Bovine Streptococcus uberis Intramammary Infections and Mastitis

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Abstract

Streptococcus (S.) uberis is a causative agent for clinical and subclinical bovine mastitis which significance for the udder health has increased over the last decades. Molecular diagnosis methods revealed that S. uberis may be subdivided into many different varieties with different epidemiological properties. In addition, some varieties were re-classified as Streptococcus parauberis and Globicatella sanguinis. The present paper reviews S. uberis and its role in modern dairy farming. This pathogen is ubiquitous for which it is considered as environment-associated. Straw bedding and pasture, but also the bovine skin and digestive mucosae are typical localizations inhabited by S. uberis. Due to its capacity to persist within the mammary tissue, some infections may eventually turn cow-associated. In other cases, the infection is short, but in any case, there is a high risk of re-infection. Although many varieties remain susceptible to most antimicrobial agents, the problem for the dairy farm lies in the high rate of re-infection. This paper also reviews risk factors, therapies and measures to control S. uberis at farm level.

Keywords: Streptococcus uberis; Microorganism; Cellobose

Introduction

Mastitis caused by Streptococcus (S.) uberis has been detected increasingly in dairy farms. This species is known to cause both clinical and subclinical infections of the bovine udder and represents the leading pathogen in a growing amount of dairy herds.

The present paper is intended as a literature review regarding the properties, the ways of diagnosis and the possible strategies to combat this microorganism. The more risk factors that favour the development of the disease are known, the more efficient advice can be provided to the farmer.

Properties and diagnosis

S. uberis [1] is classified within the order Lactobacillales and the family Streptococcaceae. There have been many ways to classify streptococci. One of the first was the Lancefield grouping which is based on serology. In the original paper [2], S. uberis (which was described in 1932) was not mentioned explicitly, but all corresponding strains (group E) originated from bovine milk. However, not all S. uberis strain can be classified as group E, so that other authors [3] consider it as a part of group G. Many Lancefield groups (including E) were later merged to a “pyogenic group”, along with other typical animal pathogens, e.g. S. dysgalactiae, S. agalactiae, S. canis, and S. equi [4]. Nowadays, molecular biology methods, e.g. DNA-DNA reassociation or 16S rDNA gene sequencing, are used to classify the different species. S. uberis, which also belongs to the pyogenic group, acts as the sister clade to the species mentioned before, as was demonstrated by Täpp et al. [5] who sequenced the RNase P RNA gene rmpB. From the clinical point of view, Facklam [4] chose another way of classification which is based on phenotype. Being human-based, S. uberis was classified as an “unusual Streptococcus species”.

The bacterium is Gram-positive, aerotolerant and anaerobial. The cells are coccoid (diameter 0.5 to 1 µm) and occur in pairs or in chains. On blood agar plates, S. uberis grows at 30 to 37°C. Colonies have a diameter of 1 to 2 mm after an incubation period of 24 to 48 h. Providing 0.1% aesculin to the medium enhances bacteria growth. Among 1,894 isolates of aesculin-positive streptococci, 82.3% were classified as S. uberis [7], no data was provided on the origin of these strains. On the other hand, some papers indicate that approx. 83% of S. uberis isolates are also aesculin-positive [8,9], while others [10,11] demonstrated that all strains are capable of hydrolysing aesculin. When growing on blood agar plates, S. uberis displays either a weak α- or γ-haemolysis [12]. Approx. 95% of S. uberis isolates are positive for β-galactosidase which can be used to tell them apart aesculin-hydrolyzing enterococci. Watts et al. [13] recommended a modified Rambach agar for identification on which S. uberis colonies grow with a blue colour. In comparison, colonies of S. agalactiae, S. dysgalactiae, Enterococcus (E.) saccharolyticus or E. faecalis do not metabolize propylenglycol (red colonies) and do not produce β-galactosidase [13]. S. uberis produces acids out of cellobiose, aesculin, glucose, fructose, galactose, inulin, maltose, mannitol, mannose, ribose, sorbitol, starch, sucrose and trehalose [9]. Its growth was observed in 4%, but neither in 6.5% NaCl nor at a pH of 9.6. Telling S. uberis from enterococci is also possible applying the Sherman criteria. S. uberis grows slowly or ceases to do so at ≤ 10°C and ≥ 45°C. Microorganisms become inactivated after heating to 60°C for 30 minutes [12]. Recently,
a scheme based on eleven biochemical tests was recommended to identify S. uberis [9], i.e. the Christie-Atkins-Munch-Petersen reaction (81% of tested S. uberis were negative), argin hydrolysis (83% negative), ascucin (89% positive) and sodium hippurate (96% positive), growth in an inulin-containing medium (64% positive), usage of mannitol (98% positive), raffinose (94% negative), salcin (94% positive) and sorbitol (96% positive) as a source for carbohydrates, growth at 45°C (98% negative) and in 6.5% NaCl (100% negative).

