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## CAN WE ERADICATE *STAPHYLOCOCCUS AUREUS* MASTITIS?

*Alfonso Zecconi*

Dipartimento Patologia Animale, Igiene e Sanità Pubblica Veterinaria, Sezione di Malattie Infettive  
Università degli Studi di Milano, Italy  
[alfonso.zecconi@unimi.it](mailto:alfonso.zecconi@unimi.it)

### 1. INTRODUCTION

*Staphylococcus aureus* is the most frequently isolated contagious udder pathogen worldwide, and it has the same role that *S. agalactiae* had in many dairy herds in '70s and '80s. Segregation has been applied to eradicate *S. agalactiae* IMI obtaining a worldwide decrease of the prevalence of this microorganism, and segregation has been also applied for *S. aureus* control, but on a smaller scale. Indeed, segregation alone is not enough and additional measures may be necessary (Fox *et al.* 1991). Moreover, the presence of intramammary infections, without large increase of SCC (Zecconi & Piccinini, 2002) induce many practitioner and farmers to think that infections has low impact on milk quality and yield.

### 2. DO WE NEED TO ERADICATE *S. AUREUS*?

A general misunderstanding of the epidemiology of *S. aureus* IMI and, sometime, inadequate objective information are common for what concerns *S. aureus* control. Insofar, a rational approach to the problem could help the practitioner and the farmer to take the correct decisions. The first step is to define correctly the problem and the second step is to apply the proper measures to control it.

To define the problem we should answer to the following questions:

- has *S. aureus* IMI an economic impact on the dairy herd?
- has *S. aureus* IMI an impact on food safety?
- could *S. aureus* IMI be efficiently cured?
- has *S. aureus* reservoirs outside the mammary gland that could affect the results of the control program?

There are scientific and practical data that allow us to answer to the previous questions and I will try to summarize this information in the following paragraphs.

## **2.1 Has *S. aureus* IMI an economic impact on the dairy herd?**

Recent studies addressed the economic impact of *S. aureus* infections. In one of these studies, a large survey in New York State, Wilson *et al.* (1997), estimated the effect of IMI on SCC and milk yield in 1601 herds (108.312 cows), where 11.3% of the cows were *S. aureus* positive. The cost of IMI was estimated by DHIA records and *S. aureus* positive cows showed a loss of 185\$ (with milk price at 13\$/cwt), when 305 mature equivalent milk (305ME) was considered and 160\$ when linear score was considered. The comparison of milk yield (305ME) showed that negative cows produced 9578 kg vs. 8929 kg produced by negative ones. More recently Grohn *et al.* (2004) evaluated the effect of pathogen-specific clinical mastitis on milk yield in 2 dairy herds. Primiparous cows, *S. aureus* positive, after clinical mastitis occurrence produced about 8.4 kg/d less milk than before the outcome of clinical mastitis. In pluriparous cows the decrease was lower with a maximum of 5.5 kg/d after clinical mastitis outcome, and the differences were not statistically significant. A field study in 1431 dairy herds in Lombardia (an Italian region) confirmed that *S. aureus* IMI had a severe economic impact on milk production (Piccinini *et al.* 2003ab). Average cow milk yield in *S. aureus* negative herds was compared with the yield in positive ones (30% of all herds), stratified by herd size. A total loss of 5.778 tons of milk/year was observed, while individual costs were estimated in the range between 55 and 113 € for each cow in *S. aureus* positive herds.

## **2.2 Has *S. aureus* IMI an impact on food safety?**

Staphylococcal food poisoning is a leading cause of foodborne illness worldwide. Center for Disease Control and Prevention in USA estimated an average of 21 outbreaks (16.4% of total number of confirmed bacterial foodborne outbreaks) yearly for the period 1973 through 1992. A worldwide surveillance of foodborne disease between 1985 and 1989, which included 17 countries, showed that the highest incidence of staphylococcal foodborne diseases was 58.5 outbreaks per 10<sup>7</sup> population in Cuba, Hungary, and Finland (Wong & Bergdoll, 2002). Moreover, De Buyser *et al.* (2001) showed that both pasteurized and unpasteurized milk and milk products were involved in the reported outbreaks.

