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FOOD MICROBIOLOGY

Circulation of *Streptococcus agalactiae* ST103 in a Free Stall Italian Dairy Farm

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ABSTRACT We report here on an outbreak of mastitis caused by *Streptococcus agalactiae*, or group B *Streptococcus*, in a northern Italy (Lombardy Region) free stall dairy farm. This outbreak was unusual because it occurred in a closed dairy herd and proved to be extremely difficult to resolve even after the application of the classical control procedures, which are specifically focused on the contagious nature of *S. agalactiae*. In order to better understand the potential origins of the pathogen and the critical points that could impair the eradication program and to investigate the possible presence of *S. agalactiae* in sources outside the mammary gland, we collected 656 individual composite milk samples, 577 samples from extramammary body sites (289 rectal, 284 vaginal, and four throat samples from milking cows, dry cows, heifers, and calves), and 81 samples from the cattle environment, including the milking parlor and the barn. Twenty-two *S. agalactiae* isolates were obtained from lactating cows or their environment. Of these, nine were isolated from milk, two were from rectal swabs, and two were from vaginal swabs, while nine were isolated from environmental samples. Based on molecular serotyping, pilus island (PI) typing and multilocus sequence typing, all isolates belonged to serotype III, pilus type PI-1/2b, and sequence type 103 (ST103), a type previously described to have an environmental transmission cycle and a potential human origin. Once the classical mastitis control measures were supplemented with environmental hygiene measures, herd monitoring using bulk tank milk revealed no further positive results for *S. agalactiae*, and the outbreak was considered resolved.

IMPORTANCE *Streptococcus agalactiae* is an important pathogen in humans and cattle. Bovine mastitis caused by this bacterium and its control are generally associated with contagious transmission between animals. More recently, the presence of a fecal-oral transmission cycle in cattle has been proposed, linked to the ability of some *S. agalactiae* strains to survive in the bovine gastrointestinal tract and environment. Based on analysis of 1,316 specimens from cattle and their environment on a single dairy farm, we demonstrate the presence of sequence type 103 (ST103), which may have an environmental mode of transmission. This possibility was supported by the fact that the mastitis outbreak could not be controlled through measures to prevent contagious transmission alone and required additional environmental hygiene measures to be brought to a halt. This case study highlights that measures to control animal disease need to evolve alongside the microorganisms that cause them.

KEYWORDS sequence type 103, ST103, *Streptococcus agalactiae*, cattle, mastitis, veterinary epidemiology

Mastitis is one of the costliest and most antibiotic-requiring diseases affecting dairy cattle (1, 2). Bacterial pathogens causing mastitis are classified as contagious or environmental (3). Bacteria belonging to the first group survive primarily or exclusively

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within the udder are mainly transmitted during milking from infected to healthy cows. Their introduction into previously negative dairy farms is generally attributed to the acquisition of infected animals from other herds. Bacteria belonging to the second group are opportunistic invaders of the mammary gland and can be found in environmental sources. To address the distinct transmission patterns, different approaches are usually taken for the control of pathogens belonging to these two groups. Traditionally, the contagious versus environmental nomenclature has been used for the main bacterial species known to cause bovine mastitis. Using molecular epidemiology approaches, however, it has since been shown that bacterial species that were traditionally known as "environmental" include strains that may spread from cow to cow, and species traditionally known as "contagious" may include strains that spread via the environment (4). Thus, preliminary classification of mastitis as contagious or environmental, based on species-level diagnosis pathogens, may need to be revised at the strain level, especially if control measures fail (5).

Among the bacterial mastitis agents, *Streptococcus agalactiae* is a reemerging pathogen in European countries, despite the implementation of control programs aimed at eradicating it, in particular in north European countries (6–9). In a veterinary context, *S. agalactiae* mastitis was considered to be strictly contagious, with the udder believed for decades to be the sole reservoir of the organism on dairy farms (10). This notion is flawed because *S. agalactiae*, known in human medicine as group B *Streptococcus*, is also able to infect humans, causing a variety of clinical manifestations (11), as well as frequent colonization of the gastrointestinal and urogenital tracts of healthy men and women (7, 12, 13). Intramammary *S. agalactiae* infection in dairy cattle usually manifests as chronic and subclinical mastitis, characterized by a high somatic cell count and a reduction in milk production, causing significant impact on milk quality and milk quantity. Jørgensen et al. (7) reported that dairy cattle can also carry *S. agalactiae* in their gastrointestinal tract and demonstrated the existence of an orofecal transmission cycle on dairy farms, which could contribute to the failure of eradication, particularly in free stall herds and proposed further measures for optimizing eradication programs to complement those aimed at preventing pathogen transmission during milking.

