

New insights of canine mastitis – a review

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Disorders of the mammary gland are frequently diagnosed in bitches of all breeds and of all ages. They usually appear in the postnatal period, during lactation peak or during false pregnancy. They may also be related to different pathologies of the mammary gland, such as mammary neoplasia. It is frequently underestimated by clinicians or misdiagnosed. A reliable diagnosis is the key to success. Conventional diagnosis consists of a clinical diagnosis together with a standard blood test, while in some cases cytological examination of the mammary gland is performed. In some cases of mastitis, especially its subclinical presentation, those methods may prove unreliable. For this reason, determination of specific inflammatory biomarkers may enable clinicians to produce a precise diagnosis, while it is very useful in treatment monitoring. A wide variety of inflammatory biomarkers have been extensively studied both in human and veterinary medicine. In this article, we will describe the most commonly evaluated inflammatory biomarkers - acute phase proteins, which seem to be promising tools in the diagnostics of canine mastitis. We hope that this paper will provide clinicians with new ideas for precise diagnosis and more specific treatment of canine mastitis.

KEY WORDS: biomarkers / bitch / dog / inflammation / mammary gland

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The mammary gland is a transformed skin gland. It is functionally connected with the secretion of ovarian hormones secretions; therefore, ovarian pathologies may have an influence on the mammary glands. Bitches usually have 5 pairs of mammary glands located symmetrically on both sides of the chest and abdomen, but abnormalities in the position and number of glands may occur. The mammary gland is basically an exocrine gland, which basic function is to produce milk to feed the offspring. It is composed of epithelial cells, which form the lining of alveoli and ducts, surrounded by myoepithelial cells. The stroma of the gland consists of blood and lymphatic vessels, fibrous and fat tissue and nerve endings. The contraction of myoepithelial cells produces milk secretion into the lumen of secretory ducts which drain in the opening in the nipple. During the puberty, mammary gland development is related to cyclic ovarian activity and hormonal changes, with the growth continued until sexual maturity is reached [Marti *et al.* 2010, Momont *et al.* 2002].

Mastitis is a medical term, which refers to an inflammation with or without an infection of the mammary gland. Therefore, we can distinguish septic and aseptic mastitis. It may involve one or more glands. Inflammation of the mammary gland is a common problem in bitches of all breeds at various ages. Despite that fact, mastitis is frequently underestimated or incorrectly diagnosed. The pathogens are usually bacteria, but some cases of fungal mastitis in endemic areas or in dogs with immunodeficiency were observed as well [Ditmyer *et al.* 2011, Murai *et al.* 2013].

Prevalence

It is more often observed in non-spayed bitches, as it frequently appears in the postnatal period, during lactation peak or during false pregnancy. However, it may also be related to different pathological states of the mammary gland, such as galactostasis, mammary hyperplasia or mammary neoplasia. Sometimes, mastitis is indistinguishable from a mammary tumor with present inflammation. There are four clinical presentations of mastitis: acute mastitis, gangrenous mastitis, chronic mastitis and subclinical mastitis. Risk factors include: poor hygienic conditions, trauma and systemic infections. The most common route of infection is the ascending route from the nipple, while trauma and haematogenous route are less frequent. Trauma may be produced by sucking puppies during lactation. In some cases, injuries involving foreign bodies or insect bites may cause similar lesions. In extreme cases the bitch may present no signs of the disease or may be already in a critical condition. The mammary gland is usually changed, but in the case of a subclinical presentation it may remain normal [Marti *et al.* 2010, Momont *et al.* 2002].

Causes of mastitis

Lactation

The term lactation refers to the beginning of milk production and secretion. It is strongly related to hormonal changes during pregnancy and parturition. Due to a decrease of progesterone secretion at the end of pregnancy, the concentration of prolactin increases. Prolactin together with other factors is responsible for the initiation of lactation. There are several factors that either stimulate (e.g. oxytocin, serotonin, oestrogens) or inhibit (e.g. dopamine, somatostatin, progestogens) prolactin release [Concannon *et al.* 1989]. Mastitis during lactation develops as a consequence of mammary gland trauma caused by sucking puppies or due to poor hygienic conditions. Trauma and formed skin lesions facilitate bacteria penetrations into the mammary tissue. Post-partum metritis through the haematogenous route may also cause mastitis. Mastitis during lactation can be life – threatening both for the bitch and the puppies [Schafer-Somi *et al.* 2003].