The role of *S. aureus* enterotoxins in food poisoning was suggested in 1914, but only in 1929 the association between strains and the diseases was demonstrated. Since then, an increasing number of Staphylococcal Enterotoxin (SE) has been described. Indeed, there are at least 11 classified SE (Fueyo *et al.* 2005). The frequency of isolates harboring one or more genes coding for enterotoxins varies from 20% to 100% depending on the source of the isolates and the geographical area (Larsen *et al.* 2002; Omoe *et al.* 2002; Zecconi *et al.* 2006b; Zschock *et al.* 2005). Moreover, as the detection methods improve, the frequency of gene-positive isolates from cow milk increases, confirming that the potential of enterotoxin production in milk is high.

These data suggest that *S. aureus* IMI could represent a risk for the presence of enterotoxins in milk and milk products.

## **2.3 Could *S. aureus* IMI be cured?**

The application of antibiotic therapy should follow the principles of a prudent use of antibiotics, as proposed by various scientific organizations. To apply a prudent and efficacious protocol for mastitis treatment, different factors should be considered (Osteras *et al.* 1999; Radostits *et al.* 1994): type of pathogen involved, antibiotic susceptibility patterns, severity of inflammatory response, duration of infection, stage of lactation and age of the cow.

These principles should be applied also to *S. aureus* IMI and on this aspect, the most comprehensive analysis of the different factors affecting cure rate for clinical, subclinical mastitis and for dry-cow therapy for *S. aureus* has been proposed by Sol and co-workers (Sol *et al.* 1994, 1997, 2000). The results of these papers are summarized to give an overview of the factors affecting *S. aureus* cure rate (Table I).

**Table I. Factors affecting *S. aureus* cure rate after therapy for clinical, subclinical mastitis and at drying-off (Sol *et al.* 1994, 1997, 2000)**

Factors	Cure rate after therapy for		
	Clinical mastitis	Subclinical mastitis	Drying off therapy
SCC	Higher < 10 <sup>5</sup> /ml	Higher < 10 <sup>6</sup> /ml	Higher < 10 <sup>6</sup> /ml
Hind vs. Front qt.	Not evaluable	Lower for hind qt.	Lower for hind qt.
Age	No significant differences	No significant differences	Decreases as age increases
Days in milk	Higher < 20 DIM	No significant differences	Not evaluable
No infected qt.	Not evaluable	Not evaluable	Lower when > 1 qt infected
Clinical signs	No significant differences	Not evaluable	Not evaluable

When these factors are considered and the therapy is applied selecting molecules in relation to the results of susceptibility and a rational and efficient protocol is followed, the cure rate could be higher than 75% both for dry cow-therapy and for treatment of fresh cows (< 30 after calving) (Sol *et al.* 1994; Zecconi *et al.* 1999).

In recent years, several therapeutical protocols based on different combination of treatments (IM + IMM, different combination of molecules, different treatment timing, prolonged administration) have been proposed. It is beyond the scope of this paper to discuss the rational and the results of these proposals. However, I would like to recommend to select the protocol after an evaluation of its efficacy and a very careful analysis of the cost-benefit ratio, when complicate and/or expensive treatment are proposed. Indeed, in many cases, the simple intramammary treatment at drying off and/or at the beginning of lactation are, by far, the most efficient and economical method to cure *S. aureus* IMI.

#### **2.4 Has *S. aureus* IMI reservoirs outside the mammary gland that could affect the results of the control program?**

Teat cup is considered the major fomite of IMI and the teat skin the major extra-mammary reservoir. Indeed, Davidson (1961) in an intensive and extensive study of sites of colonization and IMI found the udder and teat skin to be an important reservoir associated with *S. aureus* mastitis. However, there were types, recovered from the mammary gland skin that never caused mastitis. Thus colonization of the extra-mammary sites did not always lead to IMI, and some strains were more likely to be resident in extra- and intra- mammary locations.

Zadoks *et al.* (2002) suggest that the teat skin is not a likely reservoir for *S. aureus* IMI. Such an argument is opposite to that reported (Fox *et al.* 1991; Larsen *et al.* 2000; Roberson *et al.* 1994). Transmission of human strains to cattle, although possible, is not a major factor in the development of *S. aureus* IMI.