In addition to alternative transmission cycles, the issue of alternative sources needs to be considered, i.e., sources other than cattle with intramammary infection. In herds that have been free of *S. agalactiae* mastitis for many years and that do not bring any animals into the herd from elsewhere, it is difficult to explain the occurrence of *S. agalactiae* mastitis, unless sources other than the bovine mammary gland exist. The importance of this phenomenon has been quantified in Denmark, which has a national group B *Streptococcus* (GBS) control program, with annual monitoring of every dairy herd. Based on years of data from this program, approximately half of the newly infected herds in Denmark had brought in animals from other herds, but half had not (14). For those herds, the possibility of introduction of *S. agalactiae* by people needs to be considered. Strain typing of isolates from cattle and people supports this possibility, with suggestions or evidence of potential human-to-animal transmission from North America (15), South America (16), and Europe (17). Indeed, in northern Europe and in Colombia, a considerable proportion of bovine mastitis cases is caused by sequence types (ST) that are also found in people (8, 9, 16, 18).

In Italy, there is no national control program but contagious catarrhal mastitis, the disease caused by *S. agalactiae*, is subject to mandatory notification, and two regions in the north of the country, Lombardy and Emilia Romagna, have current control programs (19, 20). In Lombardy, a monitoring program for *S. agalactiae* in dairy farms has been in force since 2012, when the herd-level prevalence of the infection was estimated to be around 17.2% (13). According to the program, once a year, bulk milk samples are collected by the Official Veterinary Services from all the dairy farms of the region to check for the presence of *S. agalactiae* (20), which is similar to the approach taken in Denmark (14, 21, 22). Subsequently, farms can enroll in a voluntary eradication program. Notably, by 2018, the registered herd-level prevalence of contagious

TABLE 1 Animal and environmental source samples tested and found to be positive for *S. agalactiae* during an outbreak of mastitis on a dairy farm in the Lombardy region, northern Italy

General source	Specific source	Sample type	No. of samples	
			Tested	Positive (%)
Animal	Cow	Milk	656	9 (1.37)
		Rectal swab	271	2 (0.74)
		Vaginal swab	270	2 (0.74)
	Heifer (prepartum)	Rectal swab	14	0 (0)
		Vaginal swab	14	0 (0)
	Calf	Rectal swab	4	0 (0)
		Throat swab	4	0 (0)
	Total animals		1,233	13 (1.05)
Environmental	Barn	Floor	26	1 (3.85)
		Cubicles	22	3 (13.6)
		Water trough	26	4 (15.4)
	Milking parlor	Floor	3	1 (33.3)
		Teat cup liners	4	0 (0)
	Total environment		81	9 (11.1)

catarrhal mastitis in the Lombardy region had decreased to 7.3%, demonstrating the feasibility of eradicating *S. agalactiae* from dairy herds (13).

In 2019, our laboratory was asked to investigate an outbreak of *S. agalactiae* in a Lombardy region dairy herd which could not be resolved despite the adoption and supervised implementation of classical control measures. These measures are related to the contagious nature of *S. agalactiae* and focus on the identification and removal of the source of infection, i.e., *S. agalactiae*-positive cows, through antibiotic treatment of infected cows and culling of chronically infected cows, and on prevention of transmission from positive cows to susceptible ones through good milking hygiene and use of postmilking teat disinfection (see Materials and Methods for further information). To understand the persistence of the problem despite the implementation of specific control measures, we cultured and subtyped *S. agalactiae* isolates from bulk and individual milk, as well as from sources other than the udder, and investigated hypothetical alternative transmission patterns so that effective control measures for the eradication of *S. agalactiae* could be proposed.

RESULTS

S. agalactiae field isolates were obtained from 9 of 656 individual milk samples (1.37%, Table 1) from nine different cows (two from fresh cows and seven from animals with subclinical mastitis). Moreover, *S. agalactiae* was isolated from 2 of 271 rectal swabs (0.74%) and 2 of 270 vaginal swabs (0.74%) (Table 1). The four cows that tested positive on swabs were sampled again 2 months later, but repeat samples did not yield *S. agalactiae*. All positive samples originated from lactating cows in groups A or B, with only a single positive sample per cow. During the sampling, no vaginal lesions were observed. From the environmental samples, we isolated *S. agalactiae* from three cubicles hosting lactating cows, from the floor of lactating cow group A, from the floor of the milking parlor, and from four drinking troughs of the lactating cows, but not in any environmental samples from dry cows, young stock, or calves (Table 1).

