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Staphylococcus aureus adlb gene is associated with high prevalence of intramammary infection in dairy herds of northern Italy: A cross-sectional study

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ABSTRACT

Staphylococcus aureus is a major mastitis pathogen in dairy cattle worldwide, responsible for substantial economic losses. Environmental factors, milking routine, and good maintenance of milking equipment have been described as important factors to prevent intramammary infections (IMI). *Staphylococcus aureus* IMI can be widespread within the farm or the infection can be limited to few animals. Several studies have reported that *Staph. aureus* genotypes differ in their ability to spread within a herd. In particular, *Staph. aureus* belonging to ribosomal spacer PCR genotype B (GTB)/clonal complex 8 (CC8) is associated with high within-herd prevalence of IMI, whereas other genotypes are generally associated with individual cow disease. The *adlb* gene seems to be strictly related to *Staph. aureus* GTB/CC8, and is a potential marker of contagiousness. We investigated *Staph. aureus* IMI prevalence in 60 herds in northern Italy. In the same farms, we assessed specific indicators linked to milking management (e.g., teat condition score and udder hygiene score) and additional milking risk factors for IMI spread. Ribosomal spacer-PCR and *adlb*-targeted PCR were performed on 262 *Staph. aureus* isolates, of which 77 underwent multilocus sequence typing. In most of the herds (90%), a predominant genotype was identified, especially *Staph. aureus* CC8 (30%). In 19 of 60 herds, the predominant circulating *Staph. aureus* was *adlb*-positive and the observed IMI prevalence was relevant. Moreover, the *adlb* gene was detected only in genotypes of CC8 and CC97. Statistical analysis showed a strong association between the prevalence of *Staph. aureus* IMI, the specific CCs, and carriage of *adlb*, with the predominant circulating CC and presence of the gene alone explaining the total variation. Interestingly, the difference in the odds ratio obtained in the models for CC8 and CC97 suggests that it is carriage of the *adlb* gene, rather than the circulation of these CCs per se, that leads to higher within-herd prevalence of *Staph. aureus*. In addition, the model showed that environmental and milking management factors had no or minimal effect on *Staph. aureus* IMI prevalence. In conclusion, the circulation of *adlb*-positive *Staph. aureus* strains within a herd has a strong effect on the prevalence of IMI. Thus, *adlb* can be proposed as a genetic marker of contagiousness for *Staph. aureus* IMI in cattle. However, further analyses using whole-genome sequencing are required to understand the role of genes other than *adlb* that may be involved in the mechanisms of contagiousness of *Staph. aureus* strains associated with high prevalence of IMI.
Key words: *Staphylococcus aureus*, mastitis, *adlb* gene, dairy cattle

INTRODUCTION

Staphylococcus aureus is one of the most important pathogens causing mastitis in dairy cattle worldwide, and it results in economic losses for dairy farmers in terms of reduced milk yield and quality and increased treatment costs (Hogeveen et al., 2011). The spread of this bacteria within a herd primarily happens during milking, and management factors such as milking rou-

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tine and the good maintenance of milking equipment are important to prevent *Staph. aureus* IMI (Dufour et al., 2012). Nevertheless, it has been reported that *Staph. aureus* IMI may be widespread in many herds but not in others, where the infection is limited to a few animals, suggesting a central role of the strain circulating within the herd and involved in the IMI. Several studies have reported that different *Staph. aureus* genotypes are associated with different virulence and pathogenicity properties. In particular, *Staph. aureus* genotype B (**GTB**) is associated with high contagiousness and pathogenicity, leading to high within-herd prevalence of IMI. In contrast, other genotypes are associated with individual cow disease and rarely seem to cause herd health problems (Fournier et al., 2008; Graber et al., 2009; Cremonesi et al., 2015; Cosandey et al., 2016). Moreover, *Staph. aureus* GTB seems to be highly associated with the mammary gland (Leuenberger et al., 2019). Specific sanitation programs based exclusively on the identification and management of *Staph. aureus* GTB-positive dairy herds were successfully carried out in Switzerland (Sartori et al., 2018). Thus, discrimination between different genotypes seems to be important for *Staph. aureus*–targeted control programs (Barkema et al., 2006; Sartori et al., 2018; Exel et al., 2022). Although previous studies investigated the virulence factors and mechanisms that could facilitate *Staph. aureus* colonization of the mammary gland and its establishment and persistence in the host tissue (Monistero et al., 2018; Hoekstra et al., 2020; Pérez et al., 2020; Vaughn et al., 2020), no study to date has clearly identified a single marker or combination of markers capable of predicting *Staph. aureus* contagiousness within a herd. The possible association between the circulating *Staph. aureus* strain (in terms of genotype and virulence factors) with specific farm health parameters, including the incidence of clinical mastitis or the prevalence of subclinical IMI, has been evaluated (Dufour et al., 2012; Luini et al., 2015; Magro et al., 2017). Using a stochastic bio-economic model, Exel et al. (2022) proposed different control strategies based on the described epidemiological and clinical differences between different *Staph. aureus* strains.

Sartori et al. (2017) demonstrated that the single-copy gene *adlb* is strictly related to *Staph. aureus* GTB and may be a potential marker of contagiousness. This gene encodes the adhesion-like bovine protein and is located in the GTB-specific staphylococcal cassette chromosome SCCgtb. A study conducted in northern Italy on bulk tank milk samples confirmed the association between *Staph. aureus* GTB and the presence of *adlb*, even though some non-GTB strains also carry the gene (Gazzola et al., 2020).

Boss et al. (2016) reported that 80% of *Staph. aureus* strains isolated in 12 European countries belonged to only 6 different clonal complexes (**CC**), of which CC8, CC705, and CC97 were the most frequent. Additionally, the distribution of sequence types (**ST**) differs based on the considered country and region of interest (Boss et al., 2016; Cvetnić et al., 2021). Recently, Gazzola et al. (2020) investigated the distribution of multilocus sequence typing (**MLST**) profiles of *Staph. aureus* strains isolated in northern Italy and compared them to their ribosomal spacer-PCR (**RS-PCR**) genotypes. They found 16 CC, the most frequent being CC8, CC97, CC398, and CC1, isolated from bovine milk and reported as livestock-associated lineages (Boss et al., 2016).

Because *Staph. aureus* IMI is mainly chronic and subclinical, its contagiousness is of utmost importance in determining the economic losses for the affected herd. In this work, we aimed to investigate the prevalence of *Staph. aureus* IMI in northern Italian dairy farms and to relate the *Staph. aureus* circulating genotypes (especially the presence/absence of the *adlb* gene) as well as some farm characteristics and milking management factors to the prevalence of IMI within the herds as a marker of contagiousness of the circulating strains.

MATERIALS AND METHODS

This analysis did not require approval by an Institutional Animal Care and Use Committee because it did not involve animals used for scientific purposes as required by Directive 2010/63/EU (European Union, 2010) [Art. 2 ... 5. This Directive shall not apply to the following:... (f) practices not likely to cause pain, suffering, distress or lasting harm equivalent to, or higher than, that caused by the introduction of a needle in accordance with good veterinary practice.].

Study Design and Herd Data Collection

Between September 2011 and August 2012 and between March 2016 and March 2017, 60 dairy cattle herds with *Staph. aureus* IMI were enrolled in our study. The average size of the herds was 102 milking cows (range: 18 to 417 cows). All farms reared Holstein Friesian cattle and were located in the Lombardy, Emilia-Romagna, or Piedmont regions in northern Italy. These herds were representative, in terms of the number of lactating cows and average milk yield per cow, of a geographical area where more than 70% of Italian bovine milk is produced.