Fig. 1. Acute mastitis during lactation in a 3 years old Yorkshire Terrier.

False pregnancy

False pregnancy, also called pseudopregnancy is a physiological state of a bitch. It is connected with hormonal changes (progesterone concentration) after oestrus that are similar both in pregnant and non-pregnant bitches. The clinical signs are present usually in 6-8 weeks after oestrus and include mammary gland enlargement, beginning of milk production, change in behaviour to typical of pregnancy and lactation such as nesting behaviour, anorexia or agitation. Secretion from mammary glands may be physiological but sometimes it may even be brown and mastitis may appear. Usually treatment is necessary, especially in cases of severe lactation, galactostasis, complicated additionally by infection and inflammation [Marti *et al.* 2010, Momont *et al.* 2002].

Galactostasis

Galactostasis is the overload of the mammary gland with milk that is seen before the parturition or shortly afterwards. The enlargement of mammary gland is observed,

which leads to failure of milk let-down. Animals usually show pain and discomfort, with the progression of the disease aseptic or septic mastitis may develop. It is observed in highly lactating bitches, especially at very early weaning of puppies. The treatment consists in limited feeding of the animal for several days (fasting may be recommended during first day), cold packs or compresses with baking soda are recommended, use of diuretics and glucocorticoids may be necessary. A prolactin antagonist should only be administered in cases of galactostasis due to false pregnancy or when the offspring was weaned.



Fig. 2. Galactostasis after weaning in a young female dog.

Mammary gland tumors

Tumors of the mammary gland represent the most frequent cases of neoplasia in female dogs. Around half of them are malignant neoplasia. They occur in bitches at an older age, usually between 8 and 10 years old [Benavente *et al.* 2016, Sorenmo 2003]. It is more common in intact bitches. It is known that ovariohysterectomy before first oestrus greatly reduces the risk of mammary tumor [Beauvais *et al.* 2012]. Still, the aetiology of canine mammary tumor is unknown. Some risk factors have been identified, among which the most frequent are: hormonal, nutritional and genetic. Tumors are usually discovered when macroscopic changes of the mammary gland are visible. Inflammation is very common, especially at a prolonged process of tumor development is long. The treatment of choice is mastectomy with resection of local lymph nodes, however, because of mastitis, in some cases antibiotics together with anti-inflammatory drugs have to be administered prior to the surgery.

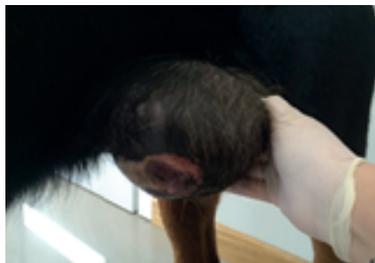


Fig. 3. Enormous mammary tumor with gangrenous mastitis.

Mammary gland hyperplasia

Mammary gland hyperplasia also called mammary hypertrophy or mammary fibroadenomatosis is a non-neoplastic proliferative process. It is more typical in queens than in bitches. It concerns young females, usually after the first oestrus, although it may also appear during pregnancy or false pregnancy. It is related to the progesterone concentration [Martin *et al.* 2000, Momont *et al.* 2002]. It is thought that the administration of progestogens plays an important role in its pathogenesis [Loretto *et al.* 2009, Marti *et al.* 2010]. Mammary glands are usually firm and enlarged, it may affect one, several or all glands. In severe cases, mastitis with ulcerative necrosis of the skin may appear. Treatment should always be undertaken.

Clinical presentation of mastitis

Acute mastitis

In the case of acute mastitis mammary glands are usually hot and painful during palpation, enlarged, swollen and erythematous [Marti *et al.* 2010]. If acute mastitis is septic, clinical signs such as fever, apathy, depression, lethargy, anorexia and neglect of puppies may appear. The puppies may present the toxic milk syndrome, which is basically a bacterial infection of puppies due to maternal milk contaminated with bacteria and their toxins. The secretion of the gland is brown, may contain small amounts of pus and blood, its smell may be changed [Marti *et al.* 2010]. In cases of acute mastitis urgent treatment is needed.