All phenotypically identified isolates (nine from individual milk, two from rectal swabs, two from vaginal swabs, and nine from environmental swabs) were confirmed to be *S. agalactiae*. All isolates belonged to PI-1/2b, MCT III, and ST103. Phenotypically, all isolates fermented lactose and showed *in vitro* resistance to tetracycline and, as expected for all *Streptococcus* strains, to kanamycin.

DISCUSSION

We describe an outbreak in a dairy cattle herd in Italy of *S. agalactiae* ST103 and confirm the likely existence of environmental pathways of infection for this pathogen. The farm under investigation was located in the Lombardy region, an area where, in the frame of an official surveillance program, the bulk tank milk had been monitored annually by the Veterinary Service since 2012. Although eradication is not compulsory, a farm positive for *S. agalactiae* is not allowed to sell animals to *S. agalactiae*-free herds. In addition, being free from *S. agalactiae* is generally considered a critical point for the application of selective dry-cow therapy (23), which is mandatory from February 2022 (regulation [EU] 2019/6) (24). For both of these reasons, the owners of the farm were very motivated to undertake an eradication strategy.

We believe the issues encountered in the eradication of the infection in this herd, despite the application of strict procedures focused on prevention of contagious transmission, could be attributable to the existence of an environmental transmission cycle for ST103. During our investigation, *S. agalactiae* isolates were recovered from extramammary body sites, including rectal swabs. The specimens (swabs from animals and environmental samples) were collected in winter, so the possibility of shedding due to heat stress was considered negligible. *S. agalactiae* ST103 has previously been detected in the gastrointestinal tract of dairy cattle (7, 25). Our findings, including detection of fecal shedding and resolution of the outbreak after improved environmental hygiene, are compatible with the existence of an environmental transmission cycle, and could involve an orofecal transmission cycle as proposed by Jørgensen et al. (7). These authors demonstrated the ability of ST103 to persist in the plaque and crusts on the surfaces of the drinking troughs. During our investigation, we also detected ST103 in water troughs and, even when the drinking water was clear, plaques of dirt on the walls of drinking troughs were visible.

In the outbreak herd, *S. agalactiae* was isolated only from the lactating cows and their environment. During our inspection of the lactating cow barn, we noticed many wet spots in the bedding and on the ground. These spots could be due to milk leakage caused by weakening of the teat sphincter, typical in animals with high milk yield. One of the swabs from bedding contaminated with milk tested positive for *S. agalactiae*, which, like fecal contamination, may result in environmental transmission. A similar phenomenon has been described for *Klebsiella* mastitis and could be classified as "contagious via the environment" because the original source is an infected dairy cow (as is the case for contagious mastitis), but the exposure of other cows happens via the barn environment (as is the case for environmental mastitis) (26). Based on the molecular typing alone, it is not possible to determine whether the predominance of a single pathogen strain, as observed here, is due to contagious transmission, point source transmission (here via the milk spots in the bedding), or environmental transmission of organisms from bovine feces. The impact of interventions, however, can show the difference between contagious and environmental transmission (7, 27). Therefore, in addition to the already-described classical measures for the eradication of *S. agalactiae* infection, further environmental hygiene measures were proposed to the farmer to break the environmental transmission cycle. Specifically, we suggested improving the hygienic conditions of drinking troughs, beds, cubicles, and floors (see "Evidence-Based Intervention" in Materials and Methods). A follow-up investigation over the 6 months after the application of the environmental hygienic measures revealed no *S. agalactiae* in bulk-milk samples that were collected twice a month. This suggests that a reduction in environmental transmission (whether from a point source in bedding, or from fecal contamination of the environment) was successful in control of an outbreak that could not be controlled with a focus on contagious transmission alone.