We identified many herds known to be infected with *Staph. aureus* during routine diagnostic activities con-

ducted during the 3 previous months on bulk tank milk samples or individual milk samples by 2 regional public health veterinary laboratories located in northern Italy (i.e., Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna and Istituto Zooprofilattico del Piemonte, Liguria e Valle D'Aosta). We considered only the farms established free from other contagious microorganisms, such as *Streptococcus agalactiae* and *Mycoplasma bovis*. Moreover, we selected only the herds that, at the time of sampling, did not follow any specific *Staph. aureus* mastitis control program and did not conduct a specific sanitation program aimed to control this pathogen. Considering the resources available for our study, the first 60 farms that fulfilled the described criteria and voluntary agreed to participate to the sampling (and eventually to take place specific actions) were enrolled in our study.

At first, composite milk samples were collected cow by cow (first sampling round) from all lactating cows of the herds, and bacteriological analysis was then performed to determine the prevalence of *Staph. aureus* IMI (Maisano et al., 2019). Then, *Staph. aureus*-positive cows were resampled 1 to 3 wk later by collecting quarter milk samples to detect the infected quarters and perform molecular characterization of the isolates (second sampling round). Finally, the proportions of *Staph. aureus*-infected cows and the average number of infected quarters per cows were determined as indicators of strain infectivity. Specific indicators of milking management, such as teat condition and udder hygiene, were evaluated as well. These 2 parameters can be considered risk factors for *Staph. aureus* transmission and, consequently, for within-herd prevalence of IMI (Zadoks et al., 2001; Graber et al., 2009), thus potentially leading to biased conclusions regarding the contagious properties of different *Staph. aureus* strains. Teat condition score (**TCS**) and udder hygiene score (**UHS**) were visually evaluated for each cow during milk sampling and assigned according to Neijenhuis et al. (2001) and Schreiner and Ruegg (2002), respectively. Herd-level TCS and UHS were calculated as the arithmetic mean of individual cow TCS and individual cow UHS. The milking routine was assessed based on a specific checklist of 8 items created by the Italian National Reference Center for milk quality. The checklist is largely in accordance with the recommendations of the National Mastitis Council (NMC, 2016). The checklist was created to specifically address Italian milking practices. For each question (**Q**), scores from 1 to 3 were assigned (Q scores), where 1 was optimal (the goal for the farmer), 2 was acceptable (not the goal but nondetrimental), and 3 was insufficient (dangerous or not allowed). A cumulative milking routine score (**MRS**) was calculated for each herd as the arithmetic mean of the 8 Q scores. The management factors and the scoring system are listed in Table 1. The TCS, UHS, and Q scores were assigned during milk sampling by 4 veterinarians experienced in mastitis control and specifically trained (by both classroom training before the study and field practice with an expert tutor as a gold standard for the internal validation of the checklist) to reduce intra- and interobserver variability. Furthermore, age of the lactating cows at the time of sampling was also evaluated in the study as a possible risk factor for IMI prevalence. The age (in days) of lactating cows was obtained from the bovine registry of farms and average herd age (**HA**) was calculated. This parameter was considered a risk factor, because older cows are generally more likely to become infected and cure rates decrease with increasing age of the cow (Barkema et al., 2006).

Sample Collection and Bacteriological Analyses

Composite milk samples were collected hygienically (after foaming predipping and drying with disposable paper towels), and the subsequent quarter milk samples were collected aseptically (by thorough disinfection of teat using denatured alcohol). All samples were kept at 4°C and bacteriological assays were performed within 48 h. Milk samples were cultured using standard methods: 10 μL of the sample was plated on esculin blood agar (**EBA**) and Baird Parker with rabbit plasma fibrinogen agar (**BP-RPF**). After incubation at 37°C for 48 h, suspected *Staph. aureus* colonies (hemolytic on EBA or displaying the typical halo on BP-RPF) were confirmed by tube coagulase test. The growth of one colony in 10 μL of inoculated milk (100 cfu/mL) was chosen as the threshold to define a sample as positive (Dohoo et al., 2011) and a cow or quarter as infected.

Molecular Analyses

Genotyping by RS-PCR. For each herd, *Staph. aureus* isolates from quarter milk samples were confirmed by a specific PCR assay targeting the *nuc* gene (Cremonesi et al., 2006) and genotyped by RS-PCR. Specifically, 5 *Staph. aureus* isolates (if present) per herd from different positive cows were randomly selected and genotyped. In case of different cultural morphologies of colonies (i.e., pigmentation and hemolysis on EBA and type of halo on BP-RPF), up to 5 isolates per morphology were selected.

DNA was extracted from strains using the DNeasy Blood and Tissue kit (Qiagen), following the manufacturer's instructions. DNA was then stored at −20°C until use. The RS-PCR was performed according to Fournier et al. (2008), based on amplification of 16S-23S rRNA intergenic spacer region. The PCR products

Table 1. Farm characteristics and specific milking management factors included in the statistical analysis as possible risk factors in the spread of IMI by *Staphylococcus aureus*

Factor	Category	Description
Q1 Hygienic level of the milking parlor	1: Optimal 2: Acceptable 3: Insufficient	Cleaning thoroughly with high-pressure hot water after each milking Cleaning with high-pressure cold water after each milking Rough cleaning with cold water
Q2 Udder and teat preparation	1: Optimal 2: Acceptable 3: Insufficient	Foremilk examination, preparation with predipping, and accurate cleaning of the teats (no signs of dirt) and drying them with disposable material (one for each cow) Preparation with good cleaning of the teats and drying teats with disposable materials (one for more than one cow) Cleaning of udder or teats with water and no drying or no use of disposable materials for each cow
Q3 Use of back-flushing	1: Optimal 2: Acceptable 3: Insufficient	Use of back-flushing system with the use of steam and disinfectants Use of back-flushing system with only hot or cold water Nonuse of back-flushing system
Q4 Post-milking teat disinfection	1: Optimal 2: Acceptable 3: Insufficient	Use of postdipping teat disinfection with a specific film product and frequent cleaning of the cups Use of postdipping teat disinfection with a specific product and occasional cleaning of the cups Nonuse of postdipping teat disinfection
Q5 Management and routine of milking procedures	1: Optimal 2: Acceptable 3: Insufficient	Correct stimulation followed by attaching the cluster within 90 s; control of the milk flow and of cluster during milking; removing the clusters, avoiding machine stripping Correct stimulation followed by attaching the cluster within 90 s; irregular or no control of the milk flow and of cluster during milking but removing the clusters, avoiding machine stripping No correct stimulation followed by rapid attaching of the cluster, no control of the milk flow or of cluster during milking or removing the clusters without attention to machine stripping
Q6 Cleaning and sanitizing of milking equipment	1: Optimal 2: Acceptable 3: Insufficient	Regular cleaning and disinfection procedure program, taking water hardness in account; no residual dirt or biofilm on the inner side of the liners Regular cleaning and disinfection procedure program; no residual dirt or biofilm on the inner side of the liners No coherent or absent cleaning and disinfection procedure program or residual dirt or biofilm
Q7 Hygienic level of milkers	1: Optimal 2: Acceptable 3: Insufficient	Milkers use clean clothing, with clean waterproof apron and disposable gloves Milkers use clean clothing and waterproof apron but use plastic nondisposable gloves Milkers use dirty clothing or no use of gloves
Q8 Maintenance of milking equipment and liner replacement	1: Optimal 2: Acceptable 3: Insufficient	Fully checked by a specialist at least once a year, and liner replacement ≤600 h of use Fully checked by a specialist at least once a year, and liner replacement between 600 and 1,000 h of use Fully checked by a specialist less than once a year or only in case of problems, or liner replacement >1,000 h of use or only replaced when damaged
Milking routine score (MRS)	Cumulative milking routine score	Calculated as the arithmetic mean of the 8 Q scores
Udder hygiene score (UHS)	Average of the scores of all cows	Hygiene of udder, flanks, and legs was scored based on a 4-point scale system, from very clean (score 1) to very dirty skin (score 4; Schreiner and Ruegg, 2002)
Teat condition score (TCS)	Average of the scores of all teats	Callosity of the teat orifice was scored based on a 4-point scale system: absent callosity = 1; a smooth callous ring around the orifice = 2; rough and very rough callous rings = 3 and 4, respectively (Neijenhuis et al., 2001)
Herd age (HA)	Age in days	Average age of cows >21 mo
adlb status of the herd (ADLB)	*Staph. aureus* circulating strain is *adlb*-positive	Yes or no

