Chapter

How Reliable Are Laboratory Test When Diagnosing Bitch Mastitis?

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Abstract

Canine mastitis represents a major threat for both pups and nursing bitch. If left untreated, it can complicate with neonatal death, sepsis, and *mastitis gangrenosa*; for this reason quick and accurate diagnosis and treatment initiation are crucial health restoration. Even though mastitis is considered to be an emergency, most of the time it is overlooked. Henceforth, clinicians should be aware of the clinical importance of mastitis and that laboratory assays such as milk pH, cytology, and biochemistry (milk and serum) are of high utility. Furthermore, milk microbiology and susceptibility tests are still important since they bring additional information about the pathogenesis and the treatment possibilities.

Keywords: canine, mastitis, milk, cytology, pH, diagnostic

1. Introduction

Mastitis is an inflammation of the mammary glands. It can affect a segment or the entire mammary gland, one or more glands at the same time. Usually, the milk secretion is found modified, with a yellow-greenish to brown color, with a modified consistency, but there are also cases where the milk secretion is grossly unmodified. The ill bitch presents local signs of illness such as painful hot, red, engorged mammary glands, with or without general signs of disease like depression, anorexia, neglecting puppies, tacky mucous membranes, dehydration, delayed capillary refill time with sepsis, and even *mastitis gangrenosa*. For the litters, the main signs consist of failure to thrive and weight gain [1].

Mastitis has also been reported in *lactatio sine graviditate* (false pregnancy) bitches, as a result of milk retention and consistent licking of the activated mammary glands. Since this condition is evolving as clinical or subclinical, usually, in pseudocyesis dams, mastitis occurs because of an allergic reaction of the mammary gland to its own milk casein [1]. However, since every bitch undergoes *lactatio sine graviditate* [2], this condition should be addressed as an atavism and not a pathological behavior.

According to the pathogenesis and clinical signs, mastitis in bitches is classified as *mastitis acuta*, *mastitis gangrenosa*, galactostasis, *mammae* congestion, and

subclinical mastitis. Furthermore, early recognition of galactostasis and *mammae* congestion is of great importance since, if not treated accordingly, it could lead to the development of mastitis.

Mastitis acuta cases are characterized by the presence of fever (40–41°C); hypertrophy of the mammary gland; extremely painful, hot, swollen mammary tissues, with a modified milk secretion (**Figures 1** and **2**); dehydration; anorexia; pyrexia; depression; and even sepsis in severe cases. Blood examination reveals neutrophilia with a left shift. Bacteriological examination of milk is positive in most cases [3].

Mastitis gangrenosa is described as the presence of severe local and systemic reaction characterized by fever (40–41°C), while affected mammary glands have a dark to purple color and are extremely painful, hot, and swollen; animals are dehydrated, anorectic, pyretic, and depressed and show signs of sepsis. Blood analysis reveals anemia, leukocytosis, and thrombocytopenia. Milk analysis shows an alkaline milk and presence of active inflammatory cells. Bacteriological examination of milk samples is also positive [3].

Subclinical mastitis is diagnosed based on clinical record, physical examination, and milk analysis (pH, cytology, and microbiology, among others). Usually, in subclinical mastitis cases, there is no visible alterations in mammary gland or milk secretion, while milk analysis indicates an alkaline pH and the increased number of

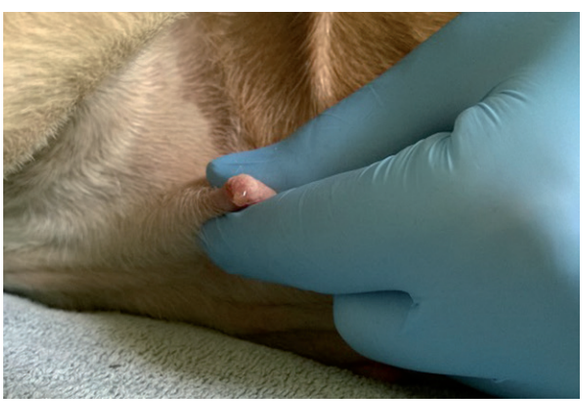

Figure 1.
A serous milk sample from a bitch suffering from mastitis acuta.

Figure 2.
A mucopurulent milk secretin from a bitch diagnosed with mastitis acuta.

somatic cell count (SCC; neutrophils, foamy cells, macrophages) (**Figures 3** and **4**). Bacteriological examinations are positive [3].

In galactostasis cases, there is no sign of infection, but the glandular tissue tends to get distended (**Figures 5** and **6**), warm, and painful. Small litters and absence of puppies will promote the buildup of a high pressure of milk into the undrained mammary glands, which will allow for the milk pH to become alkaline but will not change the milk chloride levels. Milk cytology will reveal the presence of increased count of eosinophils [3].

Bitches with *mammae congestion* can present local signs of mastitis including hardened, painful mammary glands and acidic milk pH. However, no inflammatory

Figure 3.
Clinical aspect of a healthy mammary gland.

Figure 4.
Milk secretion, from a clinically healthy mammary gland.