All ST103 isolates from the farm showed resistance toward tetracycline. Interestingly, authors from various countries reported that isolates belonging to clonal complex 103, which includes ST103, carried tetracycline resistance (4, 28–31). It has been suggested that *S. agalactiae* clones infecting humans were selected and fixed through the extensive use of tetracycline

(32). This raises the question of whether a person may have served as the source of introduction of the pathogen into the closed herd. The possibility of human-to-bovine transmission has been described before, both with epidemiological data (16, 17) and with evolutionary data (9, 33). Indeed, the presence of the tetracycline resistance gene *tet*(M) in newly emerged (last 25 years) bovine *S. agalactiae* lineages in Sweden was interpreted as an indicator of reverse zoonotic events (human-to-bovine spillover), followed by amplification and onward transmission in the bovine population (9). In a survey aimed at comparing human and bovine isolates in the Emilia Romagna region (an area of northern Italy bordering the Lombardy region), ST103 was the third most common *S. agalactiae* ST from cattle (15 of 103 strains), indicating that this type is now well established in the regional cattle population (18). Likewise, ST103 or closely related variants were among the three most common types of *S. agalactiae* in bovine milk in Denmark (4), Sweden (9), Colombia (16), and China (34). ST103 has also been reported among human *S. agalactiae* in emerging economies (31, 35), so a possible future spread of this genotype to humans from bovine or *vice versa*, as probably happened in Sweden, cannot be excluded (9). In addition, its ability to form biofilms and to survive in feces and the environment, combined with its common occurrence in dairy farms in Italy and elsewhere, implies that we must be open to the possibility of the emergence of environmental *S. agalactiae* mastitis. This is even more important now that Europe has banned blanket dry-cow treatment, which was long used in many countries to limit the prevalence of *S. agalactiae* in dairy herds (7).

MATERIALS AND METHODS

Study farm. A free stall farm that had been negative for *S. agalactiae* in bulk milk since 2012 based on annual monitoring through the regional control plan, tested positive for the first time in 2018, without having introduced animals from outside. The farm, located in the Po valley area of the Lombardy region, had an average daily milk production of 40 L per cow. The lactating herd consisted of 190 cows on average, divided into three groups. The group of the "fresh cows" was housed in cubicles with straw bedding. Animals stayed in this group for around a week. Subsequently, lactating cows were divided into two groups: group A included multiparous cows housed on solid floors managed with a scraper, and group B included primiparous cows housed on a slatted floor. Groups A and B had access to cubicles with rubber mats covered with pelleted straw. The heifers (nulliparous animals) were located a few kilometers away from the main farm in a dedicated heifer-raising facility and were raised in cubicles with straw bedding. Calves were raised in groups on straw bedding. The farm had not introduced animals from the outside for decades. The waste milk, i.e., milk from cows that were treated for mastitis or other conditions, was discarded. Unpasteurized colostrum was used for the first meal of each calf; it was then replaced by commercial milk substitutes. The bulk milk somatic cell count was consistently $\leq$200,000 cells/mL, both before and during the outbreak (2017 to 2022).

Control of mastitis. The farm recorded, on average, one case of clinical mastitis per month. Blanket dry-cow therapy with sodium cloxacillin, coupled with teat sealant, was applied. The farm was characterized by regular and programmed maintenance of the milking machine. The bulk tank milk (BTM) was tested once every 3 months for *S. agalactiae*. If the sample tested positive, the milking cows were individually tested, taking a composite sample from each cow, i.e., a sample containing equal volumes of milk from each functional udder quarter. Cows whose milk tested positive for *S. agalactiae* were treated with antimicrobials, and response to treatment was monitored by bacteriological culture of milk samples collected 2 to 3 weeks posttreatment. If still positive, a second cycle of therapy was applied. Cows that were still *S. agalactiae* positive after the second antimicrobial treatment or that showed clinical signs of chronic mastitis or high somatic cell count (SCC) were considered chronically infected, and they were culled. From 2019, a more stringent protocol was implemented, consisting of culling of any cows that tested positive for *S. agalactiae*. A standardized milking procedure was performed with the use of predipping (lactic acid plus chlorhexidine), postdipping (iodine), and a clean paper towel for each cow; latex gloves were used and disinfected after milking each animal. Notably, despite the application of these measures, aimed at controlling the contagious nature of *S. agalactiae* under veterinary supervision to ensure consistency of implementation, the outbreak was not resolved.

Sampling. In total, 656 individual composite milk samples were collected and analyzed for the presence of *S. agalactiae* (Table 1). This included two whole-herd surveys of all lactating animals, as well as samples from all fresh cows, all cows with positive a California mastitis test (an indicator of high SCC and subclinical mastitis), and all cows with clinical mastitis signs. After disinfection of teat ends and removing the first streaks of milk from the quarter, a composite milk sample from all quarters was collected into a single sterile 10-mL vials. Vials labeled with the animal identification number were stored at −20°C until they were shipped to the laboratory.