were analyzed using the miniaturized electrophoresis system DNA 7500 LabChip (Agilent Technologies), and genotypes were inferred from electrophoresis profile using Mahal 2.0 software, which is freely available online (https://mahal.vitech.dev/#/). Ribosomal spacer-PCR allows classification of isolates in several genotypes (**GT**) that can be grouped in clusters (**CL**). Each CL includes the genotype itself and its variants, differing in only one band in the electrophoretic analysis (Syring et al., 2012; Cosandey et al., 2016).

If all tested *Staph. aureus* isolates within a herd or most of them (i.e., 4 of the 5 isolates tested) belonged to the same RS-PCR genotype, this genotype was considered the predominant circulating strain likely responsible for IMI within the herd; in the remaining cases, the infection was considered "mixed" by different genotypes, none individually responsible for the herd problem (Table 2). The number of 5 isolates per herd is based on the previous studies by Fournier et al. (2008) and Cremonesi et al. (2015), which showed that either there is no variation among genotypes within one herd or it is very low, particularly when more than 5 isolates are involved in the IMI.

***adlb*-Targeted PCR.** The *adlb*-targeted real-time PCR was performed on all RS-PCR genotyped isolates according to Sartori et al. (2017).

MLST Analysis. Multilocus sequence typing analysis was performed on a subset of strains, based on the results of RS-PCR. The selection was done as follows: one strain per RS-PCR genotype per herd was analyzed by MLST. Therefore, in the herds with a unique circulating genotype, only one strain was randomly selected; in herds with different genotypes, one strain per genotype was randomly selected.

In detail, the selected strains were subjected to whole-genome sequencing on the Miseq platform (Illumina) as follows: genomic libraries were prepared using Nextera XT DNA Library Preparation Kit (Illumina) and sequenced generating paired-end reads of 250 bp. Raw reads were checked for quality using FastQC (Babraham Bioinformatics, 2018). The MLST analysis was performed on raw reads through the Center for Genomic Epidemiology online platform (Center for Genomic Epidemiology, 2020), or by submitting them to PubMLST (https://pubmlst.org). The original contributions in the present study are publicly available. Illumina raw reads have been deposited in the National Center for Biotechnology Information GenBank database under the Bioproject number PRJNA897860.

Statistical Analysis

Descriptive Analysis. The 60 herds were sorted in ascending order according to their cow prevalence for *Staph. aureus*, and then divided in 3 groups of equal size. Group 0 (herds 1 to 20), group 1 (herds 21 to 40), and group 2 (herds 41 to 60) were considered as low, intermediate, and high cow prevalence groups, respectively. For each group, data of continuous variables (HA, UHS, TCS) were expressed as minimum and maximum, mean, median, standard deviation (**SD**), and standard error, and they were plotted as box plots. The overall comparison among groups and the comparisons between 2 groups were performed by single-factor ANOVA using the Kruskal-Wallis test, and by the Mann-Whitney U test, respectively. Categorical variables [*adlb* presence (ADLB) Q1-Q8, GT, ST, CC] were instead expressed as frequencies or minimum and maximum. For graphical representation of ADLB and Q1-Q8, the mean and standard error of the mean were calculated and plotted. Comparisons among groups were computed by exact χ^2 test. All analyses were performed using Systat 13.0 software (Systat Software Inc.).

Modeling of Staph. aureus Cow Prevalence. Quasi-binomial logistic regression was applied to model the within-herd prevalence of *Staph. aureus* IMI (response variable) as a function of the explanatory variables identified in the study, by using R 3.6.3 (R Core Team, 2020) with "MASS" package. A quasi-binomial distribution was used for describing the error distribution to account for overdispersion. Specifically, we tested the effect on cow prevalence of the binary variable ADLB, the categorical variable predominant clonal complex (**pCC**), and the different continuous variables (i.e., HA, UHS, TCS), in addition to the global milking score (MRS). The MRS, defined as the mean over the variables Q1 to Q8, was introduced in the statistical model to replace the individual Q variables to avoid the problem of collinearity among various Q variables. To evaluate in the model the effect on the prevalence of the major CC observed in the population, the categorical variable pCC was introduced. In detail, to take into account only the main CCs observed in the population (and avoid estimating the effect of rare CCs using a limited amount of data), the categorical variable pCC was built by assigning to each farm its pCC under the following conditions: (1) at least 10% of the farms displayed the pCC, or (2) at least 5% of the farms displayed the pCC and the within-herd prevalence range of farms in which circulated the given pCC does not include the overall prevalence of *Staph. aureus* in the study population (i.e., 20.3%). The farms not fulfilling criterion (1) or (2) were assigned to the group "Other CCs." The "Other CCs" group (which represents a generic CC introduced in the farm) was used as the benchmark to evaluate the effect size of a given CC on within-farm prevalence.

Table 2. Characteristics of the 60 analyzed herds, in ascending order of *Staphylococcus aureus* IMI prevalence[1]