S. uberis is a serologically heterogeneous species [12]. This is a key fact to notice as this heterogeneity leads to marked differences regarding the pathogen’s epidemiological properties [14]. It is not possible to differentiate between S. uberis and S. parauberis, which was described in the year 1990 [6] using phenotypic methods [4]. As shown by Zadoks et al. [15], a few S. uberis strains isolated from milk are closely related to S. parauberis. Species-specific primers for the detection of S. uberis on species level via molecular probes reacting in PCR are described by several authors [16,17]. Forsman et al. [16] used the primer pair STRU-UbI and STRU-UbII (size of the main PCR product: 330 bp) to detect S. uberis with a high degree of reliability, while Riffon et al. [17] worked with the primers Sub 302, 396, 1546, and 2170, based on GI no. 43370 and 2668550 (23S rDNA) in order to design a culture-independent PCR diagnosis kit. Pulsed-field gel electrophoresis and random amplified polymorphic DNA fingerprinting are used to evaluate the diversity of strains of S. uberis which in turn reflects its adaptability to the udder [18-20]. In addition, multilocus sequence typing is used to characterize S. uberis populations as well as the epidemiological properties of this pathogen [21,22], i.e. the loci arc, ddi, gki, recP, ddk, tpi, yqiL, hasA, and hasC (225 to 799 bp amplicon size).

Primers used for PCR include ub-I and ub-II (for the rRNA target gene 16S), ub-23S-I and ub-23S-II (for 23S) as well as STRU-UbI and STRU-UbII (for the 16S-23S intergenic spacer [23]).

Virulence factors

The factors of virulence are not known completely and it is suggested that their expression varies from one strain to the other [24,25]. S. uberis is able to adhere to and invade in mammary epithelia cells [26-28]. Adherence and invasion can be attributed to the “S. uberis adhesion molecule” (SUAM) [29]. According to Frost et al. [30], the high prevalence of S. uberis in some dairy herds may be explained by the ability to adhere to host cells. The enzymes of S. uberis seem to affect strongly the dissemination of infections caused by it [28]. All strains produce free hyaluronidase that enhances the distribution of the pathogen within tissues [24,28]. According to Matthews et al. [26], the hyaluronidase synthesized by S. uberis is capable of preventing the proliferation of a line of udder epithelial cells. Another factor of virulence could be its capability to produce hyaluronic acid capsules [25]. Matthews et al. [25] indicated that 44% of S. uberis strains isolated from bovine udders provoked these capsules. Crowley et al. [31] showed that clinical isolates produce more biofilm biomass than a strain from a healthy cow. S. uberis binds lactoferrin to obtain iron required for bacterial growth [32]. Besides that, a plasminogen activator factor and a CAMP factor have also been recorded [33]. In fact, Tassi et al. [34] could show that S. uberis strains may also be differentiated into host-adapted, pathogenic strains, and non-adapted, basically apathogenic strains.

Epidemiology

Habitats and reservoirs

S. uberis is no mastitis pathogen that is obligatorily adapted to the udder. In fact, it is a ubiquitous microorganism which colonizes animals as well as their environment [35,36]. It has been localized on the animals' lips, tonsils and skin, inside the oral cavity, rumen, respiratory tract, rectum, on the teat orifice, in teat canals and infected udders, and in faeces and wounds. It appears that S. uberis spreads mainly via the mucosae of the digestive tract. Starting from the oral and lips mucosa, the pathogen is distributed via licking into the environment, including fur and epidermis of other cows. Furthermore, bovine faeces (and with that, the intestinal mucosa) also contribute strongly to its dissemination in the environment [36-45]. Despite the detection of S. uberis in bovine teat canals, it remains unclear if unlike cow-associated mastitis pathogens like Staphylococcus (S.) aureus and S. agalactiae the environmental-associated pathogen S. uberis is able to colonize the teat canal epithelium [46-48].