Gangrenous mastitis

Gangrenous mastitis is usually a consequence of untreated acute mastitis [Marti *et al.* 2010]. It is characterised by pus production and abscess formation may appear. Secondly, mammary glands may become ulcerated and necrosis may form. Altered glands become darker, colder and have an unpleasant putrid odour. Signs of sepsis are usually present at that time.



Fig. 4. Severe gangrenous mastitis together with mammary gland neoplasia.

Chronic mastitis

Little is known about chronic mastitis in dogs. It is typically a consequence of less severe acute cases of mastitis or are related to mammary neoplasia. During clinical examination, the gland might be slightly inflamed or swollen and its consistency during palpation may be heterogeneous. Chronic mastitis should be suspected in cases of an increased rate of falls in new-borns and when the litter is not gaining weight properly.

Subclinical mastitis

Subclinical mastitis is very difficult to diagnose, it is a challenge for clinicians as no clinical signs are seen. Moreover, this form of mastitis occurs quite often [Marti *et al.* 2010, Vasiu *et al.* 2015]. Sometimes the only presenting complaint is slow progress of the offspring growth and their limited weight gain. Additionally, puppies may also show signs of the toxic milk syndrome. It may develop and lead to systemic inflammation or sepsis. Therefore, subclinical mastitis cannot be neglected and an early and precise diagnosis must be provided. The mammary gland remains unchanged and the secretion may also be normal, so clinical diagnosis is usually unhelpful. Diagnostic imaging may appear to be useful, but not always changes in the mammary gland structure will be visible. Evaluation of specific inflammatory biomarkers in serum as well as in milk samples may represent the best non-invasive, fast and very sensitive method of mastitis diagnosis [Vasiu *et al.* 2015]. In the next section selected inflammatory biomarkers used in the diagnosis of mastitis will be described.

Diagnosis

If during a veterinary consultation we suspect mammary gland inflammation, first of all we need to obtain relevant information from the owner. We need to ask: how long the animal has been presenting this symptom, when was the parturition, whether it was its first parturition, how many puppies were born and/ or whether it is the first time the bitch presents a false pregnancy. To diagnose mastitis in a bitch full clinical examination is required [Marti *et al.* 2010]. All mammary glands should be evaluated, checking their symmetry, temperature, size, consistency and skin colour. Secondly, a blood test together with sampling of secretion/ milk from the abnormal mammary gland should be performed. In the case of secretion, we need to evaluate its colour, consistency and smell. Cytological examination should always be performed. In cases of chronic mastitis a microbiological culture of the secretion together with drug susceptibility testing may be indicated. The most common isolated pathogens are staphylococci, streptococci and *E. coli* [Murai *et al.* 2013, Seweryn *et al.* 2009]. Apart from that, X-ray should always be considered, especially if the

mammary gland neoplasia is suspected, in order to exclude metastases to the lungs. Also, ultrasonography of the mammary gland appears to be a very useful diagnostic method for mastitis as well as mammary tumor detection. It can precisely estimate the degree of inflammation as well as detect abnormalities inside the gland [Trasch *et al.* 2007]. Fine needle biopsy is usually recommended if mammary neoplasia is suspected, though the obtained results may not always be very precise. Last, but not the least, determination of specific inflammatory biomarkers in serum might prove to be highly informative.

Selected inflammatory biomarkers used in the diagnosis of mastitis

Inflammatory biomarkers are usually proteins, which can be measured in blood or other tissues (e.g. milk) and provide information on the presence of the inflammation, results of treatment or further prognosis for the patient. They are also detectable in the serum and tissues of healthy dogs, but their levels are significantly lower. During local or systemic inflammation processes, inflammatory cells secrete proinflammatory mediators such as cytokines and chemokines, which are released into systemic circulation. A wide range of compounds are classified as inflammatory biomarkers, among which acute phase proteins, T-lymphocytes, macrophages, interleukins, receptor tyrosine kinases, as well as cyclooxygenases play a particularly important role. In the case of canine mastitis only the determination of acute phase proteins has been briefly described [Vasiu *et al.* 2017]. The results of this research proved that evaluation of inflammatory biomarkers of inflammation may be very useful in the diagnosis of mastitis, especially its subclinical presentations, which remain a challenge for the clinicians. Additionally, their assay in cases of clinical mastitis may be useful in monitoring the treatment outcome.