Matos *et al.* (1991) suggest that there are several other potential sources of *S. aureus* on a dairy, as this pathogen could be isolated from bedding and air of the parlour. Flies were not a source of *S. aureus* in this study, which is in contrast to that reported by Owens & Nickerson (1998). In a more extensive study, Roberson *et al.* (1994) examined the ecology of *S. aureus* on dairies. In low-prevalence herds (< 3%), bedding, insects, and water did not yield *S. aureus*, but high-prevalence herds did on average have some of these samples that were positive. They concluded that most

likely reservoir of *S. aureus* IMI was the cow or the heifer, and that the environment although possible, a less likely reservoir for this disease, particularly when the incidence of IMI decreases, as during a control program.

To summarize the answers to our questions, based on the existing evidence we can reasonably affirm that:

- *S. aureus* has a variable, but large economic impact on dairy herd,
- *S. aureus* could represent a significant risk factor for food safety,
- efficient and rational *S. aureus* therapeutical protocols have a cure-rate not lower to the ones reported for other pathogens,
- *S. aureus* is not an obligate parasite of the mammary gland, but the importance of potential reservoirs on other body sites or in the environment significantly decreases as the prevalence of IMI decreases in the herd.

### **3. HOW *S. AUREUS* CAN BE ERADICATED?**

Once we recognize the importance of *S. aureus* IMI and the realistic chances to control it, the second step is now to develop a control strategy. The potential rational approaches are:

- apply a test-and-cull strategy,
- apply a control program based on fomites control,
- apply a control program based on segregation.

#### **3.1 Test-and-cull strategy**

Culling is often suggested as the only way to control *S. aureus* (Saperstein *et al.* 1988; Stott *et al.* 2002). However, there is poor scientific evidence either on the efficacy or on the economic return of this approach. Hoblet & Miller (1991) compared *S. aureus* control programs in 3 herds with a different culling policy. The first herd kept a yearly culling rate in the range 34-37%, the second increased from 24% to 34% and the third from 27 to 35%. At the end of the program all the three herds had a very similar culling rate, but only the third one, applying a well-managed program achieved the control of *S. aureus* IMI.

Therefore, culling could be a component of a control program, mainly as the most efficient way to remove the chronic *S. aureus* cows and to thus reducing the pressure on healthy ones. However, as a main method of control it showed to be poorly efficient and with a negative economic impact. Indeed, the presence of undetected cows allows the spread of infection among the herd, perpetuating the disease.

#### **3.2 Control program based on fomites control**

Fomites control could be mainly achieved through proper milking hygiene. The importance of a proper milking hygiene to prevent intramammary infections is well-known (Bramley & Dodd, 1984; Fox *et al.* 1991; Naive *et al.* 1969; Radostits *et al.* 1994) and all the recommendations for milking time hygiene can be applied to *S. aureus* control. The most important recommendations are: use of disposable gloves by the milker, use of single-service towel/paper to clean the teat, or pre-dip where allowed, forestripping, and use of postmilking teat disinfectants of known efficacy.

Milking time hygiene and milking procedures have a significant impact on teat tissue and teat skin conditions (Hamann, 1987; Hamann *et al.* 1994; Hillerton *et al.* 2001; Mein *et al.* 2001). Teat tissue and skin are considered the first line of defense against infections and, recently, the importance of preserving teat skin conditions has been recognized as one of the most important factors to reduce the risk of infections (Neijenhuis *et al.* 2000; Zecconi *et al.* 2006a). However, proper milking hygiene is not enough to control infections, because it reduces but does not stop the spread of infections among cows.

To overcome this problem, backflush was proposed many years ago as an effective way to clean the cluster and the liner. The system showed some efficacy in reducing the bacteria load, but it is not enough alone to control the spread of infections, and the supposed reduction in the spread of IMI do not justify the cost of the equipment (Fox *et al.* 1991; Hogan *et al.* 1984; Smith *et al.* 1985).

Milking hygiene is an essential part of a control program, but alone it does not fully control the spread of *S. aureus* IMI.