Farm no.	Group	Herd size	RS-PCR (no.)[2]	MLST (ST-CC)[3]	Predominant circulating CC	*adlb* status	*Staph. aureus* IMI prevalence (%)[4]	HA[5]	TCS[5]	UHS[5]	MRS[5]
1	0	285	C (1)	ST151-CC705	CC705	Negative	0.7	1,413	2.8	1.4	1.625
2	0	114	B (1)	ST8-CC8	CC8	Negative	0.9	1,380	1.2	2.2	2.125
3	0	158	R^{I} (2)	ST133-CC133	CC133	Negative	1.3	1,532	1.5	2.0	1.375
4	0	131	R (1)	ST352-CC97	CC97	Negative	1.5	1,477	1.7	1.7	1.625
5	0	119	S (2)	ST398-CC398	CC398	Negative	1.7	1,562	1.4	2.1	2.375
6	0	141	C (3)	ST504-CC705	CC705	Negative	2.1	1,371	2.3	1.6	1.625
7	0	47	S (1)	ST398-CC398	CC398	Negative	2.1	1,607	1.4	1.9	1.375
8	0	157	C (3)	ST504-CC705	CC705	Negative	2.6	1,466	2.2	2.0	1.625
9	0	267	S (5)	ST398-CC398	CC398	Negative	3.7	1,306	1.4	1.4	1.875
10	0	417	BJ (5)	ST1-CC1	CC1	Negative	3.8	1,500	1.7	1.6	1.75
11	0	44	B^{III} (1); C^{II} (1)	ST291-CC398; ST504-CC705	Mixed[6]	Negative	4.5	1,458	1.4	2.2	1.5
12	0	85	S (2); BE (2)	ST398-CC398; ST97-CC97	Mixed	Negative	4.7	1,596	2.1	1.3	2.125
13	0	105	C (4)	ST504-CC705	CC705	Negative	4.8	1,335	1.8	1.5	1.625
14	0	88	R (3)	ST352-CC97	CC97	Negative	5.7	1,461	1.4	1.9	1.25
15	0	52	S (1); Z (1)	ST398-CC398; ST1380-CC479	Mixed	Negative	5.8	1,621	1.3	1.6	1.375
16	0	203	BA (5)	ST398-CC398	CC398	Negative	5.9	1,392	1.4	1.9	1.25
17	0	65	C (4)	ST504-CC705	CC705	Negative	6.2	1,450	1.5	1.9	1.375
18	0	127	R (5)	ST352-CC97	CC97	Negative	6.3	1,482	1.0	2.5	1.5
19	0	134	AO (5); Z (3); C (1); BE (1)	ST97-CC97; ST1380-CC479; ST504-CC705; ST97-CC97	Mixed	Negative	7.5	1,547	1.3	2.1	1.5
20	0	40	F (2)	ST20-CC20	CC20	Negative	7.5	1,590	2.0	1.5	2.125
21	1	24	B (1); R^{III} (1)	ST8-CC8; ST133-CC133	Mixed	Negative	8.3	1,944	1.2	2.0	1.875
22	1	101	F (5)	ST9-CC9	CC9	Negative	8.9	1,539	1.6	1.2	2
23	1	59	AO (3)	ST97-CC97	CC97	Negative	10.2	1,457	1.8	1.9	2
24	1	66	Z (5)	Unknown-CC97	CC97	Negative	12.1	1,607	2.3	1.6	1.625
25	1	63	Y (5)	ST45-CC45	CC45	Negative	12.7	1,386	1.5	1.9	2.125
26	1	54	I^{I} (5)	ST6881-CC97	CC97	Negative	14.8	2,487	1.5	1.1	1.875
27	1	73	F (5)	ST389-CC389	CC389	Negative	15.1	1,488	1.7	1.8	1.5
28	1	18	BN (2)	ST71-CC71	CC71	Negative	16.7	1,979	1.8	1.8	1.625
29	1	38	BI (2); R^{I} (3)	ST97-CC97; ST30-CC30	Mixed	Negative	18.4	1,234	1.5	1.8	1.625
30	1	131	S (5)	ST398-CC398	CC398	Negative	19.1	1,534	1.6	1.5	1.625
31	1	70	F^{III} (4); Y (1)	ST9-CC9; ST9-CC9	CC9	Negative	20.0	1,485	1.4	1.4	2.25
32	1	96	F^{III} (5)	ST20-CC20	CC20	Negative	21.9	1,431	1.2	2.0	2.125
33	1	73	CJ (5)	ST9-CC9	CC9	Negative	23.3	1,463	2.2	1.3	1.875
34	1	38	B (4)	ST8-CC8	CC8	Positive	23.7	1,367	1.5	2.0	1.5
35	1	70	U (5)	ST20-CC20	CC20	Negative	24.3	1,617	1.3	1.4	1.5
36	1	82	AO (5)	ST97-CC97	CC97	Negative	25.6	1,577	1.3	1.8	2.25
37	1	95	AX (4); I^{I} (1)	ST97-CC97; ST97-CC97	CC97	Negative	26.3	1,557	1.5	2.7	1.75
38	1	45	R (5)	ST352-CC97	CC97	Negative	26.7	1,489	1.6	1.5	1.625
39	1	205	B (5)	ST8-CC8	CC8	Positive	27.3	1,556	2.0	2.6	1.625
40	2	132	BQ^{I} (5)	ST97-CC97	CC97	Positive	28.0	1,416	1.4	2.2	2.125
41	2	166	B (5)	ST8-CC8	CC8	Positive	28.3	1,626	1.5	1.3	1.75

Continued

Table 2 (Continued). Characteristics of the 60 analyzed herds, in ascending order of *Staphylococcus aureus* IMI prevalence[1]

Farm no.	Group	Herd size	RS-PCR (no.)[2]	MLST (ST-CC)[3]	Predominant circulating CC	*adlb* status	*Staph. aureus* IMI prevalence (%)[4]	HA[5]	TCS[5]	UHS[5]	MRS[5]
42	2	111	B (5)	ST8-CC8	CC8	Positive	28.8	1,405	1.2	1.5	1.625
43	2	107	BM (5); BT (1)	ST126-CC126; ST126-CC126	CC126	Negative	31.8	1,502	1.6	2.9	2.125
44	2	122	K (5)	ST6837-CC5	CC5	Negative	35.2	1,426	1.4	1.6	1.75
45	2	30	B (5)	ST8-CC8	CC8	Positive	36.7	1,677	1.4	1.9	1.5
46	2	37	B (5)	ST8-CC8	CC8	Positive	40.5	1,502	1.3	1.9	2
47	2	239	S^{II} (5)	ST126-CC126	CC126	Negative	41.8	1,317	1.4	1.6	1.375
48	2	100	R^{VI} (5)	ST97-CC97	CC97	Positive	43.0	1,448	1.3	2.5	1.875
49	2	78	B (4)	ST8-CC8	CC8	Positive	46.2	1,716	1.2	1.9	2.125
50	2	73	B^{III} (5); B (1); B^{I} (1)	ST8-CC8; ST8-CC8; ST8-CC8	CC8	Positive	46.6	1,543	1.8	2.8	1.25
51	2	99	B (5)	ST8-CC8	CC8	Positive	49.0	1,539	1.1	1.5	1.875
52	2	62	B (5); B^{I} (1)	ST8-CC8; ST8-CC8	CC8	Positive	51.6	1,587	1.9	1.4	1.875
53	2	25	B (5); B^{III} (2)	ST8-CC8; ST8-CC8	CC8	Positive	56.0	1,856	1.2	2.5	2.25
54	2	87	R^{VI} (5)	ST97-CC97	CC97	Positive	58.6	1,513	2.2	1.9	2.375
55	2	56	B (5)	ST8-CC8	CC8	Positive	60.7	1,513	1.4	1.3	2.125
56	2	142	B (5); B^{I} (1)	ST8-CC8; ST8-CC8	CC8	Positive	62.0	1,454	2.7	1.1	1.75
57	2	66	B (5); AQ (1)	ST8-CC8; ST1-CC1	CC8	Positive	62.1	1,645	1.9	1.1	2.125
58	2	81	S^{II} (5)	ST126-CC126	CC126	Negative	66.6	2,589	2.2	1.9	1.625
59	2	49	B (5)	ST8-CC8	CC8	Positive	67.4	1,605	1.4	2.2	1.625
60	2	37	B (5)	ST8-CC8	CC8	Positive	73.0	1,951	1.3	1.2	1.625

[1]The prevalence of both *Staph. aureus*-infected cows and some IMI risk factors are listed, as well as results of molecular analyses performed on the strains isolated in each farm.

[2]The number in parentheses indicates the number of isolates belonging to the corresponding genotype. The sum of the isolates per herd is the total number of isolates analyzed by ribosomal spacer (RS)-PCR in the herd. For example, 10 isolates were genotypes by RS-PCR in herd 19.

[3]Multilocus sequence typing (sequence type-clonal complex).

[4]IMI prevalence is expressed as a proportion of positive cows.

[5]HA = age of cows; TCS = teat condition score; UHS = udder hygiene score; MRS = milking routine score. See Table 1 for specifications.

[6]Mixed = within the same herd different genotypes were identified, of which none was predominant.