Hejlićek [43] encountered S. uberis in 51.6% of dairy cow skin samples and in 85.8% of environment samples. Zadoks et al. [15] found that it was present in 63% of environment samples (i.e. earth, vegetable material and bedding), in 23% of faeces samples and 4% of milk samples. During summer (grazing season), bovine faeces are more contaminated than in other seasons. Straw and other organic bedding materials enhance the growth of S. uberis [36]. Furthermore, approx. 20% of Canadian colostrum samples also contained S. uberis [49]. Since samples were drawn from the drinking bottles, the exact origin of these bacteria remains undetermined [49]. Furthermore, it is hypothesized that flies may be a potential reservoir for S. uberis [50].

Infections originated during the dry period

The dry period is the most common portion of the production cycle in which dairy cows acquire an infection with S. uberis [51,52]. Frequently S. uberis infections are manifested as acute mastitis, usually during the subsequent lactation [46]. Wilkinson [53] calculated that 56% of clinical cases had originated during the dry period. If few strains are found on a single farm, a contagious form is suspected. If several cows of a herd were infected, it is relatively improbable that all infections were caused by the same strain. Infections caused by one dominating S. uberis strain are more persistent than those by several minor strains.

Due to its biochemical abilities and the ability to invade in mammary gland cells and to its capacity to produce biofilms and capsule forming it was suggested that S. uberis can persist in infected bovine udders. This promotes the development of chronic infections of the mammary gland and allows the pathogen to turn from environment-associated to cow-associated [54].

Intramammary infections and mastitis

Mammary epithelial cells are involved in inflammatory processes [55,56]. In comparison to Escherichia (E.) coli, S. uberis induces a delayed mRNA expression of interleukin-8 by epithelial cells [56]. This cytokine is involved in the recruitment of neutrophils [57]. Tassi et al. [31] observed that neutrophils, lymphocytes and interleukin-17A may play roles in the healing of intramammary S. uberis infections.

Numerous authors stated that S. uberis produces clinical and subclinical cases of mastitis [e.g. 58,59], being classified as an...
environment-associated, major pathogen. According to Hillerton and Berry [60], environmental streptococci are responsible for one third of clinical mastitis cases. *S. uberis* enters the udder via the teat canal, and high bacterial counts in the environment raise the infection rate [35].

Reports on the duration of the infection vary considerably. While most infections are relatively short (16 to 46 days; [19,61]), some authors described prolonged periods from 2 to 20 months [43,62,63].

The infection rate of subclinically-diseased quarters seems to be low, even in herds in which *S. uberis* is the most prevalent pathogen of clinical mastitis. Accordingly, Tenhagen et al. [64] detected *S. uberis* in only 1% of quarters sampled in the German federal state of Brandenburg. Zadoks et al. [65] showed that in older cows, prevalence increased with the lactation stage, while during early lactation, there were no significant differences between primiparous and multiparous cows.

On the contrary Piepers et al. [66] pointed out that aesculin-positive cocci including *S. uberis* have become more important as cause of subclinical mastitis. Analysing quarter-milk samples obtained from Belgian dairy cows during cross-sectional dairy herd screenings performed between 2000 and 2002, they found a herd prevalence of aesculin-positive cocci of 2.6% (0-21.7%).

If *S. uberis* evolves to the leading pathogen of a dairy herd, frequent antimicrobial treatments and a series of environment-associated factors seem to promote the development of this type of mastitis [67,68]. Clinical cases caused by *S. uberis* are closely associated with hygiene conditions, feeding and machine-milking [69].

