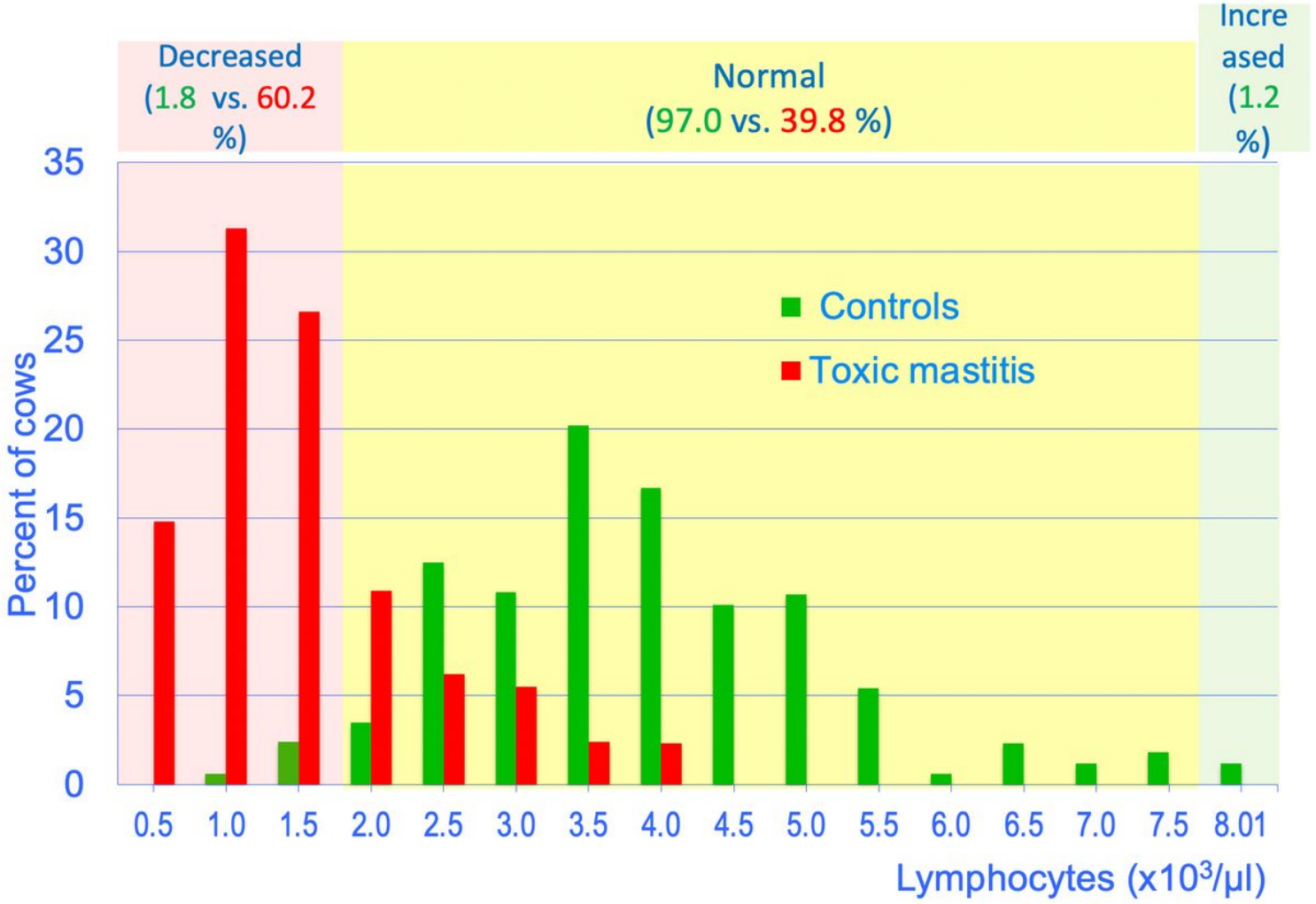

Figure 8

Frequency distributions of lymphocytes in 168 healthy control cows and in 128 cows with toxic mastitis.