### **3.3 Control program based on segregation**

Efficient control programs based on segregation follows the general principles of contagious mastitis control (White, 1965) and are based on precise diagnostic procedures and strict control of segregation of infected cows.

Recently we reported the result of a standardized and detailed mastitis control program in 9 commercial dairy herds (Zecconi *et al.* 2003).

The main steps in the control program were:

- application of a precise and consistent milking procedure that included use of disposable gloves by the milker, use of a single-service towel to clean the teat, forestripping, and use of postmilking teat disinfectants of known efficacy,
- establishment of a milking sequence to reduce infection risk, by milking healthy cows first, followed by cows and heifers that have recently entered the herd either through purchase or freshening, and then milking cows with *S. aureus* IMI last,
- after the first sampling of all lactating cows at the time of enrollment to segregate infected cows, a precise sampling schedule is used. All cows that have recently calved are sampled 7 and 14 days after enrollment, purchased cows are sampled 7 and 14 days after entry into the herd. Cows with *S. aureus* IMI are segregated and are not sampled again until they had calved. Non-infected cows are sampled again 2, 4, 7, 10, 14 and 18 months after the first sampling,
- diagnosis of *S. aureus* IMI is performed with mammary quarter milk samples via bacteriologic culture on 5% blood-agar media. Quarter samples are collected to increase the sensitivity of detection. Recovery of a single colony of *S. aureus* is considered a positive result indicating an IMI,
- all mammary quarters of all cows are treated at drying-off with a commercial antimicrobial treatment. Choice of the product is based on the susceptibility of the *S. aureus* strains isolated in the herd as determined by use of the disk diffusion method,

- treatment of infected lactating cows without clinical signs is restricted to those with  $\leq 3$  lactations and in their first 30 days of lactation, to avoid treatments with an expected poor cure-rate. These cows are moved to a hospital pen to be sampled 7 and 14 days after the end of the antimicrobial withdrawal period. Only cows that are deemed cured because of negative results of 2 consecutive bacteriologic cultures of milk samples are allowed to leave the hospital pen,
- antimicrobial and anti-inflammatory treatments are administered to cows that developed clinical mastitis, independently of their *S. aureus* infection status, and these cows are moved to the hospital pen to be sampled 7 and 14 days after the end of the withdrawal period. Only cows that were deemed cured are allowed to leave the hospital pen to come back to the group of origin,
- at the herd level, farm managers are advised to improve and maintain proper bedding hygiene,
- the control program is monitored directly by monthly visits by a trained practitioner, discussion of analysis results, and indirectly by a weekly check of samples sent to the laboratory, to ensure compliance.

Results of this field study indicated that an incidence rate  $< 1$  new IMI/100 cow-months can be achieved after approximately 10 months after the start of the control program for IMI. Initial herd prevalence appeared to influence the incidence rate of new *S. aureus* IMI, although this influence was unexpected. Indeed, low-prevalence herds had a significantly higher incidence than did high-prevalence herds up to 8<sup>th</sup> month of control. Reasons for the differences between these groups include differences in infection rates with different strains of *S. aureus* or different application of the control program procedures among the various farms.

Analysis of the relationship between parity and incidence of IMI revealed that heifers were at nearly twice the risk of new IMI during lactation, compared with that of cows with 3 or more parities, at the beginning of lactation, and nearly 3 times the risk at the end of lactation. Indeed, young cows and freshening cows are the most likely to develop new IMI among uninfected cows when segregation of infected cattle is used. Uninfected cattle should be carefully checked and specific procedures should be applied to reduce the risk of infection. It could be also suggested that heifers should be housed separately from older cows for at least 2 weeks before calving, to reduce the risk of becoming infected from older cows.

Based on our field experiences and on the scientific evidence we can responsibly answer to the question posed in the title of this paper saying: yes, *S. aureus* IMI can be eradicated from a dairy herd. It is not possible to eradicate *S. aureus* from a herd, because it is not an obligate parasite of the mammary gland. However, if the proper biosafety measures are put in place, the chance that extra-mammary *S. aureus* be again a source of IMI are negligible.

#### **4. OTHER QUESTIONS**

When such a program is proposed to practitioners and farmers two other questions are generally asked. The first one concerns the cost-benefit of the program, while the other one is about the measures to adopt when the program is concluded.