To understand possible sources of infection and to explore whether the issues encountered in eradication might be caused by alternative transmission routes, notably environmental transmission, we investigated sites other than the udder, collecting 577 samples (289 rectal, 284 vaginal, and 4 throat samples) from cattle (milking cows, dry cows, heifers, and calves), and 81 from the cattle environment. For these samples, flocked swabs in transport medium were used. Swab collection from animals and the

environment was performed as described previously (7). All samples were refrigerated during transport to the laboratory. The sampling details (number, animal category, and sources), and the relative results are reported in Table 1.

Sample analysis. Milk samples were cultured according to the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER) routine procedures, in line with the recommendation of the National Mastitis Council (36). Briefly, 10-μL samples of milk were inoculated on one plate of TKT medium (Tallium Kristalviolette Toxin, containing staphylococcal β hemolysin for visualization of the Christie–Atkins–Munch-Peterson [CAMP] reaction). Plates were incubated aerobically for 48 h at 37°C and examined at 24 and 48 h. Suspected *S. agalactiae* colonies, characterized by bluish pigmentation, the absence of esculin splitting, and the presence of a hemolytic area, were subjected to Gram staining and additional CAMP testing on blood agar supplemented with esculin (1 g/L) and iron citrate (100 mg/L).

Swabs from extramammary body sites or environmental samples were placed in 5 mL of Todd-Hewitt broth with colistin (0.01 g/L) and nalidixic acid (0.015 g/L) (Thermo Fisher Scientific, USA), vortexed, and incubated aerobically for 24 h at 37°C. Then, 10-μL portions were plated to obtain isolated colonies on modified Edwards medium with the addition of 5% sheep blood (Bioside, Italy). Plates were incubated for 24 to 48 h at 37°C and observed daily; suspected *S. agalactiae* colonies with gray to blue pigmentation were transferred onto blood agar to obtain isolated colonies and confirmed as Gram positive, esculin negative, and CAMP positive.

Molecular analysis. DNA was extracted from suspected *S. agalactiae* colonies, submitted to species-specific PCR (8), and stored at −80°C until further molecular and phenotypic analysis. This included molecular capsular typing, PI typing, multilocus sequence typing, and phenotypic lactose typing (8, 37–39). Alleles and sequence types (ST) were assigned using the *S. agalactiae* database (40; http://pubmlst.org/sagalactiae/).

Antimicrobial tests. The antimicrobial susceptibility of the isolates was evaluated using the disc diffusion method according to the Clinical and Laboratory Standard Institute (CLSI) guidelines, which are specific to animals (41). The compounds included in the test were part of the laboratory panel that is routinely used for Gram-positive mastitis pathogens at IZSLER. The compounds used are representative of their antimicrobial class, according to the guidelines of the Italian Reference Centre for Antimicrobial Resistance (https://www.izslt.it/crab/linee-guida-per-linterpretazione-delle-prove-di-sensibilita-ai-chemioantibiotici-in-vitro-per-un-utilizzo-nella-terapia-clinica/). The following antimicrobials were tested: amoxicillin-clavulanic acid (20 and 10 μg), ampicillin (10 μg), cephalothin (30 μg), ceftiofur (30 μg), erythromycin (15 μg), kanamycin (30 μg), penicillin G (10 IU), pirlimycin (2 μg), rifampicin (5 μg), sulfisoxazole (300 μg), tetracycline (30 μg), and trimethoprim-sulfamethoxazole (1.25 and 23.75 μg).

Evidence-based intervention. Because the management measures reported in the control plan failed to eradicate the outbreak and based on the results of our sampling and testing, we proposed additional control measures aimed at preventing environmental mastitis. This included an increase in the frequency of floor scraping (several times per day) and removal of dirt from the slatted floor area at least twice. Moreover, because *S. agalactiae* can survive in milk fat and in fresh water (7), we suggested the adoption of control measures aimed at reducing the presence of leaked milk in bedding areas and the humidity of passage lanes. Finally, we suggested improved cleaning of drinking troughs, including removal of dirt and plaque. To minimize the risk of introduction of *S. agalactiae* into the herd by people, we recommended strict biosecurity measures: dedicated farm personnel, with no contacts with other herds, the use of disposable gloves, and the use of clean clothes and footwear during animal handling.