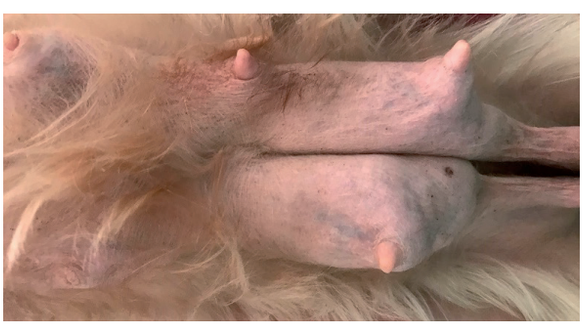

Figure 5.
Distended mammary glands with galactostasis in a bitch that just weaned. Such glands are predisposed to develop retention mastitis.

Figure 6.
Normal milk secretion from the same bitch.

cells and phagocytosis are detected in milk smears. Discomfort during nursing is sometimes present. Bacteriological examination of milk samples is positive [3].

2. Laboratory assays used in the diagnosis of lactating bitch mastitis

Bitch mastitis diagnostic is performed by laboratory assays such as chloride levels, milk pH [4], and milk cytology [5, 6] alongside microbiology susceptibility testing [7]. Hematology and biochemistry assays such as total solids (TS), total proteins (TP) [8], CRP [3], or haptoglobin (Hp) measurements [4, 9] are also taken into account.

2.1 Microbiologic analysis of bitch milk

For microbiology analysis, milk samples have to be collected aseptically into sterile vials manually [10], using sterile swabs impregnated with transport medium [7] or directly onto sterilized bacteriological loops [11]. In all cases a first milk drop has to be discarded. Some authors even describe the use of fine needle aspiration technique to collect milk samples [12], but these can be traumatic and painful.

For bacteria isolation and identification, standard microbiology techniques can be employed. Usually, milk is plated onto different media depending on the agent to isolate. For example, blood agar is used for *Staphylococcus* and *Streptococcus* spp., and MacConkey is used for *Enterobacteriaceae*. The samples are incubated for 24–72 h at 37°C in aerobic and modified atmosphere (5% CO_2) conditions [13]. The identification of bacteria can be made initially by macroscopic observation of colonies, Gram stain, cellular morphology, oxidase, catalase, mobility and oxidation-fermentation tests, and in the particular cases of *Streptococcus* spp. the type of hemolysis and in *Staphylococcus* spp. the coagulase activity [7].

For the identification of bacterial species, the commercially available biochemical incorporated analytical profile index (API) systems can be used [13]. Additionally, cultural procedures on solid media are performed by 2 days of enrichment in Thioglycollate medium fluid and Trypticase Soy Broth [13].

For the particular case of the isolation of lactobacilli, the procedures consisting in sample culture in aerobic and anaerobic conditions are recommended. The aerobic condition for lactobacilli isolation consists in sample dilution with peptone water and their culture on De Man, Rogosa and Sharpe agar (MRS) plates for 24 h

at 37°C in aerobic conditions. The anaerobic condition consists of the same sample culture on MRS supplemented with L-cysteine (0.5 g/L) and (MRS-Cys) agar plates, for 48 h at 37°C in anaerobic conditions (85% nitrogen, 10% hydrogen, 5% carbon dioxide) [10].

Recently, milk strains that are also found in human milk secretions [14] were also isolated from the milk of bitches suffering from both clinical and subclinical mastitis [15]. Along standard microbiology techniques [16], after isolation, bacterial strains were identified by using Vitek2 (bioMérieux, l'Étoile, France) technology, respecting the manufacturer's guidelines [15]. A total of 57 different strains were isolated, with *Staphylococcus* spp., *E. coli*, and *Proteus mirabilis* as main isolates (**Table 1**).

Since they are often isolated from various infection sites, *Staphylococcus pseudintermedius* and *Streptococcus canis* are of major importance in companion animals [17]. In addition, other pathogens have been isolated from milk samples of bitches with acute *mastitis*; these include hemolytic *Staphylococcus* spp., *Staph. intermedius*, *Staph. haemolyticus*, β-hem. *Streptococcus*, *Klebsiella pneumoniae*, *E. coli*, and *Proteus mirabilis* [13]. In addition, there have been some case reports that proved that *Staph. hyicus* can produce mammary gland inflammation accompanied by lymphadenitis. The pathogen was isolated from a 3-year-old pit bull female, which died after a *mastitis acuta* episode [18].

Also in cases of asymptomatic females, *Staph. intermedius*, hem. *Staphylococcus* spp., *Staph. epidermidis*, *Staph. simulans*, β-hem. *Streptococcus*, *E. coli*, *Enterococcus durans*, *P. stutzeri*, *Shigella* spp., *Acinetobacter anitratus*, and *Bacillus* spp. have been isolated [13]. Jung et al. also isolated α-hemolytic *Streptococci* and γ-hemolytic *Streptococci* [19].

