1. Introduction

Despite the efforts of farmers and technicians to improve the health of herds’ udders, colibacillary mastitis remains a major problem in many livestock farms. In farms where contagious mastitis is virtually eliminated, and that have low tank cell counts, between 20 and 40% of episodes of clinical mastitis are caused by coliforms. Escherichia coli, Klebsiella spp. and to a lesser degree Enterobacter spp. are the most commonly isolated coliforms from clinical episodes of this kind. The presentation of clinical symptoms, and costs stemming from them (discarded milk, treatment costs, replacement due to the death or slaughter of animals, etc.) is highly variable and depends primarily on factors in relation to the cow, rather than the pathogenicity of the strain involved. In this paper, we will discuss the predisposing factors and preventive measures to fight against this disease.

2. Pathogeny

Escherichia coli, and most Gram-negative bacteria, have a characteristic and essential macromolecule in their external cell membrane called lipopolysaccharide (LPS). This LPS is the major factor of pathogenicity of the bacterium. It triggers the typical set of symptoms of hyperacute coliform mastitis. The experimental intramammary injection of LPS in healthy animals causes the same symptoms, being dose dependent, and causes the death of the animal at high doses. The bacterium only enters via the teat canal; it multiplies rapidly in the cistern of the udder, and in the process of multiplication and lysis, the LPS’ toxicity and potent induction of inflammatory cytokines causes generally acute symptoms in cows. After running their course, it can cause an almost total loss of production, as well as an acute inflammation of the affected quarter and often loss of appetite, fever, listlessness, shock and sometimes death. Depending on the immune status of the cow, the presentation may be less acute. Chronic infection with recurrent clinical episodes may also occur but that is less frequent.

The ability of the immune system of the cow is a key factor to limit the rapid spread of E. coli in the udder and reducing the toxic action of LPS. Neutrophils are key players in the fight against intramammary infections. They are responsible for sequestering, killing and eliminating the pathogen. They are aided by opsonising antibodies, mainly IgG, and pro-inflammatory cytokines, which are responsible for the massive influx of neutrophils from the blood capillaries of the udder into the cistern. The rapid mobilization of neutrophils into the udder is essential in reducing the impact of clinical symptoms.
3. Predisposing factors

Most coliform intramammary infections occur in the first two weeks of the dry period and especially at peripartum. Furthermore, almost half of the cases of clinical mastitis that occur within the first 100 days in milk originate in the dry and peripartum periods. The presentation of hyperacute or acute colibacillary mastitis is not exclusive to post-partum, but a high percentage is. Intramammary coliform infections in advanced lactation cause mild or moderate cases, that a cow’s immune system is able to resolve and that often go unnoticed.

The onset of the dry period is a phase of risk due mainly to:

- Increased pressure within the udder, which sometimes causes loss of milk. Antibiotic syringes used during drying, can leave the sphincter open, so bacteria can enter.
- Bacterial proliferation on the skin of the teat, which is the result of the cessation of milking and the practice of pre-and post-dipping.
- Delay in the formation of the keratin plug. It takes days or even weeks for the teat of some cows to seal.
- Poor hygiene when applying drying intramammary cannulae, which can cause intramammary infections.

The peripartum phase is also a time of risk because the immune system is compromised by several factors:

- Calving is stressful for the cow. Plasma levels of cortisol experience a sharp physiological increase, which is needed for the development of the delivery and colostrogenesis. Cortisol inhibits the inflammatory response and adversely affects the operation of the neutrophils.
- Negative Energy Balance (NEB). There are numerous studies that relate a NEB with post-partum pathologies. Increased energy needs in post-partum along with a reduced ability to intake, induces the mobilization of fat reserves, which after metabolism in the liver can cause ketosis. The ketone bodies negatively influence the ability of migration and recruitment of neutrophils into the udder, phagocytosis and the ability of oxidation and destruction by the neutrophils.
- Others
  - Loss of milk is the result of the increase in intramammary pressure at the end of the dry period. The sphincter is open for the entry of pathogens.
  - The majority of antibiotic formulations administered through cannulae at drying do not cover the final phase of drying, especially in standard 60-day dry periods. Furthermore, the majority of products on the market have limited activity against Gram-negative bacteria.
  - Postpartum milking that is often difficult due to udder oedemas, thereby facilitating air intake during milking and the subsequent entry of pathogens into the cistern.
  - Stress. Stress factors such as heat, metabolic stress, competition, transport, etc. induce the secretion of cortisol and cause immunosuppression. Stress is a vicious circle in post-partum. Cows eat less when stressed, which lengthens or increases the NEB, and immunosuppression is enhanced.
4. Treatment

