Mini Review: Lactoferrin-binding protein of *Streptococcus* in Bovine Mastitis

Meta Iqomah[1], *Alek* Arisona[1], *Imawan* Daru Prasetya[1], *Adretta* Soedarmanto[2], *Yanuartono*[3], and *Soedarmanto* Indarjulianto[3*]

[1]Magister Sain Veteriner, Faculty of Veterinary Medicine, Universitas Gadjah Mada
[2]International Graduate of Veterinary Science and Technology Program, Faculty of Veterinary Science, Chulalongkorn University
[3]Department of Internal Medicine, Faculty of Veterinary Medicine, Universitas Gadjah Mada. Jl. Fauna 2, Karangmalang Yogyakarta, Indonesia, 55281

Abstract. Bovine mastitis is an udder inflammation mostly found in dairy cattle that causes enormous economic losses. Streptococcus is a bacterium that is often found in mastitis, including *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, and *Streptococcus uberis*. These three species have lactoferrin-binding protein (LBP) as one of their virulence factors. Lactoferrin is a host innate immune protein that acts as antibacterial, immunomodulator, anti-adhesion, and has iron-binding properties. The LBP on the surface of *Streptococcus* could bind to lactoferrin produced by host cells. Uniquely, the three Streptococcus bacteria showed different responses to lactoferrin. The lactoferrin-LBP bound on *S. agalactia*e and *S. dysgalactiae* was known to inhibit their penetration ability into the host epithelial cells, on the contrary, in *S. uberis* it could enhance their ability to invade the cells. This paper aims to review the role of the lactoferrin-binding protein of *Streptococcus* in bovine mastitis.

1 Introduction

Mastitis, an inflammation of the parenchyma mammary glands, is a multi-etiological and complex disease. It can be detected by the examination of physical appearance, presented biochemical shifts in composition of the milk secretion, and the changes in the pathological appearance of glandular tissue [1]. Mastitis is easily found worldwide and causes financial losses in many countries by affecting animal health and the milking production quality [2]. This disease is divided into two major forms, sub-clinical mastitis which does not show any clinical signs and is difficult to be detected, and clinical mastitis which alters the milk composer and appearance, decline the milk secretion, present the cardinal signs of inflammation, and is easily detected by general examination of the mammary quarters. In contrast, the absence of clinical signs in sub-clinical mastitis affects the diagnosis difficulties [3].

Streptococcus is a bacterium that is often found in mastitis, including *S. agalactiae*, *S. dysgalactiae*, and *S. uberis* [4,5]. One of the virulence factors in streptococcus is a lactoferrin-binding protein (LBP), but there are differences among these organisms, especially the pathogenesis of milk lactoferrin protein to interact with streptococcus [6]. Lactoferrin is a host innate immune protein that acts as antibacterial, immunomodulator, anti-adhesion, and has iron-binding properties [7,8,9]. On the other hand, the LBP on the surface of Streptococcus could bind to lactoferrin produced by host cells [10]. Uniquely, a previous study revealed that the three Streptococcus bacteria showed the various binding ability to lactoferrin [11]. This paper aims to review the role of the lactoferrin-binding protein of Streptococcus in bovine mastitis.

2 Mastitis Pathogens

Mastitis is mostly caused by bacteria, fungi, mycoplasma, and algae that attack the mammary glands of cows [3, 12]. *Staphylococcus aureus*, *S. agalactiae*, *Mycoplasma spp.*, and *C. bovis* are bacteria that are transmitted from cow to cow, whereas *S. uberis*, *S. dysgalactiae*, *Escherichia coli*, *Klebsiella* spp., and *Enterobacter* spp., are pathogenic agents from environmental [13, 14].

3 Bacterial Mastitis

Bovine mastitis is mostly caused by bacteria which 90% of cases are caused by environmental pathogens [15]. The more animal population, the higher humidity, and pollutants in the cow's environment which increases the bacterial and other pathogens infection in animals [16]. *Staphylococcus aureus* and *S. agalactiae* are frequently found as the main pathogens, while rarely found the C. bovis or M. bovis infections [17]. To support this, studies by [15] presented the greatest number of isolates are Streptococci and Staphylococci (38,5% and 17,9%, respectively). Moreover, *Streptococcus spp.*, Coagulase-negative staphylococci (CoNS), *S. aureus*, Gram-negative bacilli, and *Corynebacterium spp.* were the main isolates

* Corresponding author: indarjulianto@ugm.ac.id

[16]. These were supported by [15] findings, where 16.4% of coliforms, 3.3% of fungi, 31% of A. pyogenes, 0.9% of algae, and 0.8% Corynebacterium spp. were the major causes of mastitis diseases. Different results revealed by [18], *S. aureus* was identified in 10.4%, *S. agalactiae* in 4.9%, and *Streptococcus spp.* in 6.4% from of all the samples, but CoNS and E. coli was isolated (36.6% and 3.5% samples, respectively).