Nevertheless, some authors suggest that exogenous infections of the mammary glands are rare and mainly are due to endogenous pathogens [20]. This hypothesis would be supported by Martín et al., who described the presence of lactobacilli

No.	Milk bacterial pathogens	Percentage (%)
1.	*Staphylococcus* spp.	27.17
2.	*Escherichia coli*	25.00
3.	*Proteus mirabilis*	9.24
4.	*Enterococcus faecium*	7.61
5.	*Staphylococcus pseudintermedius*	7.07
6.	*Staphylococcus simulans*	5.43
7.	*Agrobacterium radiobacter*	4.89
8.	*Pseudomonas aeruginosa*	3.80
9.	*Staphylococcus xylosus*	3.80
10.	*Staphylococcus hominis* subsp. *hominis*	3.26
11.	*Bacillus* spp.	2.72
12.	*Enterococcus faecalis*	2.72
13.	*Micrococcus luteus*	2.72
14.	*Staphylococcus intermedius*	2.72
15.	*Streptococcus* spp.	2.72

Table 1.
Main milk isolates from lactating bitches in the periparturient period [15].

(*L. murinus*, *L. animalis*, *L. reuteri*, *L. johnsonii*, and *L. fermentum*) *E. faecium*, *E. faecalis*, *Strep. salivarius*, *Staph. epidermidis*, *Strep. bovis*, *Staph. simulans*, *Staph. pseudintermedius*, *W. viridescens*, and yeast in milk of healthy bitches [10]. Furthermore, many of the bacterial pathogens involved are part of the urogenital microflora and, therefore, are opportunistic pathogens that cause a disease in the presence of other predisposing factors. It should be noted that enterococci, *E. faecalis*, and *E. faecium* strains constitute a pathogenic reservoir for offsprings [21] and, thus, could be responsible for neonatal infections [22, 23].

Ascending mammary gland infections can be caused by poor hygiene and/or the exogenous transmission by both skin and oral mucosa bacteria strains of both bitch and the offsprings [24]. This kind of infection is especially frequent in *lactatio sine graviditate* cases, where the bitch frequently licks affected mammary glands [19]. Furthermore, some reports identified the same bacterial strains from the serum of puppies diagnosed with septicemia and bitch milk [23, 25]. Reproductive track disorders including pyometra, metritis, or vaginitis can predispose to inflammation of mammary gland tissue resulting in "fading puppy syndrome," endotoxemia, or septicemia development in litter and neonatal mortality [7, 13, 20, 26]. Special care should be also taken by owners and veterinary specialists when handling mammary glands of lactating bitch and puppies [27], since a risk of pathogen transfer from people to bitch milk is high [28, 29].

An adequate management of mammary gland infection is of major importance. Milk samples should be aseptically obtained for cytology analysis and microbial cultures and before initiation of therapy with antibiotics [30]. Subclinical mammary infection can result in postpartum complications (i.e., mortality in pups, antibiotic resistance); for this reason many dog breeders treat bitches before and after parturition with antibiotics to reduce the neonatal mortality risk. But this practice is not recommended since it will predispose to vagina colonization by opportunistic pathogens and will increase antibiotic resistance [25, 27, 31].

After the milk samples are collected for analysis, mastitis treatment may be empirically initiated [2, 24]. However, caution should be taken when selecting the optimal antibiotic. Weak base antibiotics, like trimethoprim-sulfonamide, distribute and get trapped into milk due to the acidic nature of the milk. As milk pH will generally become alkaline due to bacterial infections, macrolides or amoxicillin-clavulanate and cephalosporins may be used before culture results arrive [2, 24]. Irrespective of milk pH, enrofloxacin can be used to start treatment, even in patients that continue to nurse by weighing the pros and cons, regarding puppy cartilage abnormalities [24].

2.2 Bitch milk biophysical analysis

Usually, due to the low volume of milk acquired from bitches, milk pH is evaluated using litmus paper [4]. Healthy bitches have slightly acid pH of about 6.3 similar to that recorded in cows (pH = 6.63) [32]. In terms, animals suffering from *mastitis acuta* have an alkaline milk (pH = 7), while females diagnosed with *lactatio sine graviditate* present pH similar to healthy ones (pH ~ 6.7) [4, 33]. There is also a report of a milk pH value of 8, for a *mastitis obliterans* case reported in a dog with diabetes [34]. Chloride levels registered show an increase in (mean = 199 mg%) cases of mastitis. Healthy females and females with *lactatio sine graviditate* have been found to have similar results for chloride levels, 84.3 and 84.1 mg%, respectively [4].

Recent research [15] shows that milk pH value is influenced by the lactation period and each specific type of mastitis.

In the antepartum period, milk pH values ranged between 6 and 7. A total of 75% of tested samples had an acidic milk pH reaction [15].

In the postpartum period, milk pH obtained values ranged between 6 and 8.5. An alkaline milk reaction was obtained in 83% of retention mastitis cases, 80% of mammary congested and subclinical cases of mastitis, respectively, and 75% of *mastitis acuta* cases. Healthy mammary glands had an acidic milk pH in 73% of examined milk secretions [15] (**Table 2**).

Postpartum	**Acidic**	**Alkaline**	***Mammae* diagnostic**	**Acidic**	**Alkaline**
Healthy *mammae*	73%			73%	
Mastitis acuta		75%			67%
Mammary congestion		80%			80%
Subclinical mastitis		80%			81%
Retention mastitis		83%			96%

Table 2.
Milk pH reaction according to the postpartum period (left) and to the mammary gland diagnostic pH reaction (right).

From *lactatio sine graviditate* bitches, milk pH values ranged between 6.5 and 9.5. All (100%) tested samples from retention mastitis glands in this period had an alkaline milk pH reaction [15].