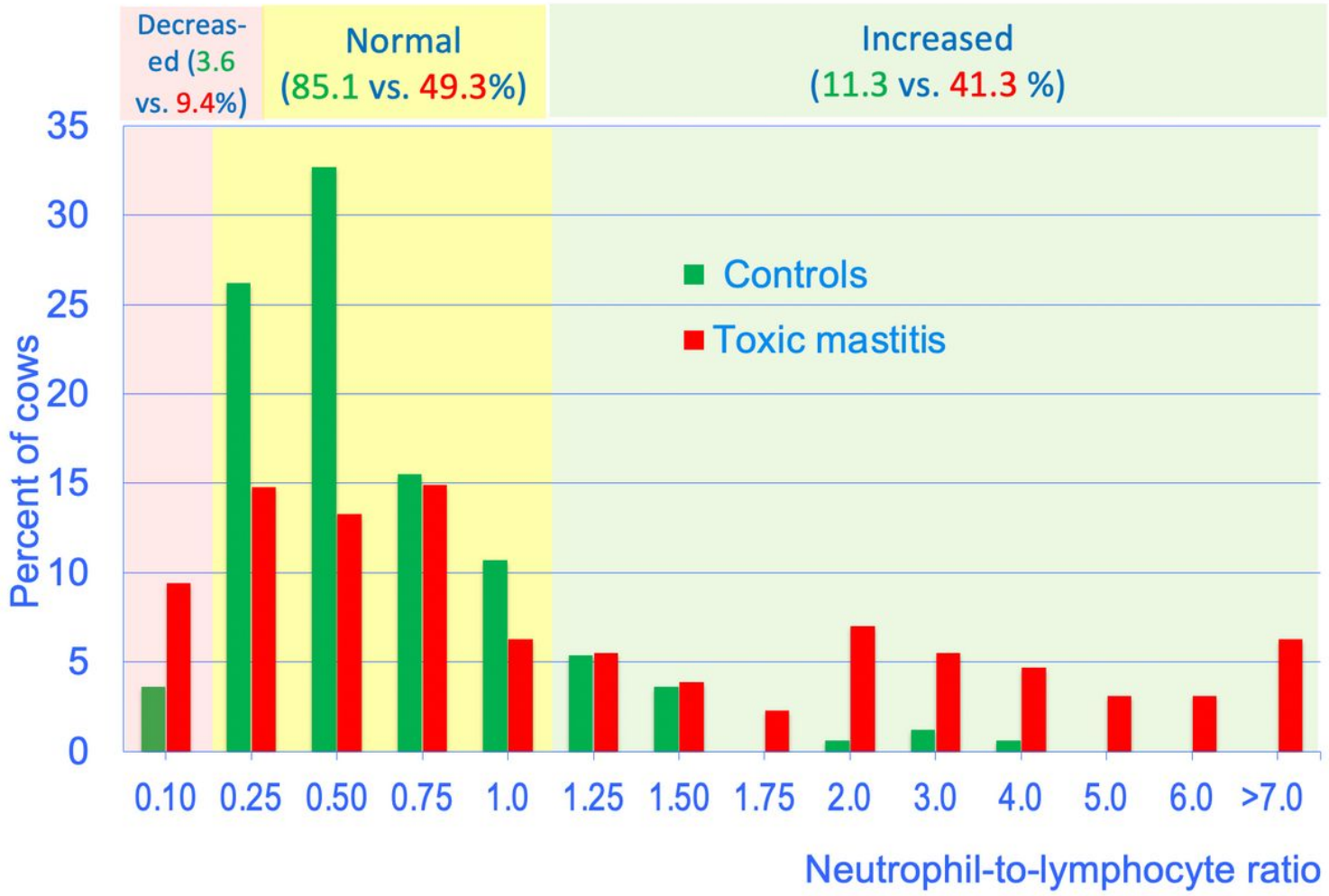

Figure 9

Frequency distributions of the neutrophil-to-lymphocyte ratios in 168 healthy control cows and in 128 cows with toxic mastitis.