4 Streptococcus

It has been previously known, more than 130 pathogens are related to bovine mastitis and some of which belong to the genus Streptococcus [19,20]. The Streptococcus genus is one of the common pathogen groups causing bovine mastitis [21]. Streptococcus belongs to the gram-positive group of bacteria, round-shaped with chain formation. Mostly, streptococcus is common bacteria found in the environment, humans, and animals, but in some cases, these bacteria can cause serious diseases such as bovine mastitis. Even though most of the Streptococci species are non-pathogenic and commensal microbiota of humans and animals, some of them are harmful and cause bovine mastitis, i.e., *S. agalactiae*, *S. dysgalactiae ssp. dysgalactiae* (or *S. dysgalactiae*) and *S. uberis* [21].

Bovine mastitis is classified into clinical, subclinical, and chronic depending on the inflammation degree [22]. The clinical signs can be found physically in clinical mastitis. The swelling of the udder, pyrexia, and the changing in milk yield with clots and sometimes reddish colored of milk. These changes cannot be seen in subclinical cases. Usually, only the decrease of milk production all of the sudden and the diagnosis could be a challenge. Subclinical mastitis can only be enforced with laboratory findings by somatic cell count (SCC). The prevalence of subclinical mastitis could be 15–40 times more than clinical mastitis [23]. Subsequently, the deprivation caused by subclinical mastitis is more subversive. Chronic mastitis lasts for a long period [5]-and its occurrence is often the result of untreated acute mastitis [24]. Streptococci that repeatedly cause chronic mastitis are *S. uberis* and *S. agalactiae* [25,26].

5 Streptococcus Virulence Factors

Streptococcus agalactiae is classified as a contagious pathogen. Biofilm is one of its virulence factors that allows this bacteria to survive in the bovine udder and cause subclinical mastitis [22]. *Streptococcus agalactiae* is distinguished into nine serotypes with their specific pathogenicity. In humans, this pathogen is recognized as the typical causal agent of bacterial meningitis in neonates [27].

Streptococcus dysgalactiae is able to endure within the host and in the environment, this uncertainty makes this bacteria counted as an intermediate pathogen [28]. Furthermore, *S. dysgalactiae* generally concurrent with the infectious problem in bovine and other ruminants. *Streptococcus uberis* mostly classified as an environmental pathogen, the nonetheless contagious case has also been studied [29]. Bovine mastitis caused by *S. uberis* was first found in 1932 and it has been identified from miscellany bovine host infections [29]. Compare with *S. agalactiae* and *S. dysgalactiae, S. uberis* has virulence factor with slight different mechanism. Virulence factors of *S. uberis* include synthesis of the protein HasC to prevent opsonization, lactoferrin-binding protein, Biofilm formation, PauA, and Sk C proteins, and the activation of plasminogen [21]. The virulence factors and their encode genes of the three streptococcus summarized in Table 1.

Table 1. Virulence factors encoded genes of streptococcus

Streptococcus	Virulence factors encoded genes	References
agalactiae	bac, bca, bibA, cylE, fbsA, fbsB, rib, scpB, cfb, cspA, hylB, sip, PI-1, PI-2a, and PI-2b	[30, 31]
dysgalactiae	streptolysin S, glyceraldehyde-3-phosphate dehydrogenase, the plasminogen-binding M-like protein PAM, and the collagen-like protein SclB, GAS bacteriophage-associated virulence genes encoding superantigens, DNase, and/or streptodornase, cfb, lmb, eno, napr, bca, scpB, cyl	[32, 33]
uberis	hasA, hasB, hasC, sua, gapC, lbp, pauA, oppF, mtuA, CAMP factor or cfu	[34, 35]

6 Lactoferrin-Binding Protein

Lactoferrin-binding protein (LBP) is one of the virulence factors of Streptococcus. Meanwhile, lactoferrin is mainly produced by neutrophils and certain glandular tissue and plays a role as an innate immune protein [30,31]. It has numerous functions such as broad-spectrum antimicrobial, immunomodulatory, antiadhesive, and has can bond with iron [7,8,9]. Lactoferrin has bacteriostatic and bactericidal effects which interfere with the invasion and the development of bacteria [32,33]. The lactoferrin was thought to facilitate the inhibition of bacteria-iron binding [34]. Moreover, the proliferation of lymphocyte-induce mitogen and alloantigen, and the inhibition of bovine mammary epithelial cell (MAC-T cells) proliferation were allowed by lactoferrin [35,36]. During the involution period of a cow, lactoferrin will be excessively produced, likewise in the infected udder [37].