Irrespective of the lactation period, an alkaline milk pH reaction was obtained in 96% of retention mastitis cases, 81% of subclinical mastitis cases, 80% of mammary gland congestion cases, and 67% of *mastitis acuta* suffering mammary glands. For healthy mammary glands, about 73% of tested samples had an acidic milk reaction (**Table 2**).

For the antepartum period, the normal mean pH value was 6.5, followed by the postpartum period with a mean normal pH value of 6.57 [15].

In the antepartum period for *mastitis* acuta, mean pH value was 6. In the postpartum period, the mean pH value for congested mammary glands was 6.6, for subclinical cases it was 7, and for *mastitis acuta*, the mean value was 7.04, while for retention mastitis, the mean value was 7.5 [15].

For *lactatio sine graviditate*, the mean pH value for retention mastitis was 7.9 [15].

Results show that an alkaline milk pH is prone to be developed in undrained mammary glands, in cases of retention mastitis and pseudopregnancy or in cases of small litters [6], where milk spends more time in the glandular tissue, thus explaining why, in some cases of *mastitis acuta*, milk pH is still acidic during the time of examination.

Care should be taken whenever one evaluates bitch milk pH reaction, since interpretation of results by litmus paper is very subjective and can be very deceiving to the naked eye. Future research should better adapt to the morphophysiological particularities of the bitch mammary gland in order to obtain irrefutable results whenever one should interpret bitch milk pH reaction [6].

2.3 Bitch milk cytology analysis

Only few studies focused on bitch milk cytology and only two of them evaluated canine milk cytology in deep [5, 6].

Smears for milk cytology are carried out using squash technique [35]. The squash technique is a procedure used for semisolid, mucus-like, or pelleted by

centrifugation cytology specimens. In case of milk analysis, a drop (~20 μL) of milk is plated onto objective glass slide, approximately 1 cm from the frosted end, covered by the second slide, gently but firmly compressed, and the sample is extended over the bottom slide by pulling the top one along the surface. In this way, the sample is redistributed, turning a multicellular mass into a thin monolayer ideal for stain penetration and optical assessment of individual cell morphology by a microscope. Properly prepared smears present a feather-shaped area with a monolayer end referred to as the "sweet spot" [35].

Smears are stained by using the Giemsa [11], Wright-Giemsa [5], Wright-Leishman [12], and Romanowsky-type stains; these last ones are preferred due to easy use and rapid turnaround [35]. Stained smears permit evaluation of different types of inflammatory cells and calculation of their amounts in percent within the smear [11].

For total SCC in milk determination, white blood cell (Unopette—Becton Dickinson) and hemocytometer counting chambers are used [5]. Diff-Quick solution II was recommended to be used in order to differentiate inflammatory milk cells from fat droplets. Total SCC of milk samples are expressed as cells/μL of milk [5].

In milk of healthy bitches, although cytology variations among mammary glands of the same animal are described, commonly somatic cells, accompanied by a high amount of cellular debris, many squamous epithelial cells (**Figure 7**), few neutrophils, macrophages, erythrocytes, and foamy cells are detected [6]. Polymorphonuclear leukocytes with pyknotic nuclei can be also present [5]. SCC is lower after delivery and increases afterward (mean SCC 2 weeks after delivery, 3 × 106/mL; 3–5 weeks after, <3.8 × 106 mL/L; and 6–8 weeks after delivery, <4.9 × 106/mL) [4]. Milk samples collected from milking bitches present more fat droplets than samples collected from females' resorbing milk. Usual findings in milk smears from healthy bitches are bacteria, resulting from skin contaminants. However, caution should be taken when bacteria are detected, since if this observation is accompanied by increased SCC, it would indicate infection of mammary gland [5, 36].

In dams with *mammae* congestion, the milk smear is characterized by few numbers of somatic cells, accompanied by increased numbers of cellular debris (**Figure 8**), few neutrophils, inactivated macrophages, and epithelial cells [6].

In subclinical mastitis cases, the presence of somatic cells is moderate to high, accompanied by slightly elevated numbers of degenerated neutrophils, many foamy (**Figure 9**) and epithelial cells along with activated macrophages, bacteria, and phagocytosis. Scattered cellular debris, erythrocytes, and eosinophils can also be encountered [6].

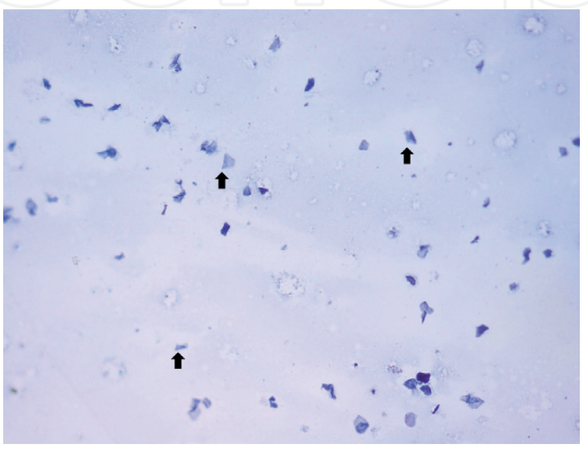

Figure 7.
Presence of many squamous epithelial cells on a basophilic background (black arrows) (MGG, ×10) [6].