##### **4.1 The control of *S. aureus* IMI is economically sustainable?**

One of the most obvious side effects of the successful adoption of contagious mastitis control procedures, is the decline in the prevalence of non-contagious mastitis, therefore also SCC and clinical mastitis incidence is reduced (Zecconi *et al.* 2003, 2004).

Moreover, the available results support both the efficacy and the positive economic return of implementing a control program (Hoblet & Miller, 1991; Wilson *et al.* 1995) report the data on 76 dairy herds with an initial prevalence of *S. aureus* IMI greater than or equal to 10%. Criteria for inclusion were that herds did not change teat disinfection or dry cow treatment practices, did not segregate cows that were positive for *S. aureus* at the initial visit, and did not cull > 50% of cows found to be positive on the initial visit. During a follow-up period (6 to 24 mo), segregation or separate milking of cows that were positive for *S. aureus* reduced prevalence from 29.5 to 16.3% and bulk tank SCC from 600,000 to 345,000/ml. Prevalence of *S. aureus* mastitis was unchanged for herds that did not segregate cows, and the reduction in SCC for non-segregated herds was also smaller (from 698,000 to 484,000 cell/ml).

To assess the cost-benefit ratio of the control program described in this paper, a simulation analysis was developed for a 4-year program (including 2 years for the full program plus 2 years when only freshening heifers are checked), following the principles and procedures described by Dijkhuizen & Morris (1997).

For the preliminary analysis the following parameters were considered:

- discount rate: 3%,
- costs: first 2 years 50 €/cow per year, next 2 years 15 €/cow per year (only heifers testing),
- program management: 1500 €/year,
- increased replacement rate: ¼ of *S. aureus* prevalence per year,
- benefits: increase in milk yield 300 kg/cow per year,
- increased SCC premium: range 0.25-1.5 €/100 kg.

The increase of milk yield was the minimum increase observed in the 9 herds between negative and positive cows; the SCC premium is based on the different policy applied by the dairies. We assumed also that the changes in dry-cow therapy, teat-dip and therapy during lactation products costs would be negligible.

The cost-benefit analysis shows that the break-even point is reached at different levels, depending mainly on the level of quality premiums (Zecconi *et al.* 2001). As the premium increases, the break-even point is reached at lower level of prevalence. When the most common premium levels are considered (1.0 and 1.5 € for 100 kg of milk), the positive cost-benefit ratio is reached when *S. aureus* prevalence is respectively higher than 20% and 10%. Only when no quality premium is paid, the break-even point was estimated at a prevalence of 50%.

#### **4.2 What to do once *S. aureus* IMI are controlled (eradicated)?**

The complete control (eradication) of *S. aureus* IMI implies that consistent measures of biosafety and monitoring should be applied, once the program has been completed. Individual SCC, clinical mastitis monitoring and, at least, yearly quarter milk sampling of the whole herd could be simple and unexpensive measures to monitor the herd health. The application of a biosafety program is often less obvious and simple to apply. In the case of *S. aureus* IMI the biosafety measures are aimed to heifers and purchased cows. These animals represent a potential threat for herd health (not only for *S. aureus* IMI) and we don't recommend this practice. If unavoidable, purchased cows should be sampled twice at 7 and 14 days after calving or after entering into the herd (quarter milk samples), and milked last.

The role and the control of heifers in the epidemiology of *S. aureus* IMI are more controversial. Indeed, traditionally the prevalence of mastitis in heifers pre-partum has been underestimated, given the concept that heifers without a developed gland are not susceptible to IMI. Fox *et al.* (1995) summarize some recent studies showing a prevalence estimated as low as approximately 1% of all heifers with *S. aureus* IMI at parturition with some estimates approaching 10%. Therefore, heifers could be important risk factor acting both as a reservoir and as a fomite for *S. aureus*. Middleton *et al.* (2002) report that herds that purchased replacement heifers had a higher prevalence of *S. aureus* mastitis than other herds. Herds purchasing replacement heifers had more new strains of *S. aureus* that did not import foreign cattle, closed herds. In the extended study by Roberson *et al.* (1994), there was a significantly greater percentage of *S. aureus* isolated from epidermal and mucosal sites of heifers in high prevalence herds. In particular, isolation was twice as common from the mammary skin of heifers in high herds, emphasizing the possible importance of the mammary gland area as reservoir of this disease. Overall, heifers colonized with *S. aureus* in the mammary gland area were 3.4 times as likely to calve with *S. aureus* IMI, and this was the largest risk factor of disease.