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REFERENCES

1. Halasa T, Huijps K, Østerås O, Hogeveen H. 2007. Economic effects of bovine mastitis and mastitis management: a review. Vet Q 29:18–31. https://doi.org/10.1080/01652176.2007.9695224.
2. Krömker V, Leimbach S. 2017. Mastitis treatment-reduction in antibiotic usage in dairy cows. Reprod Domest Anim 52(Suppl 3):21–29. https://doi.org/10.1111/rda.13032.
3. Ruegg PL. 2017. A 100-year review: mastitis detection, management, and prevention. J Dairy Sci 100:10381–10397. https://doi.org/10.3168/jds.2017-13023.
4. Zadoks R, Middleton J, McDougall S, Katholm J, Schukken Y. 2011. Molecular epidemiology of mastitis pathogens of dairy cattle and comparative relevance to humans. J Mammary Gland Biol Neoplasia 16:357–372. https://doi.org/10.1007/s10911-011-9236-y.
5. Gurjar A, Gioia G, Schukken Y, Welcome F, Zadoks R, Moroni P. 2012. Molecular diagnostics applied to mastitis problems on dairy farms. Vet Clin North Am Food Anim Pract 28:565–576. https://doi.org/10.1016/j.cvfa.2012.07.011.
6. Katholm J, Bennedsgaard TW, Koskinen MT, Rattenborg E. 2012. Quality of bulk tank milk samples from Danish dairy herds based on real-time polymerase chain reaction identification of mastitis pathogens. J Dairy Sci 95:5702–5708. https://doi.org/10.3168/jds.2011-5307.
7. Jørgensen HJ, Nordstoga AB, Sviland S, Zadoks RN, Sølverød L, Kvitle B, Mørk T. 2016. *Streptococcus agalactiae* in the environment of bovine dairy herds: rewriting the textbooks? Vet Microbiol 184:64–72. https://doi.org/10.1016/j.vetmic.2015.12.014.
8. Lyhs U, Kulkas L, Katholm J, Waller KP, Saha K, Tomusk RJ, Zadoks RN. 2016. *Streptococcus agalactiae* serotype IV in humans and cattle, northern Europe. Emerg Infect Dis 22:2097–2103. https://doi.org/10.3201/eid2212.151447.
9. Crestani C, Forde T, Lycett S, Holmes M, Fasth C, Persson-Waller K, Zadoks R. 2021. The fall and rise of group B *Streptococcus* in dairy cattle: reintroduction due to human-to-cattle host jumps? Microb Genom 7:000648. https://doi.org/10.1099/mgen.0.000648.
10. Keefe G. 2012. Update on control of *Staphylococcus aureus* and *Streptococcus agalactiae* for management of mastitis. Vet Clin North Am Food Anim Pract 28:203–216. https://doi.org/10.1016/j.cvfa.2012.03.010.
11. Le Doare K, Heath PT. 2013. An overview of global GBS epidemiology. Vaccine 31(Suppl 4):D7–D12. https://doi.org/10.1016/j.vaccine.2013.01.009.