Episodes of galactostasis are characterized by the presence of eosinophils (**Figure 10**), activated macrophages, foamy cells, and degenerated neutrophils, accompanied by bacteria and phagocytosis. Cellular debris and erythrocytes had also been identified [5, 6].

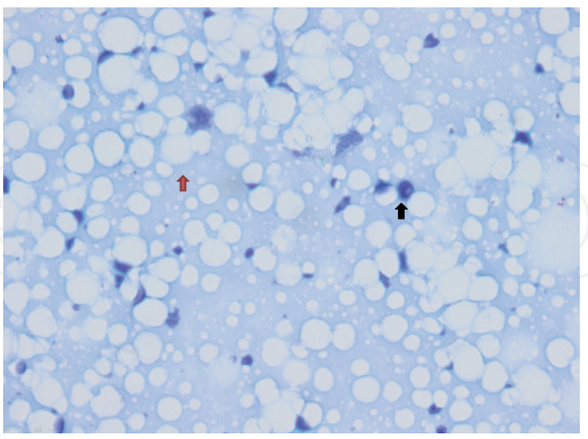

Figure 8.
Presence of cellular debris on a basophilic background (red arrow) with variable-sized lipid droplets (black arrow) (MGG, ×40) [6].

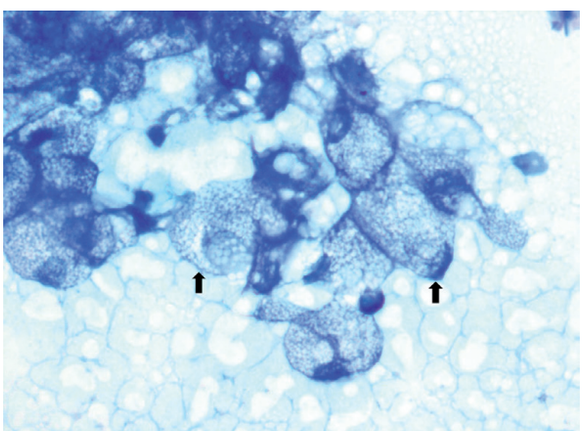

Figure 9.
Presence of agglutinated foamy cells on a vacuolar background (black arrows) (MGG, ×100) [6].

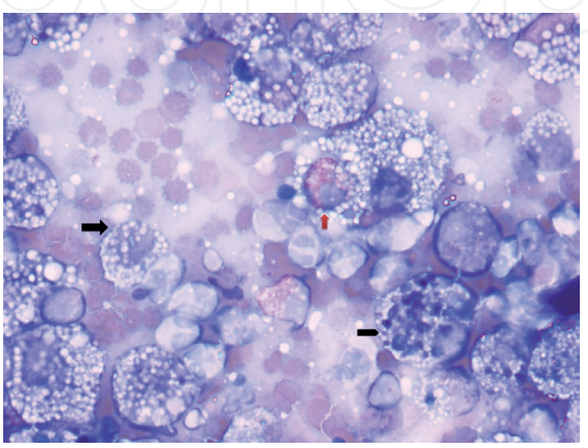

Figure 10.
Presence of eosinophils (red arrow), erythrocytes, and foamy cells (black arrow) with hemosiderin phagocytosis (arrow head) (MGG, ×100) [6].

Episodes of *mastitis acuta* (including *mastitis gangrenosa*) are accompanied by an increase in SCC and are characterized by an increased number of foamy cells, degenerated neutrophils (**Figure 11**), cellular debris, and bacteria accompanied by phagocytosis (**Figures 12–14**). The presence of small numbers of activated macrophages, eosinophils, and erythrocytes was also reported [6, 12].

In human medicine, in cases of breast inflammatory lesions due to acute *actinomycosis*, numerous polymorphonuclear leukocytes, scattered ductal cells, and necrotic material are found on milk smears [37], while in chronic pathologies, such as breast tuberculosis lesions, columnar, oddly shaped, and multinucleated giant cells surrounded by inflammatory infiltrate can be detected in milk smears [38].

Experimental inoculation of *Staph. intermedius* in the mammary gland of six female Beagle dogs resulted in increased SCC in milk 12 h after in infected and control glands [11]. At initial stages, smears performed with milk from affected mammary glands predominantly contained neutrophils (>75%). From day 3 post-challenge, degenerated neutrophils were present on smears, and on day 6, lymphocytes were also present (20%) that began to predominate from day 14. Smears performed with milk obtained from control glands showed similar findings [11]. Besides these evidences, some authors still opine that cytology of bitch milk has no clinical importance [24, 39, 40]. Nevertheless, the use of milk cytology has proven

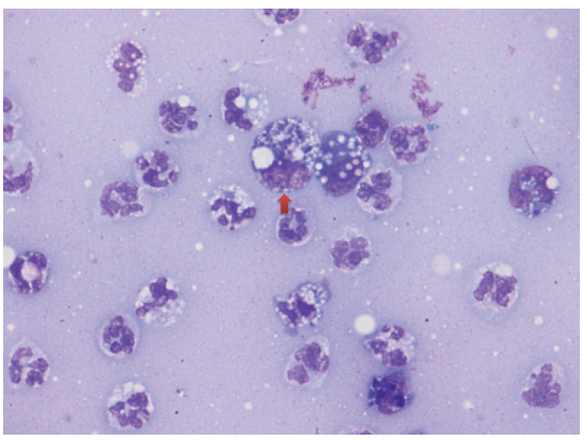

Figure 11.
Presence of many neutrophils and foamy cells (red arrow) on a basophilic background with discrete lipid droplets (MGG, ×100) [6].