Streptococcus is believed to be more insusceptible to the antimicrobial effects of lactoferrin since it has a minimum necessity of iron [38,39] or maybe due to the ability to extort the metal component other protein comprising iron [40]. Undeniably, several species of streptococcus express the lactoferrin-binding proteins, i.e., *S. uberis*, *S. agalactiae*, and *S. dysgalactiae*, implying an objective of lactoferrin with streptococcal infections which may have a crucial indication for Intra Mammary

Infection (IMI) pathogenesis [10]. However, there is a distinction in lactoferrin-binding protein molecular mass among streptococcus due to the diversity of their surface characteristic [41].

In detail, *S. uberis* isolated from bovine mastitis is insusceptible to the antimicrobial response of lactoferrin [37,42]. This was proven by research conducted by [41], the *S. uberis* bind to the purified bovine milk lactoferrin by gaining iron from lactoferrin regardless of its low iron necessity. The in vitro study by [43] showed that *S. uberis* bind to lactoferrin, presented as a connecting molecule between its surface and the lactoferrin receptors on epithelial cells of the mammary gland. In *S. uberis*-infected sub-clinical mastitis, the level of bovine milk lactoferrin significantly increased compared to the uninfected one and it was hypothesized that lactoferrin activity was not able to interrupt the invasion of *S. uberis* and the high level of lactoferrin in bovine milk precisely reinforce the pathogenesis of *S. uberis* [11]. Interestingly, the increase of lactoferrin level did not correspond with other bacteria such as *Staphylococcus aureus*, *S. dysgalactiae*, and *Streptococcus coagulase-negative* in sub-clinical mastitis. This study points out that the response to lactoferrin expression in the bovine mammary gland is pathogen-specific. Meanwhile, the study by [44] points that bovine lactoferrin connects with *S. agalactiae* by the definite reciprocal action of lactoferrin with the bacterial surface which may have crucial consequences associated to host defense or the virulence of bacteria. Also, a study of the binding between bovine lactoferrin-binding protein and *S. dysgalactiae* taken by [10] exposed *S. dysgalactiae* is binding to lactoferrin and was not interrupted by molecule components of lactoferrin such as mannose, galactose, and lactose. The lactoferrin-binding protein is conveyed by *S. agalactiae* and *S. dysgalactiae* [10]. The Study by [6] revealed that bovine intramammary is challenged with *S. dysgalactiae* significantly increase in milk lactoferrin protein compared with control. The in vitro studies confirmed that milk lactoferrin acted as the bacteriostatic antimicrobial that limits the adhesions and invasions of *S. dysgalactiae* into epithelial cells of the mammary gland. This suggests the lactoferrin and *S. dysgalactiae* communications and demonstrated the bacteria-lactoferrin binding. Compiled studies indicate that milk lactoferrin levels were increasing as a response of the bovine immune system and apparently by restraint the invasion of *S. dysgalactiae* and by decreasing the adherence to the host cell. Identical with this study, [45] exposed that lactoferrin was able to interfere with the adherence of *S. dysgalactiae* to the mammary epithelial cells.

The study by [11] is to determine possible changes in bovine milk lactoferrin (bLF) concentration, which could establish the mammary gland response to mastitis caused by several bacteria and to evaluate the relation among the possible response and also the pathogen susceptibility to bLF in infected quarters compared with uninfected quarters. This determination declared that this raise was mostly caused by *S. uberis* intramammary infection, moreover, the study proved that *S. aureus* and *E. coli* were sensitive to bLF and that *S. uberis* came to be resistant. Previous studies showed that bLF can enhance the adherence of *S. uberis* to the animal's cells and increase its invasiveness [39,46]. Those results and the ones presented by [11] suggest that *S. uberis* might transform to get benefit from the existence of bLF.

7 Conclusion

Lactoferrin-binding protein is expressed by *S. agalactiae, S. dysgalactiae*, and *S uberis*. For *Streptococcus agalactiae* and *S. dysgalactiae*, lactoferrin bind to both receptors and have the ability to inhibit the invasion of both organism and in contrast for *S. uberis*, this binding bridges bacterial invasion with host epithelial cells. In conclusion, the response of Streptococcus to the presence of lactoferrin-binding protein is pathogen-specific.

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