The response variable was modeled for dependence on multiple explanatory variables by using a forward stepwise selection procedure (Venables and Ripley, 2002) with a drop-in-deviance test statistic based on quasi-likelihood inference (Roback and Legler, 2021) to define the model providing the best prediction. The drop-in-deviance test comparing Model 1 (with p parameters) and Model 2 (with q parameters, and $q < p$) was performed using the statistic

$$F = \frac{1}{\hat{\phi}} \times \frac{D_2 - D_1}{p - q},$$

where $\hat{\phi}$ represents the overdispersion parameter for the variance, D_1 and D_2 represent the residual deviance for Model 1 and Model 2, respectively, and $p - q$ represents the difference in the number of parameters between the models (Roback and Legler, 2021). Then, the test statistic was compared with an F-distribution with $p - q$ and $n - p$ degrees of freedom (where n represents the sample size). We used the odds ratio (**OR**) as effect size statistics in quasi-binomial logistic regressions. All data included in the statistical analyses are listed in Table 2.

RESULTS

Sample Collection and Bacteriological Analyses

During the first sampling round, a total of 6,079 composite milk samples from as many cows were collected from the 60 selected herds. Overall, 1,233 cows were *Staph. aureus*-positive, and within-herd prevalence ranged from 0.7 to 73%.

Because some cows were slaughtered or dried off between the first and second samplings (an interval of 1 to 3 wk), 1,228 positive cows were resampled during the second round, collecting a total of 4,912 sterile quarter milk samples. All cows resampled were positive for at least one quarter. The proportion of infected quarters per cow within a herd ranged from 1 to 3 in the different herds (Table 2). Teat condition score and UHS ranged from 1.0 to 2.8 and from 1.1 to 2.9, respectively; HA ranged from 1,234 to 2,589 d (Table 2). The results referring to the 8 Q scores of the milking routine check list are shown in Supplemental Table S1 (https://data.mendeley.com/datasets/3z2vnckwg3/1; Romanò et al., 2022); MRS are reported in Table 2.

Molecular Analyses

Genotyping by RS-PCR and MLST. Ribosomal spacer-PCR was performed on 262 *Staph. aureus* isolates from the 60 investigated herds. The number of genotyped isolates per herd ranged from 1 to 10, depending on the number of isolates obtained and on their morphological characteristics, as described in Materials and Methods. A predominant genotype was detected in 54 herds: it was a unique genotype in 46 herds, whereas in 8 herds it was predominant (i.e., 4 of the 5 strains tested belonged to the same RS-PCR genotype). In the 6 remaining herds, up to 4 different genotypes were isolated, of which none was predominant ("mixed" isolates; Table 2).

Seventy-seven out of these 262 strains were also analyzed by MLST; 19 different ST, grouped into 15 CC, were identified, including 2 previously unknown profiles. The results are shown in Table 3. The most frequent MLST profiles were CC8-ST8 (n = 23; 30%), CC97-ST97 (n = 11; 14%), CC398-ST398 (n = 7; 9%), and CC705-ST504 (n = 6; 8%). When a predominant genotype circulated within a herd (n = 54), it was CC8 in 17 herds (31%), CC97 in 12 (22%), CC705 and CC398 in 5 (9%), and CC20, CC9, and CC126 in 3 (6%) herds. In the 6 remaining herds with a predominant genotype, we isolated a CC that was not isolated in any of the other analyzed herds (Table 2). Comparing the results of MLST and RS-PCR, all CC8-ST8, CC398-ST398, and CC705 *Staph. aureus* strains belonged to CLB, GTS (RS-PCR genotype S), and CLC (RS-PCR genotypic cluster C), respectively.

adlb-Targeted PCR. The *adlb*-targeted PCR was performed on the same 262 isolates that underwent RS-PCR. Eighty-five of 87 CLB strains were *adlb*-positive (75 GTB, 7 GTB^{III}, and 3 GTB^{I}), whereas *adlb* was detected in only 15 of the non-CLB circulating strains (10 GTR^{VI} and 5 $GTBQ^{I}$; Table 2). Among the 77 strains analyzed with MLST, the *adlb* gene was present in 22 of 23 CC8 (96%) and 3 of 17 CC97 (18%). The remaining CCs did not harbor the gene.

Nineteen herds were considered *adlb*-positive because the predominant strain carried this gene. Conversely, 41 herds were identified as *adlb*-negative, because none of the isolated strains carried the gene. Our results show that IMI prevalence was always considerably higher in *adlb*-positive herds compared with *adlb*-negative herds. The relationship between prevalence of *Staph. aureus* IMI and circulation of *adlb*-positive strains within the herd is displayed in Figure 1 and Figure 2. No *adlb*-positive strain was isolated in herds with an IMI prevalence <23%, and the effect of the carriage of *adlb* gene on IMI prevalence is represented in Figures 1, 2, and 3.

Statistical Analyses

Descriptive Analysis. An association was observed among the 3 groups of prevalence (group 0/low preva-

Table 3. Distribution of clonal complexes (CC) and sequence type (ST), and their relation with ribosomal spacer (RS)-PCR genotypes and the presence of *adlb*, of the 77 strains analyzed with multilocus sequence typing (MLST)

No. of strains	CC[1]	ST	ST (% of total)	Genotype (GT)	*adlb*-positive strains
23	CC8	ST8 (23)	30	B (18), B^{I} (3), B^{III} (2)	B (17), B^{I} (3), B^{III} (2)
17	CC97	ST97 (11)	14	AO (3), R^{VI} (2), BE (2), AX (1), BI (1), BQ^{I} (1), I^{I} (1)	R^{VI} (2), BQ^{I} (1)
		ST352 (4)	5	R (4)	
		ST6881 (1)	1	I^{I} (1)	
		Unknown (1)	1	Z (1)	
8	CC398	ST398 (7)	9	S (6), BA (1)	
		ST291 (1)	1	B^{III} (1)	
7	CC705	ST504 (6)	8	C (5), C^{II} (1)	
		ST151 (1)	1	C (1)	
4	CC9	ST9 (4)	5	CJ (1), F (1), F^{III} (1), Y (1)	
4	CC126	ST126 (4)	5	S^{II} (2), BM (1), BT (1)	
3	CC20	ST20 (3)	4	F^{III} (1), F (1), U (1)	
2	CC1	ST1 (2)	3	AQ (1), BJ (1)	
2	CC133	ST133 (2)	3	R^{I} (1), R^{III} (1)	
2	CC479	ST1380 (2)	3	Z (2)	
1	CC5	ST6837 (1)	1	K (1)	
1	CC30	ST30 (1)	1	R^{I} (1)	
1	CC45	ST45 (1)	1	Y (1)	
1	CC71	ST71 (1)	1	BN (1)	
1	CC389	ST389 (1)	1	F (1)	

[1]All strains belonging to the same CC were isolated in different herds.

lence; group 1/intermediate prevalence; group 2/high prevalence) and GT, ST, and CC ($P < 0.001$). In particular, all CC705/GTC strains and most of the herds with various GTs ("mixed") were observed in group 0. *Staphylococcus aureus* belonging to CC398/ST398, and CC97/ST352 were the most prominent in group 0 as well. In contrast, less variability was observed in group 2: *Staph. aureus* strains mainly belonged to CC8/ST8/CLB, and CC126/ST126/GTS^{II} strains were isolated exclusively in this group (Table 2).