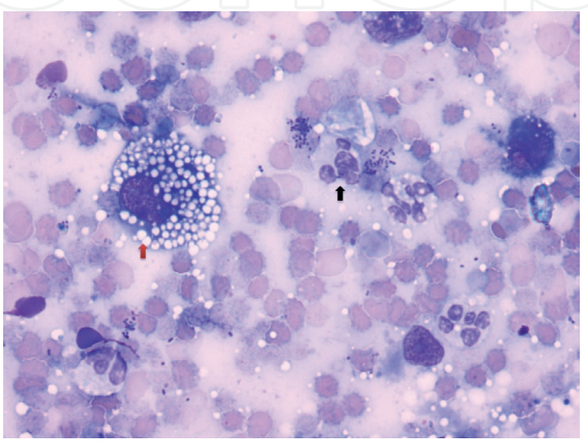

Figure 12.
Presence of foamy cells (red arrow), erythrocytes, and degenerated neutrophils with bacterial phagocytosis (black arrow) on an eosinophilic, vacuolar background (MGG, ×100) [6].

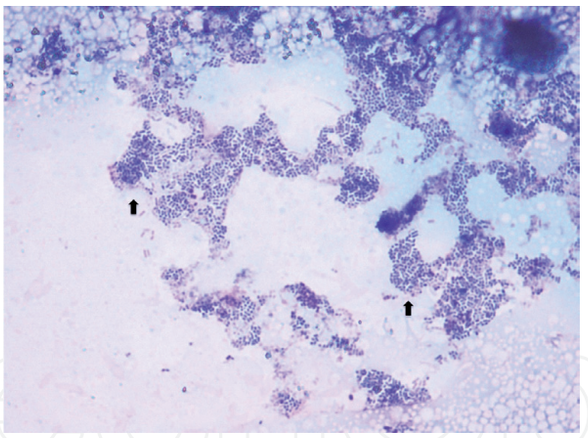

Figure 13.
Presence of cluster-organized round-shaped bacteria (black arrow) on a milk smear from a septic mammary gland (MGG, ×100) [6].

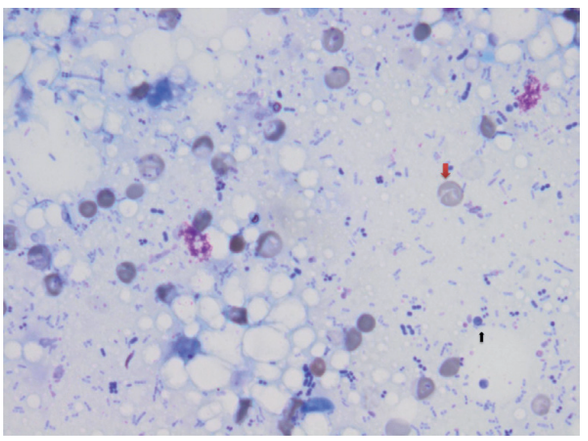

Figure 14.
Presence of erythrocytes (red arrow), cell debris, and many rod-shaped, respectively, round bacteria (black arrow) in a milk sample from a septic mammary gland (MGG, ×100) [6].

to be of aid whenever a mastitis diagnostic protocol was required [6, 41, 42]. Milk cytological evaluation can be helpful in diagnosing mammary gland inflammations, by helping clinicians to confirm the presence of clinical mastitis and avoid agalactia in the most developed mammary glands as well as secondary bacterial infections in infrequently milked mammary glands [6].

Milk cytology can be of help in parasitic disease diagnosis, e.g., nematodes (*Dirofilaria repens* of about 350 μm in length) were detected in nipple secretion fluid smears of a 6-year-old Bernese female dog. Glandular cell clusters and inflammatory cell including macrophagic foamy cells, neutrophils, and eosinophils were also detected in milk of this dog [43].

Furthermore, milk cytology helps to discriminate between mammary cancer and mastitis cases. While the prognosis is guarded to poor in cancer [44], it is usually good in mastitis, except for gangrenous cases. Thus, it is of high importance to differentiate the two pathologies, and a correct interpretation of mammary or milk cytology will lead to a correct diagnosis [27].

2.4 Hematological and biochemical analyses

Hematological and biochemical analyses are recommended to evaluate the general health status of the patient and specially to evaluate and monitor the degree of inflammation that can be done by the study of the leukocytes in the hematology

and the acute-phase proteins and electrophoretic separation of the proteins in the biochemical profile.