Acute phase proteins

Acute phase proteins (APP) are serum proteins, which concentrations change as a part of innate host defence systemic response to infection, inflammation or trauma [Ceron *et al.* 2005, Eckersall *et al.* 2010, Paltrinieri *et al.* 2007]. APP can be divided into positive, moderate and negative APP, with the concentration of the former increasing after inflammation, the levels of moderate increasing slightly, whereas the concentration of negative APP is reduced after inflammation. The most commonly evaluated positive APP are C-reactive protein (CRP) and serum amyloid A (SAA), whereas, albumin is the main negative APP. An APP assay may be helpful in the diagnostics of the inflammation, in treatment monitoring of inflammation as well as in diagnostics of some neoplastic diseases [Ceron *et al.* 2005, Eckersall *et al.* 2010, Planellas *et al.* 2009, Tecles *et al.* 2009]. APP production is rapid (being detectable within several hours after the beginning of the inflammatory process), intense, but also unspecific for the disease. They can be induced by any inflammatory stimulus

or even by pathophysiological conditions (tumors, stress, pregnancy) [Eckersall *et al.* 2010]. For these reasons, due to its low specificity its clinical importance is limited. On the other hand, it gives us a clear sign, that the organism is suffering from some pathological process, which can possibly be life-threatening. Therefore, APP may be very useful in the detection of particularly subclinical mastitis as well as for monitoring patients during recovery.

C-reactive protein

C-reactive protein (CRP) is a very well-studied inflammatory biomarker, it has been described in cases of canine pyometra, systemic inflammatory diseases and mammary tumors etc. [Christensen *et al.* 2015, Dąbrowski *et al.* 2007, Dąbrowski *et al.* 2015, Planellas *et al.* 2009, Tecles *et al.* 2009]. Its biggest advantage is that its concentration increases rapidly after the onset of the tissue damage and decreases with its recovery [Vasiu *et al.* 2017]. As it was mentioned before, CRP is a non-specific acute phase protein and its concentration may be increased due to any disease. However, an elevated CRP concentration in milk is evidence of mastitis. A recent study confirmed that CRP concentrations in both serum and milk samples from bitches presenting mastitis were higher than in healthy bitches [Vasiu *et al.* 2017]. A positive correlation was observed between serum and milk CRP concentrations. Interestingly, no differences were noticed between subclinical and clinical presentations of mastitis. The serum values in bitches suffering from mastitis ranged from 0.3 to 162.3 µg/ml, while in healthy bitches it was 2-8.6 µg/ml, thus in diseased bitches the CRP concentration was as high as 20-fold higher in comparison to healthy bitches. Milk CRP levels in bitches suffering from mastitis ranged from 0.3 to 40.0 µg/ml, while in healthy females it was 0.1-4.9 µg/ml, so in diseased bitches the concentration was even 8 times higher than in healthy bitches. Still the range of recorded concentration values was wide, moreover, other on-going pathologies were not excluded and finally the number of patients was relatively low. Nevertheless, it seems to be a very good biomarker for early mastitis diagnosis, especially in cases of subclinical mastitis, which is difficult to diagnose clinically.

Serum amyloid A

Serum amyloid A is another very important positive APP, assayed in many systemic diseases in dogs [Christensen *et al.* 2014, Dąbrowski *et al.* 2007, Jiptean *et al.* 2014]. Some studies indicated that SAA may be a more sensitive marker of systemic inflammation than CRP, as its concentration may be increased, while CRP concentration would remain normal [Christensen *et al.* 2014, Jiptean *et al.* 2014]. Also, the recorded concentration ranges in patients presenting the disease turned out to be narrower in the case of SAA in comparison to CRP [Jiptean *et al.* 2014], which was in contrast to the data from a previous study [Christensen *et al.* 2014]. Therefore, it

seems to be a more sensitive and more precise inflammatory biomarker. Nevertheless, a simultaneous assay of both SAA and CRP is usually recommended. Still, to the best of the authors' knowledge no studies have been conducted on the determination of SAA in the case of canine mastitis.