12. van der Mee-Marquet N, Fourny L, Arnault L, Domelier AS, Salloum M, Lartigue MF, Quentin R. 2008. Molecular characterization of human-colonizing *Streptococcus agalactiae* strains isolated from throat, skin, anal margin, and genital body sites. J Clin Microbiol 46:2906–2911. https://doi.org/10.1128/JCM.00421-08.
13. Bergseng H, Bevanger L, Rygg M, Bergh K. 2007. Real-time PCR targeting the sip gene for detection of group B *Streptococcus* colonization in pregnant women at delivery. J Med Microbiol 56:223–228. https://doi.org/10.1099/jmm.0.46731-0.
14. Mweu MM, Nielsen SS, Halasa T, Toft N. 2014. Spatiotemporal patterns, annual baseline and movement-related incidence of *Streptococcus agalactiae* infection in Danish dairy herds: 2000–2009. Prev Vet Med 113:219–230. https://doi.org/10.1016/j.prevetmed.2013.10.020.
15. Dogan B, Schukken Y, Santisteban C, Boor K. 2005. Distribution of serotypes and antimicrobial resistance genes among *Streptococcus agalactiae* isolates from bovine and human hosts. J Clin Microbiol 43:5899–5906. https://doi.org/10.1128/JCM.43.12.5899-5906.2005.
16. Cobo-Angel CG, Jaramillo-Jaramillo AS, Palacio-Aguilera M, Jurado-Vargas L, Calvo-Villegas EA, Ospina-Loaiza DA, Rodriguez-Lecompte JC, Sanchez J, Zadoks R, Ceballos-Marquez A. 2019. Potential group B *Streptococcus* interspecies transmission between cattle and people in Colombian dairy farms. Sci Rep 9:14025. https://doi.org/10.1038/s41598-019-50225-w.
17. Sørensen UBS, Klaas IC, Boes J, Farre M. 2019. The distribution of clones of *Streptococcus agalactiae* (group B streptococci) among herdspersons and dairy cows demonstrates lack of host specificity for some lineages. Vet Microbiol 235:71–79. https://doi.org/10.1016/j.vetmic.2019.06.008.
18. Carra E, Russo S, Micheli A, Garbarino C, Ricchi M, Bergamini F, Bassi P, Prosperi A, Piva S, Cricca M, Schiavo R, Merialdi G, Serraino A, Arrigoni N. 2021. Evidence of common isolates of *Streptococcus agalactiae* in bovines and humans in Emilia Romagna region (northern Italy) Front Microbiol 12:673126. https://doi.org/10.3389/fmicb.2021.673126.
19. Tamba M, Pallante I, Petrini S, Feliziani F, Iscaro C, Arrigon iN, Di Sabatino D, Barberio A, Cibin V, Santi A, Ianniello M, Ruocco L, Pozzato N. 2021. Overview of control programs for twenty-four infectious cattle diseases in Italy. Front Vet Sci 8:665607. https://doi.org/10.3389/fvets.2021.665607.
20. Zanardi G, Vitali A, Bertocchi L, Bolzoni G. 2020. Infezione da *Streptococcus agalactiae* in bovine da latte: epidemiologia e piano di controllo di regione Lombardia. Large Animal Rev 25:207–213.
21. Mweu MM, Nielsen SS, Halasa T, Toft N. 2012. Annual incidence, prevalence and transmission characteristics of *Streptococcus agalactiae* in Danish dairy herds. Prev Vet Med 106:244–250. https://doi.org/10.1016/j.prevetmed.2012.04.002.
22. Churakov M, Katholm J, Rogers S, Kao RR, Zadoks RN. 2021. Assessing potential routes of *Streptococcus agalactiae* transmission between dairy herds using national surveillance, animal movement data and molecular typing. Prev Vet Med 197:105501. https://doi.org/10.1016/j.prevetmed.2021.105501.
23. Bradley A, De Vliegher S, Farre M, Jimenez LM, Peters T, de Leemput ES, van Werven T. 2018. Pan-European agreement on dry cow therapy. Vet Rec 182:637. https://doi.org/10.1136/vr.k2382.
24. European Union. 2019. Regulation (EU) 2019/6 of the European Parliament and of the Council of 11 December 2018 on veterinary medicinal products and repealing Directive 2001/82/EC). European Union, Brussels, Belgium.
25. Cobo-Ángel C, Jaramillo-Jaramillo AS, Lasso-Rojas LM, Aguilar-Marin SB, Sanchez J, Rodriguez-Lecompte JC, Ceballos-Márquez A, Zadoks RN. 2018. *Streptococcus agalactiae* is not always an obligate intramammary pathogen: molecular epidemiology of GBS from milk, feces and environment in Colombian dairy herds. PLoS One 13:e0208990. https://doi.org/10.1371/journal.pone.0208990.
26. Munoz MA, Welcome FL, Schukken YH, Zadoks RN. 2007. Molecular epidemiology of two *Klebsiella pneumoniae* mastitis outbreaks on a dairy farm in New York State. J Clin Microbiol 45:3964–3971. https://doi.org/10.1128/JCM.00795-07.