M*astitis gangrenosa* in bitch was characterized by the presence of leukocytosis (36,300/μL); increased α-, β-, and γ-globulins; anemia; and increase in plasma analytes (alkaline phosphatase and creatine phosphokinase) and electrolytes (sodium and potassium) [33]. In an experimental infection, a significant increase in total leukocyte counts and neutrophils that returned to pre-challenge values after the sixth day has been reported [11]. In addition to total leukocyte and neutrophil increases, other changes that can occur in analytes in mastitis are monocytosis (for chronic cases), hypoalbuminemia, hyperglobulinemia, and mild dilutional normochromic, normocytic, and non-regenerative anemia due to precedence of pregnancy, eosinophilia, and changes in markers of the liver and kidney and in electrolytes. In severe cases that develop SIRS or sepsis, hypoglycemia, hypoalbuminemia, and leukopenia with consecutive coagulopathies are also encountered [36, 40].

Recent data [8] shows that hematology and other blood tests such as packed cell volume (PCV) and TP are not very reliable ($p > 0.05$) when trying to diagnose or evaluate the mammary gland health status in bitch [8]. However, there are isolated cases of *mastitis acuta* described in bitch that evolved with lymphocytosis with a degenerative left shift [41] or with normocytic normochromic anemia, lymphopenia, and reactive thrombocytosis [42].

In the last years, acute-phase proteins (APPs) that can be major such as serum amyloid A (SAA) and C-reactive protein (CRP); moderate such as α1-acid glycoprotein (AGP), fibrinogen (Fb), and haptoglobin (Hp); and negative such as albumin [45] had started to be used to assess and monitor inflammatory diseases in veterinary medicine [46]. Acute-phase proteins are sensitive and non-specific markers of inflammation. They indicate the existence of an inflammatory stimulus [45, 46] and cannot diagnose a specific pathology; thus the final diagnostic should be pronounced according to other laboratory assays (**Table 4**) and clinical status.

The acute-phase response is induced by any type of tissue injury or infections that stimulate the release of different cytokines from macrophages and monocytes [47] that induce the synthesis of APPs by hepatocytes [46, 47]. In dogs, increases in APPs (SAA, CRP, Hp, Fb, and ceruloplasmin) in response to ovariohysterectomy in both healthy and with pyometra bitches have been reported. In the same sense, altered APP levels in pregnancy and estrus have been described in healthy female dogs [48–52].

Recently, by using the time-resolved immunofluorometric assay (TR-IFMA) method [53], CRP levels in bitch milk and serum were evaluated [3]. In both clinical and subclinical mastitis (**Table 2**), CRP milk (0.3–40.0 μg/mL) and serum levels (0.3–162.3 μg/mL) were elevated than levels obtained from milk (0.1–4.9 μg/mL) and serum (2.0–8.6 μg/mL) of healthy bitches. Research shows that milk CRP levels are more specific than blood levels. However, there were no differences detected in this APP between clinical and subclinical cases of mastitis.

Taking into account this novel finding, an increase in milk and serum CRP level reveals the presence of both systemic and local inflammations (mastitis), thus providing a new specific and noninvasive method of diagnosis [3] (**Table 3**).

Hp was also studied in milk of bitches [4]. The Hp peaks on 3–5 days after tissue injury with values between 4 and 9 mg/mL [4, 46, 54]. Hp concentration reflects the intensity of inflammation and is correlated with the degree of tissue damage [52]. Since its concentrations increase two- to threefold in response to injury, Hp in dogs is considered a moderate APP [45, 55]. In serum of healthy dogs, circulating Hp levels range between 0 and 1.69 mg/mL.

In cases of *mastitis acuta*, the serum Hp concentrations range between 5.3 and 7.5 mg/mL, while in bitches with *mastitis cronica* between 2.2 and 2.5 mg/mL. In terms, in cases of *lactatio sine graviditate,* Hp presents normal values [4]. On the other

Mammary gland diagnostic	Milk CRP levels	Serum CRP levels
Healthy	2.1 (0.1–2.5)	5.6 (3.9–8.0)
Subclinical mastitis	11.3 (2.9–20.7)	21.9 (8.1–67.9)
Mastitis acuta	6.5 (2.4–23.7)	7.1 (8.1–67.9)
Mammary retention	6.3 (3.8–10.9)	14.6 (4.1–51.8)
Mammary congestion	1.7 (0.7–3.7)	11.4 (8.2–39.0)
Mastitis gangrenosa	8.0 (7.9–8.0)	113.4 (112.2–113.4)

Table 3.
Mean value of milk and serum CRP levels (μg/mL) in bitches suffering from clinical and subclinical mastitis [3].

hand, recent study could not find correlation between serum Hp levels in bitches with mastitis and to the extent of the mammary gland inflammation degree [9].

In ruminants, Hp is considered a major APP and a valuable marker of inflammatory disease [47, 56]. In healthy cows, Hp levels range between 22 and 47 mg/L. During experimental *E. coli* mastitis infection in cows, the Hp level started to increase 12 h after challenge and reached the maximum (52 folds) 48–120 h after the inoculation [57]. It was also shown that Hp levels have predictive accuracy [58].

However, serum Hp level does not always correlate with the intensity of inflammation of mammary glands and could not discriminate the fatal *E. coli* cases [59]. Furthermore, rapid increase of somatic cells in milk after repeated challenges shows that the laboratory methods which have been adaptable for milk examination and used for early diagnosis in cow mastitis cells are primed by previous *E. coli* challenge. Subsequent Hp challenges do not increase at the same level as in the first challenge scenarios [57].