Cyclooxygenases

Cyclooxygenases (COX) are inflammatory biomarkers of inflammation basically assayed in neoplastic diseases [Benavente *et al.* 2016, Carvahlo *et al.* 2016]. The cyclooxygenase enzyme catalyses the prostaglandin biosynthesis from arachnoid acid. There are two isoforms: Cox-1 and Cox-2, but they have different biological functions. Cox-1 is expressed in normal tissues and is responsible for the control of renal function, reproduction and cytoprotection of stomach, among other things. Cox-2 is undetectable in normal tissues, as it is expressed in tissue due to inflammatory reactions, growth factor, tumor promoters or oncogenes. Many studies confirmed that prostaglandins play an important role in tumor's development. COX-2 expression was found to be increased in cases of malignant mammary tumors [Benavente *et al.* 2016, Carvahlo *et al.* 2016]. Determination of COX-2 in the cases of mastitis may be interesting, especially in order to distinguish between mastitis and mammary tumors. However, no such studies have been conducted to date.

Treatment

All the above-mentioned presentations of mastitis require treatment: firstly, stabilisation of patients by fluid administration targeted at correcting metabolic disturbances. This is followed by therapy involving broad-spectrum antibiotics, selected based on drug susceptibility testing, while milk pH should also be considered. If the milk pH is lower than serum pH (<7,3), trimethoprim/ sulfathiazine (15-30 mg/kg orally BID during 21 days), erythromycin (10 mg/kg orally TID during 21 days), lincomycin (15 mg/kg orally TID during 21 days) should be used. When pH is >7.4, ampicillin (20 mg/kg intramuscularly TID during 21 days) or cephalexin (30 mg/kg orally BID during 21 days) are administered [Jiptean *et al.* 2014]. Some antibiotics may reach therapeutic concentrations in milk regardless its pH, but they are usually not recommended due to their negative effect on the puppies' growth. In the case of acute mastitis, the selected antibiotic is not that important as the integrity of the milk/serum barrier has already been broken [Marti *et al.* 2010].

Moreover, additional therapy consisting in manual emptying of abnormal mammary glands to avoid the accumulation of secretion is very important. Keeping the area clean by washing and disinfecting is also necessary. Application of warm compresses at least twice a day may help to reduce the inflammation of the gland. In some cases, local application of antibacterial and anti-inflammatory tubes used in cows for intramammary treatment of mastitis (e.g. Tetra Delta, Zoetis) may be helpful.

Whether to separate puppies from their mother or not remain disputable. In cases of severe mastitis with the presence of abscesses and gangrenous inflammation it is strongly advised against to continue nursing of puppies, as ingestion of toxic or antibiotic-containing milk may produce health problems among the offspring. The owner needs to hand-raise the neonates. In the case of slight mastitis, natural feeding can be maintained, but it is necessary to remember that during mastitis milk composition will deteriorate and weight gains among the puppies will be lower.

It is recommended to perform a surgical drainage in cases of gangrenous inflammation; sometimes drains have to be left in the tissue for several days. In some very severe cases mastectomy is the method of choice.

Prognosis

If sepsis has not been developed, mastitis can be treated without any complications. Mastitis is usually resolved with appropriate antibiotic therapy, although it takes some time. The function of the mammary gland remains unaffected, unless it was seriously damaged due to abscesses or necrosis. Bitches may suffer from mastitis during every lactation and every false pregnancy, probably because the mammary gland anatomy facilitates introduction of pathogens through the nipples. This phenomenon must be mentioned to the owner and pros and cons of bitch castration should be discussed.

Conclusions

Disorders of the mammary gland are frequent health problems in bitches of various ages. Among them mastitis is relatively common, especially in lactating bitches or in false pregnancy. When left untreated it can be life-threatening, so proper diagnosis and specific treatment are of great importance. Standard diagnostic methods, involving clinical examination, blood tests and cytological examination of mammary gland secretion may be insufficient, especially in the case of subclinical mastitis. In order to provide a rapid and more precise diagnosis inflammatory biomarkers need to be assayed. Acute phase proteins (CRP or SAA) detected in serum and milk samples seem to be the most accurate inflammatory biomarkers to diagnose canine mastitis, as their concentrations are significantly higher in bitches suffering from mastitis than in healthy bitches. However, more studies need to be conducted to evaluate differences in concentrations between different mastitis presentations (clinical vs. subclinical). Identification of differences between clinical and subclinical presentations may promote a more adequate treatment. Other inflammatory biomarkers such as cyclooxygenases or interleukins seems to be promising both in diagnosis of mastitis and differentiation between mastitis and mammary tumours. However, before it is clinically feasible, further studies on these biomarkers have to be carried out to obtain more specific data.

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