Treatment should be focused towards the cow, not the bacteria. *E. coli* rapidly multiply in the udder reaching peak concentration in less than 12 hours (Erskine et al. 1989). The recognition of clinical signs of colibacillary mastitis normally occurs after the maximum bacterial concentration in the udder is reached. This idea questions the appropriateness of treating colibacillary mastitis with antibiotics. In addition, there are many studies demonstrating the poor efficacy of treatment with antibiotics against gram-negative mastitis. Therefore, we will focus on symptomatic treatment:

1. IV Hypertonic saline serum. Cows must have free access to clean fresh water.
2. NSAIDs for controlling fever and inflammation.
3. Calcium, iron and vitamins A, D, and E to enhance neutrophil function.
4. Frequent milking and oxytocin. The pain and inflammation inhibits milk drop. Oxytocin helps better emptying of the udder, thus removing more bacteria.
5. Antibiotics active against Gram-negative bacteria by parenteral route (as a preventive measure against sepsis, not for curing the infection).

5. Prevention

Given the low efficiency of any treatment against the hyperacute colibacillary mastitis, prevention is the best possible treatment. Knowing the periods of greatest risk and predisposing factors, prevention strategies, are mainly focused on 2 routes:

1. Minimize exposure of the teat tip to bacteria present in the environment:
   - Maximize hygiene in areas where cows rest, especially drying yards, during pre- and post-partum since these are the periods of maximum risk of intramammary infection by coliforms. Clean dry cubicles or beds are key to prevent the proliferation of *E. coli* in resting areas. Inert materials such as sand or marble dust are more suitable when compared to materials such as straw, sawdust or husks because bacteria proliferate less.
   - Milk clean dry teats.

2. Increase the animal’s resistance to infection:
   - Minimize stress of any kind.
   - Rations and feeding strategies that minimize the NEB and its duration. The objective is to maximize intake of dry matter.
   - Securing the necessary intake of Vitamin E and Se in the ration; they are important for the immune system and increasing the phagocytary activity of neutrophils. A state of deficiency of these elements increases the likelihood of suffering from mastitis, as well as the severity and duration of infection.
   - Vaccination. Vaccination against colibacillary mastitis is a commonly implemented strategy on dairy farms in the United States (between 40-65% of the farms apply vaccination). The most widely used vaccines are based on the J5 strain of *E. coli*. This strain is a mutant that lacks the O-polysaccharide chain of the LPS, leaving the LPS antigen core exposed to the immune system. Unlike the O-polysaccharide chain, the composition and structure of the antigen core is highly conserved among the various Gram-negative bacteria, so vaccines with J5 induce opsonising “anti-core” antibodies with cross-immunity against different strains of *E. coli* and other Gram-negative bacteria. The efficacy of vaccination in protecting against acute colibacillary mastitis has been demonstrated in several field studies. In many references, it is clear that immunization with J5 does not prevent coliform intramammary infection.
infections, but does reduce the severity, the onset of clinical cases and economic losses from death or slaughter.

According to economic studies conducted in the USA, a vaccine against this type of mastitis is economically profitable if more than 1% of lactations are affected by colibacillary mastitis. According to the literature, vaccination can be an important tool in preventing mastitis caused by Gram-negative bacteria on farms where there is such a problem. Considering that the post-partum period is the most critical and where there are most cases for the reasons already mentioned, the goal should be to strengthen immunity in that period by vaccinating animals in the drying period and revaccinating before calving. A booster dose during the first months of lactation may be appropriate to extend the duration of immunity. In hot and humid climates where the incidence can be high in the summer months, a booster dose to all the animals could also protect the herd.

## Conclusions

Colibacillary mastitis is an important pathological condition on many farms due to the economic impact it implies. Prevention is the best tool to control this problem. The management of the dry period and the peripartum phase is crucial. Cows housed in clean, dry and comfortable cubicles and yards will have reduced intramammary coliform infections. In addition, feeding strategies to minimize the NEB in the post-partum period and reducing stress on the cow will help face hyperacute mastitis. Finally, it should be noted that a vaccination protocol in the drying period can help to prevent clinical cases of coliform on farms where there is a problem.

## Bibliographic references