Antimicrobial treatment before calving has been suggested to decrease the infection rate in heifers (Oliver *et al.* 2003; Zeconi *et al.* 1999), but this treatment could further decrease the sensitivity of bacteriologic testing after calving, lowering the concentration of bacteria in milk and it is not economically justified (Borm *et al.* 2005). Therefore, we suggest that this practice should be discontinued when a program based on segregation and frequent testing is used.

As recommended for purchased cows, heifers should be checked twice at 7 and 14 days after calving (quarter milk samples), at least for two years after the end of the program in a closed herd. If heifers are purchased or raised off-site, they must be always be sampled twice at 7 and 14 days after calving (quarter milk samples).

## **5. CONCLUSIONS**

*S. aureus* affects many dairy herds worldwide, which justify the adoption of a control program because of the cost of the disease, the risk for milk safety and, not last, for the decrease in cow welfare. Therefore, both from ethical and economic point of view practitioner should advise farmers to adopt a rational and consistent control program based on segregation, efficient hygienic and management procedures and rational therapeutical protocols. Results of our field studies suggest that within the proposed control program, the most important control procedure is precise and consistent sampling to identify new *S. aureus* IMI and to segregate infected cattle.

To be successful this program needs a motivated and cooperative farmer with a motivated and well-trained practitioners that will insure the application of the different control measures and the consistent monitoring of the herd.

## **6. SUMMARY**

*Staphylococcus aureus* is the most frequently isolated contagious udder pathogen worldwide. The need to apply a control program is often put under discussion in relation to diagnostic, therapeutical and economical concerns. In this paper these aspects were discussed and the characteristics of a rational and economically sustainable control program are described.

## **7. KEY WORDS**

*Staphylococcus aureus*, mastitis, control program.

## 8. RESUME

*Staphylococcus aureus* est l'agent pathogène contagieux le plus fréquent en élevage laitier dans de nombreux pays. La nécessité et la possibilité de contrôler ces infections sont souvent mises en doute à cause de problèmes diagnostiques, thérapeutiques et économiques. Cette étude présente les aspects en faveur ou contre l'application d'un programme de contrôle. De plus, sont illustrés les caractéristiques d'un programme rationnel et les bénéfices économiques dérivant d'une telle application.

## 9. MOTS CLES

*Staphylococcus aureus*, mammite, programme de contrôle.

## 10. ZUSAMMENFASSUNG

*Staphylococcus aureus* ist der häufigsten kontagiose Erreger von Euterinfektionen in milcherzeugenden Betrieben in vielen Ländern. Die Notwendigkeit aber und die Möglichkeit diese Entzündungen zu kontrollieren wird oft bestreitet, aus diagnostischen, therapeutischen und wirtschaftlichen Gründen. Die verschiedenen Aspekte für oder gegen die Anwendung eines Kontroll-Programms, zusammen mit der Rationale solches Programms und dem folgenden Gewinn, werden besprochen.

## 11. SCHLÜSSELWÖRTER

*Staphylococcus aureus*, Mastitiden, Kontroll-Programms.

## 12. RESÚMEN

*Staphylococcus aureus* es el patogeno contagioso mas difundido en los hatos lecheros, en numerosos paises del mundo. A menudo se presentan dudas sobre la necesidad y posibilidad de controlar estas infecciones, por factores diversos como son: diagnostico certero, aspectos terapeuticos y economicos, principalmente. En este trabajo se plantean varios puntos, unos a favor y otros en contra de la aplicacion de un programa estricto de control de *Staphylococcus aureus*, en los hatos lecheros. Y se especificarán las características de un programa racional y los beneficios economicos de la aplicacion del mismo.

## 13. PALABRAS CLAVES

*Staphylococcus aureus*, programa de control, infecciones intramamarias.

## 14. REFERENCES

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