The presence of *Staph. aureus* strains carrying the *adlb* gene was highly group-dependent ($P < 0.001$). In fact, they were never observed in group 0, whereas they were found in 3 herds of group 1 (15%) and in 16 herds (80%) of group 2. Based on the exact χ^2 test, the presence of at least one *Staph. aureus* carrying the *adlb* gene in a herd was highly dependent on the GT of the strain itself ($P < 0.001$). In fact, only *Staph. aureus* strains belonging to CC8/ST8/CLB and CC97/ST97 harbored the gene. Among CC97/ST97 strains, *adlb* was found only in strains belonging to GTR^{VI} and $GTBQ^{I}$.

As for HA, medians did not differ among groups ($P = 0.105$). Groups 1 and 2 showed increased standard deviation, as a result of one herd in each group having considerably older cows. The UHS ($P = 0.756$) and TCS ($P = 0.759$) values were very similar among the groups (Table 2; Figure 4A; Supplemental Table S2; https://data.mendeley.com/datasets/3z2vnckwg3/1; Romanò et al., 2022).

For some of the Q variables (Q1, Q2, Q3, Q4, Q8), all 3 defined levels (optimal, acceptable, and insufficient) were observed, whereas insufficient values were never detected for the remainder (Q5, Q6, Q7). A significant association was observed between Q7 and the group variable ($P = 0.032$): the hygienic level of the milkers worsened as the prevalence of *Staph. aureus* IMI increased. As for all remaining Q variables, no significant association among groups was observed.

Modeling of Staph. aureus Cow Prevalence. The statistical analysis of factors affecting *Staph. aureus* within-herd prevalence, performed through quasi-binomial logistic regression, revealed that the pCC present in the farm and ADLB (the presence/absence of the *adlb* gene in the circulating strains) were the only explanatory variables included in the best model obtained from the forward stepwise selection (see Table 4). In Supplemental Table S3 (https://data.mendeley.com/datasets/3z2vnckwg3/1; Romanò et al., 2022), we show the effect size (expressed as OR) associated with the parameters estimated in the 1-variable and 2-variable best models selected through the model selection process. The model selection process identified the pCC as the main explanatory variable in explaining the observed difference in *Staph. aureus* within-herd prevalence (see the 1-variable best model in Table 4 and Supplemental Table S2; https://data.mendeley.com/datasets/3z2vnckwg3/1). Specifically, the 1-variable best model predicted a significantly different OR with respect to the benchmark for 4 of 5 of the main CC observed in the study (i.e., CC8, CC97, CC705, and CC126; see Supplemental Table S2). However, the 2-variable best model (which provided the best fit in

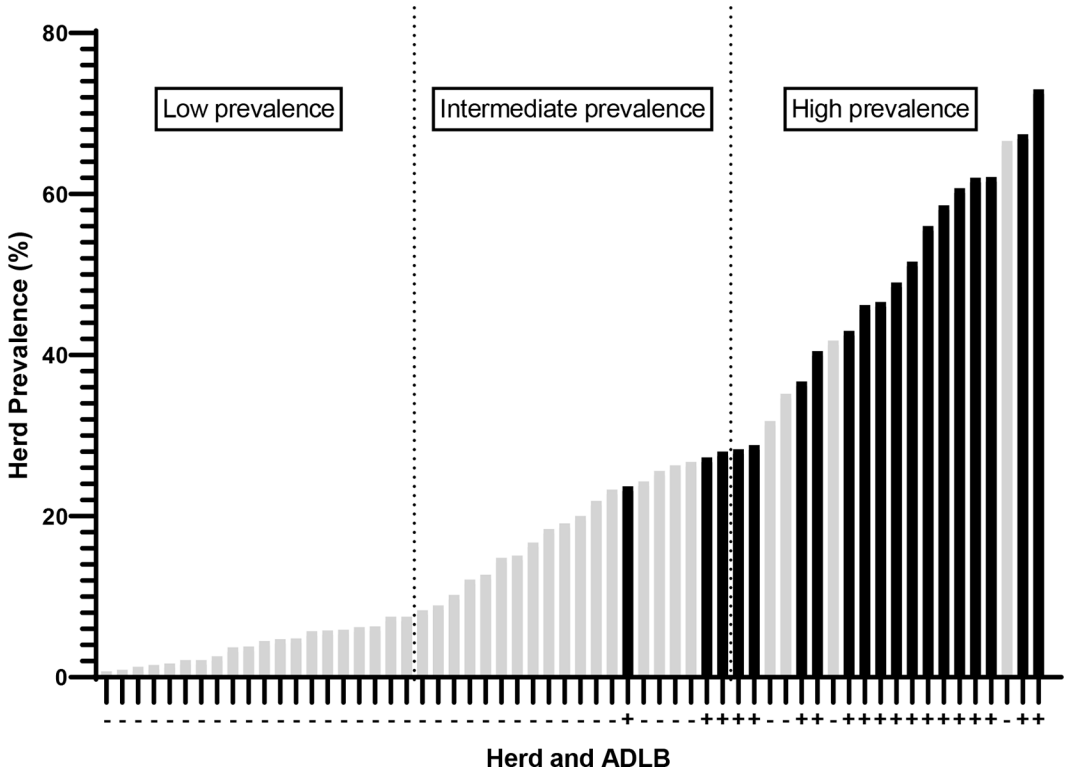

Figure 1. Distribution of *Staphylococcus aureus* IMI prevalence in the 60 farms in relation to their *adlb* status. The vertical lines show the 3 groups of 20 farms, arbitrarily defined as characterized by low (group 0), intermediate (group 1), and high (group 2) prevalence for statistical analysis. Gray (−) = *adlb*-negative farms (gray); black (+) = *adlb*-positive farms.

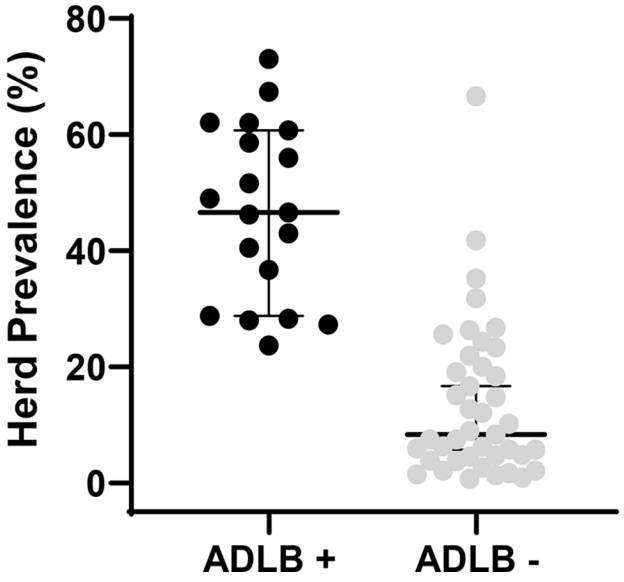

Figure 2. *Staphylococcus aureus* IMI prevalence in the 60 herds based on their *adlb* status. Gray = *adlb*-negative farms; black = *adlb*-positive farms. The central line is the median and the whiskers are 95% CI.