27. Sommerhäuser J, Kloppert B, Wolter W, Zschöck M, Sobiraj A, Failing K. 2003. The epidemiology of *Staphylococcus aureus* infections from subclinical mastitis in dairy cows during a control programme. Vet Microbiol 96:91–102. https://doi.org/10.1016/s0378-1135(03)00204-9.
28. Oliveira IC, de Mattos MC, Pinto TA, Ferreira-Carvalho BT, Benchetrit LC, Whiting AA, Bohnsack JF, Figueiredo AM. 2006. Genetic relatedness between group B streptococci originating from bovine mastitis and a human group B *Streptococcus* type V cluster displaying an identical pulsed-field gel electrophoresis pattern. Clin Microbiol Infect 12:887–893. https://doi.org/10.1111/j.1469-0691.2006.01508.x.
29. Yang J, Xu D, Yin LQ, Zhu BQ, Wang AH. 2013. Neonatal group B *Streptococcus* infection in the children's hospital of Gansu province through PCR array. Zhonghua Er Ke Za Zhi 51:688–691.
30. Carvalho-Castro GA, Silva JR, Paiva LV, Custódio DAC, Moreira RO, Mian GF, Prado IA, Chalfun-Junior A, Costa GM. 2017. Molecular epidemiology of *Streptococcus agalactiae* isolated from mastitis in Brazilian dairy herds. Braz J Microbiol 48:551–559. https://doi.org/10.1016/j.bjm.2017.02.004.
31. Botelho ACN, Ferreira AFM, Fracalanzza SEL, Teixeira LM, Pinto TCA. 2018. A perspective on the potential zoonotic role of *Streptococcus agalactiae*: searching for a missing link in alternative transmission routes. Front Microbiol 9:608. https://doi.org/10.3389/fmicb.2018.00608.
32. Da Cunha V, Davies MR, Douarre PE, Rosinski-Chupin I, Margarit I, Spinali S, Perkins T, Lechat P, Dmytruk N, Sauvage E, Ma L, Romi B, Tichit M, Lopez-Sanchez MJ, Descorps-Declere S, Souche E, Buchrieser C, Trieu-Cuot P, Moszer I, Clermont D, Maione D, Bouchier C, McMillan DJ, Parkhill J, Telford JL, Dougan G, Walker MJ, Holden MTG, Poyart C, Glaser P, DEVANI Consortium. 2014. *Streptococcus agalactiae* clones infecting humans were selected and fixed through the extensive use of tetracycline. Nat Commun 5:4544. https://doi.org/10.1038/ncomms5544.
33. Richards VP, Velsko IM, Alam T, Zadoks RN, Manning SD, Pavinski Bitar PD, Hasler HB, Crestani C, Springer G, Probert B, Town CD, Stanhope MJ. 2019. Population gene introgression and high genome plasticity for the zoonotic pathogen *Streptococcus agalactiae*. Mol Biol Evol 36:2572–2590. https://doi.org/10.1093/molbev/msz169.
34. Yang Y, Liu Y, Ding Y, Yi L, Ma Z, Fan H, Lu C. 2013. Molecular characterization of *Streptococcus agalactiae* isolated from bovine mastitis in eastern China. PLoS One 8:e67755. https://doi.org/10.1371/journal.pone.0067755.
35. de Aguiar EL, Mariano DC, Viana MV, Benevides Le J, de Souza Rocha F, de Castro Oliveira L, Pereira FL, Dorella FA, Leal CA, de Carvalho AF, Santos GS, Mattos-Guaraldi AL, Nagao PE, de Castro Soares S, Hassan SS, Pinto AC, Figueiredo HC, Azevedo V. 2016. Complete genome sequence of *Streptococcus agalactiae* strain GBS85147 serotype of type Ia isolated from human oropharynx. Stand Genomic Sci 11:39. https://doi.org/10.1186/s40793-016-0158-6.
36. Adkins P, Middleton J. 2017. Laboratory handbook on bovine mastitis. National Mastitis Council, Inc, New Prague, MN.
37. Poyart C, Tazi A, Réglier-Poupet H, Billoët A, Tavares N, Raymond J, Trieu-Cuot P. 2007. Multiplex PCR assay for rapid and accurate capsular typing of group B streptococci. J Clin Microbiol 45:1985–1988. https://doi.org/10.1128/JCM.00159-07.
38. Springman A, Lacher D, Waymire E, Wengert S, Singh P, Zadoks R, Davies H, Manning S. 2014. Pilus distribution among lineages of group B *Streptococcus*: an evolutionary and clinical perspective. BMC Microbiol 14:159. https://doi.org/10.1186/1471-2180-14-159.
39. Jones N, Bohnsack J, Takahashi S, Oliver K, Chan M, Kunst F, Glaser P, Rusniok C, Crook D, Harding R, Bisharat N, Spratt B. 2003. Multilocus sequence typing system for group B *Streptococcus*. J Clin Microbiol 41:2530–2536. https://doi.org/10.1128/JCM.41.6.2530-2536.2003.
40. Jolley K, Bray J, Maiden M. 2018. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. Wellcome Open Res 3:124. https://doi.org/10.12688/wellcomeopenres.14826.1.
41. Clinical and Laboratory Standards Institute. 2018. VET01: performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animal. CLSI, Wayne, PA.