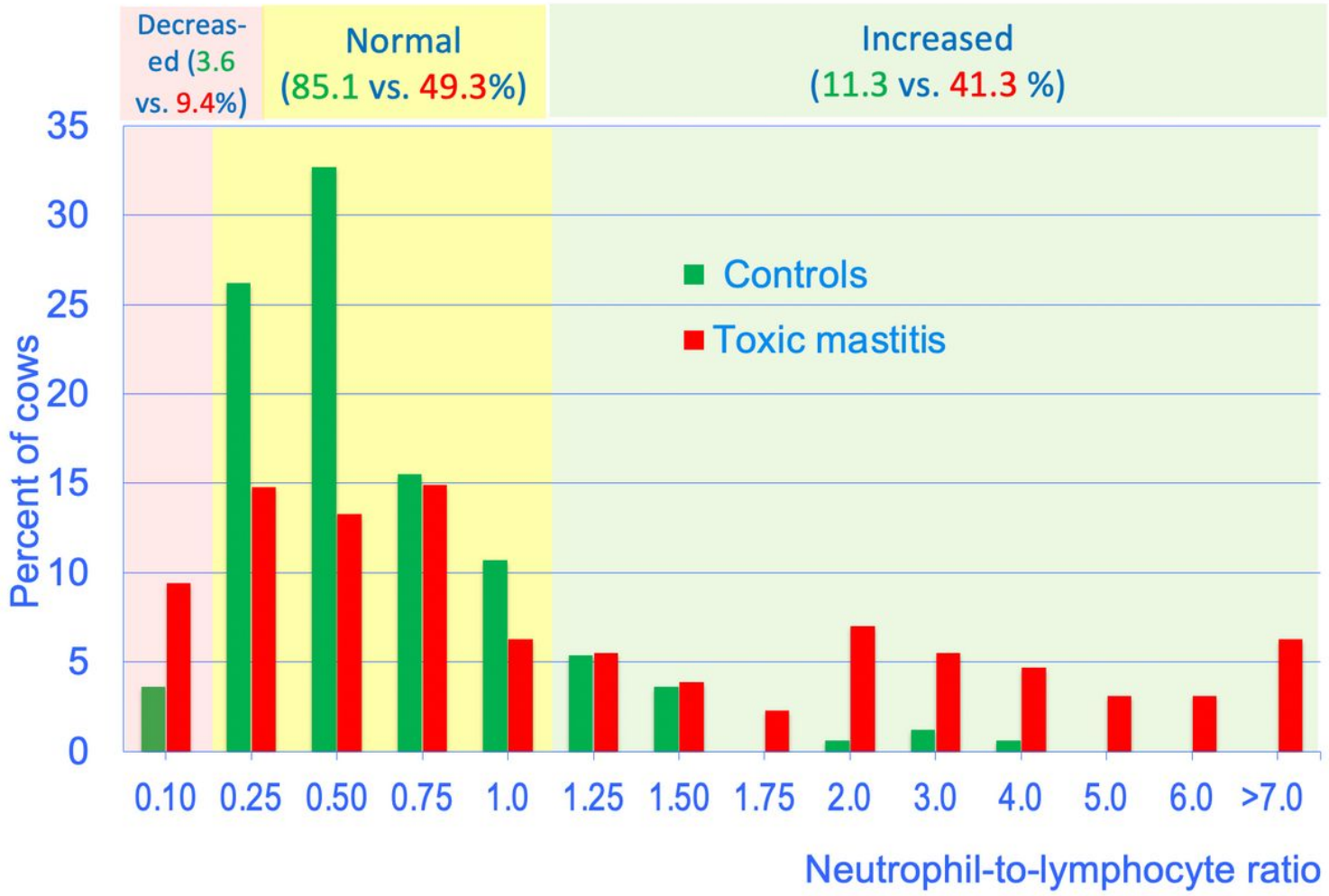

Figure 9

Frequency distributions of the neutrophil-to-lymphocyte ratios in 168 healthy control cows and in 128 cows with toxic mastitis.