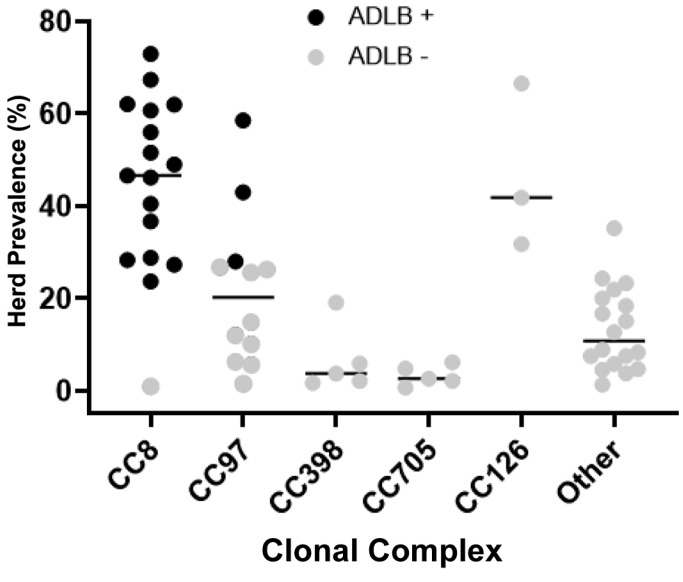

Figure 3. Distribution of *Staphylococcus aureus* IMI prevalence in the 60 farms in relation to the clonal complex and *adlb* status of the predominant circulating strains. Gray = *adlb*-negative farms; black = *adlb*-positive farms. Lines indicate the median.

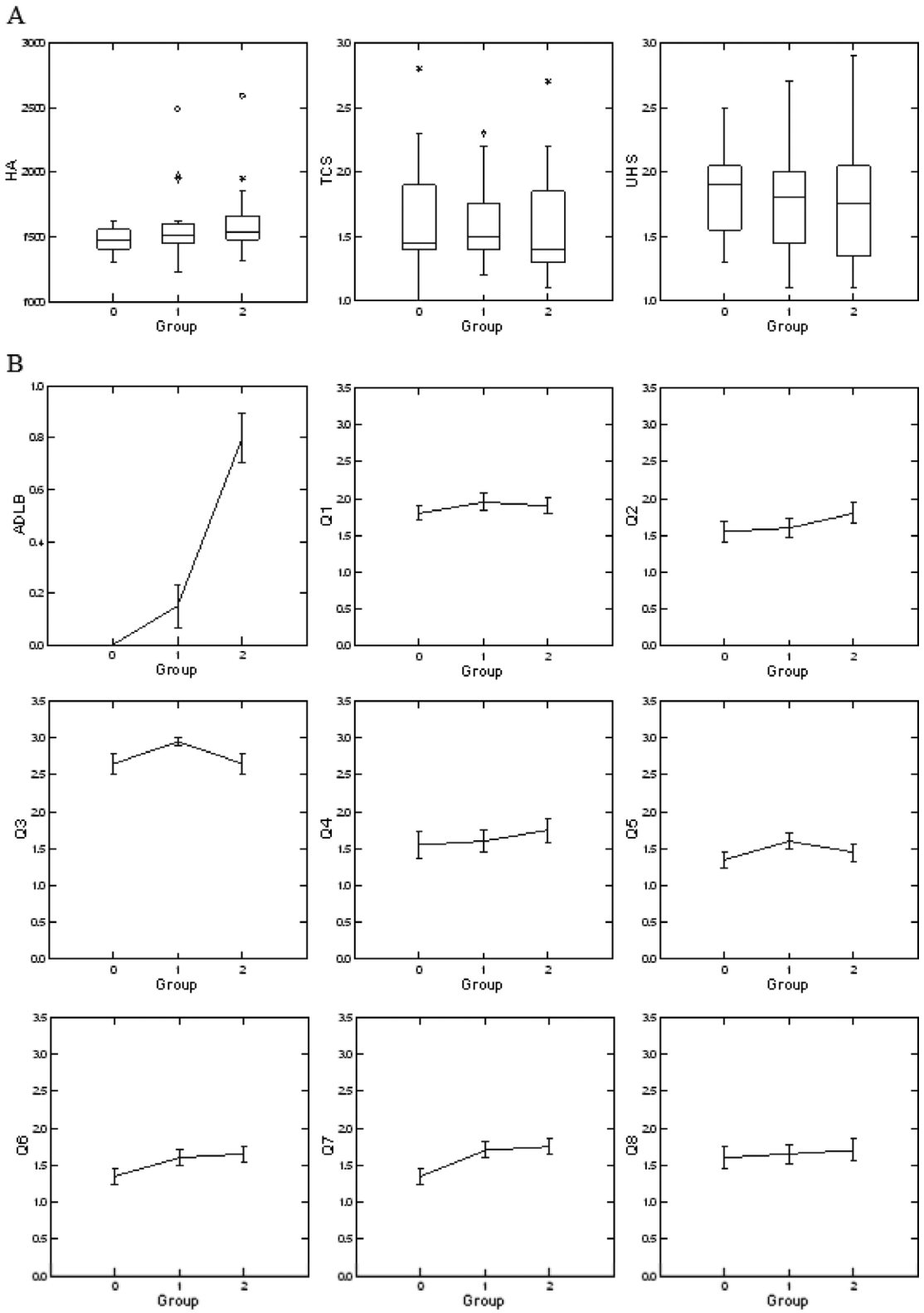

Figure 4. Graphical representation of the mean and SEM describing the relationships between different groups of prevalence (0 = low, 1 = intermediate, 2 = high) and the studied variables. (A) Continuous variables: herd age (HA), teat condition score (TCS), and udder hygiene score (UHS); (B) categorical variables: *adlb* status (ADLB) and questions (Q) 1 to 8 (Q1–Q8). A: The box is the area between the 25th and 75th percentiles, the line is the median, the whiskers are the limits (minimum and maximum), and the asterisks are outliers. The circles are the extreme outliers.

Table 4. Forward stepwise model selection for within-herd prevalence (Herd Prev) obtained from quasi-binomial regression[1]

Response variable	v-variable best model[2]	ϕ	Residual deviance	k	P-value
Herd Prev	~1[3]	14.8	961.6	1	—
	~pCC	6.9	401.2	6	9.5×10^{-10}
	~pCC + ADLB	5.4	287.1	7	2.8×10^{-5}
	~pCC + ADLB + HA	5.4	276.4	8	0.16

[1]Models were compared using drop-in-deviance tests. The best models for v explanatory variables are shown, with the dispersion parameter (ϕ), the residual deviance, the number of parameters (k), and the P-value of the comparison with the $v - 1$ variable best model (P-tests: <0.05 as the inclusion and exclusion criteria).

[2]pCC = predominant clonal complex; ADLB = *adlb*-positive strain; HA = herd age. Other explanatory variables included in the full model but not selected in the v-variable best models were udder hygiene score, teat condition score, and milk routine score.

[3]Null model.

the selection process) predicted a significantly higher *Staph. aureus* prevalence in farms where the pCC was CC126 (OR = 3.85, 95% CI: 2.26–6.54), and a significantly lower *Staph. aureus* prevalence in farms where the pCC was CC705 (OR = 0.21, 95% CI: 0.07–0.66) with respect to the benchmark. Instead, it did not predict significant differences in *Staph. aureus* prevalence with respect to the benchmark where the pCC was CC8 or CC97. Additionally, the 2-variable best model predicted a significantly higher *Staph. aureus* prevalence in farms where the circulating strains harbored the *adlb* gene compared with farms in which *Staph. aureus* strains were *adlb*-negative (OR = 4.06, 95% CI: 2.16–7.63).

DISCUSSION

Because *Staph. aureus* IMI is mainly chronic and subclinical, the contagiousness of this pathogen is of utmost importance in determining economic losses for the affected dairy farms. We investigated *Staph. aureus* IMI prevalence in 60 dairy farms located in northern Italy, with known *Staph. aureus* IMI and without other contagious microorganisms. Our study showed very variable prevalence of *Staph. aureus* IMI in the different herds, ranging from 0.7 to 73%. Of these, about one-third had a prevalence <8% and one-third >28%, and in 15% of the herds, prevalence was >50%. This is in line with previous Italian data (Luini et al., 2015; Magro et al., 2017) and confirms that very different situations can be found depending on the single farm considered. Indeed, in certain herds, *Staph. aureus* IMI are reported to remain confined to a few cows, whereas in many others, the infection appears to be widespread with up to 70 to 80% of cows infected, leading to serious economic losses and management problems (Cremonesi et al., 2015; Luini et al., 2015; Cosandey et al., 2016; Gazzola et al., 2020).