Teat and udder surface contamination between milkings is the first step for the development of mastitis by *S. uberis*. Construction of the resting areas, the available space per cow, the bedding material and its frequency of renewing, cleaning and disinfection and the time the animal spends in the cubicles are factors that determine the infection between milkings [70]. Sources for *S. uberis* are found in loose housing-as well as in pasture systems and also include water for livestock. Increased bacterial counts in bedding materials contribute to rising infection rates which are maximized during warm seasons [61,71].

Previous and persisting infections favour new infections. After the infection by *S. uberis* had healed, an increased risk of reinfection (particularly by *S. uberis*) was observed [65]. The infection rates were also increased if quarters were affected by Trueperella pyogenes, enterococci or *S. aureus*.

Infections with minor pathogens such as *Corynebacterium* (C.) bovis were thought, for a long time, to reduce the risk of becoming affected by *S. uberis*. However, Hogan et al. [72] could demonstrate that udder inflammations due to environmental streptococci were 3.9 times more frequent in quarters infected by *C. bovis* than in uninfected ones; regarding *Staphylococcus*, the infection risk increased by the factor 2.6. To explain this difference to the common assumption, the authors referred to the elevated sampling frequency and to the fact, that 69% of infections with *S. uberis* last fewer than 30 days.

Apart from the transmission by means of the contamination of teat and udder skin between milkings, Zadoks et al. [50] suggested a contagious way of transmission. The pathogen could be detected in a milk swab sample from a liner rubber directly after milking a cow infected with *S. uberis* [18]. Chronically affected animals could contribute particularly to the transmission of the bacterium during milking [73]. As stated above, *S. uberis* could also be isolated from the oral cavity, so that reciprocal sucking of the teats might also pose one risk of infection [74]. According to Smith et al. [71], the new infection rate rises by the factor of 5 between the first and the fourth lactation. The results of Zadoks et al. [65] support these finding by stating that the infection rate regarding environmental streptococci is lower in animals of the first and second lactation than in older animals.

Zadoks et al. [65] did not found a relation between teat end roughness and *S. uberis* mastitis. In contrast, Breen et al. [75] showed that very rough callous rings increase the risk of clinical *S. uberis* mastitis. Furthermore, the absence of callous rings may be associated with an increased mastitis risk. Paduch et al. [48] could show that the teat canal microbial load of *S. uberis* is associated with teat end hyperkeratosis. In the study of Moyes et al. [76] regarding the negative energy balance, udders were infected experimentally with *S. uberis*. One result was that most genes necessary for the modulation of an immune response were suppressed in the case of animals that presented a negative energy balance. However, no significant associations between body condition score (BCS) and infection rate [65] or clinical mastitis risk [75] for *S. uberis* were found. No further details are known regarding the specific interaction between this pathogen and tissue damages produced by inadequate machine milking except that there is a relation between the degree of hyperkeratosis of the teat tip and the microbial load of *S. uberis* [48].

**Control of *S. uberis***

Implementing the five-point-plan developed by Bramley and Dodd [46] to control contagious mastitis (i.e. teat disinfection after milking, dry-off using antibiotics, culling of animals resistant to therapy, therapy of clinical mastitis, maintenance and correct application of the milking machine) affects udder health only marginally in cases where the pathogen is environment-associated [77]. This implies that treatment measures only are not enough to improve the situation of infections.

The goal is rather to minimize the exposure of the teat canal to *S. uberis*. This is obtained by an optimisation of the environment hygiene and the udder preparation. Concerning the latter however, it remains unclear what precisely improves the status of the infection. Hogan et al. [72] focused on the inhibiting interaction between *S. uberis* and *C. bovis* (see above), which would suggest that a high degree of hygiene at milking may reduce the colonization of the teat skin with environment streptococci and *C. bovis* alike. Thus, the protective effect of *C. bovis* would be diminished (as would be the risk of getting infected via teat skin contamination). An udder preparation including humidified, single-use udder towels or a pre-dipping with 0.1% iodophor solution can achieve a significant reduction of the infection rate [78], with pre-dipping being more effective than udder towels soaked in water.

Teat disinfection after milking reduces the prevalence of new infections with *S. agalactiae* and *S. aureus*, but not that of other streptococci [79].