Based on literature data, it is known that in the course of subclinical infections, the levels of pro-inflammatory cytokines increase. Henceforth, determinations of APPs in slaughter houses are very common as indicators of food safety. Furthermore, mean Hp herd levels were suggested to be related to the hygiene level [54, 60, 61].

3. Conclusions

Evaluating laboratory assays such as milk and serum CRP, milk microbiology, cytology, and pH proves to be of great help when trying to early diagnose, treat, or even avoid episodes of clinical or subclinical mastitis in bitches (**Table 4**).

Healthy mammary glands are characterized by the presence of low levels of both milk (2.1 μg/mL) and serum (5.6 μg/mL) CRP, with a normal blood count; with the presence of somatic cells, cellular debris, squamous cells, and few neutrophils and macrophages; with an acidic milk pH; and with the presence of different milk bacterial strains.

Mammary congestion is characterized by slightly elevated levels of serum (11.4 μg/mL) CRP, with normal blood counts; with the presence of low numbers of somatic cells, high amount of cellular debris, few neutrophils, and inactivated macrophages; with an acidic milk pH; and with the presence of different milk bacterial strains.

Subclinical mastitis is characterized by the presence of elevated milk (11.3 μg/mL) and serum (21.9 μg/mL) CRP levels, with a modified blood count; with the presence of moderate to high levels of somatic cells; with the presence of degenerated neutrophils, many activated (foamy) macrophages and epithelial cells, bacteria, and

	Median CRP μg/mL Milk/serum	Hematology	Cytology	pH	Milk microbiology
Healthy *mammae*	2.1/5.6	—	Few somatic cells and cellular debris	<6.5	*Staph. simulans; B. cereus; E. faecalis; Acinetobacter ursingii*
Mammary congestion	1.7/11.4	—	Increased number of cellular debris	<6.5	*E. faecium*; *Staph. pseudintermedius*
Subclinical mastitis	11.3/21.9	Leukocytosis with left shift	Increased cell counts	≥7	*Staph. xylosus; Agrobacterium radiobacter; E. coli*
Retention mastitis	6.3/14.6	Leukocytosis with left shift	Increased cell counts	>7	*P. vulgaris*; *B. pumilus*
Mastitis acuta	6.5/7.1	Leukocytosis with left shift	Increased cell counts	>7	*E. coli; Staph. intermedius; Ps. aeruginosa*

Table 4.
Laboratory values of different bitch mastitis diagnostics.

phagocytosis; with an alkaline milk pH; and with the presence of many different milk bacterial strains.

Retention mastitis is characterized by the presence of elevated milk (6.3 μg/mL) and serum (14.6 μg/mL) CRP levels, with a modified blood count; with the presence of erythrocytes, eosinophils, foamy cells, degenerated neutrophils, bacteria, and phagocytosis; with an alkaline milk pH (up to 9.5); and with the presence of different milk bacterial strains.

Mastitis acuta (including *Mastitis gangrenosa*) episodes are characterized by the presence of elevated levels of both milk (6.5 μg/mL) and serum (7.1 μg/mL) CRP, with a modified blood count and with the presence of high numbers of foamy cells, degenerated neutrophils, cellular debris, bacteria, phagocytosis, and few numbers of erythrocytes.

The use of APPs in the diagnostic strategy of mastitis in bitch proves to be of great benefit since it provides a precise, quick, and noninvasive tool of diagnostic. Milk cytology evaluation helps to establish a definitive diagnostic, by characterizing the type of inflammatory response on milk smears.

Milk pH evaluation, alongside milk microbiology and susceptibility tests, helps establish a correct treatment strategy, by choosing the correct type of antibiotic according to its pH asset (acidic/alkaline) and antibiogram susceptibility results.

Mammary infections of bitches are widely overlooked; thus extra care should be taken to adequately revise status of mammary glands during postpartum since bacterial pathogens can migrate from the uterus and vagina to the mammary glands and cause their infection (hematogenous transmission) [39].

Laboratory analysis including determination of milk pH together with performance of biochemistry, hematology, milk cytology, and microbiology tests should be a golden standard for mammary gland infection management in dogs [27]. Taking into account the scarce milk quantities obtained from lactating bitches, further research should be carried out, in order to establish a more applicable method to evaluate bitch milk pH values. Different APP (Hp, SAA, AGP, Fb, etc.) diagnostic potentials should also be assessed, in order to complete the picture that CRP levels started to draw, regarding this novel, noninvasive diagnostic protocol.

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Conflict of interest

The authors of this chapter declare that they have no conflict of interest, whatsoever.

Dedication

My dear family, Cristina, Rebeca, and Filip.

In loving memory of the one we once called mother, our beautiful, kind, and gentle LIVIA.

Abbreviations

AGP	α1-acid glycoprotein
API	analytical profile index
APPs	acute-phase proteins
CRP	C-reactive protein
Fb	fibrinogen
Hp	haptoglobin
MGG	May-Grünwald Giemsa
MRS	De Man, Rogosa and Sharpe agar
PCV	packed cell volume
SAA	serum amyloid A
TP	total proteins
TR-IFMA	time-resolved immunofluorometric assay
TS	total solids

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