Our results are consistent with previous studies about the circulation of one predominant genotype within a farm; indeed, in most cases, when we isolated different genotypes within the same herd, only one of them was predominant (Joo et al., 2001; Capurro et al., 2010; Leuenberger et al., 2019). Interestingly, in herds with high prevalence of *Staph. aureus* IMI, we observed few genotypes, mostly CC8, whereas most of the different genotypes were isolated in the remaining herds.

To date, no study has clearly identified a single marker or combination of markers capable of predicting *Staph. aureus* contagiousness within a herd and that is universally valid in all geographical and farming conditions. In our study, we investigated the relationship between the prevalence of *Staph. aureus* IMI and environmental and management factors generally considered predisposing to IMI from contagious pathogens, such as the age of animals and the average number of milking cows (Cicconi-Hogan et al., 2013). In addition, we considered other factors related to hygiene and quality of milking, including UHS and TCS, as well as those strictly related to the milking routine, such as the hygienic level of the milking parlor and the milkers, udder preparation, the quality of pre- and postdipping, the use of back-flushing, the routine of milking procedure, and the cleaning and maintenance of milking equipment. Previously, Dufour et al. (2012) investigated manageable risk factors for *Staph. aureus* IMI incidence and prevalence, reporting that they seemed to be mostly related to milking procedures in herds where postmilking teat disinfection and blanket dry-cow therapy had already been implemented. In particular, wearing gloves during milking, adequate teat-end condition, and use of premilking teat disinfection were associated with lower IMI incidence and prevalence, highlighting the importance of good milking practices (Dufour et al., 2012). The association between TCS and mastitis in dairy cows has been the subject of a systematic review, which showed that only

severe teat condition was associated with the incidence or prevalence of *Staph. aureus* IMI (Pantoja et al., 2020). To avoid possible bias, we enrolled herds that did not practice segregation or culling of infected cows and that did not implement a specific dry-cow therapy, even if these remain best practices for the control and eradication of *Staph. aureus* (NMC, 2016).

Our descriptive analyses did not identify a significant association between most of the considered variables and the prevalence of *Staph. aureus* IMI, except for the udder and teat preparation for milking (Q7). In contrast, at least for Italian farms, some of the Q variables could not be individually used as predictors, because they were highly associated with each other as they reflect the farmer's attitude. For example, we noted that if the farmers performed a good milking procedure (Q5), they also wanted to maintain a clean milking parlor (Q1); if the personal hygienic level of the farmers was high (Q7), udder and teat preparation for milking (Q7) was also good, resulting in a general cleanliness; if the farmers cared for cleaning the milking equipment (Q6), they also maintained it in good condition (Q8) and performed good postmilking teat disinfection (Q4). Considering the global milking variable, that is, the combination of all Q variables, it had no or only a minimal impact on *Staph. aureus* cow prevalence within a herd. The HA had an effect, as reported by Barkema et al. (2006), but this small effect was not significant in the best model analysis. Our study showed a strong association between the prevalence of *Staph. aureus* IMI and the presence of the *adlb* gene ($P < 0.001$) in both the univariable and multivariable models. In the multivariable analysis, the model providing the best prediction included pCC and ADLB as the only significant predictors. Interestingly, the difference in OR obtained in the 1-variable and 2-variable models for CC8 and CC97 (the only CCs where *adlb* was detected) suggests that it is the presence of the *adlb* gene, not the circulation of these CCs in a herd per se, that leads to higher *Staph. aureus* within-herd prevalence. The within-herd prevalence of *Staph. aureus* IMI was always higher than the population average in farms in which CC8 or CC97 were the predominant CCs when they carried the *adlb* gene, whereas it was lower than the population average when the predominant CC8 and CC97 did not carry the gene.

These results demonstrate that the genetic properties of the *Staph. aureus* circulating within a herd may affect IMI prevalence and play a crucial role in the resultant herd problem. In particular, our findings show that the presence of a strain harboring *adlb* may be associated with the within-herd prevalence of IMI. As for herds with intermediate or high prevalence of IMI caused by *adlb*-negative *Staph. aureus*, such as CC126, on top of the standard factors HA, UHS, TCS, and MRS, other genetic factors may explain the increased cow prevalence. Other genotypes, such as CC705, seem to be associated with low within-herd prevalence of IMI and to behave similarly to environmental mastitis pathogens (Leuenberger et al., 2019). Interestingly, our results also suggest that, in spite of different environmental influences, intermediately contagious subtypes may occur as well, as in the case of CC97. They may have specific genetic properties that differ from both noncontagious and highly contagious types. However, as this was a cross-sectional study, we cannot exclude the possibility that cow prevalence in group 1 would have increased over time, reaching prevalence typically observed in group 2. Additional genomic and field studies are required to support the hypothesis of intermediate contagiousness.

These findings, together with our previous observations (Fournier et al., 2008; Graber et al., 2009; Cremonesi et al., 2015; Luini et al., 2015), indicate that bovine *Staph. aureus* per se is not contagious, but that it acquires this property most likely by horizontal transfer of appropriate genetic elements. In fact, our bioinformatics studies demonstrate that the *adlb* gene is part of a staphylococcal cassette chromosome that is known to be transferable (Malachowa and DeLeo, 2010). Based on these observations, the transfer of the *adlb* gene among different CCs is theoretically probable. Indeed, the present study showed that such a transfer can happen because the *adlb* gene was observed in both CC8 and CC97. However, further analyses are required to confirm and fully describe this gene transfer.

This study has potential limitations. Because we conducted a cross-sectional study, we cannot rigorously define the contagiousness of the strains circulating in the farms, which would require longitudinal studies that measure the incidence of infection over time. Considering the available resources and the need for farmers' consent, we preferred to conduct a cross-sectional study enrolling a greater number of herds (i.e., 60), rather than a longitudinal one on a restricted number of herds. Above all, this allowed us to collect a greater number of *Staph. aureus* isolates from different farms for the molecular analyses, with a cost-benefit ratio favorable to the informativeness of the study.

The second limitation concerns the checklist of 8 questions: although it is only internally validated, it is based on the experience of the Italian National Reference Center for milk quality to specifically address Italian milking practices, and it is largely inspired by the National Mastitis Council's Recommended Mastitis Control Program.

CONCLUSIONS

Our study showed the crucial role of the genetic properties of *Staph. aureus*, especially the *adlb* gene, in determining the prevalence of IMI within a herd. Environmental and management factors, which have long been considered predisposing to the spread of contagious mastitis (i.e., caused by *Strep. agalactiae*, *Mycoplasma bovis*, or *Staph. aureus*), may be less relevant if the disease is caused by a *Staph. aureus adlb*-positive strain. For these reasons, use of a molecular test such as *adlb*-targeted PCR in the diagnostic routine is of paramount importance. However, without a specific molecular characterization of the circulating *Staph. aureus*, hygienic and management measures for prevention of contagious mastitis should not be neglected, because they play a fundamental role in *Staph. aureus* mastitis control and eradication programs. Longitudinal studies may be useful to confirm the role of *adlb* in the mechanisms of contagiousness; further analyses using whole-genome sequencing could highlight other genes involved in the high prevalence of *Staph. aureus* IMI caused by *adlb*-negative strains, such as CC126.

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