Godinho and Bramley [80] investigated the efficiency of post-milking, dipping disinfectants containing ethanol, iodophores or chlorhexidine on teats which previously had been contaminated with *S. uberis* and *Escherichia coli*. All substances displayed a bactericidal effect, but the persistency of this effect on the teat surface varied. According to this study, a long persistence is beneficial to control environmental pathogens.
The cows’ coat may be contaminated with *S. uberis* and, since reciprocal licking is part of the social behaviour of the animals, the pathogen may be distributed readily from one animal to another. Clipping the udder of the animals reduces the surface available for pathogens, and frequent clipping was associated with low bulk SCC [69]. The same is true for providing clean water for drinking. Zadoks et al. [14] encountered *S. uberis* in water and faeces at irregular intervals, so an oral infection seems possible.

Improving the hygienic conditions of the cows also includes the maintenance of the cubicles and the choice of an adequate bedding material. Zadoks et al. [50] postulated that bedding management may play a role in outbreaks of *S. uberis* mastitis. As stated by Hughes [52], *S. uberis* is not able to grow in bedding materials at pH values above 9.5. Paduch et al. [45] showed that the alkalisation of sawdust bedding reduces teat skin bacterial counts of *S. uberis*. However, associations between the alkalisation and teat canal bacterial counts could not be found for *S. uberis*. In general, environmental pathogens like *S. uberis* and *E. coli* could be controlled by teat cleaning before milking and housing and bedding management practices [81].

If reducing the risk of exposition is one way to reduce the new infection rate, improving the immune status of the animals is another one. As concluded by O’Rourke [81], deficiencies in nutrition are generally associated with the suppression of the immune system which promotes the risk of clinical mastitis. According to a study of Todhunter et al. [61], approx. 50.5% of intramammary infections initiate during the dry period, just as Smith et al. [71] recorded more infections during dry period than during lactation. Applying dry cow treatment is effective to reduce the number of infections by *S. uberis*, especially during the first quarter of the dry period [71]. Therefore, in order to maintain a high level of protection also during the rest of the period, measures beyond drying-off with antibiotics will be necessary. Prepartum teat disinfection with an iodophore dip alone however was not sufficient [82].

Cattell [73] in turn claimed that *S. uberis* as a herd problem originates from a bad choice of dry-off substance and omitting to treat clinical cases. This leads to chronic infections that serve as a reservoir for reinfection which ultimately will increase the presence of this pathogen in the environment. To cope with the latter, the author recommends thorough stripping extraction including a control of this pathogen may be distributed readily from one animal to another. As S. uberis colonizes the ducts before spreading into the mammary parenchyma, intra-cisternal treatment is the treatment of choice. While Sandgren et al. [84] could not find any statistical difference between parenteral and local treatments (five days each), Hillerton and Kliem [85] were able to obtain the best results by applying a sufficient dose of antibiotics intra-cisternally over a prolonged time. Another study [86] treated clinical mastitis due to streptococci using either parenteral applications of penethamate hydroiodine or a combination of cloxacillin and ampicillin locally, each for three days. The bacteriological healing rates were 71 and 74%, resp., i.e. comparable values to those obtained by Hillerton and Kliem [85]. Although these rates did not differ significantly, the parenteral application lead to a reduction of high somatic cell counts in adjacent, culture-negative quarters.

Several studies demonstrated that the extension of the therapy is advantageous [85,87-90]. Oliver et al. [88,90] investigated the effect of time using the third-generation cephalosporin cefotiofur to treat *S. uberis* mastitis for two, five, and eight days. Bacterial cure rates for naturally-occurring, subclinical cases were to 17, 56, and 67%, resp. (no significant differences), while for experimental infections treated intra-cisternally, values were 43, 88 and 100%, respectively. However, in the last trial the somatic cell counts were only reduced when a bacteriological cure had actually taken place.

Hillerton and Kliem [85] also infected cows experimentally with this pathogen. If the clinical cases were left untreated, parenchyma tissue damage was extreme and lead to the loss of the given quarter. If an intensive local treatment (three days, twice a day) was initiated, clinical cure rates amounted to 70% (after three days) and even 100% (after six days). Using a similar design (but with procaine penicillin and dihydrostreptomycin applied parenterally) lead to clinical cure rates of 18 and 91%, respectively. Bacteriological cure was obtained in 80% of quarters in both designs. By combining local and parenteral treatment, clinical cure rates were 61 and 100%, respectively. However, the bacteriological cure rate of 72% ranged below the values obtained for the single treatments. One intra-cisternal application per day over a period of three days reduced clinical (27 and 91%, resp.) and bacteriological cure rates (64%) alike.

Administering oxytoci in intramuscularly twice a day for three days as an alternative did not produce any clinical cure, neither alone nor in combination with a once-a-day intra-cisternal treatment with antibiotics. Even after six days, this treatment combination only yielded 10% of clinical healing.

McDougall et al. [91] applied 5 g tylosin base daily for three days and obtained a bacteriological healing in 89.8% of quarters. 87.7% of quarters were healed if 5 g of penethamate hydroiodine were used instead; results did not differ significantly.

Cattell et al. [92] applied lincosamides (pirlimycin hydrochlorine) daily for two days into the cistern to treat clinical and subclinical mastitis due to streptococci and obtained bacteriological cure rates of 48% (cow level) and 70% (udder level).

As demonstrated by Hillerton and Kliem [85], applying oxytocin alone to treat clinical cases by *S. uberis* is unsatisfactory. 20 IU of this hormone administered to cows with a suspected case of mastitis eliminated 25% of cases; thus, 75% of animals still developed clinical mastitis [93]. Comparing three consecutive intracisternal applications of antibiotics (at each milking time i.e. over a period of 1,5 days) with 10 applications (i.e. over a period of 5 days) the prolonged treatment
regimen leads in cases of moderate an severe clinical mastitis cases to higher bacteriological and cytological cleaning rates [89].

In a study of Roberson et al. [94] frequent milk-out appeared to be detrimental for managing mild to moderate clinical mastitis caused by environmental streptococi.

Dry cow treatment

As stated above, new infections are most frequent during the dry period, and dry-off treatment leads to a marked reduction of problems during the first phase of the dry period. Cure rates of 87 to 95% were observed when administering semi-synthetic penicillins [43]. Yet, the last two weeks remain unprotected (in many cases, the teat canal open up, the keratin plug gets lost and the periparturient immune suppression commences).

Usage of dry cow antibiotics plays an important role in the reduction of mastitis incidence due to S. uberis. In a study of Williamson et al. [59] the administration of a dry cow antibiotic to uninfected quarters reduced the incidence of new infections with S. uberis from 12.3% (untreated quarter) to 1.2% (treated quarter). This reduction was significant for dry-period as well as post-calving infections.

Furthermore the usage of external or internal teat sealers should lower the infection rate with environmental germs like S. uberis and E. coli. Lim et al. [95,96] showed that the efficiency of an external teat sealer varies with the time frame of adherence (longer adherence on longer teats, in cold climate or after double application). In the review of Rabiee and Lean [97], internal teat sealer alone or in combination with antibiotic dry cow therapy reduced the risk of acquiring new intramammary infections after calving by 25%. Compared with untreated cows, internal teat sealer reduced the risk by 73% although there could be stated a huge heterogeneity in the results. The reduction of clinical mastitis was calculated with 29% and 48%.

Vaccination

Since Hill [98] showed that previous exposure to S. uberis could provide some resistance to infection against the same strain, several trials with a broad variety in efficiency were performed to identify potential vaccine candidates against S. uberis mastitis: i.e. the plasminogen activator PauA as a total antigen as well as a PauA depleted antigen [99], recombinant S. uberis GapC or a chimeric CAMP antigen [100], recombinant S. uberis adhesion molecule [101] as well as S. uberis bacterin [102] etc. Until now there is still a lack of vaccine which is able to control infection without the participation of a marked inflammatory response.

Conclusion

Controlling S. uberis-infections remains an important task. The germ is commonly found in manure and other organic matter. Inadequate stall or pasture management e.g. dirty and wet bedding material or muddy areas as well as improper milking procedures lead to an increased infection risk. As infections are difficult to cure, emphasis needs to be placed on prevention of these infections. Still further research is necessary to allow a ranking of management methods regarding their efficiency to reduce contamination with these bacteria.

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